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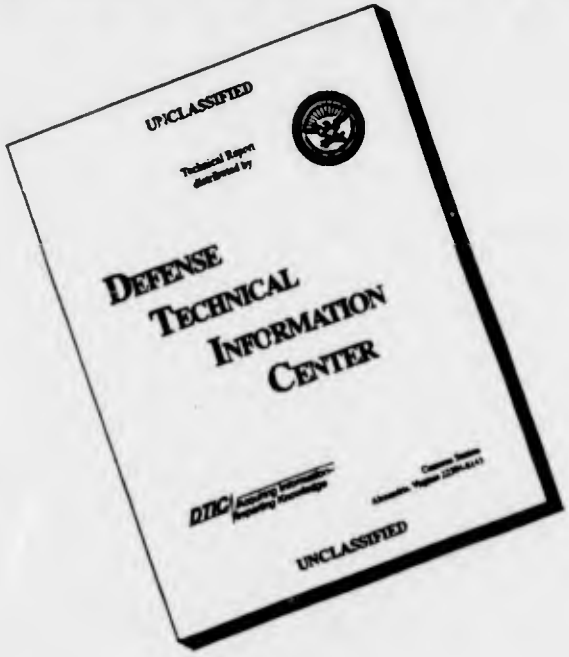

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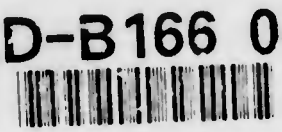

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TITLE: RESEARCH IN DRUG DEVELOPMENT AGAINST VIRAL DISEASES OF MILITARY IMPORTANCE (LARGE-SCALE ANTIVIRAL DRUG SCREENING)

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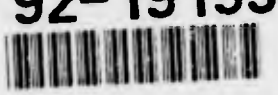

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19. ABSTRACT The purpose of this program was to evaluate the efficacy of candidate antiviral compounds against a spectrum of viruses of military importance. This program involved (a) prescreen testing of natural products for antiviral efficacy *in vitro*, (b) primary testing of synthetic compounds and active natural products *in vitro* against an expanded spectrum of viruses of military importance, (c) confirmatory testing of all active lead compounds from the prescreen and primary screen, (d) secondary *in vitro* and *in vivo* studies of selected active compounds with best broad spectrum antiviral activity. The target viruses for *in vitro* testing were Vaccinia Virus (VV), Yellow Fever Virus (YF), Japanese Encephalitis Virus (JE), Venezuelan Equine Encephalomyelitis Virus (VE), Punta Toro Virus (PT), Sandfly Fever Virus (SF) and Human Immunodeficiency Virus (HIV). The secondary *in vitro* and *in vivo* testing was carried out with three major classes of viruses a) Filoviruses, b) Arenaviruses and c) Bunyaviruses. Approximately 5500 (2762 prescreen compounds and 2713 primary screen compounds) were received for *in vitro* evaluation and over 34,000 assays were performed on this contract. Compounds were identified in nearly all virus systems that have confirmed antiviral activity equal or exceeding that of the various positive control compounds (Ribavirin, Selenazofurin, Ara-A, ddC and AZT). Many of these compounds represent potent and selective new antiviral agents.

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The prescreen protocol (YF, VE and PT viruses) successfully identified potential active materials (~5%) for further confirmatory testing. Confirmatory testing of these potential active compounds were carried out in the primary screen against a broader range of more virulent viruses (VV, YF, JE, VE, PT and SF). Approximately seventy percent of the active prescreen compounds showed some degree of activity against one or more of these virulent viruses.

During this ten-month contract period, a number of compounds were found to exhibit highly significant activity against VV *in vitro*. Compounds AVS-9480, 9589, 9607, 9590 and 9795 demonstrated the greatest *in vitro* promise, having Selectivity Indices (SI) that ranged from 7 - 26. Nineteen excellent lead compounds were found against the Yellow Fever Virus *in vitro* and 12 compounds appeared to have excellent *in vitro* antiviral potential against the Japanese Encephalitis Virus. Fifteen compounds demonstrated excellent *in vitro* antiviral activity against Venezuelan Equine Encephalomyelitis Virus. At least 20 excellent lead compounds were found against the Punta Toro Virus *in vitro* and 38 compounds produced excellent *in vitro* antiviral activity against Sandfly Fever Virus. AVS-6724 demonstrated broad spectrum activity against YF, JE, SF, PT, VV and VE Viruses.

Anti-HIV *in vitro* activity was observed with 35 AVS compounds. Three of these compounds produced significant anti-HIV activity with selectivity indices of > 100.

In vivo data indicates that the SCID mouse model is suitable for evaluating potential active compounds against filoviruses. Several compounds which are S-Adenosylhomocysteine Hydrolase Inhibitors showed good activity. AVS-0303 and AVS-4275 appeared to be the most active. Ribavirin has shown to be moderately effective both *in vitro* and *in vivo* against Venezuelan Hemorrhagic Fever. Initial success has also been obtained with *in vivo* studies against the Hantaan Virus with S-Adenosylhomocysteine Hydrolase Inhibitors.

Due to the conflict in the Gulf (Desert Storm) in 1991 and lack of funding for these antiviral studies, this contract was cancelled (10 months into the work) on 12/31/91.

FOREWORD

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_____For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45 CFR 46.

_____In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

William M. Shannon
PI Signature

January 27th 1992
Date

Executive Summary

This is the Final Progress Report on SRI Project No. 7289, Contract No. DAMD17-91-C-1050. It covers the progress of the research program during the report period from February 1, 1991 to December 31, 1991.

The goal of this program was to provide *in vitro* prescreen/confirmatory testing of large numbers of compounds from a variety of sources for antiviral efficacy against multiple viruses of specific importance to the military. The program consisted of five major task areas: a) Chemical acquisition, drug preparation and database management performed at USAMRIID, Fort Detrick, Maryland, b) Antiviral prescreen testing of approximately 10,000 submitted test materials (chemical compounds/natural product extracts). This included confirmatory testing of active compounds (from prescreen) using the primary screen protocol and recommending compounds most active from the primary screen for secondary evaluations *in vitro* and *in vivo* at USAMRIID, c) Secondary antiviral evaluation (both *in vitro* and *in vivo*) against an expanded spectrum of viruses of military importance at USAMRIID, Ft. Detrick, Maryland, d) Maintenance of a centralized cell culture and drug prep laboratory support service for operations at SRI-Birmingham, e) Management of large-volume database, reporting these data to the suppliers in a timely manner and tracking the flow of compound testing at SRI-Birmingham and SRI-USAMRIID.

During the abbreviated period of this contract (stop work order issued in August 1991, with an orderly phase-out of operations by December 31, 1991), SRI tested 11,555 materials for antiviral efficacy in *in vitro* prescreen assays. Of this materials, we identified 455 potentially active antiviral agents (~5% of the materials screened) for confirmatory testing. These agents were included with earlier identified actives from the prescreen and confirmatory testing was carried out in the more quantitative primary *in vitro* screen using a broader range of viruses: Vaccinia Virus (VV), Yellow Fever Virus (YF), Japanese Encephalitis Virus (JE), Venezuelan Equine Encephalomyelitis (VEE), Punta Toro Virus (PT), Sandfly Fever Virus (SF) and Human Immunodeficiency Virus (HIV). Approximately 70% of these actives from the prescreen exhibited some degree of antiviral activity against one or more of these target viruses in the primary screen.

The results of our large-scale antiviral screening program for USAMRIID during the period February 1, 1991 to September 30, 1991, were that 41 compounds were identified with activity against VV (8 of these compounds exhibited highly significant antiviral effects in which virus-induced cytopathic effects were reduced by $\geq$95%), 144 compounds showed activity against YF virus with 19 of these compounds exerting highly significant antiviral effects ($\geq$95% reduction in CPE), 82 compounds were active against JE virus with 12 of these exhibiting excellent antiviral potential ($\geq$95% reduction in CPE), 174 compounds demonstrated antiviral efficacy against VEE virus with 15 of the compounds producing highly potent antiviral effects ($\geq$95% reduction in CPE), 161 compounds exhibited activity against PT virus with 20 compounds showing the greatest activity ($\geq$95% reduction in CPE), 230 compounds were identified active against SF virus with 38 of these compounds exhibiting excellent antiviral potential ($\geq$95% reduction in CPE). The program also included primary testing of compounds against HIV-1, the etiologic agent of AIDS. During the period covered by this final report, SRI performed 856 *in vitro* assays with HIV-1 and identified 35 compounds with activity ($\geq$50% reduction in HIV-induced CPE). Three (3) of these compounds were identified to have excellent potential as anti-AIDS agents ($\geq$95% reduction in HIV-induced CPE with selectivity indices > 100).

Extensive work was performed at USAMRIID by SRI technical personnel to further pursue active candidate antiviral leads in secondary *in vitro* studies and in available animal models. Developmental work conducted during this report period demonstrated that an ELISA assay was suitable for performing *in vitro* antiviral studies with filoviruses such as Ebola virus. In addition, a SCID mouse model was employed for the evaluation of antiviral agents against Ebola virus *in vivo*. Several compounds were identified that have excellent antiviral efficacy against this virus *in vitro* and *in vivo*. These compounds should be evaluated in primates as candidate antiviral drugs for potential clinical use.

During this report period, it was also shown that the antiviral drug ribavirin is effective against a new arenavirus, Venezuelan Hemorrhagic Fever Virus (VHF), both *in vitro* and *in vivo*. This virus was isolated recently in an outbreak that caused substantial mortality and morbidity in Venezuela. Thus, the program has identified an antiviral agent that should be effective in the treatment of this lethal viral infection.

It is hoped that funds will be forthcoming in FY 1993 to restore this important program at USAMRIID. There is currently no other agency, public or private, that is conducting similar research work to identify and develop drugs against these target viruses of military importance.

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1. INTRODUCTION

This is the Final Progress Report on SRI Project No. 7289, Contract No. DAMD17-91-C-1050. It covers the progress of the research program during the report period from February 1, 1991 to December 31, 1991.

The goal of this program was to provide *in vitro* prescreen/confirmatory testing of large numbers of compounds from a variety of sources for antiviral efficacy against multiple viruses of specific importance to the military. The program consists of five major task areas: a) chemical acquisition, drug preparation and database management performed at USAMRIID, Fort Detrick, Maryland, b) antiviral prescreen testing of approximately 10,000 submitted test materials (chemical compounds/natural product extracts). This included confirmatory testing of active compounds (from prescreen) using the primary screen protocol. Recommend compounds most active from primary evaluation for secondary evaluations *in vitro* and *in vivo* at USAMRIID, c) secondary antiviral evaluation (both *in vitro* and *in vivo*) against an expanded spectrum of viruses of military importance at USAMRIID, Ft. Detrick, Maryland, d) maintain a Centralized Cell Culture and Drug Prep Laboratory support service for operations at SRI-Birmingham, e) management of large volume data, reporting these data to the suppliers in a timely manner and tracking the flow of compound testing at SRI-Birmingham and SRI-USAMRIID.

One of the primary missions of the U.S. Army Medical Research and Development Command is to perform studies on the pathogenesis, diagnosis, epidemiology, prophylaxis, and treatment of infectious diseases of military importance. The Army's infectious disease research program, conducted by the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) at Fort Detrick, is primarily concerned with medical defense against (a) naturally-occurring infectious diseases that could seriously interrupt U.S. military operations such as troop mobilization and deployment and (b) the threat of infectious diseases or toxic effects caused by the potential field use of biological warfare (BW) agents, either conventional BW agents or altered agents, by an unfriendly force.

The U.S. Army has a recognized need for new chemical compounds that will be useful as prophylactic or therapeutic antiviral drugs to treat U.S. military personnel who are at risk of exposure to, or who might become infected with, naturally-occurring viruses or altered viruses for which there exists no effective protection or therapy at the present time. The development of selective antiviral drugs for use in the successful treatment of infections with certain exotic RNA viruses (togaviruses, bunyaviruses, arenaviruses, rhabdoviruses, and other, unclassified RNA viruses) is of particular importance to the Army because there are no other research efforts being conducted, either by the government or by the private sector, which are directed toward the control of these virus diseases of military relevance.

In 1973, USAMRIID initiated a research and development program to identify and to pursue new compounds with activity against these exotic RNA viruses. Approximately 1500 compounds have already been screened *in vitro* for selective antiviral effects against these target viruses and a number of the compounds which were found active in cell culture have been evaluated for antiviral efficacy *in vivo*. Several of these compounds (e.g., Ribavirin, Selenazole, and Pyrazofurin) have been extensively tested for efficacy against lethal RNA virus infections in various animal model systems at USAMRIID. To date, the most promising antiviral drug with demonstrated, broad-spectrum activity against these viruses of military importance, both *in vitro* and *in vivo*, appears to be Ribavirin, its prodrug derivatives, and its carboxamidine derivative (AVS-206). Ribavirin has been evaluated in humans infected with Sandfly Fever (SF) Virus, Lassa Fever Virus, and Korean Hemorrhagic Fever (KHF) Virus and has demonstrated marked clinical efficacy against these particular virus infections. This drug will be further developed for general use in military personnel. There is a real need, however, for more potent and more selective antiviral drugs to combat these virus diseases which represent serious threats throughout the world.

Troops in the field are threatened not only by infectious diseases of natural origin, but also by the possibility of BW attack. The commercial development of antiviral drugs for the treatment of the more common respiratory virus, enteric virus, and herpesvirus infections may not solve the problems which are unique to the Armed Forces. These potential problems of encountering exotic viruses and BW agents will not be sufficiently addressed by depending solely on the possibility that antiviral drugs originally developed for the treatment of acute respiratory diseases, enterovirus infections, and herpesvirus infections might also be useful in the treatment of these virus diseases of military relevance. A more direct approach, and one which is clearly indicated, is to focus on antiviral drug development efforts designed to attack these particular virus disease threats that are unique to the Armed Forces.

The U.S. Army Medical Research Institute of Infectious Diseases has, for a number of years, been involved in conducting a unique and ambitious antiviral drug research and development program, primarily directed toward the chemical control of exotic RNA virus infections of military importance. Potential antiviral agents have been synthesized and evaluated against a number of target viruses both *in vitro* and *in vivo*. Program emphasis is currently on the development of antivirals for use in the treatment of infections with alphaviruses, flaviviruses, bunyaviruses, arenaviruses, and other viruses which are capable of eroding combat strength in troops deployed in overseas areas. In addition, current efforts are also being directed toward the development of antivirals for use in the treatment of AIDS through an Inter-Agency Agreement with the National Institutes of Health (NIH).

Members of the *Togaviridae* and *Flaviviridae* families (alphaviruses and flaviviruses) are capable of producing serious hemorrhagic or encephalitic diseases in humans. Infections with alphaviruses [Eastern equine encephalomyelitis (EEE), Western equine encephalitis (WEE), and Venezuelan equine encephalitis (VEE)] have occurred in epidemic proportions in the Americas. Chikungunya and O'nyong-nyong viruses also continue to cause epidemic disease on the African continent. The flaviviruses include several members which cause significant disease in humans. Dengue viruses types 1-4 are prevalent causes of acute illness in the tropics and subtropics of the world. Available vaccines are inadequate to control these infections effectively. Other members such as St. Louis encephalitis virus, Japanese B Encephalitis (JE) virus, and West Nile encephalitis virus cause mild to severe encephalitis diseases in humans. The tick-borne encephalitis virus group, represented by Russian Spring-Summer Encephalitis Virus, has caused widespread encephalitic disease in the U.S.S.R. and Northern Europe with high mortality rates. Yellow Fever (YF), in either the urban or jungle form, continues to be a threat, although the use of the 17-D vaccine is quite effective as a prophylactic measure against this disease. The Army's program is interested in controlling infections caused by the dengue viruses, Japanese encephalitis virus, Russian Spring-Summer encephalitis virus, Yellow Fever virus, and West Nile encephalitis virus.

A number of bunyaviruses have caused epidemic disease in many areas of the world: Rift Valley Fever virus was responsible for a major epizootic in Egypt in 1977-79 with considerable losses in domestic animals (sheep and cattle) and significant mortality among those humans infected with the virus. Sandfly fever virus has also been recognized as an important cause of epidemics in the Mediterranean area, in the Middle East, and in Central Europe. Oropouche, La Crosse, and California encephalitis viruses have all caused significant disease in the Americas. Oropouche virus, for example, has been associated with a number of large human epidemics in Brazil over the past twenty years. Hantaan virus, the causative agent of Korean hemorrhagic fever, causes appreciable mortality and is widely distributed in Asia. It has only recently been shown to belong to the *Bunyaviridae* family. Of the *Bunyaviridae* family, the USAMRIID program has initiated studies with Sandfly fever virus, Rift Valley fever virus, Korean hemorrhagic fever virus, and Punta Toro virus.

Of the *Arenaviridae* family, current interest includes Lassa fever virus, an agent which causes significant lethal disease among infected humans in Africa, especially in Sierra Leone. Other arenaviruses

under current investigation include Junin and Machupo viruses, the causative agents of Argentine hemorrhagic fever and Bolivian hemorrhagic fever, respectively. These agents are found endemic in wide areas of South America. Pichinde (PIC) virus has been used in antiviral studies as a representative of this important family of viruses. Vesicular stomatitis virus is currently employed as a representative of the *Rhabdoviridae* family. Vaccinia Virus is currently employed as a representation of the DNA Virus (Poxviridae). This agent poses a threat to the military personnel as well as the general population because of the lack of antibody protection since no World Health Vaccination Program are now required. VV Virus is also commonly used as a carrier virus for genetic engineering technology, therefore posing an ever-present threat to a laboratory modification of any genetically engineered virulent agent.

Other viruses which cause sporadic but severe hemorrhagic fever in Africa are Marburg and Ebola viruses. These two closely-related agents have been placed in a new family (*Filoviridae*).

The above viruses are those which might be encountered in exotic troop locations and against which troops would not be expected to have any pre-existing immunity. With few exceptions, specific vaccines do not exist for these agents and some of the agents encountered may be poorly classified or may even be unclassified viruses which have not been seen previously. The antiviral chemoprophylaxis/chemotherapy approach may be the best modality to defend against this threat at the present time.

Another recurring problem with naturally-occurring virus infections exists in military boot camps where new recruits are assembled. These troops often develop infections with adenoviruses, influenza viruses, and parainfluenza viruses, sometimes in epidemic proportions. Infections with the adenoviruses have been a distinct military problem for years and multivalent vaccines have been prepared for use in new recruits. Nevertheless, an effective antiviral drug for treatment of adenovirus infections would be quite useful and therefore this virus group is also a target for antiviral chemotherapy in the Army's program.

A number of naturally-occurring viruses could be developed by an adversary into potent biological weapons for use in the field against U.S. forces. Many of the exotic RNA viruses are also potential BW agents, since their dissemination in an area where they are already indigenous could be employed as a means of disguising the source of the infection. In addition, other agents such as smallpox virus could be used very effectively against a susceptible civilian population prior to and during military operations to disrupt logistics and support activities and to create panic and chaos. The threat of BW attack poses some of the same problems as those to be addressed in the defense against naturally-occurring virus infections. Again, a broad-spectrum antiviral drug with selective activity against the RNA viruses could be the only real line of defense against such attach with those particular type of agents. The vaccine approach will only be effective in affording protection against a very limited group of these agents which number in the hundreds of different antigenic types.

With the advances made in the field of molecular genetics, it is now technically possible to genetically engineer altered viruses with enhanced virulence, communicability, drug resistance, and overall threat potential. An example of such misuse of advanced biotechnology would be the insertion of genes for highly toxic peptides such as snake venom toxins, potent bacterial toxins, or other low molecular weight toxins of military importance into the genome of a highly communicable virus such as influenza. The feasibility of inserting foreign genes into vaccinia virus and obtaining expression of those genes in the host cell has already been demonstrated. Recombinant DNA technology has made it possible to insert and express heterologous genes in a variety of different viruses. Effective defense against

possible altered viruses used as BW agents may well depend upon the development of antiviral drugs active against the vector viruses.

The Department of Antiviral Studies at USAMRIID is responsible for the acquisition, identification, and development of potential new antiviral drugs which are effective against viruses of military relevance and which might be useful in the treatment of AIDS. The program is therefore, broad-based and involves the synthesis, primary and secondary testing, and further characterization of novel antiviral compounds with regard to their possible biochemical mechanisms of action, pharmacokinetics, metabolism, optimal formulation, optimal combination with other drugs, and safety in animal model systems. The Department also directs studies in support of IND applications to the FDA for clinical testing of active antiviral agents for use in man.

Since the establishment of its antiviral testing program, the Department of Antiviral Studies, USAMRIID, has evaluated approximately 3,000 compounds in its primary *in vitro* screen. Fewer compounds have been evaluated *in vivo*. Selenazole was reported to have broad-spectrum activity against the exotic RNA viruses *in vitro* and is significantly more potent than Ribavirin against the togaviruses (VEE, YF, JE), bunyaviruses (RVF, SF, KHF), and arenaviruses (PIC) *in vitro*. The activity of this compound against YF virus is most impressive with an ED_{50} of 0.005 mg/ml in cell culture. The evaluation of Selenazole for therapeutic antiviral efficacy *in vivo*, however, yielded disappointing results and pharmacological problems may be responsible for the lack of efficacy in animal model systems. Ribavirin, on the other hand, has been shown in laboratory animal models to have significant antiviral efficacy against the bunyaviruses (RFV, Punta Toro and KHF) and the arenaviruses (PIC, Junin, Machupo, and Lassa Fever). Clinical trials with Ribavirin in patients with Lassa Fever virus or Hantavirus infections have yielded good results.

Ribavirin has also been shown to be effective in the treatment of Sandfly fever virus in human volunteers. Good progress has been made toward developing this particular antiviral drug for general clinical use by the Army, but new agents with higher potency and selectivity against the exotic RNA viruses will hopefully be identified in the expanded USAMRIID antiviral program. Because of the lag time in diagnosing viral disease, treatment with broad-spectrum antiviral agents offers the best hope to successfully defend against both naturally-occurring disease and against possible BW agents in the field. It is unlikely, however, that a single drug will be found that is effective against all of these exotic RNA virus infections, so additional antiviral agents must be developed. There is also a need to explore the efficacy of immunopotentiators, biological response modifiers, interferons, combination chemotherapy, and new approaches to drug delivery to enhance the antiviral efficacy of these agents.

The basic contract at Southern Research Institute was established to enable USAMRIID to evaluate approximately 10,000 test materials for efficacy against 3 different target viruses using the *in vitro* antiviral prescreen testing protocol, 1500 - 2000 compounds for efficacy against a spectrum of six viruses using the *in vitro* primary screening protocol and several candidate antiviral agents against an expanded spectrum of viruses using secondary screening protocols.

Due to the conflict in the Gulf (Desert Storm) in 1991 and lack of funding for these antiviral studies, this contract was cancelled (10 months into the work) on 12/31/91.

This report summarizes our progress in implementing the antiviral research program and includes summaries of antiviral test data collected from February 1, 1991 through December 31, 1991.

2. CURRENT SCOPE OF WORK

This section describes the research objectives and the scope of work for each of the main tasks being performed by Southern Research Institute (SRI) on this contract during this reporting period. These tasks are: (a) Prescreen testing of natural products for antiviral efficacy *in vitro*, (b) Primary testing of synthetic compounds and active natural products *in vitro* against an expanded spectrum of viruses of military importance, (c) Confirmatory testing of all active lead compounds from the prescreen and primary screen, (d) Secondary *in vitro* and *in vivo* studies of selected active compounds with best broad spectrum antiviral activity.

2.1 *In Vitro* Antiviral Evaluations

SRI conducted the antiviral screening of chemical compounds and natural products which were furnished by the Department of Antiviral Studies, USAMRIID, for antiviral efficacy in cell culture against representative viruses from the Togaviridae, Flaviviridae, Bunyaviridae, Arenaviridae, Poxviridae, and Retroviridae families. The test viruses consist of the following: (1) Vaccinia (VV) Virus, (2) Yellow Fever (YF) Virus, (3) Japanese Encephalitis (JE) Virus, (4) Venezuelan Equine Encephalomyelitis (VE) Virus, (5) Punta Toro (PT) Virus, (6) Sandfly (SF) Virus, and (7) Human Immunodeficiency (HIV) Virus. At USAMRIID containment facilities other viruses of military importance were tested according to the priorities set forth by the Sponsor.

A prescreen procedure has been developed which uses MTT and evaluates five compounds (at four-$\log_{10}$-dose levels) per virus per 96-well plate. SRI was scheduled to evaluate approximately 3000 compound against three prescreen viruses using the prescreen protocol. Compounds deemed active from the prescreen program were tested further in the primary screening program against the six Exotic Viruses listed above.

SRI was scheduled to evaluate approximately 2000 compounds per year against each of these six viruses *in vitro*, using MTT assays, to determine the drug cytotoxicity (TC_{50}%) and the drugs antiviral effect (IC_{50}%) and the overall antiviral efficacy index ($A.I_{50}$). Also specific antiviral parameters (SI and TAI) were calculated for each drug virus test.

Screening data was reported to USAMRIID essentially as it was obtained in hardcopy form and/or on floppy diskettes.

3. EXPERIMENTAL METHODS

3.1 Cell Culture

A centralized cell culture facility for provision of high-quality cell culture for all of the virus screening laboratories on this project was located at SRI home-site. This centralized cell culture facility consisted of two laboratories (one laboratory approximately 225 sq. ft. and the other approximately 250 sq. ft.). Each laboratory had a laminar flow hood and each laboratory was fully equipped for the maintenance and propagation of cell cultures. Each laminar flow hood was inspected and certified by the University of Alabama Occupational Health and Safety Department and these inspections and certifications were done on a yearly basis.

The centralized cell culture facility propagated and maintained the Vero (ATCC) cell culture line for this project.

Vero (ATCC) cells were used for seeding 96-well plates for the virus assays and delivered to the BL-3 facility on a standing order schedule. Cell culture plates for the other virus laboratories were supplied as requested. Vero cells in T75 or T150 cell culture flasks for virus production were supplied as requested. In addition, Vero cells seeded in 96-well plates were supplied as requested for special studies, and developmental procedures.

The Centralized Cell Culture Facility prepared and supplied cell culture media and other solutions to the BL-3 facility as was needed.

For quality control, cell culture lines were routinely monitored for any change in their growth parameters. The cells seeded in 96-well plates were microscopically inspected before delivery to the laboratories to ensure proper cell distribution in the wells and cell integrity.

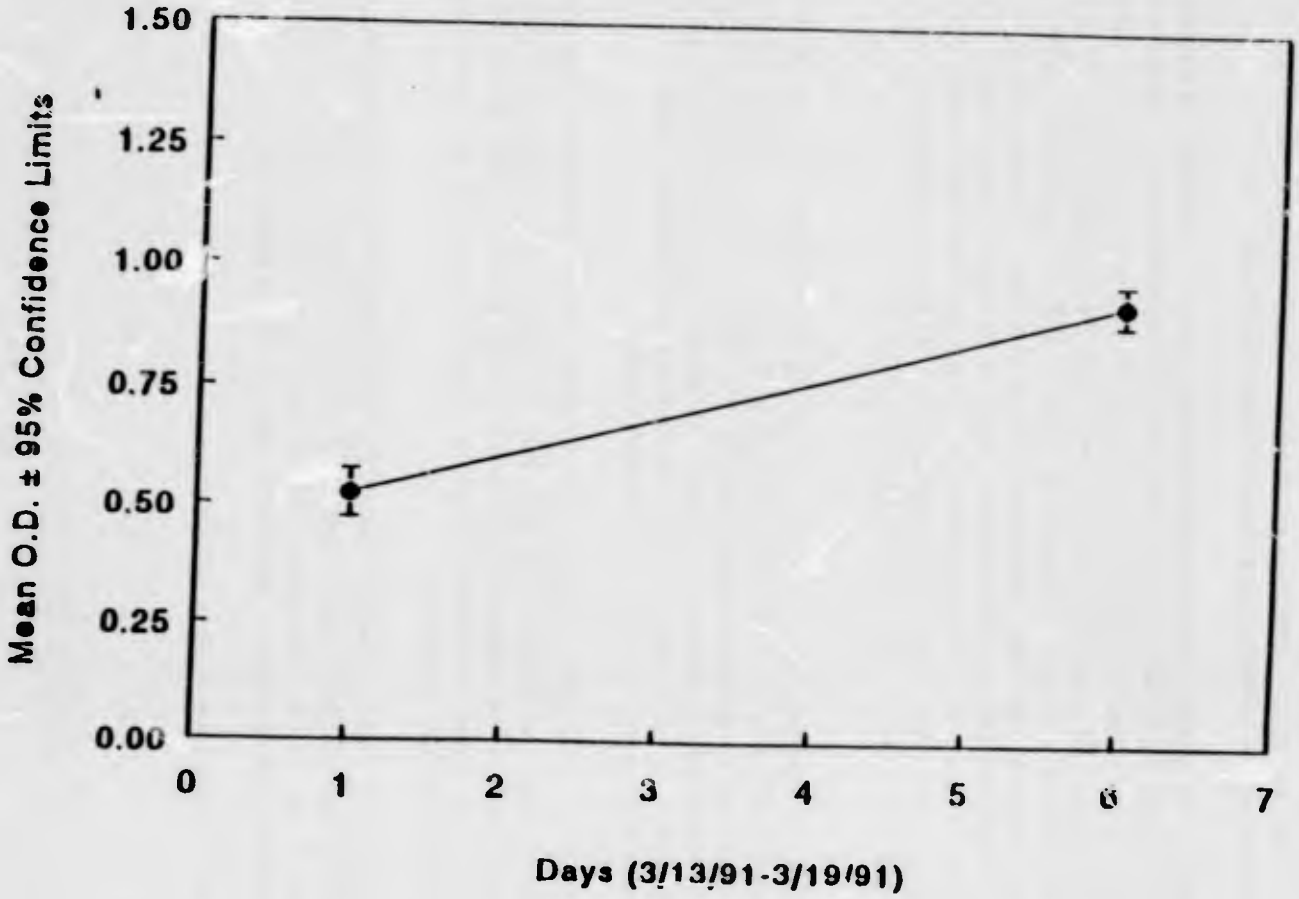
In addition to the quality control procedures described above, we evaluated, on a routine basis, the precision of preparing the 96-well plates and the proliferation of the Vero cells in the 96-well plates. Control 96-well Vero cell plates were selected at random on Day 1 (Day 1 of the prescreen and primary *in vitro* MTT Assays) and the optical densities (O.D.) were read the following day (for the Day 1 base line) and on Day 7 (Day 6 in culture). We used three plates for each plate configuration (Prescreen and Primary) for Day 1 in culture and three plates each for Day 6 in culture. The mean optical density for each plate was determined and the 95% confidence limits of the mean were calculated. The 95% confidence limits of the mean O.D. for each plate (n = 72 wells per plate) was less than 3.0% of the mean. This indicates an excellent precision in preparing the plates since the experimental error $\left\{ \frac{SD}{Mean} X\ 100 \right\}$ is only 5 to 6%. (SD is the standard deviation.)

Growth curves were constructed by plotting the mean O.D.'s for Day 1 and Day 6 in culture for each set of plate configurations and determining the slope of the curve (the increase in O.D. per day). See **Figure 1**. The slope for this particular growth curve was 0.068. The growth of the Vero cells in the 96-well plates were relatively constant. For example, the slopes of growth curves for the period from March 6 through March 26, 1991, were 0.064 $\pm$ 0.006 (mean $\pm$ 95% confidence limits, n = 25). The 95% confidence limits were only 9.4% of the mean for this period.

Sterility cultures were performed on all media and solutions used in the cell culture laboratory. At the time each reagent was made, 0.5 ml from each bottle or flask of reagent was added to an individual tube of thioglycollate medium and Sabouraud medium and the sterility culture tubes were incubated at 37°C for 48 hours before the solutions were released for use. The culture tubes were held 14 days before being discarded as negative.

At approximately 3-month intervals, samples from cell culture lines which were maintained in the Centralized Cell Culture Facility were sent to the ATCC for mycoplasma testing. These samples were monitored by use of the bisbenzamide DNA-fluorochrome stain and also by cultivating in mycoplasma broth and agar media under aerobic and anaerobic culture conditions.

Cell culture stocks were stored in liquid nitrogen.



Growth Curve of Vero Cells (96 well plates) with the MTT Assay
Figure 1

3.2 Test Compounds

3.2.1 Receipt, Cataloging, and Storage

During this report period, samples were received from the suppliers and processed at SRI-USAMRIID and shipped to SRI-Birmingham for testing. From February 1, 1991 through December 19, 1991, 3015 primary AVS samples were prepared for RNA and Vaccinia virus testing, and 2976 prescreen samples were prepared for RNA virus testing, including retests and positive control samples. Also, 438 primary AVS samples were prepared for HIV virus testing, including retests and positive control samples.

The drug samples submitted for testing were shipped to the centralized drug preparation laboratory by the SRI-Information Technology (IT) group at the USAMRIID Facility. The drugs were checked against the enclosed shipping list, and stored in numerical order in the drug repository facility under the appropriate conditions, according to the information supplied by SRI-IT. Drug samples submitted for prescreen testing were shipped by Dr. G. R. Petit, Cancer Research Institute, Arizona State University, Tempe, Arizona.

The daily drug inventory of compounds received and amount on-hand at all times were handled on the Drug Tracking Computer System. Results, including compound associated documents and all related archived antiviral data has been transferred to the Department of Antiviral Studies at USAMRIID and uploaded into the USAMRIID VAX computer.

Requests for daily drug preparation were required to be delivered to the centralized drug preparation laboratory five working days prior to the testing date. The drugs were solubilized and delivered fresh to the designated laboratory on the day of testing.

After the termination of this contract, all compounds connected with this project and in the possession of SRI will be transferred to Walter Reed Drug Repository, Washington, D.C., under the direction of Col. George Childs.

3.2.2 Determination of Drug Solubility

If no drug solubility or stability information was provided by the supplier, the following procedure was used to determine drug solubility:

A 1 mg sample was weighed into a homogenizer vessel and 1 ml of H_2O was added, which was the first solvent on the priority list. If the drug was not immediately soluble in H_2O, it was heated in a H_2O bath to 40°C. If the drug was not in solution after heating, it was homogenized with a hand homogenizer. The procedure was repeated with a freshly weighed 1 mg sample for each solvent in order of priority, until a suitable solvent was found. The priority list of solvents was as follows:

	Solvent	Volume (ml)		Solvent	Volume (ml)
1)	H_2O	1	4)	DMSO	0.1
2)	MeOH	0.1	5)	Acetone	0.1
3)	EtOH	0.1			

If the drug was insoluble in all of the above listed solvents, it was tested as a suspension in cell culture assay medium with the aid of a hand homogenizer or a vortex mixer. The final concentration of solvent in the starting drug concentration did not exceed 1% (preferably < 1%).

3.2.3 Compound Preparation for Testing

The following procedure was employed for the drug preparation:

1. Drugs for Primary Testing

All drugs were weighed in specified amounts, and the pre-determined solvent was added as required for the starting drug concentration.

Polystyrene snap-cap tubes were used for the weighed samples except for acetone-soluble drugs, then polypropylene tubes were used. Polypropylene cryotubes were also used.

2. Pre-screen Drugs (Plant Extracts)

Plant extracts were received in the repository preweighed in amounts of 200 mg (± 10%) in 2 ml cryovials with an o-ring seal.

On Day 0 (24 hours prior to testing) 200 µl of a specified solvent was added to each sample. Each sample was homogenized and placed in an ultrasonic bath for 10 - 12 minutes. The samples were extracted for 18 hours at 22 - 24° C.

On Day 1 (day of testing), 800 µl of sterile-deionized water was added to each sample and thoroughly mixed. If a drug was insoluble, it was further homogenized with a tissue tearer until a homogenous suspension was obtained. An additional 1 ml of sterile-deionized water was added to each sample, bringing the total volume in each cryovial to 2 ml.

3.3 *In Vitro* Antiviral Screening: DNA, Exotic RNA Viruses and Retroviruses

The viruses and host cell lines used in our *in vitro* assays are listed in **Table 1**. The antiviral activity of a compound was defined as a measure of its ability to inhibit the cytopathogenic effect (CPE) of the virus on its host cell.

During this contract period antiviral testing was done using the MTT assay. The MTT Assay System, measures the degree of cell viability (and therefore CPE and drug cytotoxicity) as determined by MTT uptake of the viable cells. This procedure is based upon the reduction of the tetrazolium salt, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial enzymes of viable host cells to MTT formazan. The blue color of the MTT formazan is measured spectrophotometrically. This protocol has proven to be most economical, efficient and suitable for a large-scale screening program. Plate layouts and data printouts are included in the Methods Section of this report.

Positive control drugs (**Table 1**) were included in each antiviral assay to validate the test conditions used in the antiviral assays.

The details of the assay procedures for each virus are described on the following pages.

Table 1

In Vitro Antiviral Screening Models

Virus	Strain	Classification	Cell Line	Positive Control Compound
Vaccinia Virus	Lederle Chorio-allantoic	Poxviridae	Vero	Selenazole (AVS-0253) and Arabinofuranosyladenine (AVS-1752)
Adenovirus Type 2	Adenoid 6	Adenoviridae	HEp-2	Ribavirin (AVS-0001), Selenazole (AVS-0253)
Yellow Fever Virus	Asibi	Flaviviridae	Vero	Selenazole (AVS-0253)
	17D (Prescreen)	Flaviviridae	Vero	Selenazole (AVS-0253)
Japanese Encephalitis Virus	Nakayama	Flaviviridae	Vero	Selenazole (AVS-0253)
Venezuelan Equine Encephalomyelitis Virus	Trinidad Donkey	Togaviridae	Vero	Selenazole (AVS-0253)
Punta Toro Virus	Adames	Bunyaviridae	Vero	Ribavirin (AVS-0001)
Sandfly Fever Virus	Sicilian	Bunyaviridae	Vero	Ribavirin (AVS-0001)
Hantaan Virus	76-118	Bunyaviridae	Vero (VSC-6)	Ribavirin (AVS-0001)
Pichinde Virus	4763	Arenaviridae	Vero	Ribavirin (AVS-0001)
Vesicular Stomatitis Virus	Indiana	Rhabdoviridae	L929	Carbocyclic-3-deaza-adenosine (AVS-0303)
Human Immunodeficiency Virus	IIIB	Retrovirus	ATH8,MT-2 CEM	3'-Azido-3'-deoxythymidine (AZT) (AVS-1603) 2',3'-Dideoxycytidine (ddC) (AVS-2639)

3.4 Prescreen Assays (YF, VE, PT):

Large numbers of plant extracts are available for screening for *in vitro* antiviral activity. We were requested to develop an assay that would: 1) allow more compounds to be evaluated per microtiter plate than in the regular antiviral screen and 2) prescreen plant extracts as well as other compounds for activity against two indicator viruses. The three viruses selected by USAMRIID for this purpose were the attenuated, vaccine strain (17D) of Yellow Fever virus (YF) and Punta Toro virus (PT) and Venezuelan Equine Encephalomyelitis virus (VE). The prescreen assay should select candidate compounds for screening against the Asibi strain of YF and the other target RNA viruses in the full screen.

A prescreen procedure was developed which utilizes MTT and evaluates five compounds per virus per 96-well plate.

Host Cells. Vero cells are seeded as monolayer cultures in COSTAR 96-well plates at 18,000/0.2 ml/well in MEM + 10% heat-inactivated fetal bovine serum (Δfbs). The plates are incubated approximately 24 hours prior to use.

Challenge Virus. The 17D strain of YF virus was originally obtained by Dr. Lori Brando from the Alabama Public Health Department in Birmingham, Alabama. The Adames strain of PT virus was obtained from our regular screen. Stock virus pools were prepared by passaging and titrating each virus in Vero cell monolayers. For use in the prescreen assays, PT virus is diluted in experiment medium (MEM + 2% Δfbs) to yield an inoculum of 32 $CCID_{50}$ per well. The challenge dose of YF virus had to be increased from 32 to 64 $CCID_{50}$/well in order to obtain sufficient CPE and cell-kill by 6 days post-infection. An antiviral screening assay as long as 7 or 8 days would require replacing the culture fluids with fresh drug during the incubation period, thus increasing the cost of the assay.

Test compounds are dissolved or suspended in DMSO or H_2O, then diluted in serial tenfold dilutions in experiment medium to yield final concentrations of 1000, 100, 10 and 1 μg/ml in the plate wells. Selenazofurin (AVS-0253) is utilized as a positive control drug for YF and VE; the control drug for PT is Ribavirin (AVS-0001). AVS-6724 (2-Thio-6-Azauridine) has been tested against all three viruses as a possible candidate positive control drug.

Assay Procedure. The assay is designed to evaluate four 1.0 $\log_{10}$ concentrations of each compound in duplicate against the challenge virus. MEM + 2% Δfbs serves as the experiment medium. Cell culture medium is removed from the plate wells. To each of duplicate test wells containing replicate cell monolayers, 100 μl of each test drug solution (or suspension) and 100 μl of the challenge virus (diluted in experiment medium) are dispensed. Cell controls (6/plate) containing 200 μl medium, virus-infected, untreated cell controls (6/plate) containing virus and medium and drug cytotoxicity controls (1/drug concentration) containing cells, medium and test drug are included on each plate. Medium (Reagent) color controls (no cells), and drug colorimetric controls (drug + medium -- no cells) accompany each test. The covered plates are incubated at 37°C in a humidified atmosphere containing 2% CO_2. When CPE reach 100%, 6 days post infection, 20 μl aliquots of MTT (5 μg/ml solution in PBS) are added to each well. The plates are incubated at 37°C for six hours to allow reduction of the MTT to the formazan form. Then 40 μl aliquots of a 30% solution of SDS in 0.02 NHCl are added to the plate wells. The plates are incubated overnight to allow the SDS to lyse the cells and to dissolve the MTT formazan crystals. The absorbance (570 nm) of the contents of each well is determined by a plate reader. The plate reader is interfaced with a computer programmed to capture the optical density (O.D.) measurements from the reader and calculate mean optical densities, indices such as IC_{50}, TC_{25}, SI, and plot the percents cell viability and CPE reduction. The actual O.D. readings, the indices, and bar graphs expressing cell viability and CPE reduction are automatically printed on individual compound data sheets.

An example of a data sheet with results of a prescreen assay is shown as **Figure 2.** Section I of the data sheet gives the compound and test identification and the actual raw data (O.D. readings) for each assay. Printouts of pertinent Control and Test Results are in Section II. Calculations of the test results are the same as those for assays in the regular screen. Section III displays a bar graph plotted from the computed values in Section II.

IN VITRO ANTIVIRAL RESULTS

EXOTIC RNA VIRUSES — PRE-SCREEN ASSAY — DRUG: AVS 0001

SI: >5.04

PLATE NUMBER	0C9	TEST DATE	08/24/89
PROJECT	5975-4	DATE READ	08/31/89
SPONSOR	USAMRIID		
CELLS	VERO	Satisfactory	
VIRUS	PT		
STRAIN	ADAMS		

Section I

	1	2	3	4	5	6	7	8	9	10	11	12
	drug A - 0001											
	tox	experimental		conc								
A	1.384	1.327	1.463	0.158								
B	1.471	0.310	0.684	1.135								
C	1.537	0.231	0.206	0.176								
D	1.386	0.223	0.227	0.157								
									virus control			reagent
E									0.211	0.361	0.220	0.175
F									0.249	0.278	0.225	0.179
G									1.455	1.493	1.536	0.174
H									1.462	1.474	1.483	0.177
										cell control		blank

tox=cell toxicity — BOLD = highest drug conc

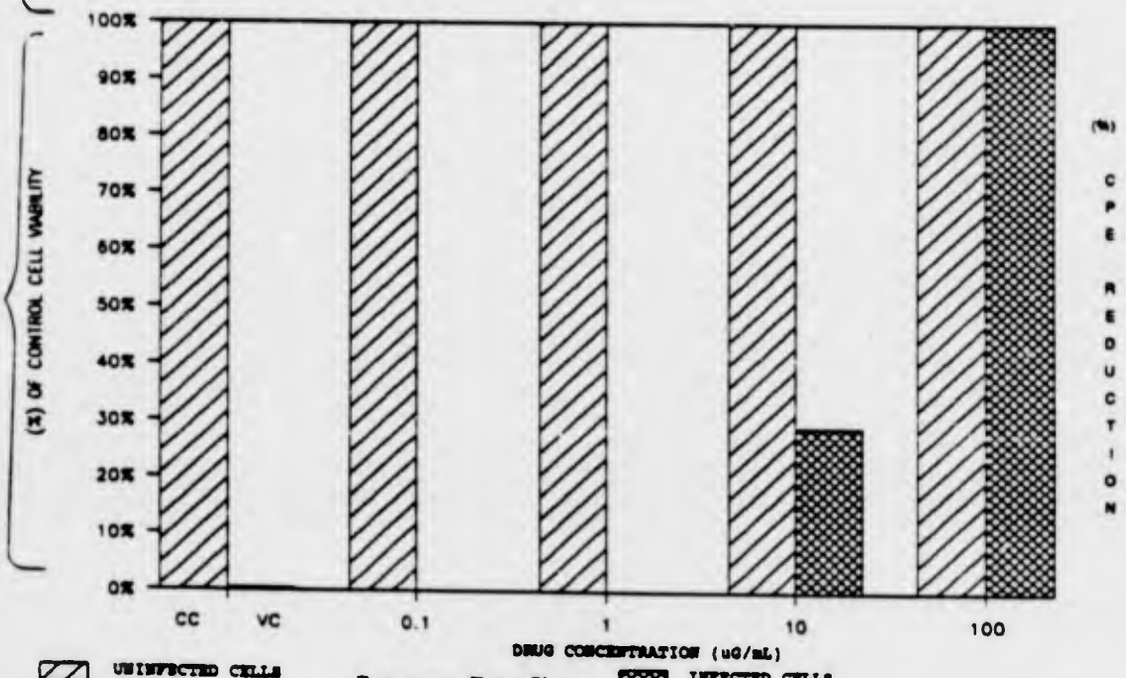
AVS 0001 vs. PT (08/24/89)

Section II

ROW OR PLATE	CONC. (uG/mL)	UNINFECTED CELL VIABILITY MEAN	UNINFECTED CELL VIABILITY PERCENT	INFECTED CPE REDUCTION MEAN	INFECTED CPE REDUCTION PERCENT
A	100	1.399	100%	1.247	100%
B	10	1.316	100%	0.346	29%
C	1	1.359	100%	-0.057	0%
D	0.1	1.349	100%	-0.030	0%

REAGENT		0.176
VIRUS CONTROL		0.098
CELL CONTROL		1.300
DIFFERENTIAL		1.202
IC50		19.80
TC25	>	100.00
SI	>	5.04

Section III



Prescreen Data Sheet

Figure 2

3.5 Primary Screen Assays:

This section describes the research methods used for testing the various viruses using the primary screening protocol. An example of the plate layout and the data printout used with this MTT Assay are included in **Figure 3**.

EXAMPLE OF A REPORT OF MTT ASSAY

PLATE PUJ — **IN VITRO ANTIVIRAL RESULTS** — **DRUG: AVS 0001**

DRUG 0001 — MTT ASSAY — TAI: >30.31 SI: >8.53

Section I

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plate background					
A	0.126	0.122	0.138	0.122	0.114	0.116	0.039	0.039	0.039	0.039	0.040	0.041
	blk	curve	drug 0001 experimental			blk					curve	
B	1.603	1.575	0.494	0.305	0.276	1.514					2.407	
C	1.581	1.554	0.452	0.406	0.274	1.514					1.537	
D	1.622	1.578	0.634	0.634	0.525	1.555					1.520	
E	1.578	0.502	1.017	0.949	1.021	1.616					0.523	
F	1.626	0.468	1.554	1.539	1.542	1.592					0.500	
G	1.498	0.456	1.403	1.403	1.403	1.392					0.56[illegible]	
	drug 0001 cytotoxicity background											
H	0.119	0.113	0.112	0.131	0.115	0.116						

blk = blank; curve = curve; BOLD = highest drug conc

Section II

VIRUS: SF
CELLS: VERO P193
SHIPMENT NUMBER: 01

PROJECT #: 5975-1
SPONSOR: USAMRIID
TEST DATE: 04/20/89
DATE READ: 05/01/89

REAGENT	0.123
VIRUS CONTROL	0.380
CELL CONTROL	1.572
DIFFERENTIAL	1.193

DRUG 0001	25%	50%	99%
TC (ug/mL)	> 100.00	> 100.00	> 100.00
IC (ug/mL)	5.48	11.70	------
ANTIVIRAL INDEX (AI)	> 18.20	> 8.53	------

DRUG 0001		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW OR PLATE	CONC. (ug/mL)	MEAN O.D.	% VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.32	-.137	100%	1.443	92%	-.007
C	1	-.117	100%	1.433	91%	-.008
D	3.2	0.094	92%	1.458	93%	0.008
E	10	0.521	56%	1.485	94%	-.011
F	32	1.053	12%	1.496	95%	-.010
high G *	100	0.905	24%	1.326	84%	-.004

* highest drug concentration tested

values shown are final adjusted numbers

Section III

SUMMARY GRAPH

AVS 0001 vs. SF (04/20/89)

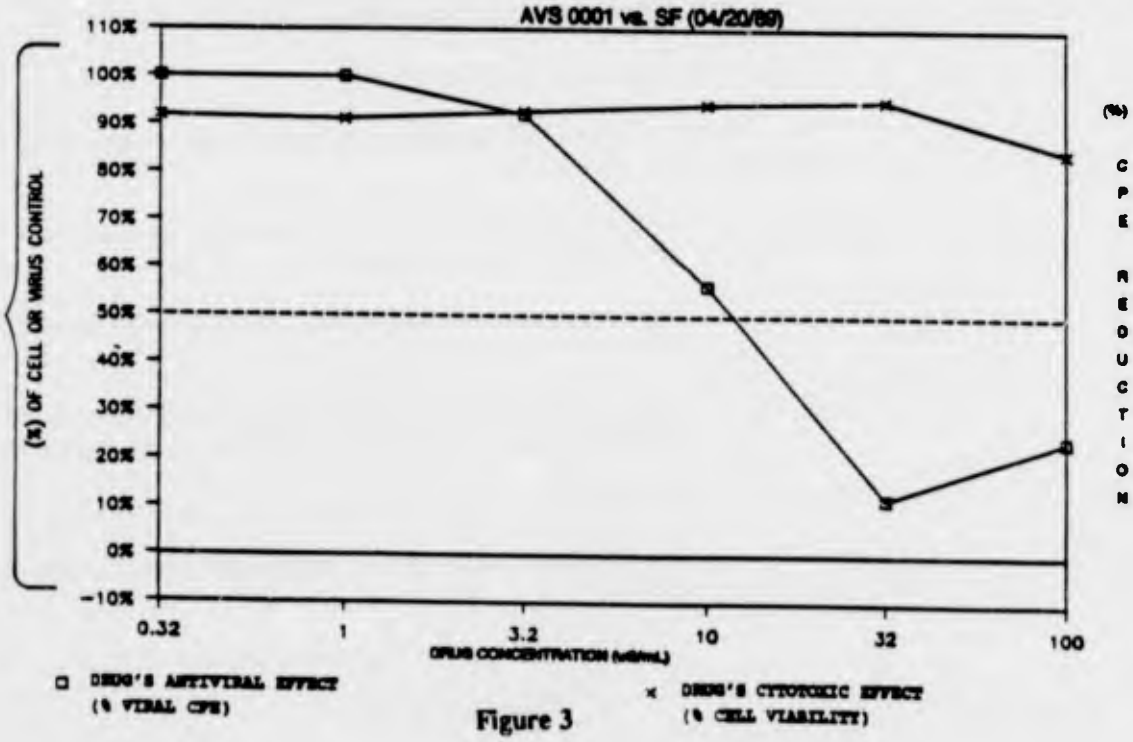


Figure 3

EXPLANATION OF *IN VITRO* ANTIVIRAL RESULTS FORM (MTT ASSAY)

The *In Vitro* Antiviral Results form (Figure 1) has three sections:

Section I. Sample and Test Identification and the actual raw data (optical density readings) collected for each 96-well plate.

The Test Identification and Raw Data Section specifies the compound that was tested, a unique plate number (assigned by the computer) and the actual optical density readings for the virus control (vc), cell control (cc), drug alone (tox) and the drug plus virus (drug experimental). Background readings are also taken for the container (plastic), reagent (culture medium) and drug colorimetric.

Section II. Printouts of pertinent Control and Test Results computed from the raw data are in this section.

Tabular Dose Response Test Results are calculated as follows:

a. Mean Medium O.D. is subtracted from means of virus and cell control O.D.s.

b. Drug colorimetric O.D. is subtracted from each infected and uninfected value at the corresponding drug dilution.

c. The Differential is the mean O.D. attributable to virus kill (CPE).

Differential = (mean O.D. cell control) - (mean O.D. Virus control).

d. For infected cells at each drug concentration,

% CPE = the reciprocal of:
[(mean O.D. infected wells - mean O.D. drug colorimetric wells) -(mean O.D. virus control - mean reagent O.D.)]/Differential; expressed .n percent.

e. For uninfected cells at each drug dilution,

% Cell Viability = [(mean O.D. uninfected drug-treated wells) - (mean drug colorimetric wells)]/(mean O.D. cell control).

Quantitation of Viral Cytopathic Effect (CPE) and Drug Activity are displayed in the shaded area of Section II. These values are defined below:

$TC_{25,50,95}$ = The drug concentration (μg/ml) that reduced cell viability by 25%, 50% or 95%.

$IC_{25,50,95}$ = The drug concentration (μg/ml) that inhibited CPE by 25%, 50% or 95% calculated by using a regression analysis program for semilog curve fitting.

$AI_{25,50,95}$ = Antiviral Index, calculated by dividing $TC_{25,50}$ or $_{95}$ by $IC_{25,50}$ or $_{95}$.

TAI = Total Antiviral Index - the area between the cytotoxicity and the antiviral curves.

SI = Selectivity Index, calculated by dividing the TC_{25} by the IC_{50}.

Figure 3 (Cont'd)

Section III. The Graphic Results Summary Section displays a plot or graphic illustration from computed values in Section II.

The line connecting the square symbols depicts the percentage of viral CPE in virus-infected cells treated with the test compound (at the indicated concentrations) relative to the Differential. This line expresses the *in vitro* anti-viral activity of the sample.

The line connecting the X symbols depicts the percentage of surviving uninfected cells treated with the test compound relative to the uninfected, untreated control (cell control). This line expresses the cytotoxicity of the drug at the various concentrations, or percent cell viability. The dotted line is just a reference line at 50%.

Figure 3 (Cont'd)

3.5.1 Vaccinia Virus (VV)

Vaccinia virus, strain Lederle CA, was obtained from Dr. Wilton Rightsel, formerly with Parke, Davis and Company, Detroit, Michigan. We have serially passed VV in HEp-2 (continuous-passage human carcinoma of the larynx) and Vero (continuous-passage African green monkey kidney) cell monolayer cultures. The VV used to screen compounds for USAMRIID was propagated and assayed in Vero cell monolayer cultures in Eagle's Minimal Essential Medium (MEM) supplemented with 2% heat-inactivated fetal bovine serum (Δfbs) and 50 μg/ml of gentamicin. Virus stocks were titrated according to the procedure of **Reed and Muench (1938)** and diluted in culture medium to 100 $CCID_{50}$ per 0.1 ml.

During the time period covered by this report, compounds were screened for activity against VV in Vero cell monolayer cultures in 96-well plates, employing a CPE-inhibition assay procedure.

Subsequent to 12/15/88 and beginning with Shipment 42, all compounds have been screened for activity against VV by the MTT assay procedure, in which the degree of cell viability (and therefore CPE and drug cytotoxicity) is determined by MTT uptake. This procedure is based on the reduction of the tetrazolium salt, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial enzymes of viable host cells to MTT formazan. The blue color of the MTT formazan is measured spectrophotometrically. Vero cells are seeded into 96-well plates at a density of 3×10^4 cells in 0.2 ml per well in MEM + 5% bovine calf serum. The plates are incubated at 37°C overnight. The next day our CPE-inhibition assay is set up according to the format shown in **Figure 3**. MEM + 2% heat-inactivated fetal bovine serum (Δfbs) serves as experiment medium. To each of triplicate test wells containing replicate cell monolayers, 100 μl of each test drug solution (or suspension) and 100 μl of experiment virus are dispensed. Six 0.5 $\log_{10}$ concentrations of each drug beginning with 320 μg/ml are routinely used. Compounds are solubilized or suspended and diluted in culture medium the day of use. Cell controls containing 200 μl of medium, virus controls containing cells, medium and virus, and duplicate drug cytotoxicity controls containing cells, medium and each drug concentration are included on each plate. Blank wells, medium (Reagent) control wells, and drug colorimetric controls (drug + medium + MTT + SDS - no cells) accompany each test. The covered plates are incubated at 37°C in a humidified atmosphere containing 2% CO_2.

When CPE reach 100%, 5 days postvirus infection (p.i.), 20 μl of MTT (a 5 mg/ml solution in PBS) are added to each well. The plates are incubated at 37°C for six to seven hours to allow reduction of the MTT to the formazan form. Then 40 μl aliquots of a 30% solution of SDS in 0.02*N* HCl are added to the plate wells. The plates are incubated overnight to allow the SDS to lyse the cells and dissolve the MTT formazan crystals. The absorbance of the contents of each well is determined by a plate reader employing dual filters of 570 and 650 nm. The plate reader is interfaced with a computer programmed to capture the optical density (O.D.) measurements from the reader and calculate indices such as the $IC_{25, 50, 95}$, $TC_{25, 50, 95}$ and to plot the percents of viral CPE and cell viability of drug-treated cultures.

3.5.2 Yellow Fever Virus (YF)

We obtained our original stock of Yellow Fever Virus, Asibi strain, from Dr. Andrew J. Main, Jr., of the Yale Arbovirus Research Unit, New Haven, Connecticut.

To grow virus stocks, Vero cells (ATCC) were infected at an moi of about 0.1 PFU/cell in MEM containing 10% inactivated fbs. Virus was allowed to adsorb for 1 hour, after which a minimal volume of growth medium was added and the cells were incubated at 37°C. The culture fluid was collected at 5 days post infection and the clarified by centrifugation (5000 rpm, 15 minutes, 4°C) in a Sorvall SA-600 rotor. The supernate was dispensed into 0.5 ml aliquots and then frozen and stored at -84°C. One aliquot was used to determine the $TCID_{50}$ and PFU titers for the stock.

The standard operating procedure is an MTT-based assay as follows:

Vero cells are seeded in 96-well tissue culture plates at a density of 1.8×10^4 cells/well in 100 µl complete EMEM and incubated overnight at 37°C/5% CO_2.

The following day, the medium is aspirated out of the wells and 50 µl of virus (diluted to a virus load of 32 $TCID_{50}$/well) are added to the test and virus control wells. The toxicity and cell control wells receive 50 µl of complete EMEM. The plates are incubated for 30 minutes at 37°C/5% CO_2 to allow virus to adsorb.

After virus adsorption, the test wells receive an additional 50 µl of a 2X concentration of drug and the control wells receive an additional 50 µl of complete EMEM. The total volume per well = 100 µl. Generally, the standard drug test concentration range is from 1 µg/ml to 320 µg/ml.

The plates are incubated at 37°C/5% CO_2 until the virus control wells exhibit maximum CPE (+4 = 100%) by visual scoring under the microscope (40X). Generally, the YF virus (with 32 $TCID_{50}$ virus load) requires 6-7 days of incubation to achieve maximum CPE. At this time, all wells (excluding plastic control wells) receive 50 µl of MTT solution (1.50) mg/ml, prepared in complete EMEM).

The plates are incubated 7 hours at 37°C/5% CO_2 (in the dark) to allow reduction of the tetrazolium salt to the formazan product, after which the cells are lysed and the formazan is solubilized by the addition of 100 µl of a lysing solution (10% SDS-0.01N HCl) to all wells generally. If the MTT solution is added in the morning, the lysing agent is added at the end of the day, and lysis occurs overnight (14 - 15 hours in the dark).

3.5.3 Japanese Encephalitis Virus (JE)

We obtained our original stock of Japanese Encephalitis Virus, Nakayama strain, from Dr. George R. French of the Salk Institute, Swiftwater, Pennsylvania.

To grow virus stocks, Vero cells (ATCC) were infected at an moi of about 0.1 PFU/cell in MEM containing 5% fbs. Virus was allowed to adsorb for 1 hour, then a minimal volume of growth medium was added and the cells were incubated at 37°C. The culture fluid was collected at 4-5 days post infection and clarified by centrifugation (5000 rpm, 15 minutes, 4°C) in a Sorvall SA-600 rotor. The supernate was dispensed into 0.5 ml aliquots and then frozen and stored at -84°C. One aliquot was used to determine $TCID_{50}$ and PFU titers of the stock.

The standard operating procedure is an MTT-based assay as follows:

Vero cells are seeded in 96-well tissue culture plates at a density of 1.8×10^4 cells/well in 100 µl complete EMEM and incubated overnight at 37°C/5% CO_2.

The following day, the medium is aspirated out of the wells and 50 µl of virus (diluted to a virus load of 32 $TCID_{50}$/well) are added to the test and virus control wells. The toxicity and cell control wells receive 50 µl of complete EMEM. The plates are incubated for 30 minutes at 37°C/5% CO_2 to allow virus to adsorb.

After virus adsorption, the test wells receive an additional 50 µl of a 2X concentration of drug and the control wells receive an additional 50 µl of complete EMEM. The total volume per well = 100 µl. Generally the standard drug test concentration range is from 1.0 µg/ml to 520 µg/ml.

The plates are incubated at 37°C/5% CO_2 until the virus control wells exhibit maximum CPE (+4 = 100%) by visual scoring under the microscope (40X). Generally, the JE virus (with 32 $TCID_{50}$ virus load) requires 6-7 days of incubation to achieve maximum CPE. At this time, all wells (excluding plastic control wells) receive 50 µl of MTT solution (1.50) mg/ml, prepared in complete EMEM).

The plates are incubated 7 hours at 37°C/5% CO_2 (in the dark) to allow reduction of the tetrazolium salt to the formazan product, after which the cells are lysed and the formazan is solubilized by the addition of 100 µl of a lysing solution (10% SDS-0.01N HCl) to all wells. If the MTT solution is added in the morning, the lysing agent is added at the end of the day, and lysis occurs overnight (14 - 15 hours in the dark).

3.5.4 Venezuelan Equine Encephalomyelitis Virus (VE)

We obtained our original stock of Venezuelan Equine Encephalomyelitis Virus, Trinidad Donkey strain, from Dr. George R. French of The Salk Institute, Swiftwater, Pennsylvania.

The virus was propagated in Vero cells (ATCC) by infection at a moi of <0.1 PFU/cell. Fluids from infected cultures were collected three days post-infection and clarified by centrifugation (5000 rpm, 15 min, 4°C) in a Sorvall SA-600 rotor. The supernatant fluid was dispensed into 0.5-ml aliquots, quick frozen in a dry ice-ethanol bath and stored at -84°C. One aliquot was used to determine $TCID_{50}$ and PFU titers of the stock.

The standard operating procedure is an MTT-based assay as follows:

Vero cells are seeded in 96-well tissue culture plates at a density of 1.8×10^4 cells/well in 100 µl complete EMEM and incubated overnight at 37°C/5% CO_2.

The following day, the medium is aspirated out of the wells and 50 µl of virus (diluted to a virus load of 32 $TCID_{50}$/well) are added to the test and virus control wells. The toxicity and cell control wells receive 50 µl of complete EMEM. The plates are incubated for 30 minutes at 37°C/5% CO_2 to allow virus to adsorb.

After virus adsorption, the test wells receive an additional 50 µl of a 2X concentration of drug and the control wells receive an additional 50 µl of complete EMEM. The total volume per well = 100 µl. Generally the standard drug test concentration range is from 1.0 µg/ml to 320 µg/ml.

The plates are incubated at 37°C/5% CO_2 until the virus control wells exhibit maximum CPE (+4 = 100%) by visual scoring under the microscope (40X). Generally, the VEE (with 32 $TCID_{50}$ virus load) requires 3 days of incubation to achieve maximum CPE. At this time, all wells (excluding plastic control wells) receive 50 µl of MTT solution (1.50) mg/ml, prepared in complete EMEM).

The plates are incubated 7 hours at 37°C/5% CO_2 (in the dark) to allow reduction of the tetrazolium salt to the formazan product, after which the cells are lysed and the formazan is solubilized by the addition of 100 µl of a lysing solution (10% SDS-0.01N HCl) to all wells. If the MTT solution is added in the morning, the lysing agent is added at the end of the day, and lysis occurs overnight (14-15 hours in the dark).

3.5.5 Punta Toro Virus (PT)

We obtained our original stock of Punta Toro virus (Adames strain) from Dr. Robert Sidwell of Utah State University. His virus stock originated from Dr. Dominique Pifat of USAMRIID.

To grow virus stocks, Vero cells (ATCC) were infected at a low multiplicity of infection (moi; $\leq$0.1) in MEM supplemented with 10% inactivated fbs. Culture fluid was collected at five days post-infection and clarified by centrifugation (5000 rpm, 15 minutes, 4°C) in a Sorvall SA-600 rotor. The supernate was dispensed into 0.5-ml aliquots, then frozen and stored at -84°C. One aliquot was used to determine a $TCID_{50}$ titer and a plaque forming unit (PFU) titer for the stock.

The standard operating procedure is an MTT-based assay as follows:

Vero cells are seeded in 96-well tissue culture plates at a density of 1.8×10^4 cells/well in 100 µl complete EMEM and incubated overnight at 37°C/5% CO_2.

The following day, the medium is aspirated and all cells are treated with 100 µl of pretreatment solution for 30 minutes at 37°C/5% CO_2. The pretreatment solution is composed of 1% DMSO from a 100% DMSO stock solution, 1% DEAE-Dextran from a 2.0 mg/ml stock solution in sterile water, and 98% Hank's Balanced Salt Solution.

After this the pretreatment medium is aspirated out of the wells and 50 µl of virus (diluted to a virus load of 32 $TCID_{50}$/well) are added to the test and virus control wells. The toxicity and cell control wells receive 50 µl of complete EMEM. The plates are incubated for 30 minutes at 37°C/5% CO_2 to allow virus to adsorb.

After virus adsorption, the test wells receive an additional 50 µl of a 2X concentration of drug and the control wells receive an additional 50 µl of complete EMEM. The total volume per well = 100 µl. Generally the standard drug for concentration range is from 1 µg/ml to 320 µg/ml.

The plates are incubated at 37°C/5% CO_2 until the virus control wells exhibit maximum CPE (+4 = 100%) by visual scoring under the microscope (40X). Generally, the PT virus (with 32 $TCID_{50}$ virus load) requires 6-7 days of incubation to achieve maximum CPE. At this time, all wells (excluding plastic control wells) receive 50 µl of MTT solution (1.50) mg/ml, prepared in complete EMEM).

The plates are incubated 7 hours at 37°C/5% CO_2 (in the dark) to allow reduction of the tetrazolium salt to the formazan product, after which the cells are lysed and the formazan is solubilized by the addition of 100 µl of a lysing solution (10% SDS-0.01N HCl) to all wells. Generally if the MTT solution is added in the morning, the lysing agent is added at the end of the day, and lysis occurs overnight (14 - 15 hours in the dark).

3.5.6 Sandfly Fever Virus (SF)

We obtained our original stock of Sandfly Fever virus, Sicilian strain (TC adapted), from Dr. George R. French of the Salk Institute, Government Services Division, Swiftwater, Pennsylvania.

To grow virus stocks, Vero-76 cells (Dr. French, Salk Institute) or Vero cells (ATCC), depending upon availability, were pre-treated with DEAE-dextran (25 µg/ml) and 1% DMSO in growth medium (MEM containing 10% inactivated fbs) for 30 minutes at 37°C. This was removed and then the cells were infected with SF virus in growth medium at an moi of 0.1. Virus was allowed to adsorb for one hour at 37°C. A minimal volume of growth medium was then added and the cells were incubated at 37°C. The culture fluid was collected at four days post infection and clarified by centrifugation (5000 rpm, 15 min, 4°C). The supernate was dispensed into 0.5 ml aliquots, then frozen and stored at -84°C. One aliquot was used to determine $TCID_{50}$ and PFU titers for the stock.

The standard operating procedure is an MTT-based assay as follows:

Vero cells are seeded in 96-well tissue culture plates at a density of 1.8×10^4 cells/well in 100 µl complete EMEM and incubated overnight at 37°C/5% CO_2.

The following day, the medium is aspirated and all cells are treated with 100 µl of pretreatment solution for 30 minutes at 37°C/5% CO_2. The pretreatment solution is composed of 1% DMSO from a 100% stock solution, 1% DEAE-Dextran from a 2.5 mg/ml stock solution in sterile water, and 98% HBSS.

After this, the pretreatment medium is aspirated out of the wells and 50 µl of virus (diluted to a virus load of 32 $TCID_{50}$/well) are added to the test and virus control wells. The toxicity and cell control wells receive 50 µl of complete EMEM. The plates are incubated for 30 minutes at 37°C/5% CO_2 to allow virus to adsorb.

After virus adsorption, the test wells receive an additional 50 µl of a 2X concentration of drug and the control wells receive an additional 50 µl of complete EMEM. The total volume per well = 100 µl. Generally the standard drug concentration range is from 1 µg/ml to 320 µg/ml.

The plates are incubated at 37°C/5% CO_2 until the virus control wells exhibit maximum CPE (+4 = 100%) by visual scoring under the microscope (40X). Generally, the SF virus (with 32 $TCID_{50}$ virus load) requires 6-7 days of incubation to achieve maximum CPE. At this time, all wells (excluding plastic control wells) receive 50 µl of MTT solution (1.50) mg/ml, prepared in complete EMEM).

The plates are incubated 7 hours at 37°C/5% CO_2 (in the dark) to allow reduction of the tetrazolium salt to the formazan product, after which the cells are lysed and the formazan is solubilized by the addition of 100 µl of a lysing solution (10% SDS-0.01N HCl) to all wells. Generally if the MTT solution is added in the morning, the lysing agent is added at the end of the day, and lysis occurs overnight (14 - 15 hours in the dark).

3.5.7 Human Immunodeficiency Virus (HIV):

3.5.7.1 Standard Screening Assay In CEM and MT-2 Cells

a. Compound dilution and delivery to the plates

Drugs were solubilized in the appropriate vehicle such as distilled water, DMSO, methyl alcohol, or any other vehicle as requested by the Project Officer or as determined at SRI. The maximum solubility was determined as appropriate. Latex gloves, lab coats, and masks were used during all phases of the handling process to prevent exposure to potentially harmful agents. At highest solubility, the drug was prepared and stored at -20°C until used by the screening laboratory. The first dilution of each compound was made in a dilution tube with medium to yield a concentration two-fold that of the highest test concentration. Sterile titer tubes were then used to make serial one half log dilutions of each compound. Following drug dilution, the diluted compound was added to the appropriate well of a 96-well microtiter plate. Up to 12 dilutions could conveniently assayed in triplicate on a single plate with all appropriate controls, including cell control, virus control, toxicity control, drug color control, medium control and plastic (background) control. When testing included only six dilutions, two drugs could be assayed on a single microtiter plate. The drugs were added to the plate in a final volume of 100 microliters.

b. Cells and virus

During the time that the drug dilutions were prepared, cells were washed and counted. Viability was monitored by trypan blue dye exclusion and assays were not performed if the viability fell below 90%. Cells were maintained in an exponential growth phase and were split 1:2 on the day prior to assay to assure exponential growth rate. For the primary screen, the cell lines utilized were CEM and MT-2. Unless otherwise indicated the medium used was phenol red-free RPMI 1640 with 10% heat-inactivated fetal calf serum (FBS), glutamine and antibiotics. Cells were propagated at 37°C in an atmosphere of 5% CC_2 in air. The virus employed for this work was HIV-1 isolates IIIB and/or RF prepared by an acute infection process, **Buckheit, *et al.*, (1991) and Cloyd, et al., (1990)**. Briefly, virus infected cells were pelleted on a daily basis beginning at three days post-infection until the virus had killed all of the cells in the culture. Reverse transcriptase activity and p24 ELISA were used to identify pools with the greatest amount of virus. These 24-hour harvests were pooled, filtered and frozen at -90°C. Prior to use in the assay the infectious pool of virus was titered on all available cell lines in order to determine the amount of virus required in the anti-viral assay. In general, pools produced by the acute virus method required the addition of one microliter of infectious virus per well resulting in the screening of drugs at a multiplicity of infection of 0.01. In this manner enough virus was prepared and frozen to complete over one thousand microtiter plates, allowing the testing of up to two thousand compounds from a single stock of infectious virus. The use of a single stock of virus for a long period of testing has very favorable effects on the repeatability of the assay systems. Virus infection of the CEM cells was carried in a bulk infection process. The appropriate number of cells required to complete the assay were mixed with infectious virus in a conical centrifuge tube in a small total volume of 1 - 2 milliliters. Following a one hour incubation the infected cells were brought to the appropriate final concentration of 5×10^4 cells per milliliter with fresh tissue culture medium and 100 microliters were added to the appropriate experimental and virus control wells. Uninfected cells at the same concentration were plated for the toxicity controls and for the cell controls. Assays could also be performed using in well infection methods. In this case, drug, cells and virus were added to the well individually. In each case the MOI was adjusted to give complete cell killing in the virus control wells by day 6.

c. Evaluation of CPE-inhibition

Following the addition of cells and drugs to the microtiter plate the plate was incubated for 6 days at 37°C. Experience had determined that incubation for longer periods of time (7 - 8 days) or the use of higher input cell numbers (1×10^4) resulted in significant decreases in cell control viability and a narrowing in the differential in optical density between cell and virus controls upon staining with MTT.

The method of evaluating the antiviral assay involved the addition of 20 microliters of the tetrazolium salt MTT at 5 mg/ml to each well of the plate for 4 - 8 hours. After this incubation period the cells were disrupted by the addition of 50 μl of 20% SDS in 0.01N HCl. The metabolic activity of the viable cells in the culture resulted in a colored reaction product which was measured spectrophotometrically in a Molecular Devices Vmax plate reader at 570nm. The optical density (O.D.) value was a function of the amount of formazan product, which is proportional to the number of viable cells. The plate reader was on-line to the screening laboratory microcomputer which evaluates the plate data and calculates plate data. The plate report provided a rundown of all pertinent information including the raw O.D. values, the calculated mean O.D.'s and the percent reduction in viral CPE as well as calculations including TC_{50}, IC_{50} and antiviral and specificity indices. Finally, the results included a plot which visually depicts the effect of the compound on uninfected cells (toxicity) and the protective or nonprotective effect of the compound on the infected cells. A representative example of a plate report is given in **Figure 4**.

d. Interpretation of results and quality control

The results shown for AZT (**Figure 4**) illustrate a frequently observed pattern for an active compound. At the concentrations tested (6 one-half log dilutions), the highest concentration of AZT (2.67 μg/ml) is always toxic as shown by the low O.D. values for both uninfected and infected cells (see mean O.D. results, TC results, and the plot). The next three successive dilutions of AZT show 100% protection. At AZT concentrations below 0.0834 μg/ml the level of protection decreases while the toxicity to uninfected cells shows no toxic effect of AZT. Patterns similar to this are recognized as active.

A drug could have much more or much less toxicity, and the uninfected and infected curves must always coincide at the toxic concentrations. Those that did not were considered unsatisfactory and were retested. A drug could be active at one concentration or across a range of concentrations. However, if it was active at a given concentration it must be active at all higher concentrations unless those concentrations were toxic. When apparent activity at one concentration was lost at a higher non-toxic concentration, that pattern was not recognized as meaningful and a repeat was performed. Generally, a valid test was one in which the virus control and cell control values differed by a significant margin. When they did not, the cause could be poor viral infectivity or poor cell growth. In these cases, assays were judged unsatisfactory. Assays were also suspect when there was widespread non-agreement between duplicate wells, when contamination occurred at key doses, and when background was not consistent with that expected.

A compound was judged inactive when no recognized pattern of protection was observed in an otherwise valid assay. When a low level of activity was observed the compound was considered possibly active and was rescheduled for testing. Protection at only high doses of the drug or low level activity across a range was not a mark of a particularly useful drug, but the information was valuable from the standpoint of the development of possible analogs of that compound and the structure of compounds with activity.

PLATE ODD
DRUG AZT

IN VITRO ANTIVIRAL RESULTS
XTT ASSAY

DRUG: AZT
TAI: >76.45 SI: 111.90

	1	2	3	4	5	6	7	8	9	10	11	12
	reagent background						plastic background					
A	0.113	0.110	0.107	0.103	0.103	0.100	0.025	0.026	0.026	0.025	0.025	0.026
	tox	cc/vc	drug AZT experimental			tox					cc/vc	
B	1.168	1.179	0.322	0.347	0.323	1.235					1.089	
C	1.198	1.150	0.945	0.964	0.833	1.382					1.047	
D	1.210	1.154	1.099	1.031	1.161	1.508					1.040	
E	1.172	0.137	1.151	1.139	1.273	1.413					0.131	
F	1.151	0.136	1.145	1.191	1.088	1.220					0.119	
G	0.515	0.142	0.496	0.544	0.562	0.578					0.118	
	drug AZT colorimetric background											
H	0.109	0.103	0.100	0.103	0.103	0.104						

tox=cell toxicity cc=cell control vc=virus control BOLD = highest drug conc values shown are optical densities

VIRUS	HIV
CELLS	CEM
STRN	38 1:33
REAGENT	0.106
VIRUS CONTROL	0.025
CELL CONTROL	1.004
DIFFERENTIAL	0.979

Satisfactory; Active; Retest

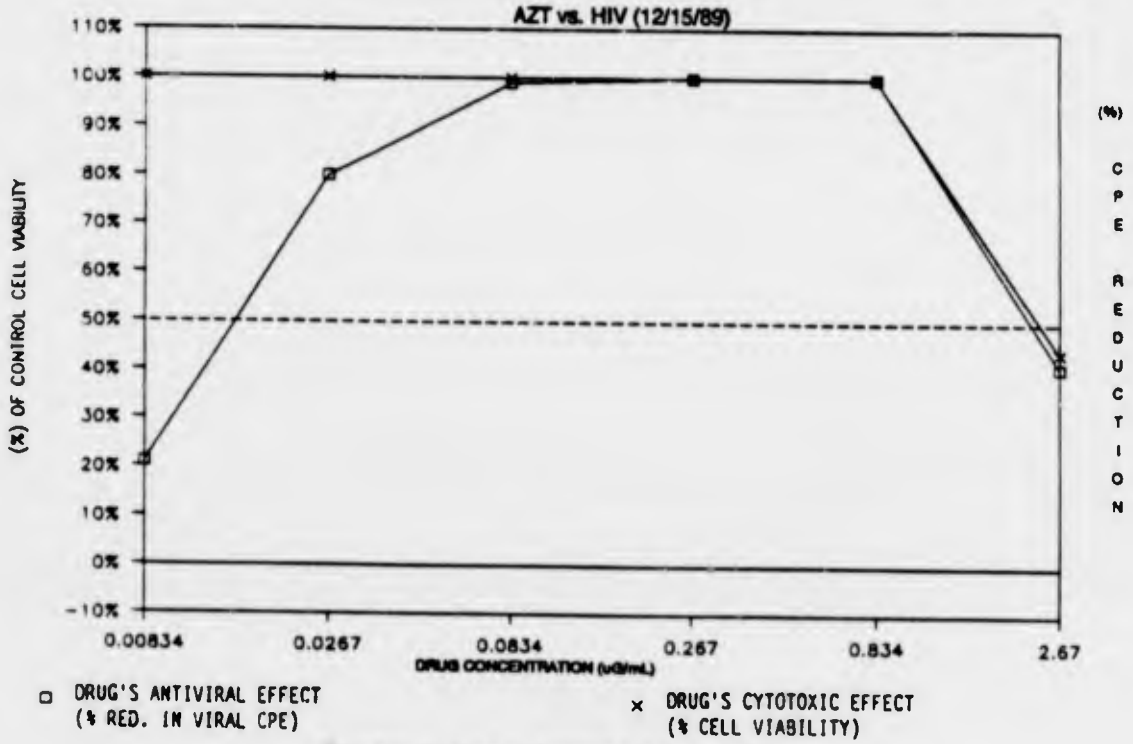
PROJECT #	615
SPONSOR	B. BUCKHEIT
TEST DATE	12/15/89
DATE READ	12/21/89

DRUG AZT	25%	50%	95%
TC (uG/mL)	1.65	2.47	> 2.67
IC (uG/mL)	0.009030	0.01	0.07
ANTIVIRAL INDEX (AI)	183.18	167.35	> 40.68

DRUG AZT		ANTIVIRAL TEST VALUES		CYTOTOXICITY TEST VALUES		
ROW ON PLATE	CONC. (uG/mL)	MEAN O.D.	% RED. IN VIRAL CPE	MEAN O.D.	% CELL VIABILITY	COLORIMETRIC CONTROL
low B	0.00834	0.202	21%	1.098	100%	-.002
C	0.0267	0.786	80%	1.187	100%	-.003
D	0.0834	0.970	99%	1.256	100%	-.003
E	0.267	1.063	100%	1.192	100%	-.006
F	0.834	1.014	100%	1.083	100%	-.003
high G *	2.67	0.401	41%	0.438	44%	0.003

* highest drug concentration tested values shown are final adjusted numbers

SUMMARY GRAPH



Example of Primary Screening Report (AZT)
Figure 4

PRINTED 05/28/91

Southern Research Institute

4. RESULTS

4.1 *In Vitro* Antiviral Evaluations: DNA, Exotic RNA and Retrovirus Viruses:

During this reporting period from February 1, 1991 to December 31, 1991, a total of 5475 (2713 primary screen and 2762 prescreen) test compounds were received at SRI for evaluation in the *in vitro* antiviral screens (prescreen and primary). A cumulative summary of the compound shipments received, and the number of compounds in each shipment, are presented in **Tables 2-A and 2-B**. Compounds received in amounts too small for appropriate evaluation against all of the viruses were screened according to a priority list (determined by the sponsor) of the target viruses from the primary screen. In some instances, the testing of compounds against the exotic viruses was coordinated with the testing against Human Immunodeficiency Virus (HIV). *In vitro* primary screening evaluations were carried out against Vaccinia Virus (VV), Yellow Fever Virus (YF), Japanese Encephalitis Virus (JE), Venezuelan Equine Encephalomyelitis Virus (VE), Punta Toro Virus (PT), and Sandfly Virus (SF). The HIV virus was also to be tested under the primary screen. Secondary screening was carried out against selected viruses (as prioritized by the staff at Ft. Detrick) at the Ft. Detrick Military Base at Frederick, Maryland.

Approximately 34,000 *in vitro* antiviral assays were performed during this contract period. This number includes quality control as well as unsatisfactory tests (**Figure 5**). Positive control drugs, as specified in **Table 1** were tested in parallel in each assay. Several internal virus load and cell load quality control tests were performed but are not included in the above total (34,000). Results of the cell controls and virus controls were monitored to test for viability, consistency and repeatable results during the day-to-day operation. **Tables 2-A and 2-B** illustrates the number of compounds that were received to be tested in the prescreen and primary screen.

The results for compounds found active are summarized in the following sections for each virus. A cumulative summary presenting all of the *in vitro* antiviral tests results (both positive and negative) is included in **Appendix A** (Prescreen Data), **Appendix B** (Primary Screen Data) and **Appendix C** (HIV Data).

Table 2-A

Prescreen *In Vitro* Antiviral Screen
Cumulative Summary of Number of Compounds Received Per Shipment Through December 31, 1991

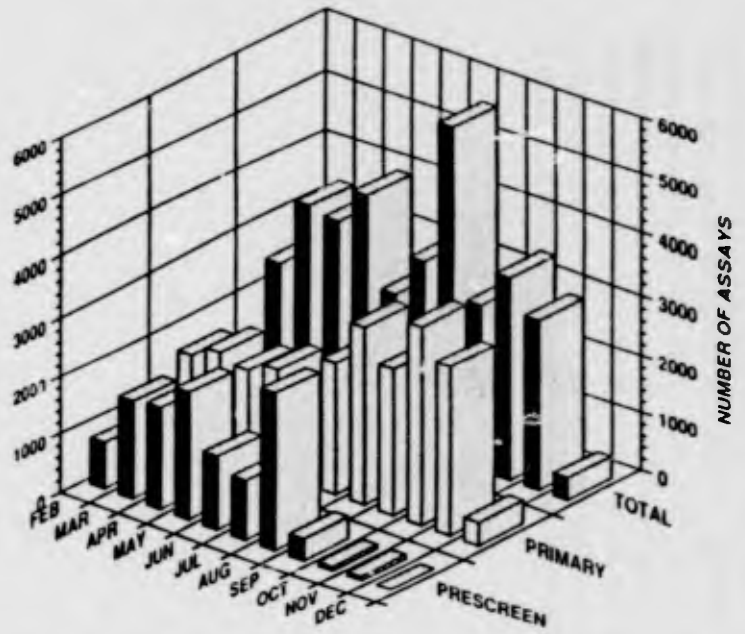
Shipment Number	Compounds Per Shipment	Date Received
1P	598	06/08/89
2P	12	07/20/89
3P	695	08/26/89
4P	38	02/01/90
4P	707	03/15/90
5P	168	06/07/90
6P	183	07/05/90
7P	152	08/22/90
8P	111	08/28/90
9P	200	09/25/90
10P	200	10/15/90
11P	200	11/16/90
12P	189	12/03/90
13P	161	12/17/90
14P	195	01/24/91
15P	6	02/08/91
16P	193	02/21/91
17P	191	03/04/91
18P	298	03/20/91
19P	200	03/29/91
20P	198	04/15/91
21P	139	04/11/91
22P	197	04/26/91
23P	211	05/09/91
24P	200	05/24/91
25P	200	06/10/91
26P	200	07/01/91
27P	352	07/15/91
28P	177	08/16/91
Total	**6571**	

Table 2-B

Primary *In Vitro* Antiviral Screen
Cumulative Summary of Number of Compounds Received Per Shipment Through December 31, 1991

Shipment Number	Compounds Per Shipment	Date Received
1	28	05/02/86
2	27	05/23/86
3	32	06/03/86
4	19	06/20/86
5	22	07/10/86
6	26	07/24/86
7	26	07/31/86
8	27	08/22/86
9	42	09/12/86
10	28	09/25/86
11	32	10/17/86
12	40	11/3-11/4/86
13	23	11/13/86
14	1	11/13/86
15	26	12/05/86
16	32	12/12/86
17	30	01/13/87
18	32	01/30/87
19	101	03/02/87
20	71	03/18/87
21	75	04/14/87
22	49	05/05/87
23	–	06/03/87
24	77	06/03/87
25	8	06/12/87
26	71	06/26/87
27	86	07/21/87
28	102	08/10/87
29	116	08/19/87
30	75	09/03/87
31	86	09/11/87
32	123	10/09/87
33	107	11/03/87
34	49	11/25/87
35	80	12/22/87
36	65	01/29/88
37	48	03/09/88
38	5	03/15/88
39	68	03/23/88
40	57	04/26/88
41	30	05/13/88
41B	30	05/13/88
41C	4	05/13/88
42	66	05/13/88
43	140	07/08/88
44	89	08/10/88
45	76	09/13/88
46	126	10/06/88
47	2	10/20/88
48	101	11/02/88
49	–	11/11/88
50	–	11/16/88
51	84	01/10/89
52	80	02/09/89
53	99	03/15/89
54	71	04/19/89
56	103	05/12/89
57	133	06/07/89
58	73	07/13/89
59	49	08/11/89
60	78	09/07/89
61	138	10/06/89
62	166	11/21/89
63	135	02/07/90
64	64	02/27/90
65	125	03/16/90
66	220	04/10/90
67	95	05/09/90
68	201	05/30/90
69	161	06/27/90
70	156	06/29/90
71	5	07/18/90
72	122	07/18/90
73	142	09/11/90
74	166	10/04/90
75	187	10/25/90
76	320	12/06/90
77	279	01/29/91
78	144	03/20/91
79	568	04/11/91
80	126	06/06/91
81	437	06/26/91
82	561	07/26/91
83	352	08/30/91
84	370	10/09/91
85	161	11/14/91
Total	8847	

TOTAL NUMBER OF *IN VITRO* ANTIVIRAL ASSAYS (PRESCREEN AND PRIMARY SYSTEMS)



Total No. Virus	FEB	MARCH	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	Tests/ Virus
Prescreen												
YF	270	475	720	795	420	185	1220	175	35	35	0	4330
VE	235	710	485	720	385	320	700	70	0	0	0	3625
PT	275	480	510	630	385	440	705	105	35	35	0	3600
Subtotal	780	1665	1715	2145	1190	945	2625	350	70	70	0	11555
Primary Screen												
VV	160	126	120	188	110	100	160	158	300	142	0	1564
YF	222	356	256	300	240	450	450	410	638	512	94	3928
JE	270	232	242	372	240	450	450	444	632	510	96	3938
VE	180	326	242	300	240	360	544	442	504	640	114	3892
PT	270	234	272	270	240	424	654	570	638	512	64	4148
SF	240	262	312	270	240	450	450	474	664	544	0	3906
Subtotal	1342	1536	1444	1700	1310	2234	2708	2498	3376	2860	368	21376
AIDS												
HIV	247	0	0	128	45	42	320	74	0	0	0	856
Monthly Total	**2369**	**3201**	**3159**	**3973**	**2545**	**3221**	**5653**	**2922**	**3446**	**2930**	**368**	
(All Tests)												**Grand Total = 33787**

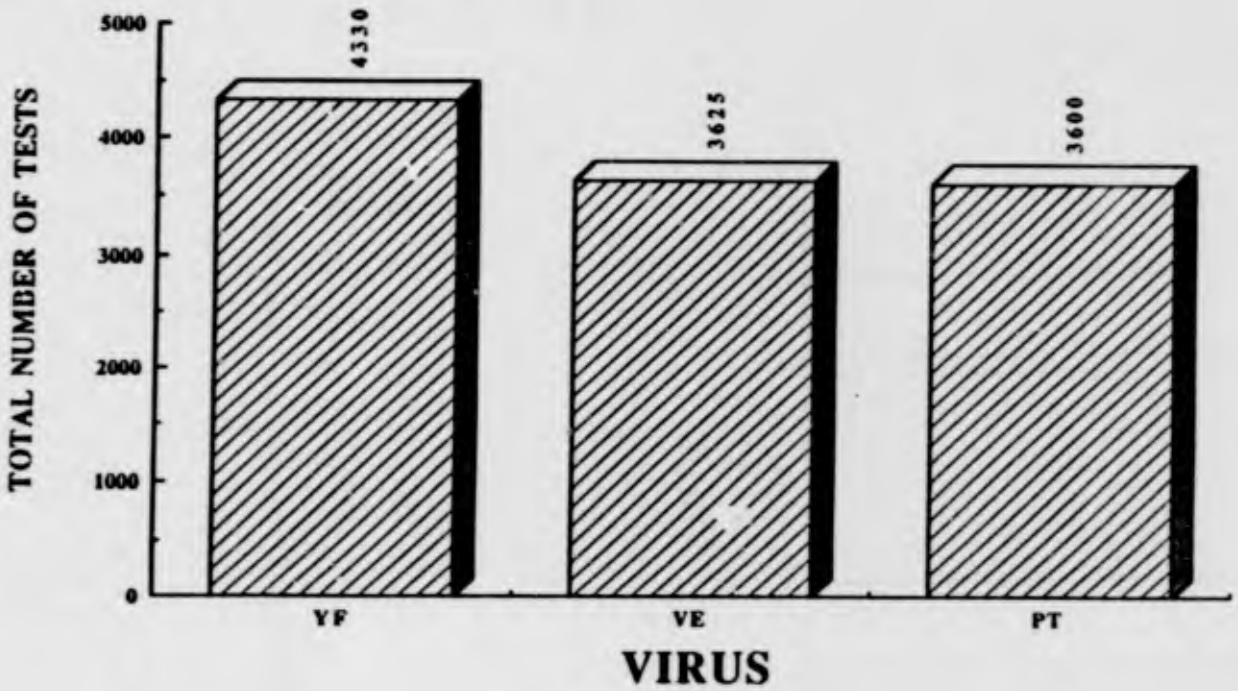
Figure 5

4.2 *In Vitro* Antiviral Evaluations: Exotic RNA Viruses (Prescreen Protocol):

A total of 2762 test compounds were received during this contract period (February 1, 1991 - December 31, 1991) at Southern Research Institute for evaluation in the *in vitro* antiviral prescreen system. The compounds were evaluated *in vitro* against Yellow Fever Virus (YF), Venezuelan Equine Encephalomyelitis Virus (VE) and Punta Toro Virus (PT) as they were received. A cumulative summary of the compound shipments received and the number of compounds in each shipment are presented in **Table 2-A**.

Approximately 11555 *in vitro* prescreen antiviral assays were performed this reporting period (**Figure 6**). Out of 8633 accepted single drug tests, 455 demonstrated antiviral activity at $\geq$50% reduction levels. This represents ~5% of the tested natural products having *in vitro* antiviral activity against these viruses (YF, VE, and PT). The remainder 8178 (95%) are to be considered inactive with the prescreen assay protocol. The antiviral activity results of the prescreen assays are summarized in **Figure 7**. These active compounds were tested (or scheduled to be tested) against the expanded battery of five primary screen viruses using the primary screening protocol. The results for compounds found to be active are summarized in the following sections for each virus. Results of all prescreen testing during this quarter (active test and inactive tests) are presented in **Appendix A**. Based upon the prescreen confirmatory criteria, the correlation between prescreen actives and confirmed actives from the primary screen has been approximately 70% during the previous quarter (February - April). Due to the project being cancelled early, we were unable to confirm some of the active prescreen compounds in the five primary screen viruses.

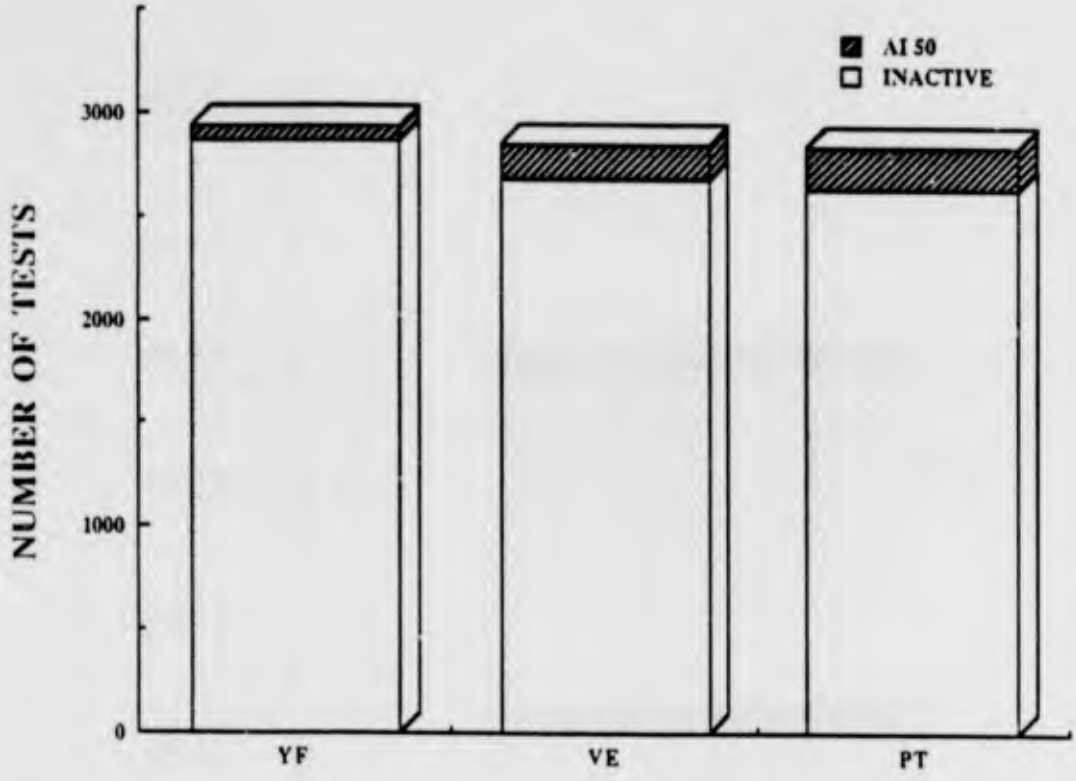
TOTAL NUMBER OF *IN VITRO* ANTIVIRAL ASSAYS TESTED IN THE PRESCREEN SYSTEM DURING THIS REPORTING PERIOD



Total No. Virus	FEB	MARCH	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	Tests/ Virus
Prescreen											
YF	270	475	720	795	420	185	1220	175	35	35	4330
VE	235	710	485	720	385	320	700	70	0	0	3625
PT	275	480	510	630	385	440	705	105	35	35	3600
Total	780	1665	1715	2145	1190	945	2625	350	70	70	11555

Figure 6

ACTIVE COMPOUNDS FROM THE PRESCREEN



Activity Status				Activity Totals
AI_{50}	63	176	216	455
Inactive	2868	2681	2629	8178
Total	2931	2857	2845	*8633

* Grand Total of Accepted Single Drug Tests (Excluding Positive Control Tests)

Figure 7

4.2.1 **Prescreen Assay (Yellow Fever Virus [YF]):**

The total output of YF prescreen-testing during this reporting period is summarized in monthly increments in **Figure 8**. During this period 4330 tests were performed against YF-virus with the MTT-assay format. Out of these, 166 were control compound assays-Selenazofurin (AVS-0253) and 100 were control compound assays-2-Thio-6-Azauridine (AVS-6724). Four hundred nine tests were internal (+ + +) virus load, cell load, and other quality control tests. Seven hundred twenty-four tests were considered unsatisfactory based on the preliminary criteria of the quality controls set during this reporting period. The rest, totaling 2931 were actual single drug tests. The total number of accepted single drug tests (2931) reflects 5.4% effort beyond our yearly contractual obligation. The 724 unsatisfactory tests represent a 17% rejection rate based on the present quality control parameters used for the YF virus.

Out of the 2931 test compounds, 63 demonstrated antiviral activity at greater than 50% reduction levels. This represents around 2% of the tested compounds having *in vitro* antiviral activity against YF-virus. The remainder, 2868 compounds (98%), are to be considered inactive with the present quality control and assay protocols.

TOTAL NUMBER OF TESTS AGAINST YELLOW FEVER VIRUS
PRESCREEN PROTOCOL

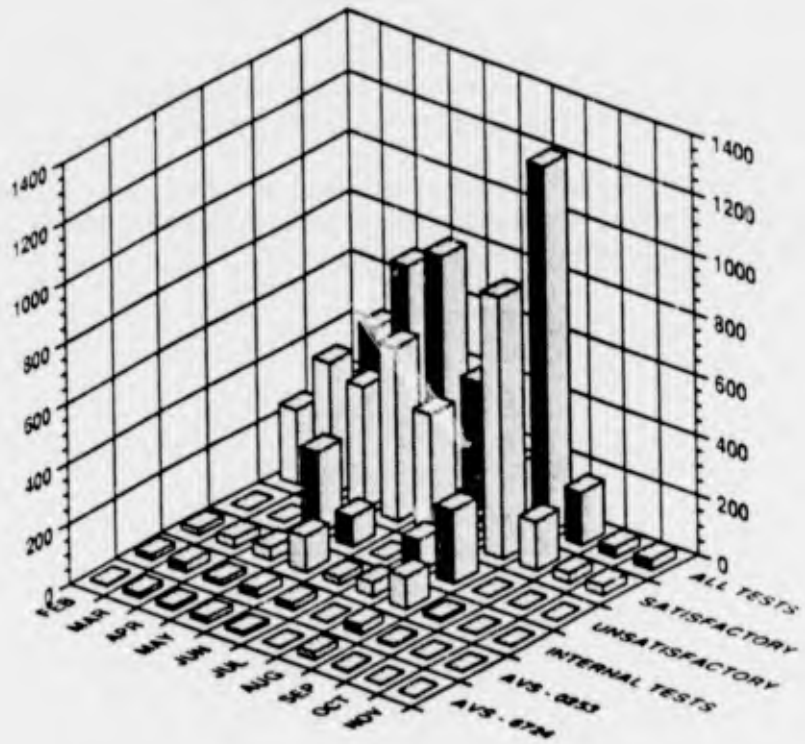


Figure 8

Quality Control Tests =	KPC (Positive Control - 2-Thio-6-Azauridine) =	100
	KPC (Positive Control - Selenazofurin) =	166
	(+ + + +) (Internal Virus and Cell Load Controls) =	409
	(UT) Unsatisfactory Test (QC rejects) =	724
Accepted Single Drug Tests =		2931
Total number of YF Tests =		4330

4.2.1.1 **Prescreen YF-Quality Controls:**

4.2.1.1.1 **Antiviral Activity of Selenazofurin vs YF Virus:**

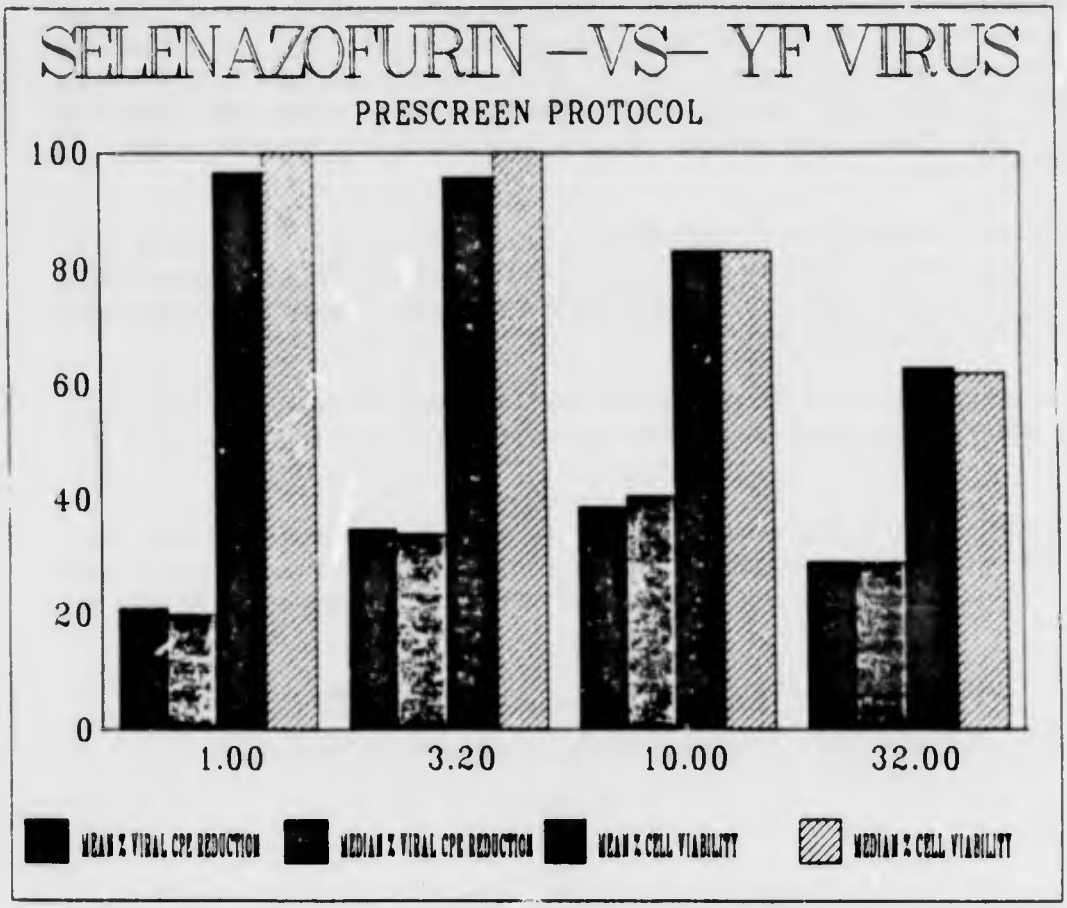
Control Compound-Antiviral Performance: Selenazofurin (AVS-0253) has been the primary control compound against YF in these MTT-assay prescreens. The mean and median antiviral inhibition and cytotoxicity patterns of the positive control drug (Selenazofurin) are illustrated in **Figure 9-A.**

The 166 control tests performed with Selenazofurin gave a mean **Selectivity Index (SI)** of 2.7 (SD ± 5.8) and the median value was 0 (range = 0 - >32), indicating poor antiviral selectivity for Selenazofurin. The reason for this discrepancy is that Selenazofurin does not consistently reach the 50% antiviral reduction level, thus SI calculations cannot be executed properly. SI is calculated by dividing the TC_{25} by the IC_{50}.

The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 2.7 μg/ml (SD ± 5.4). The median IC_{50} value was 0 μg/ml (range = 0 - >32 μg/ml). This indicates that Selenazofurin does reach 50% antiviral reduction levels. The TC_{25} and IC_{50} values can be measured relatively consistently at the 1 - 32 μg/ml (0.5 $\log_{10}$) drug concentration scale.

The average maximum antiviral inhibitory level of 166 Selenazofurin tests (**Figure 9-A**) was reached at 10 μg/ml of the compound with ~40% antiviral effect. Maximum antiviral effect (~40%) was found with a simultaneous ~17% cytotoxic suppression. Above 10 μg/ml concentration Selenazofurin starts to lose its antiviral potency with increasing cytotoxicity. An increase of the concentration of Selenazofurin to 32 μg/ml does not improve the antiviral activity (**Figure 9-A**). Actually antiviral activity decreases from 40% (at 10 μg/ml) to ~30% (at 32 μg/ml).

In the present, 166 control assays we tested Selenazofurin at 0.5 $\log_{10}$ scale concentrations (1 - 32 μg/ml) to maximize the correct measurements of its antiviral and cytotoxicity effects. This enabled us to monitor our quality control parameters more accurately.



CONCENTRATION (μg/ml)

	% Viral CPE Reduction				% Cell Viability			
Conc. (μg/ml)	1	3.2	10	32	1	3.2	10	32
Mean	21	35	39	29	97	96	83	63
Median	20	34	41	30	100	100	83	62
Std. Dev.	0.11	0.14	0.21	0.23	0.06	0.07	0.11	0.13

Figure 9-A
Average Antiviral and Cytotoxicity Values for 166 Positive Control Compound Assays

4.2.1.1.2 **Maximum Antiviral Effect of Selenazofurin vs YF Virus:**

A bar graph scatter plot (**Figure 10-A**) depicts the distribution of the maximum antiviral reduction values of 166 control compound prescreen assays for Selenazofurin. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 47% (SD ± 14.56) reduction levels. The maximum reduction levels vary from 24 - 100% but remain quite consistently around the median of 45%. The assay control values give a bell-shaped distribution curve around the median 45% reduction level. This indicates quite a consistent day-to-day performance of the control compound in the YF prescreen-MTT assay.

During this period the positive control compound performance criteria for Selenazofurin versus the YF virus in the pre-screen format has not been set to a definite endpoint. We have collected data in order to find out what would constitute a reasonable quality control endpoint and the data indicates that it could be set at the 25% reduction level.

In order to measure the maximum antiviral endpoints of Selenazofurin correctly, the concentration scale and the highest concentration to be used, must be evaluated at the proper (semi-log) scale as seen in **Figure 9-A.**

Selenazofurin is active *in vitro* against YF virus and functions as a reasonable quality control compound. On the other hand, regardless of the performance of the YF-quality control drug Selenazofurin, around 63 other compounds have equal to or better antiviral activity against YF virus than AVS-0253.

Variation of the Maximum Antiviral Effect
YF Virus - VS - Selenazofurin (Prescreen Protocol)

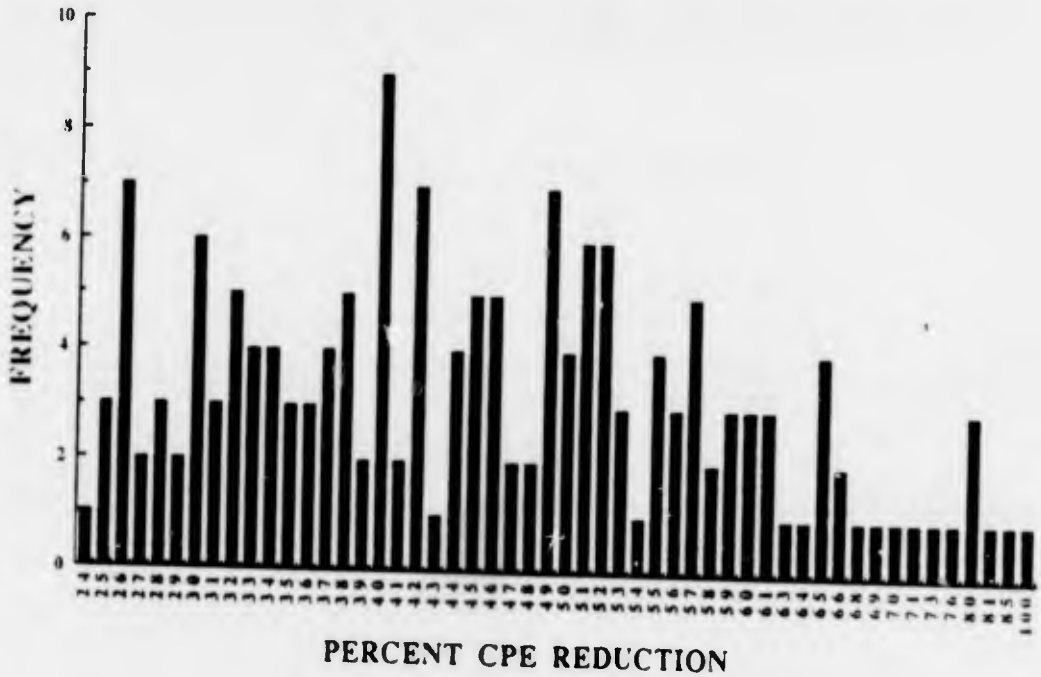


Figure 10-A
Maximum Antiviral CPE Reduction (%).
Summary of 166 Control Tests.

4.2.1.1.3 Cellular Cytotoxicity of Selenazofurin vs YF Virus:

YF-Control Compound-Cytotoxicity Performance: The 166 cytotoxicity values of the positive control compound Selenazofurin are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 19.13 μg/ml (SD ± 9.19) and the median was 18.4 μg/ml (range of 0.57 - >63.6 μg/ml).

As can be seen in **Figure 9-A**, a definite TC_{25} toxicity value can be measured with 1 - 32 μg/ml 0.5 $\log_{10}$ scale. Further increase in the concentration of Selenazofurin would be needed to consistently evaluate the maximum cytotoxicity of Selenazofurin.

Also, **Figure 9-A** indicates that when the cytotoxicity reaches ~17% at 10 μg/ml, the control compound (Selenazofurin) has reached simultaneously its maximum antiviral effect. The cytotoxic effect of Selenazofurin is insignificant below 3.2 μg/ml. The average maximum cytotoxicity reached ~37% at 32 μg/ml, which was the highest Selenazofurin concentration tested.

Selenazofurin has a definite cytotoxic suppression on cellular metabolism and growth. The TC_{25} toxicity can be achieved with the 32 μg/ml concentration of Selenazofurin. Therefore, a readjustment to 100 μg/ml (as being the highest Selenazofurin concentration tested) is not needed. However, at this concentration (32 μg/ml) the TC_{50} cannot always be measured consistently.

4.2.1.1.4 **YF-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (Selenazofurin):**

YF-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 166 control assays is plotted in **Figure 11-A**. The results indicate that the cell O.D. readings reached a mean 1.276 (SD ± 0.120) with a median of 1.276 (range of 0.579 - 1.626). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

YF-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 166 control assays is presented in **Figure 12-A**. The results indicate that the average virus load O.D. reading is 0.471 (SD ± 0.206) with a median of 0.505 (range of 0.111 - 0.899). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

YF-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 166 control assays is provided in **Figure 13-A**. The results indicate that the average differential O.D. reading is 0.805 (SD ± 0.206) with a median of 0.767 (range of 0.387 - 1.364). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 80% measurement accuracy.

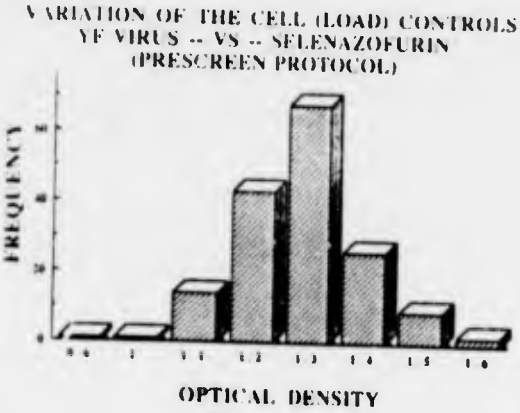


Figure 11-A

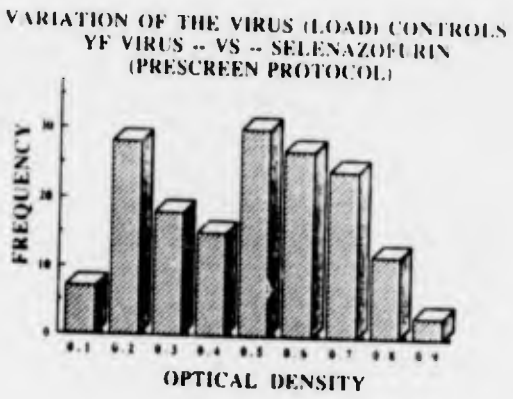


Figure 12-A

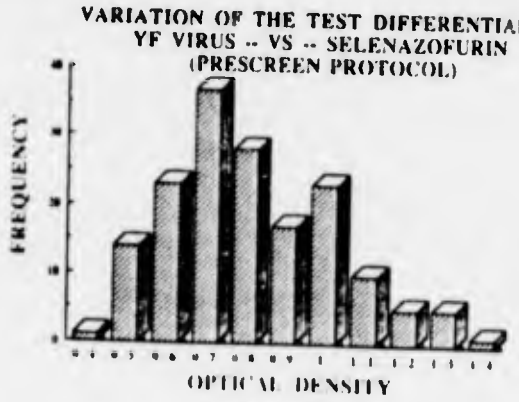


Figure 13-A

4.2.1.1 Prescreen YF-Quality Controls:

4.2.1.1.1 Antiviral Activity of 2-Thio-6-Azauridine vs YF Virus:

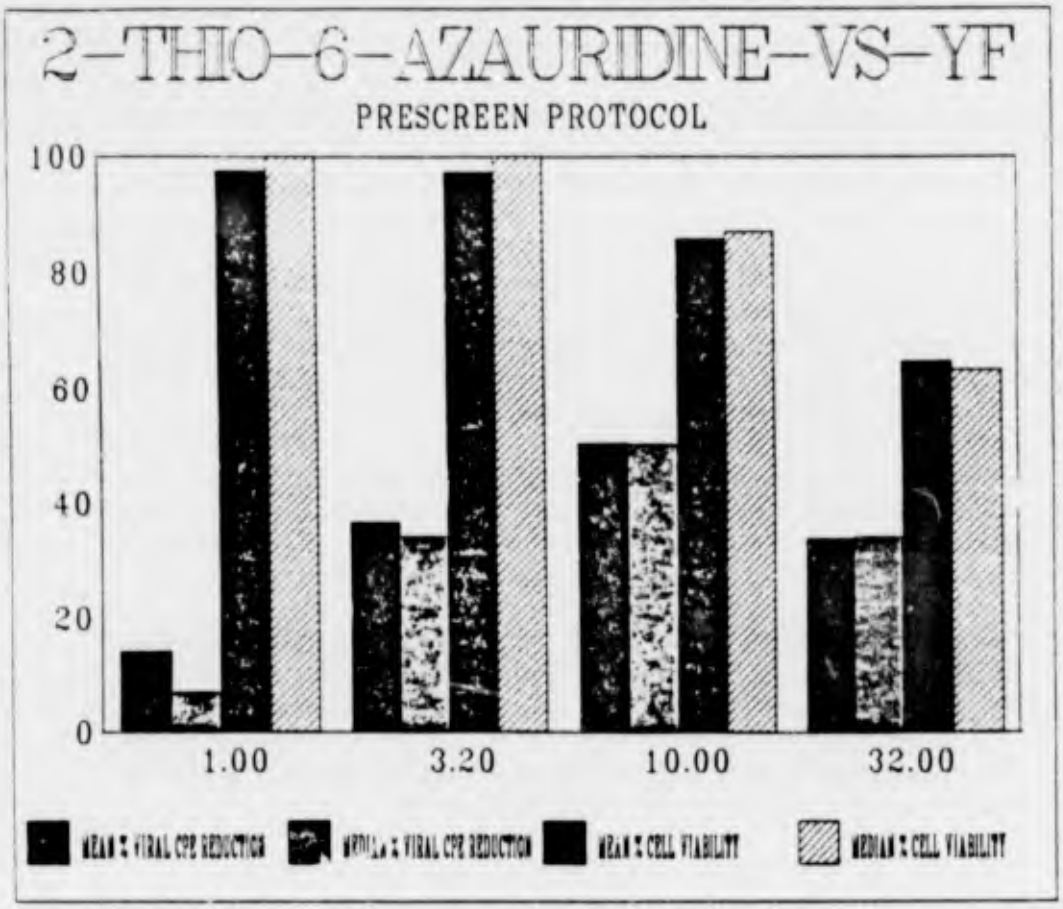
Second Control Compound-Antiviral Performance: 2-Thio-6-Azauridine (AVS-6724) has been tested as a second control compound against YF in these MTT-assay prescreens. The mean and median antiviral inhibition and cytotoxicity patterns of the second positive control drug are illustrated in **Figure 9-B**.

The 100 control tests performed with 2-Thio-6-Azauridine gave a mean **Selectivity Index (SI)** of 4.98 (SD ± 6.65) and the median value was 2.69 (range = 0 - >32), indicating moderate antiviral selectivity for 2-Thio-6-Azauridine. The reason for this discrepancy is that even at 32 - 100 μg/ml the maximum cytotoxic effect does not consistently reach 25% reduction level, thus SI calculations cannot be executed properly. SI is calculated by dividing the TC_{25} by the IC_{50}.

The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 3.09 μg/ml (SD ± 5.04). The median IC_{50} value was 2.03 μg/ml (range = 0 - 32). This indicates that 2-Thio-6-Azauridine does reach 50% antiviral reduction levels consistently at 10 μg/ml concentration. The TC_{25} and IC_{50} values can be measured with relative consistency at the 1 - 32 μg/ml (0.5 Log_{10}) drug concentration scale.

The average maximum antiviral inhibitory level of 100, 2-Thio-6-Azauridine tests **(Figure 9-B)** was reached at 10 μg/ml of the compound with 50% antiviral effect. Maximum antiviral effect (~50%) was found with a simultaneous ~14% cytotoxic suppression. Above 10 μg/ml concentration 2-Thio-6-Azauridine starts to lose its antiviral potency with increasing cytotoxicity. An increase of the concentration of 2-Thio-6-Azauridine to 32 μg/ml does not improve the antiviral activity **(Figure 9-B)**. The antiviral activity decreases from 50% (at 10 μg/ml) to 34% (at 32 μg/ml).

In the present 100 assays we tested 2-Thio-6-Azauridine at 0.5 log_{10} scale concentrations (1 - 32 μg/ml) to maximize the correct measurements of its antiviral and cytotoxicity effects. This enabled us to monitor our quality control parameters more accurately.



	% Viral CPE Reduction				% Cell Viability			
Conc.(μg/ml)	1	3.2	10	32	1	3.2	10	32
Mean	14	37	50	34	98	97	86	65
Median	7	34	50	34	100	100	87	63
Std. Dev.	0.17	0.16	0.29	0.29	0.05	0.05	0.14	0.13

Figure 9-B
Average Antiviral and Cytotoxicity Values for 100 Positive Control Compound Tests

4.2.1.1.2 **Maximum Antiviral Effect of 2-Thio-6-Azauridine vs YF Virus:**

A bar graph scatter plot (**Figure 10-B**) depicts the distribution of the maximum antiviral reduction values of all 100 control compound prescreen assays for 2-Thio-6-Azauridine. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 57% (SD ± 23.12) reduction levels. The maximum reduction levels vary from 19 - 100% but remain quite consistently around the median of 56%. The assay control values give a bell-shaped distribution curve around the median 56% reduction level. This indicates quite a consistent day-to-day performance of the control compound in the YF prescreen-MTT assay.

During this period the positive control compound performance criteria for 2-Thio-6-Azauridine versus the YF virus in the pre-screen format has not been set to a definite endpoint. We have collected data in order to find out what would constitute a reasonable quality control endpoint and the data indicates that it could be set at the 50% reduction level.

In order to measure the maximum antiviral endpoints of 2-Thio-6-Azauridine correctly, the concentration scale and the highest concentration to be used, must be evaluated at the proper (semi-log) scale (1 - 32 μg/ml) as seen in **Figure 9-B**.

2-Thio-6-Azauridine is active *in vitro* against YF virus and functions as a better quality control compound than our present control, Selenazofurin.

Variation of the Maximum Antiviral Effect
YF Virus - VS - 2-Thio-6-Azauridine (Prescreen Protocol)

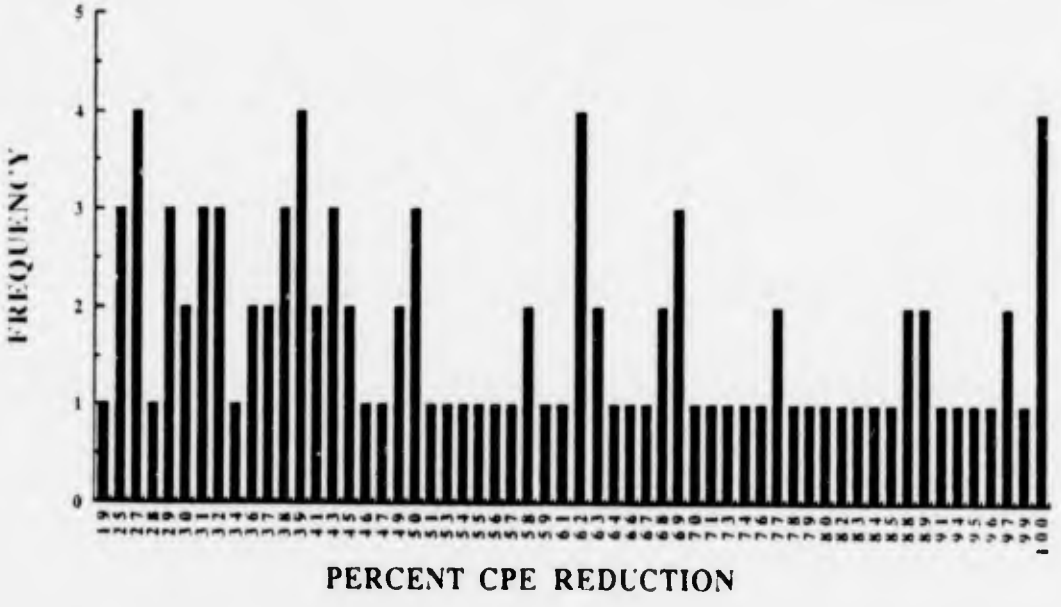


Figure 10-B
Maximum Antiviral CPE Reduction (%).
Summary of 100 Control Tests.

4.2.1.1.3 **Cellular Cytotoxicity of 2-Thio-6-Azauridine vs YF Virus:**

YF-Control Compound-Cytotoxicity Performance: The 100 cytotoxicity values of the positive control compound 2-Thio-6-Azauridine are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 20.98 μg/ml (SD ± 10.12) and the median was 21.85 μg/ml (range of 4.38 - >32 μg/ml).

As can be seen from **Figure 9-B**, a definite TC_{25} toxicity value can be consistently measured with a 1 - 32 μg/ml $\log_{10}$ scale. Further increase in the concentration of 2-Thio-6-Azauridine would be needed to properly evaluate the maximum cytotoxicity of 2-Thio-6-Azauridine.

Figure 9-B, indicates that when the cytotoxicity reaches ~13% at 10 μg/ml, the control compound (2-Thio-6-Azauridine) has reached simultaneously its maximum antiviral effect. The cytotoxic effect of 2-Thio-6-Azauridine is insignificant below 3.2 μg/ml. The average cytotoxicity reached ~35% at 32 μg/ml, which was the highest 2-Thio-6-Azauridine concentration in most tests.

2-Thio-6-Azauridine has a definite cytotoxic suppression on cellular metabolism and growth. However, the TC_{25} and TC_{50} toxicity could not be consistently achieved with the 32 μg/ml concentration of 2-Thio-6-Azauridine. Therefore, a readjustment to 100 μg/ml (as being the highest 2-Thio-6-Azauridine concentration tested) would be needed to properly evaluate the TC_{25} endpoint. However, at this concentration (100 μg/ml) the IC_{50} cannot be measured consistently.

4.2.1.1.4 **YF-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (2-Thio-6-Azauridine):**

YF-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 100 control assays is plotted in **Figure 11-B**. The results indicate that the cell O.D. readings reacned a mean of 1.286 (SD ± 0.128) with a median of 1.285 (range of 0.579 - 1.626). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

YF-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 100 control assays is presented in **Figure 12-B**. The results indicate that the average virus load O.D. reading is 0.504 (SD ± 0.199) with a median of 0.546 (range of 0.111 - 0.899). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

YF-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 100 control assays is provided in **Figure 13-B**. The results indicate that the average differential O.D. reading is 0.783 (SD ± 0.206) with a median of 0.752 (range 0.438 - 1.364). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 78% measurement accuracy.

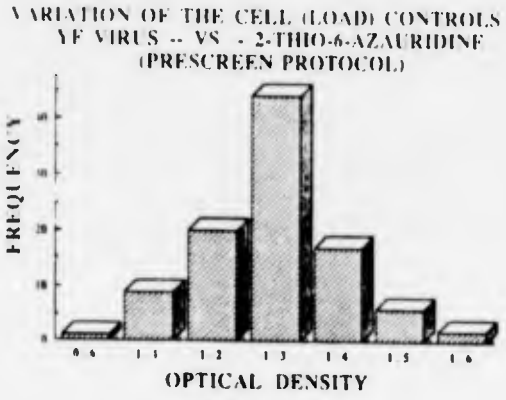


Figure 11-B

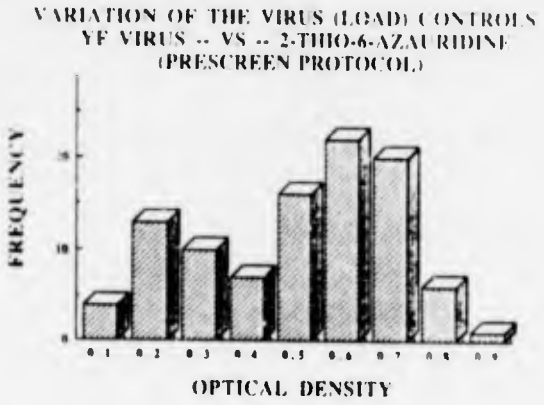


Figure 12-B

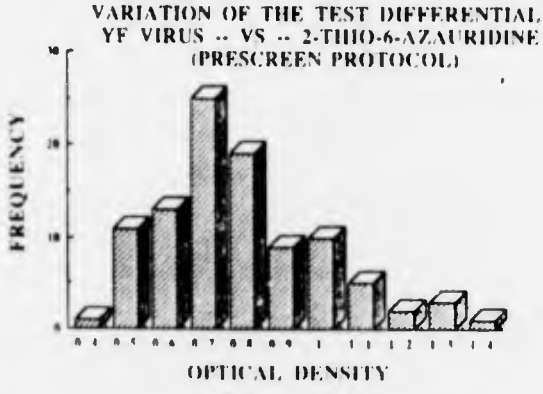


Figure 13-B

4.2.1.1.5 **Overall YF-Assay Plate Quality Controls:**

YF-Overall-Cell Load Performance: A bar graph scatter plot of the overall mean cell control (O.D. reading) of 2931 accepted assays is plotted in **Figure 11-C**. The results indicate that the overall cell O.D. readings reached a mean 1.260 (SD ± 0.106) with a median of 1.256 (range of 0.579 - 1.626). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

YF-Overall-Virus Load Performance: A bar graph scatter plot of the overall mean virus load O.D. readings of the 2931 accepted assays is presented in **Figure 12-C**. The results indicate that the overall average virus load O.D. reading is 0.494 (SD ± 0.182) with a median of 0.531 (range of 0.111 - 1.044). This demonstrates that a reasonable cell destruction was taking place and a uniform load of virus (32 $TCID_{50}$) was administered on the cell monolayer with consistent viral CPE results.

YF-Overall-Assay Differential Performance: A bar graph scatter plot of the overall mean O.D. differential values of the 2931 accepted assays is provided in **Figure 13-C**. The results indicate that the average overall differential O.D. reading is 0.767 (SD ± 0.189) with a median of 0.737 (range 0.387 - 1.364). The single bell-shaped curve is reasonably sharp and uniform. **This reflects that overall the assay plates were executed consistently and were repeatable during day-to-day operation with close to 77% measurement accuracy.**

GRAPHIC ILLUSTRATION OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ALL ACCEPTED YF PLATE DATA

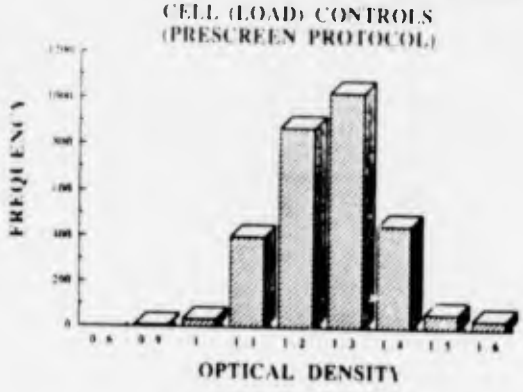


Figure 11-C

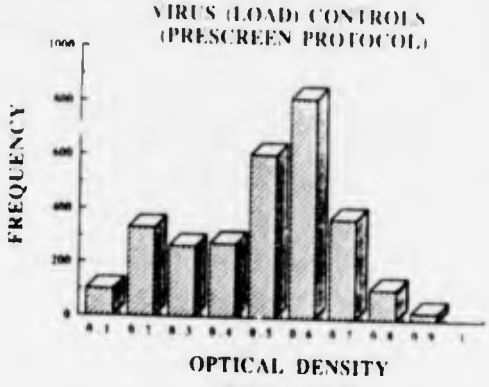


Figure 12-C

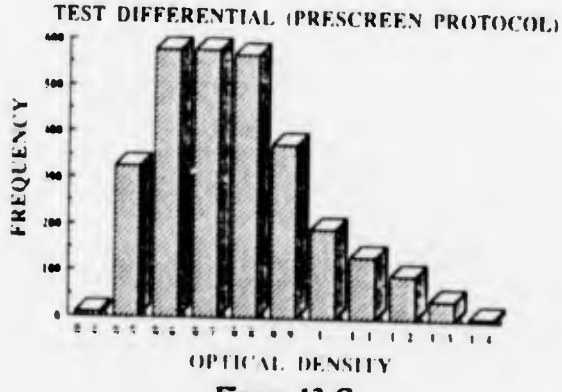


Figure 13-C

4.2.1.2 **Prescreen YF-Antiviral Activity Results:**

New Drugs with 50% Antiviral Reduction Levels: Out of the 2931 accepted single drug tests, 63 new compounds demonstrated antiviral activity, having antiviral reduction values better than 50%. This represents around 2% of the test compounds being active at this antiviral reduction level. These compounds are summarized in **Table 3** according to the highest **Selectivity Index (SI)**. Two compounds, GRP23193 and GRP21860, demonstrated the best antiviral promise having SI's of > 1000 and > 692, respectively. Twelve compounds demonstrated good antiviral activity, having SI's from 161 - > 398. The rest (49) demonstrated moderate to minimal antiviral selectivity with SI's that ranged from 0.8 - 77.

Table 3

New Prescreen Drugs that Produced 50% Antiviral Reduction Against YF Virus

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP23193	YF	4UQ	18P	04/23/91	> 1000.0	< 1.00	> 1000.0
GRP21860	YF	855	27P	08/08/91	692.00	< 1.00	> 691.96
GRP21555	YF	93R	28P	08/29/91	398.00	< 1.00	> 398.44
B724287	YF	5A2	20P	05/03/91	482.00	1.26	383.72
GRP21807	YF	69V	23P	06/07/91	333.00	< 1.00	> 333.47
B724062	YF	4U2	19P	04/18/91	> 1000.0	3.00	> 333.16
B724354	YF	5FW	20P	05/08/91	> 1000.0	3.04	> 328.67
GRP21906	YF	8F4	27P	08/13/91	325.00	< 1.00	> 325.00
B724063	YF	4U2	19P	04/18/91	> 1000.0	4.22	> 237.17
B723918	YF	5TZ	22P	05/23/91	> 1000.0	4.54	> 220.23
B723888	YF	6IC	22P	06/14/91	> 1000.0	4.79	> 208.86
GRP23194	YF	4UQ	18P	04/23/91	191.00	1.00	190.93
GRP21840	YF	851	27P	08/08/91	> 1000.0	5.90	> 169.50
B724251	YF	57H	20P	05/01/91	> 1000.0	6.19	> 161.44
GRP23270	YF	52F	18P	04/26/91	285.00	3.71	76.98
GRP21821	YF	913	27P	08/27/91	406.00	6.19	65.60
B724358	YF	5FX	20P	05/08/91	> 1000.0	18.30	> 54.70
B724137	YF	4Y8	19P	04/24/91	325.00	7.16	45.40
B724130	YF	4XU	19P	04/23/91	> 1000.0	42.40	> 23.57
B724298	YF	5A4	20P	05/03/91	770.00	47.50	16.22
GRP23078	YF	97E	28P	09/04/91	414.00	31.10	13.28
GRP23079	YF	97E	28P	09/04/91	334.00	29.30	11.40
GRP23412	YF	4TG	18P	04/16/91	> 1000.0	92.00	> 10.87
B724140	YF	4Y9	19P	04/24/91	18.50	1.84	10.02
GRP18066	YF	363	14P	02/13/91	325.00	39.20	8.30
GRP23728	YF	67N	23P	06/04/91	294.00	36.90	7.98
GRP23729	YF	67O	23P	06/04/91	274.00	37.80	7.26
DN1611220	YF	5MQ	21P	05/15/91	> 1000.0	199.00	> 5.03
GRP23235	YF	5NR	18P	05/16/91	> 1000.0	230.00	> 4.34
B723954	YF	5UL	22P	05/24/91	419.00	100.00	4.19
GRP23293	YF	4UW	18P	04/23/91	> 1000.0	246.00	> 4.06
GRP23202	YF	4US	18P	04/23/91	325.00	83.60	3.89
B724145	YF	4Y9	19P	04/24/91	325.00	87.10	3.73
B724268	YF	59L	20P	05/02/91	> 1000.0	280.00	> 3.57
B724313	YF	5EZ	20P	05/07/91	336.00	95.40	3.53
GRP21889	YF	93K	27P	08/28/91	326.00	93.50	3.48
B723881	YF	5LV	20P	05/14/91	> 1000.0	289.00	> 3.46
B724271	YF	59M	20P	05/02/91	> 1000.0	306.00	> 3.27
GRP23245	YF	529	18P	04/25/91	> 1000.0	311.00	> 3.21
B723912	YF	5TY	22P	05/23/91	> 1000.0	340.00	> 2.94
GRP21479	YF	90X	28P	08/27/91	> 1000.0	345.00	> 2.90
GRP21730	YF	68L	23P	06/05/91	224.00	84.90	2.64
B723917	YF	5TZ	22P	05/23/91	> 1000.0	381.00	> 2.63
GRP23816	YF	8IU	27P	08/15/91	> 1000.0	395.00	> 2.53
GRP23608	YF	6ML	24P	06/19/91	> 1000.0	411.00	> 2.43
B724338	YF	5FT	20P	05/08/91	> 1000.0	415.00	> 2.41
B724230	YF	56T	19P	04/30/91	> 1000.0	443.00	> 2.26
GRP21844	YF	851	27P	08/08/91	> 1000.0	541.00	> 1.85

Table 3 (Cont'd)

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP21511	YF	AS2	28P	10/16/91	> 1000.0	560.00 >	1.78
B723957	YF	5Z0	22P	05/29/91	> 1000.0	570.00 >	1.75
GRP23589	YF	6LG	24P	06/18/91	> 1000.0	570.00 >	1.75
GRP21550	YF	93Q	28P	08/29/91	> 1000.0	588.00 >	1.70
B678018/F244	YF	3IK	15P	02/26/91	64.10	38.70	1.66
GRP23222	YF	5NO	18P	05/16/91	> 1000.0	610.00 >	1.64
GRP21668	YF	4BK	18P	03/27/91	> 1000.0	654.00 >	1.53
B723911	YF	5TY	22P	05/23/91	> 1000.0	722.00 >	1.38
GRP23408	YF	4TF	18P	04/16/91	104.00	81.00	1.28
GRP21519	YF	AS3	28P	10/16/91	798.00	626.00	1.28
GRP21376	YF	7F3	25P	08/20/91	> 1000.0	823.00 >	1.21
B724299	YF	5A4	20P	05/03/91	747.00	675.00	1.11
B723826	YF	5GG	20P	05/09/91	739.00	693.00	1.07
B724275	YF	59M	20P	05/02/91	> 1000.0	1000.0 >	1.00
B718876	YF	30G	14P	02/05/91	471.00	609.00	0.77

The *in vitro* antiviral activity of the compounds in **Table 3** should be further confirmed. Verification of the antiviral activity of these prescreen actives were scheduled to be tested using the primary screening (confirmatory) protocol. Because of the discontinuation of this antiviral program by the Sponsor, some of the active compounds in **Table 3** have not been confirmed in the primary screening protocol due to lack of funding.

4.2.2 Prescreen Assay (Venezuelan Equine Encephalomyclitis Virus [VE]):

The total output of VE prescreen-testing during this reporting period is summarized in monthly increments in **Figure 14**. During this period 3625 tests were performed against the VE-virus with the MTT-assay format. Out of these, 202 were control compound assays - Selenazofurin (AVS-0253) and 69 were control compound assays - 2-Thio-6-Azauridine (AVS-6724). Four hundred fifty-one tests were internal (+ + +) virus load, cell load, and other quality control tests. Forty-six (46) tests were considered unsatisfactory based on the preliminary criteria of the quality controls set during this reporting period. The rest, totaling 2857, were actual single drug tests. The total number of accepted single drug tests (2857) reflects 3% effort beyond our yearly contractual obligation. The 46 unsatisfactory tests represents a 1.3% rejection rate based on the present quality control parameters used for the VE virus.

Out of the 2857 test compounds, 176 demonstrated antiviral activity at greater than 50% reduction levels. This represents around 6% of the tested compounds having *in vitro* antiviral activity against VE-virus. The remainder, 2681 compounds (94%), are to be considered inactive with the present quality control and assay protocols.

TOTAL NUMBER OF TESTS AGAINST VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS PRESCREEN PROTOCOL

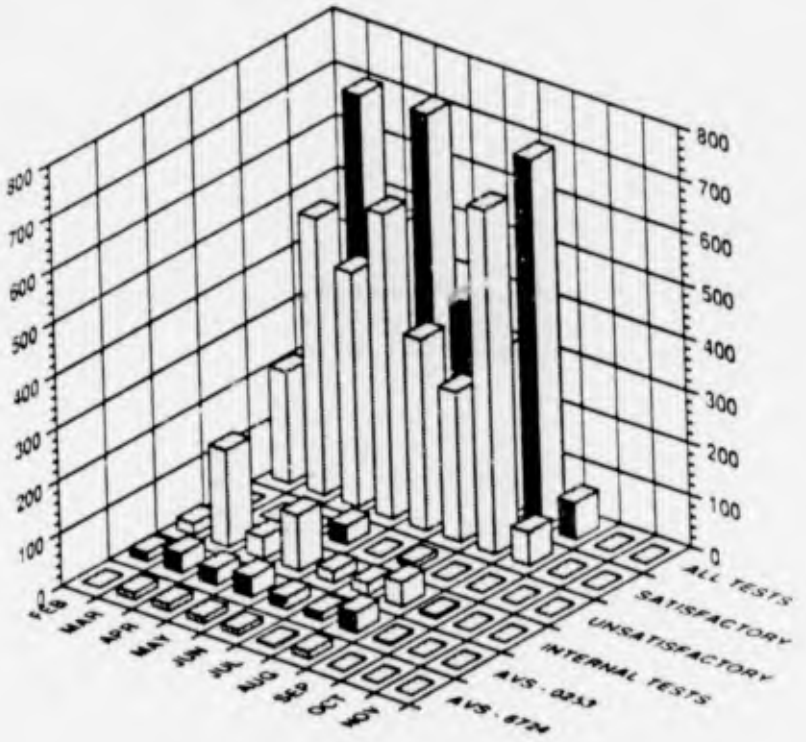


Figure 14

	KPC (Positive Control - 2-Thio-6-Azauridine) =	69
Quality Control Tests =	KPC (Positive Control - Selenazofurin) =	202
	(+ + + +) (Internal Virus and Cell Load Controls) =	451
	(UT) Unsatisfactory Test (QC rejects) =	46
Accepted Single Drug Tests =		2857
Total number of VE Tests =		3625

4.2.2.1 Prescreen VE-Quality Controls:

4.2.2.1.1 Antiviral Activity of Selenazofurin vs VE Virus:

Control Compound-Antiviral Performance: Selenazofurin (AVS-0253) has been the primary control compound against VE in these MTT-assay prescreens. The mean and median antiviral inhibition and cytotoxicity patterns of the positive control drug (Selenazofurin) are illustrated in **Figure 15-A.**

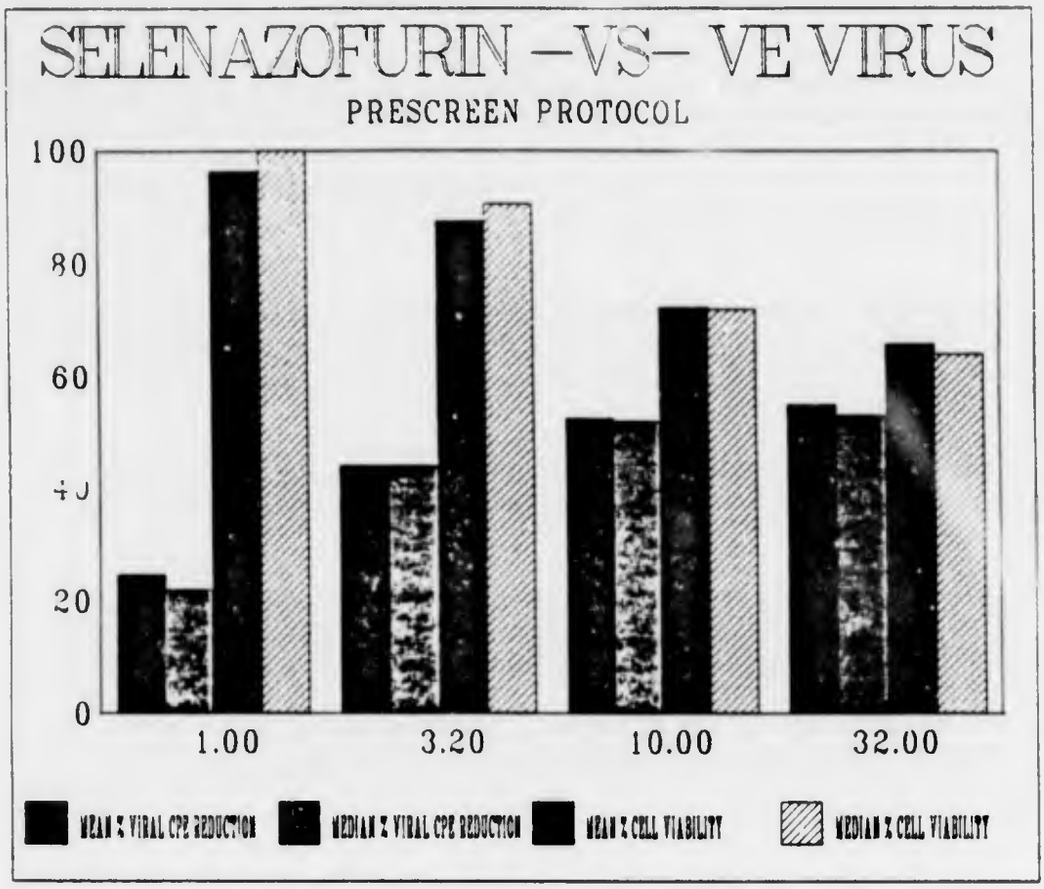
The 202 control tests performed with Selenazofurin gave a mean **Selectivity Index (SI)** of 4.67 (SD ± 8.54) and the median value was 1.1 (range = 0 - >32), indicating poor antiviral selectivity for Selenazofurin. The reason for this discrepancy is that Selenazofurin does not consistently reach 50% antiviral reduction level thus SI calculations cannot be executed properly. SI is calculated by dividing the TC_{25} by the IC_{50}.

The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 6.23 μg/ml (SD ± 9.10). The median IC_{50} value was 2.40 (range = 0 - 32 μg/ml) μg/ml. This indicates that Selenazofurin does reach 50% antiviral reduction levels. The TC_{25} and IC_{50} values can be measured relatively consistently at the 1 - 32 μg/ml (0.5 $\log_{10}$) drug concentration scale **(Figure 15-A).**

The average maximum antiviral inhibitory level of 202 Selenazofurin tests **(Figure 15-A)** was reached at 32 μg/ml of compound with 56% antiviral effect. Maximum antiviral effect (~56%) was found with a simultaneous ~34% cytotoxic suppression. Above 32 μg/ml concentration Selenazofurin starts to lose its antiviral potency with increasing cytotoxicity. An increase of the concentration of Selenazofurin to 100 - 320 μg/ml does not improve the antiviral activity. Actually (based on previous data) antiviral activity decreases from 56% (at 32 μg/ml) to ~45% (at 100 μg/ml) and remains at this level even at higher concentrations.

As reported previously, a different maximum antiviral value is obtained depending upon which concentration scale is used (log or semilog scale).

In the present, we tested 202 control assays with Selenazofurin at 0.5 $\log_{10}$ scale from 1 - 32 μg/ml to maximize the correct measurements of its antiviral and cytotoxicity effects. This enabled us to monitor our quality control parameters more accurately.



CONCENTRATION (μg/ml)

	% Viral CPE Reduction				% Cell Viability			
Conc. (μg/ml)	**1**	**3.2**	**10**	**32**	**1**	**3.2**	**10**	**32**
Mean	25	44	53	56	96	88	73	66
Median	23	44	53	54	100	90	72	64
Std. Dev.	0.17	0.19	0.17	0.12	0.07	0.13	0.15	0.14

Figure 15-A
Average Antiviral and Cytotoxicity Values for 202 Positive Control Compound Assays

4.2.2.1.2 **Maximum Antiviral Effect of Selenazofurin vs VE Virus:**

A bar graph scatter plot (**Figure 16-A**) depicts the distribution of the maximum antiviral reduction values of all 202 control compound prescreen assays for Selenazofurin. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 58% (SD ± 13.6) reduction levels. The maximum reduction levels vary from 34 - 100% but remain quite consistently around the median of 56%. The assay control values give a reasonable bell-shaped distribution curve toward the median 56% reduction level. This indicates quite a consistent day-to-day performance of the control compound in the VE prescreen-MTT assay.

During this period the positive control compound performance criteria for Selenazofurin versus the VE virus in the pre-screen format has not been set to a definite endpoint. We have collected data in order to find out what would constitute a reasonable quality control endpoint and the data indicates that it could be set at the 50% reduction level.

In order to measure the maximum antiviral endpoints of Selenazofurin correctly, the concentration scale and the highest concentration to be used, must be evaluated at the proper (semi-log) scale as seen in **Figure 15-A**.

Selenazofurin is active *in vitro* against VE virus and functions as a reasonable quality control compound. On the other hand, regardless of the performance of the VE-quality control drug Selenazofurin, around 176 other compounds have equal or better antiviral activity against VE virus than AVS-0253.

VARIATION OF THE MAXIMUM ANTIVIRAL EFFECT
VE VIRUS - VS - SELENAZOFURIN (PRESCREEN PROTOCOL)

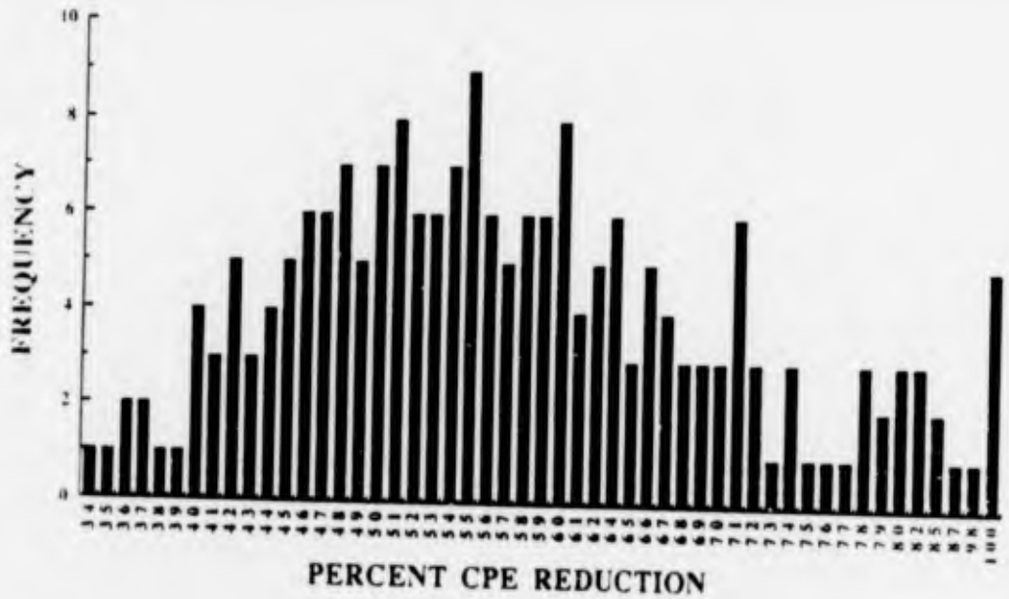


Figure 16-A
Maximum Antiviral CPE Reduction (%).
Summary of 202 Control Tests.

4.2.2.1.3 **Cellular Cytotoxicity of Selenazofurin vs VE Virus:**

VE-Control Compound-Cytotoxicity Performance: The 202 cytotoxicity values of the positive control compound Selenazofurin are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 14.79 μg/ml (SD ± 11.89) and the median was 8.95 μg/ml (range of 1.0 - >32 μg/ml). The reason for this discrepancy is that at the 1.0 - 32 μg/ml scale, neither the TC_{25} cytotoxicity or the IC_{50} cannot always be measured accurately.

As can be seen from **Figure 15-A**, a definite TC_{25} toxicity value can be measured with a 1 - 32 μg/ml (0.5 Log_{10}) scale. Further increase in the concentration of Selenazofurin would be needed to consistently evaluate the maximum cytotoxicity of Selenazofurin.

Also, **Figure 15-A** indicates that when the cytotoxicity reaches ~34% at 32 μg/ml, the control compound (Selenazofurin) has reached simultaneously its maximum antiviral effect. The cytotoxic effect of Selenazofurin is insignificant below 3.2 μg/ml. The average maximum cytotoxicity reached ~34% at 32 μg/ml, which was the highest Selenazofurin concentration in most tests.

Selenazofurin has a definite cytotoxic suppression on cellular metabolism and growth. The TC_{25} and TC_{50} toxicity can be achieved with reasonable consistency at 32 μg/ml concentration of Selenazofurin. Therefore, a readjustment to 100 μg/ml (as being the highest Selenazofurin concentration tested) is not needed. However, at this concentration (100 μg/ml) the IC_{50} cannot always be measured accurately.

4.2.2.1.4 **VE-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (Selenazofurin):**

VE-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 202 control assays is plotted in **Figure 17-A**. The results indicate that the cell O.D. readings reached a mean 1.167 (SD ± 0.168) with a median of 1.182 (range of 0.476 - 1.591). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

VE-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 202 control assays is presented in **Figure 18-A**. The results indicate that the average virus load O.D. reading is 0.066 (SD ± 0.078) with a median of 0.037 (range of -0.032 - 0.536). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

VE-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 202 control assays is provided in **Figure 19-A**. The results indicate that the average differential O.D. reading is 1.101 (SD ± 0.186) with a median of 1.125 (range 0.497 - 1.615). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 90% measurement accuracy.

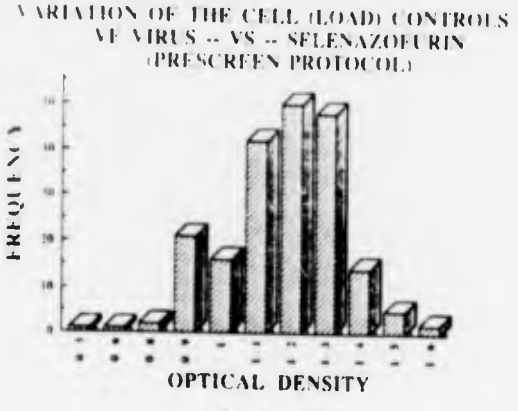


Figure 17-A

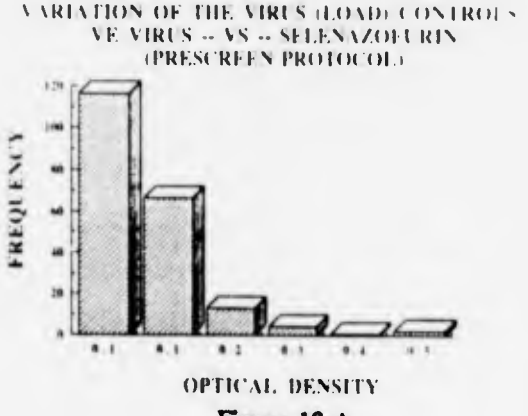


Figure 18-A

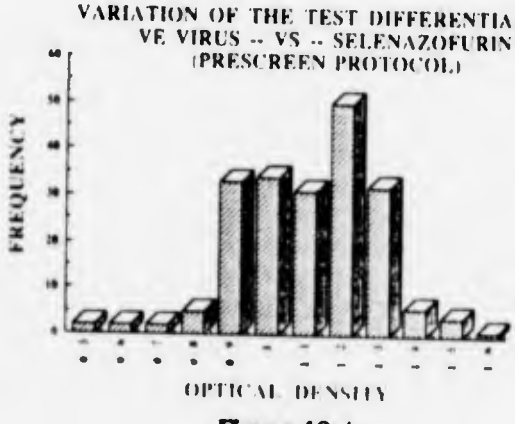


Figure 19-A

4.2.2.1 **Prescreen VE-Quality Controls:**

4.2.2.1.1 **Antiviral Activity of 2-Thio-6-Azauridine vs VE Virus:**

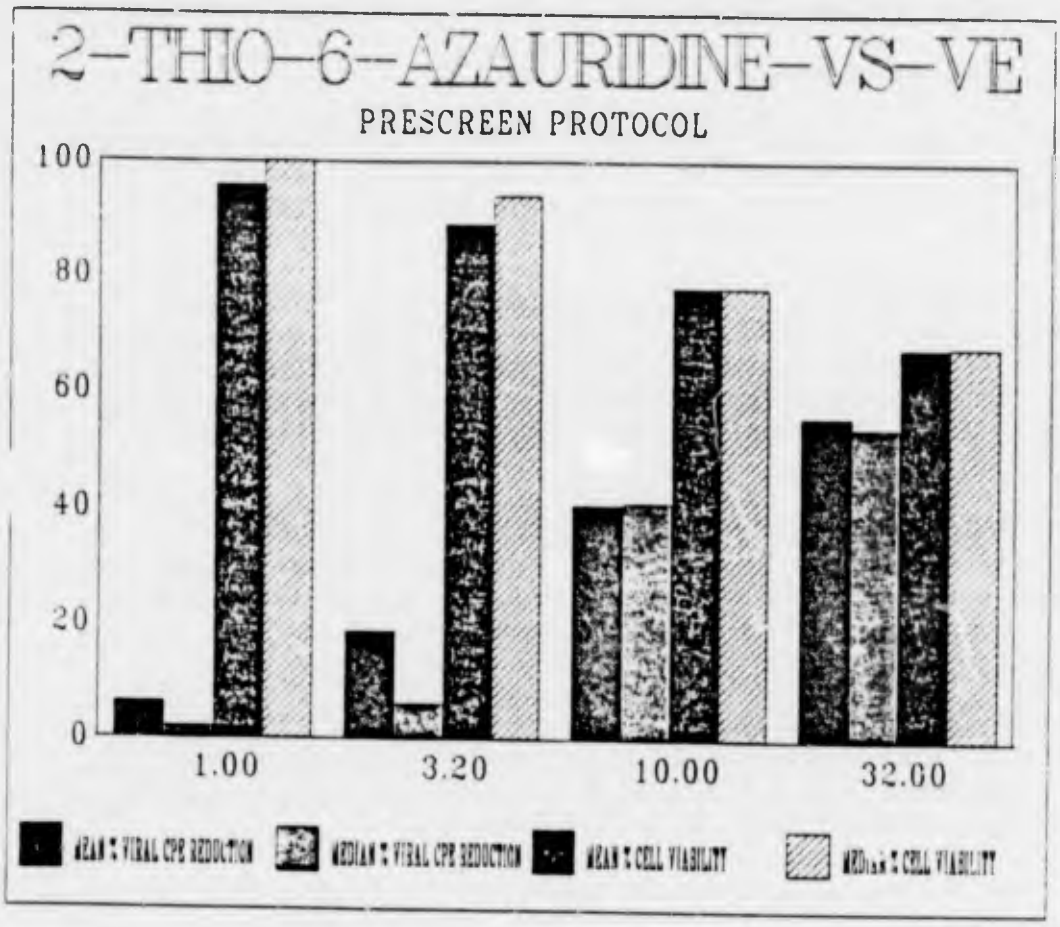
Second Control Compound-Antiviral Performance: 2-Thio-6-Azauridine (AVS-6724) has been tested as a second control compound against VE in these MTT-assay prescreens. The mean and median antiviral inhibition and cytotoxicity patterns of the second positive control drug are illustrated in **Figure 15-B**.

The 69 control tests performed with 2-Thio-6-Azauridine gave a mean **Selectivity Index (SI)** of 2.66 (SD ± 5.00) and the median value was 0.850 (range 0 - >32), indicating moderate antiviral selectivity for 2-Thio-6-Azauridine. The reason for this occasional discrepancy is that even at higher (100 µg/ml) drug concentrations the maximum antiviral effect does not always reach 50% reduction level, thus in this situation, the SI calculations cannot be executed properly. SI is calculated by dividing the TC_{25} by the IC_{50}.

The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 9.68 µg/ml (SD ± 11.37). The median IC_{50} value was 5.20 µg/ml (range = 0 - 52 µg/ml). This indicates that 2-Thio-6-Azauridine doe reach 50% antiviral reduction levels, with reasonable consistency at 32 µg/ml maximum concentration. The TC_{25} and IC_{50} values can be measured with relative consistency at 1 - 32 µg/ml (0.5 $\log_{10}$) drug concentration scale.

The average maximum antiviral inhibitory level of 69 2-Thio-6-Azauridine tests **(Figure 15-B)** was reached at 32 µg/ml of the compound with 56% antiviral effect. Maximum antiviral effect (~56%) was found with a simultaneous ~32% cytotoxic suppression. Above 32.0 µg/ml concentration 2-Thio-6-Azauridine starts to lose its antiviral potency with increasing cytotoxicity.

In the present 69 assays, we tested 2-Thio-6-Azauridine at 0.5 $\log_{10}$ scale concentrations from 1 - 32 µg/ml to maximize the correct measurements of its antiviral and cytotoxicity effects. This enabled us to monitor our quality control parameters more accurately.



	% Viral CPE Reduction				% Cell Viability			
Conc.(μg/ml)	1	3.2	10	32	1	3.2	10	32
Mean	6	19	41	56	96	89	78	68
Median	2	6	41	51	100	94	78	68
Std. Dev.	0.12	0.25	0.27	0.14	0.09	0.14	0.16	0.12

Figure 15-B
Average Antiviral and Cytotoxicity Values for 69 Positive Control Compound Tests

4.2.2.1.2 Maximum Antiviral Effect of 2-Thio-6-Azauri line vs VE Virus:

A bar graph scatter plot (**Figure 16-B**) depicts the distribution of the maximum antiviral reduction values of all 69 control compound prescreen assays for 2-Thio-6-Azauridine. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 60% (SD ± 16.5) reduction levels. The maximum reduction levels vary from 28 - 100% but remain quite consistently around the median of 58%. The assay control values give a reasonable bell-shaped distribution curve toward the median 58% reduction level. This indicates quite a consistent day-to-day performance of the control compound in the VE prescreen-MTT assay.

During this period the positive control compound performance criteria for 2-Thio-6-Azauridine versus the VE virus in the pre-screen format has not been set to a definite endpoint. We have collected data in order to find out what would constitute a reasonable quality control endpoint and the data indicates that it could be set at the 25% reduction level.

In order to measure the maximum antiviral endpoints of 2-Thio-6-Azauridine correctly, the concentration scale and the highest concentration to be used, must be evaluated at the proper (semi-log) scale of (1 - 32 μg/ml) as seen in **Figure 15-B**.

2-Thio-6-Azauridine is active *in vitro* against VE virus and functions as a reasonable quality control compound similar to the present control compound Selenazofurin.

VARIATION OF THE MAXIMUM ANTIVIRAL EFFECT
VE VIRUS - VS - 2-THIO-6-AZAURIDINE (PRESCREEN PROTOCOL)

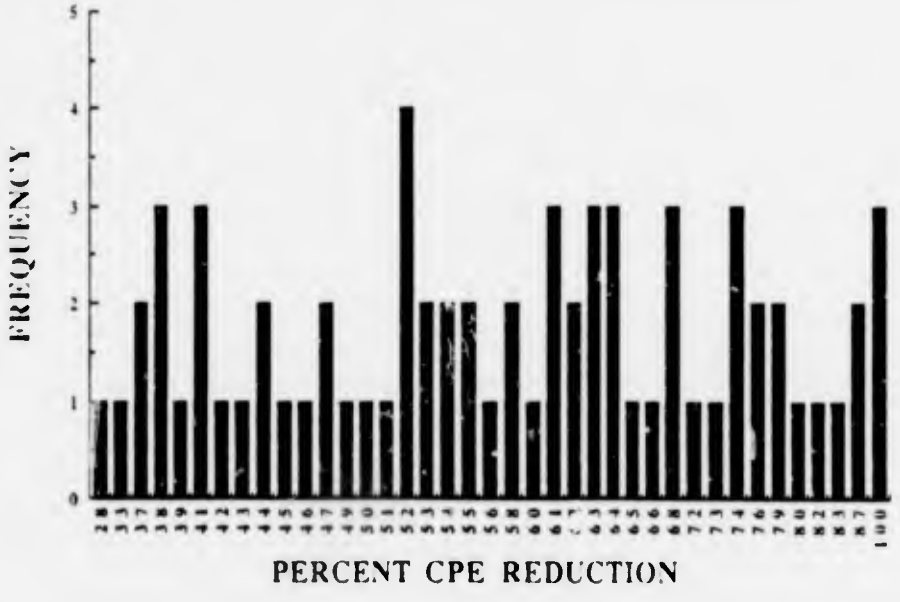


Figure 16-B
Maximum Antiviral CPE Reduction (%).
Summary of 69 Control Tests.

4.2.2.1.3 Cellular Cytotoxicity of 2-Thio-6-Azauridine vs VE Virus:

VE-Control Compound-Cytotoxicity Performance: The 69 cytotoxicity values of the positive control compound 2-Thio-6-Azauridine are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 19.06 μg/ml (SD ± 14.79) and the median was 19.60 μg/ml (range of < 1.0 - > 100 μg/ml).

As can be seen from **Figure 15-B**, a definite TC_{25} toxicity value can be consistently measured with 1 - 32 μg/ml at 0.5 $\log_{10}$ scale. Further increase in the concentration of 2-Thio-6-Azauridine would be needed to properly evaluate the maximum cytotoxicity effect.

Figure 15-B indicates that when the cytotoxicity reaches ~32% at 32 μg/ml, the control compound (2-Thio-6-Azauridine) has reached simultaneously its maximum antiviral effect. The cytotoxic effect of 2-Thio-6-Azauridine is insignificant below 3.2 μg/ml. The average cytotoxicity reached ~32% at 32 μg/ml, which was the highest 2-Thio-6-Azauridine concentration in most tests.

2-Thio-6-Azauridine has a definite cytotoxic suppression on cellular metabolism and growth. However the TC_{25} and TC_{50} toxicity cannot be consistently measured at 32 μg/ml concentration of 2-Thio-6-Azauridine. Therefore, a readjustment to 100 - 320 μg/ml (as being the highest 2-Thio-6-Azauridine concentration tested) would be needed to properly evaluate the TC_{25} and TC_{50} endpoints. However, at this concentration (100 μg/ml) the IC_{50} cannot be measured consistently.

4.2.2.1.4 **VE-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (2-Thio-6-Azauridine):**

VE-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 69 control assays is plotted in **Figure 17-B**. The results indicate that the cell O.D. readings reached a mean 1.169 (SD ± 0.165) with a median of 1.175 (range of 0.593 - 1.514). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

VE-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 69 control assays is presented in **Figure 18-B**. The results indicate that the average virus load O.D. reading is 0.085 (SD ± 0.088) with a median of 0.061 (range of -0.025 - 0.536). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

VE-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 69 control assays is provided in **Figure 19-B**. The results indicate that the average differential O.D. reading is 1.084 (SD ± 0.185) with a median of 1.062 (range 0.504 - 1.496). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 93% measurement accuracy.

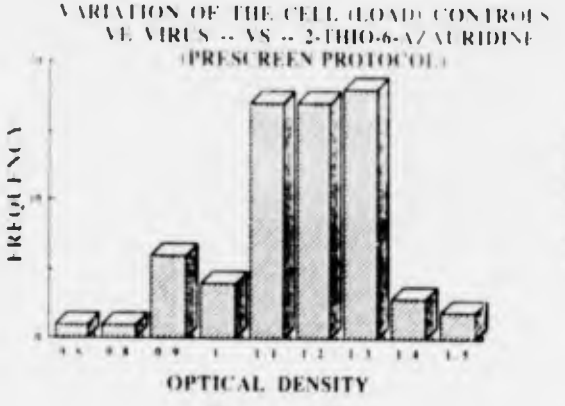


Figure 17-B

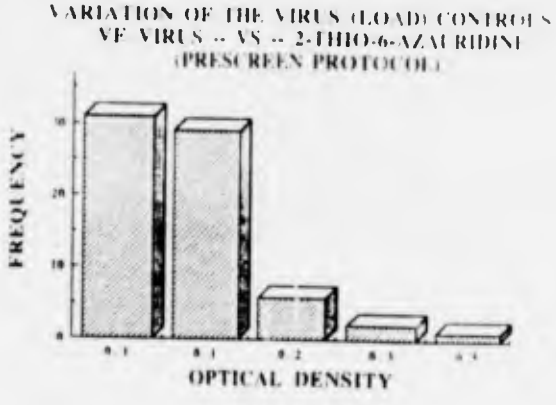


Figure 18-B

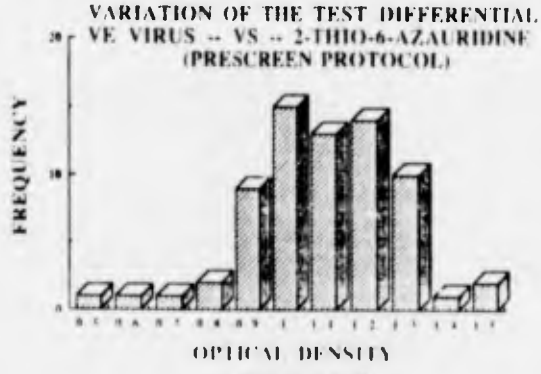


Figure 19-B

4.2.2.1.5 **Overall VE-Assay Plate Quality Controls:**

VE-Overall-Cell Load Performance: A bar graph scatter plot of the overall mean cell control (O.D. reading) of 2857 accepted assays is plotted in **Figure 17-C**. The results indicate that the overall cell O.D. readings reached a mean of 1.147 (SD ± 0.153) with a median of 1.152 (range of 0.476 - 1.619). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

VE-Overall-Virus Load Performance: A bar graph scatter plot of the overall mean virus load O.D. readings of the 2857 accepted assays is presented in **Figure 18-C**. The results indicate that the overall average virus load O.D. reading is 0.069 (SD ± 0.075) with a median of 0.040 (range of 0.001 - 0.536). This demonstrates that a reasonable cell destruction was taking place and a uniform load of virus (32 $TCID_{50}$) was administered on the cell monolayer with consistent viral CPE results.

VE-Overall-Assay Differential Performance: A bar graph scatter plot of the overall mean O.D. differential values of the 2857 accepted assays is provided in **Figure 19-C**. The results indicate that the average overall differential O.D. reading is 1.081 (SD ± 0.173) with a median of 1.100 (range 0.426 - 1.615). The single bell-shaped curve is reasonably sharp and uniform. **This reflects that overall the assay plates were executed consistently and were repeatable during day-to-day operation with close to 90% measurement accuracy.**

GRAPHIC ILLUSTRATION OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ALL ACCEPTED VE PLATE DATA

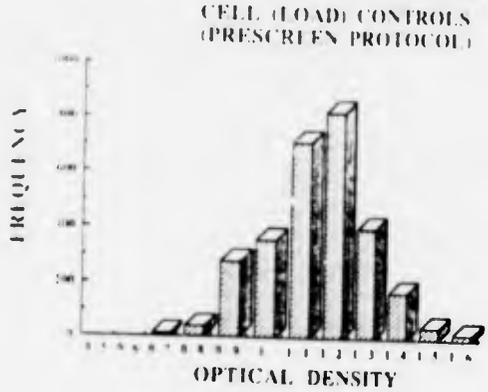


Figure 17-C

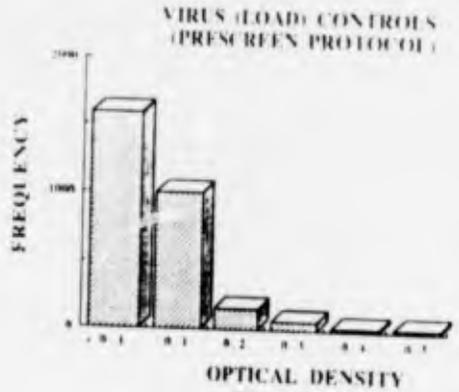


Figure 18-C

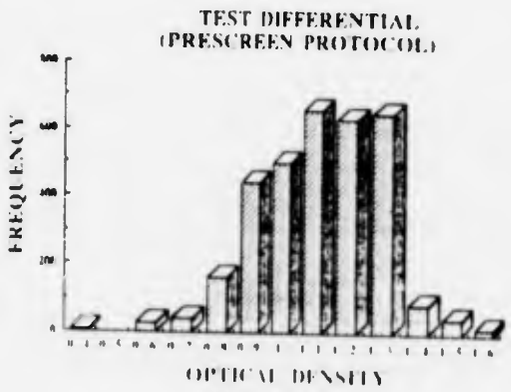


Figure 19-C

4.2.2.2 **Prescreen VE-Antiviral Activity Results:**

New Drugs with 50% Antiviral Reduction Levels: Out of the 2857 accepted single drug tests, 176 new compounds demonstrated antiviral activity, having antiviral reduction values better than 50%. This represents around 6% of the test compounds being active at this antiviral reduction level. These compounds are summarized in **Table 4** according to the highest **Selectivity Index (SI)**. GRP23817 demonstrated the best antiviral promise having a SI of around >32. GRP21459 and B723831 demonstrated good antiviral activity, having SI's of 25 and 10, respectively. Twenty-two other compounds demonstrated moderate antiviral activity, having SI's of 3.0 - 6.0. The other 151 compounds showed some degree of activity having SI's that ranged from 0.01 - 2.9.

Table 4

New Prescreen Drugs that Produced 50% Antiviral Reduction Against VE Virus

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP23817	VE	8EL	27P	08/16/91	> 1000.0	31.60 >	31.62
GRP21459	VE	902	28P	08/30/91	24.80 <	1.00 >	24.84
B723831	VE	5FO	20P	05/10/91	319.00	31.60	10.07
GRP23804	VE	8EI	27P	08/16/91	> 1000.0	167.00 >	5.99
GRP23814	VE	8EK	27P	08/16/91	> 1000.0	183.00 >	5.46
GRP21234	VE	3P8	16P	03/08/91	> 1000.0	209.00 >	4.79
GRP23810	VE	8EJ	27P	08/16/91	> 1000.0	239.00 >	4.19
GRP23515	VE	6EN	23P	06/14/91	> 1000.0	253.00 >	3.95
GRP23528	VE	6EP	23P	06/14/91	> 1000.0	263.00 >	3.80
GRP23800	VE	8EH	27P	08/16/91	> 1000.0	267.00 >	3.74
GRP19809	VE	6WX	25P	07/12/91	> 1000.0	289.00 >	3.46
GRP23235	VE	4FP	18P	04/05/91	> 1000.0	290.00 >	3.45
B849192	VE	602	22P	05/31/91	206.00	59.80	3.45
GRP23856	VE	8EU	27P	08/16/91	> 1000.0	298.00 >	3.35
GRP23043	VE	7OE	26P	08/02/91	> 1000.0	304.00 >	3.29
B724252	VE	575	20P	05/03/91	> 1000.0	310.00 >	3.23
B723957	VE	5YT	22P	05/31/91	> 1000.0	309.00 >	3.23
GRP23035	VE	7OD	26P	08/02/91	> 1000.0	310.00 >	3.23
GRP23563	VE	6EX	24P	06/14/91	> 1000.0	313.00 >	3.20
B724111	VE	4X2	19P	04/26/91	> 1000.0	315.00 >	3.17
GRP19994	VE	7O5	26P	08/02/91	> 1000.0	315.00 >	3.17
B723833	VE	5FO	20P	05/10/91	> 1000.0	323.00 >	3.10
B724114	VE	4X3	19P	04/26/91	> 1000.0	329.00 >	3.04
GRP21253	VE	3PD	16P	03/08/91	> 1000.0	331.00 >	3.02
GRP23867	VE	8LN	27P	08/23/91	> 1000.0	332.00 >	3.01
GRP23236	VE	4FP	18P	04/05/91	> 1000.0	340.00 >	2.94
B723909	VE	5QN	22P	05/24/91	182.00	63.20	2.89
B724219	VE	56F	19P	05/03/91	> 1000.0	347.00 >	2.88
GRP23258	VE	4FU	18P	04/05/91	> 1000.0	350.00 >	2.86
GRP21503	VE	908	28P	08/30/91	> 1000.0	351.00 >	2.85
GRP21790	VE	69K	23P	06/07/91	> 1000.0	355.00 >	2.81
GRP23004	VE	7O6	26P	08/02/91	> 1000.0	360.00 >	2.78
GRP21879	VE	843	27P	08/09/91	> 1000.0	371.00 >	2.70
B724230	VE	56H	19P	05/03/91	> 1000.0	393.00 >	2.55
GRP21264	VE	3PF	16P	03/08/91	> 1000.0	398.00 >	2.51
GRP23805	VE	8EI	27P	08/16/91	> 1000.0	401.00 >	2.50
GRP19686	VE	425	17P	03/22/91	> 1000.0	403.00 >	2.48
GRP23380	VE	4KC	18P	04/12/91	938.00	381.00	2.46
B724113	VE	4X2	19P	04/26/91	> 1000.0	412.00 >	2.43
GRP23538	VE	6ER	23P	06/14/91	> 1000.0	413.00 >	2.42
GRP21821	VE	7YC	27P	08/09/91	> 1000.0	415.00 >	2.41
GRP19816	VE	6WZ	25P	07/12/91	> 1000.0	418.00 >	2.39
GRP21519	VE	90B	28P	08/30/91	740.00	309.00	2.39

Table 4 (Cont'd)

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP19804	VE	6WW	25P	07/12/91	> 1000.0	421.00 >	2.37
GRP23243	VE	4FR	18P	04/05/91	> 1000.0	428.00 >	2.34
GRP23487	VE	6EG	23P	06/14/91	> 1000.0	427.00 >	2.34
GRP23779	VE	8EC	27P	08/16/91	> 1000.0	436.00 >	2.30
GRP23255	VE	4FU	18P	04/05/91	> 1000.0	448.00 >	2.23
B724015	VE	60W	22P	05/31/91	> 1000.0	449.00 >	2.23
B723865	VE	69Y	20P	06/07/91	> 1000.0	451.00 >	2.22
GRP19743	VE	6PG	24P	07/03/91	> 1000.0	460.00 >	2.18
GRP21757	VE	68Z	23P	06/07/91	949.00	441.00	2.15
GRP23906	VE	8LW	27P	08/23/91	> 1000.0	469.00 >	2.13
B718640	VE	2X0	14P	02/01/91	> 1000.0	476.00 >	2.10
GRP21846	VE	7YI	27P	08/09/91	> 1000.0	480.00 >	2.08
GRP23386	VE	4KD	18P	04/12/91	> 1000.0	484.00 >	2.07
GRP19970	VE	7NS	26P	08/02/91	> 1000.0	486.00 >	2.06
GRP23388	VE	4KE	18P	04/12/91	> 1000.0	487.00 >	2.05
B720392	VE	317	14P	02/08/91	> 1000.0	491.00 >	2.04
GRP23374	VE	4KA	18P	04/12/91	> 1000.0	490.00 >	2.04
B724010	VE	60V	22P	05/31/91	> 1000.0	489.00 >	2.04
GRP19806	VE	6WX	25P	07/12/91	> 1000.0	498.00 >	2.01
B723922	VE	5QQ	22P	05/24/91	> 1000.0	499.00 >	2.00
B723970	VE	5YW	22P	05/31/91	> 1000.0	507.00 >	1.97
GRP23015	VE	7O9	26P	08/02/91	> 1000.0	512.00 >	1.95
GRP23549	VE	6EV	23P	06/14/91	> 1000.0	518.00 >	1.93
GRP23860	VE	8LL	27P	08/23/91	> 1000.0	520.00 >	1.92
B718574	VE	2WV	14P	02/01/91	> 1000.0	523.00 >	1.91
GRP23545	VE	6EU	23P	06/14/91	900.00	474.00	1.90
GRP19655	VE	42C	17P	03/22/91	> 1000.0	530.00 >	1.89
B723885	VE	5LW	20P	05/17/91	> 1000.0	528.00 >	1.89
GRP23808	VE	8EJ	27P	08/16/91	> 1000.0	535.00 >	1.87
GRP21205	VE	3JI	16P	03/01/91	947.00	512.00	1.85
B849342	VE	7Y7	27P	08/09/91	> 1000.0	540.00 >	1.85
GRP23265	VE	4FW	18P	04/05/91	> 1000.0	547.00 >	1.83
GRP23398	VE	4KG	18P	04/12/91	> 1000.0	551.00 >	1.81
GRP23396	VE	4KF	18P	04/12/91	942.00	521.00	1.81
B723826	VE	5FM	20P	05/10/91	> 1000.0	552.00 >	1.81
GRP21255	VE	3PD	16P	03/08/91	997.00	567.00	1.76
GRP23970	VE	8MA	27P	08/23/91	801.00	460.00	1.74
GRP23397	VE	4KG	18P	04/12/91	> 1000.0	584.00 >	1.71
B724210	VE	4XO	19P	04/26/91	> 1000.0	586.00 >	1.71
GRP23632	VE	6OL	24P	07/02/91	> 1000.0	594.00 >	1.68
GRP23854	VE	8ET	27P	08/16/91	> 1000.0	595.00 >	1.68
GRP23400	VE	4KG	18P	04/12/91	> 1000.0	600.00 >	1.67
GRP19783	VE	6WK	24P	07/12/91	> 1000.0	600.00 >	1.67
GRP23836	VE	8EQ	27P	08/16/91	> 1000.0	600.00 >	1.67
B723965	VE	5YV	22P	05/31/91	> 1000.0	606.00 >	1.65
GRP21720	VE	68C	23P	06/07/91	756.00	460.00	1.65
GRP19985	VE	7O3	26P	08/02/91	> 1000.0	605.00 >	1.65
B723864	VE	69Y	20P	06/07/91	> 1000.0	619.00 >	1.62
B718969	VE	30R	14P	02/08/91	> 1000.0	633.00 >	1.58
GRP19993	VE	7O4	26P	08/02/91	> 1000.0	632.00 >	1.58
GRP23845	VE	8ER	27P	08/16/91	550.00	349.00	1.58
DN181178Y	VE	5MB	21P	05/17/91	> 1000.0	655.00 >	1.53
GRP23376	VE	4KA	18P	04/12/91	873.00	575.00	1.52
GRP23602	VE	6OE	24P	06/28/91	682.00	456.00	1.49
DN181172S	VE	5M9	21P	05/17/91	413.00	279.00	1.48
GRP23589	VE	6OB	24P	06/28/91	> 1000.0	686.00 >	1.46
GRP23885	VE	8LQ	27P	08/23/91	> 1000.0	683.00 >	1.46
GRP23248	VE	4FS	18P	04/05/91	> 1000.0	716.00 >	1.40
GRP19984	VE	7O3	26P	08/02/91	> 1000.0	743.00 >	1.35
B720398	VE	318	14P	02/08/91	643.00	479.00	1.34
GRP23825	VE	8EM	27P	08/16/91	> 1000.0	749.00 >	1.34

Table 4 (Cont'd)

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP19490	VE	3J9	16P	03/01/91	811.00	610.00	1.33
GRP21789	VE	69K	23P	06/07/91	871.00	670.00	1.30
GRP23608	VE	6OF	24P	06/28/91	> 1000.0	768.00 >	1.30
GRP19962	VE	7NQ	26P	08/02/91	> 1000.0	771.00 >	1.30
GRP23777	VE	8EC	27P	08/16/91	> 1000.0	778.00 >	1.29
B724249	VE	574	19P	05/03/91	752.00	600.00	1.25
GRP19955	VE	7NP	26P	08/02/91	> 1000.0	806.00 >	1.24
B724221	VE	56G	19P	05/03/91	> 1000.0	836.00 >	1.20
GRP19753	VE	6PI	24P	07/03/91	> 1000.0	833.00 >	1.20
GRP23784	VE	8ED	27P	08/16/91	656.00	548.00	1.20
GRP19982	VE	7O2	26P	08/02/91	> 1000.0	843.00 >	1.19
GRP23252	VE	4FT	18P	04/05/91	> 1000.0	857.00 >	1.17
GRP23428	VE	4SW	18P	04/19/91	849.00	726.00	1.17
GRP21737	VE	68G	23P	06/07/91	> 1000.0	865.00 >	1.16
B724040	VE	5ZX	22P	05/31/91	> 1000.0	870.00 >	1.15
GRP19722	VE	6PD	24P	07/03/91	835.00	729.00	1.15
GRP19857	VE	6X7	25P	07/12/91	> 1000.0	872.00 >	1.15
GRP23695	VE	7KE	26P	07/26/91	> 1000.0	877.00 >	1.14
GRP19973	VE	7O2	26P	08/02/91	> 1000.0	876.00 >	1.14
GRP23271	VE	4FY	18P	04/05/91	> 1000.0	886.00 >	1.13
GRP23961	VE	8M8	27P	08/23/91	> 1000.0	882.00 >	1.13
B724366	VE	5FI	20P	05/10/91	> 1000.0	904.00 >	1.11
GRP19757	VE	6PI	24P	07/03/91	877.00	789.00	1.11
B724025	VE	5ZU	22P	05/31/91	> 1000.0	911.00 >	1.10
GRP23458	VE	6DI	23P	06/14/91	817.00	744.00	1.10
GRP21732	VE	68F	23P	06/07/91	> 1000.0	928.00 >	1.08
GRP23852	VE	8ET	27P	08/16/91	317.00	292.00	1.08
GRP23218	VE	4FL	18P	04/05/91	> 1000.0	936.00 >	1.07
DN181499X	VE	5QE	21P	05/24/91	> 1000.0	932.00 >	1.07
GRP23967	VE	8MA	27P	08/23/91	730.00	686.00	1.06
GRP19967	VE	7NR	26P	08/02/91	871.00	829.00	1.05
GRP23391	VE	4KE	18P	04/12/91	842.00	812.00	1.04
GRP19721	VE	6PD	24P	07/03/91	809.00	802.00	1.01
GRP23507	VE	6EK	23P	06/14/91	> 1000.0	1000.0 >	1.00
GRP23935	VE	8M2	27P	08/23/91	> 1000.0	1000.0 >	1.00
GRP23383	VE	4KD	18P	04/12/91	551.00	559.00	0.98
GRP23020	VE	7OA	26P	08/02/91	638.00	656.00	0.97
GRP21460	VE	902	28P	08/30/91	653.00	681.00	0.96
B724181	VE	4XI	19P	04/26/91	628.00	663.00	0.95
B724340	VE	5FB	20P	05/10/91	946.00	1000.0	0.95
GRP23863	VE	8LM	27P	08/23/91	676.00	728.00	0.93
GRP23250	VE	4FT	18P	04/05/91	796.00	863.00	0.92
GRP23031	VE	7OC	26P	08/02/91	831.00	909.00	0.91
GRP19800	VE	6WW	25P	07/12/91	893.00	1000.0	0.89
GRP23501	VE	6EJ	23P	06/14/91	652.00	745.00	0.88
GRP23267	VE	4FX	18P	04/05/91	594.00	693.00	0.86
GRP23678	VE	7HR	26P	07/26/91	423.00	512.00	0.83
GRP19996	VE	7OS	26P	08/02/91	699.00	882.00	0.79
GRP19671	VE	42E	17P	03/22/91	606.00	789.00	0.77
GRP21833	VE	7YE	27P	08/09/91	65.30	84.50	0.77
GRP21248	VE	3PC	16P	03/08/91	735.00	1000.0	0.74
GRP23021	VE	7OA	26P	08/02/91	627.00	862.00	0.73
GRP23184	VE	4AO	18P	03/29/91	404.00	578.00	0.70
B724078	VE	4TS	19P	04/19/91	601.00	895.00	0.67
B720399	VE	318	14P	02/08/91	587.00	930.00	0.63
DN181181C	VE	5MB	21P	05/17/91	622.00	1000.0	0.62
GRP19634	VE	42K	17P	03/22/91	576.00	1000.0	0.58
GRP23785	VE	8ED	27P	08/16/91	567.00	1000.0	0.57
GRP23922	VE	8MO	27P	08/23/91	398.00	740.00	0.54
B724257	VE	576	20P	05/03/91	334.00	635.00	0.53
B724224	VE	56G	19P	05/03/91	307.00	661.00	0.46

Table 4 (Cont'd)

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP23039	VE	7OD	26P	08/02/91	72.10	386.00	0.19
GRP23675	VE	7HQ	26P	07/26/91	63.90	371.00	0.17
GRP23371	VE	4K9	18P	04/12/91	50.20	324.00	0.15
GRP21478	VE	905	28P	08/30/91	71.20	571.00	0.12
B723838	VE	5FP	20P	05/10/91	77.10	970.00	0.08
GRP23656	VE	7HL	26P	07/26/91	43.70	961.00	0.05
GRP23370	VE	4K9	18P	04/12/91	5.11	200.00	0.03
GRP23711	VE	7KH	26P	07/26/91	23.20	919.00	0.03
GRP19631	VE	42K	17P	03/22/91	4.52	272.00	0.02
GRP21564	VE	90M	28P	08/30/91	6.58	341.00	0.02
GRP19647	VE	42N	17P	03/22/91	6.53	651.00	0.01

The *in vitro* antiviral activity of the compounds in **Table 4** should be further confirmed. Verification of the antiviral activity of these prescreen actives were scheduled to be tested using the primary screening (confirmatory) protocol. Because of the discontinuation of this antiviral program by the Sponsor some of the active compounds in **Table 4** have not been confirmed in the primary screening protocol due to lack of funding.

4.2.3 Prescreen Assay (Punta Toro Virus [PT]):

The total output of PT prescreen-testing during this reporting period is summarized in monthly increments in **Figure 20**. During this period 3600 tests were performed against the PT-virus with the MTT-assay format. Out of these, 196 were control compound assays - Ribavirin (AVS-0001) and 90 were control compound assays - 2-Thio-6-Azauridine (AVS-6724). Two hundred fifty-four (254) tests were internal (+ + +) virus load, cell load, and other quality control tests. Two hundred fifteen (215) tests were considered unsatisfactory based on the preliminary criteria of the quality controls set during this reporting period. The rest, totaling 2845 were actual single drug tests. The total number of accepted single drug tests (2845) reflects 3% effort beyond our yearly contractual obligation. The 215 unsatisfactory tests represent a 6% rejection rate based on the present quality control parameters used for the PT virus.

Out of the 2845 test compounds, 216 demonstrated antiviral activity at greater than 50% reduction levels. This represents around 8% of the tested compounds having *in vitro* antiviral activity against the PT-virus. The remainder, 2629 compounds (92%), are to be considered inactive with the present quality control and assay protocols.

TOTAL NUMBER OF TESTS AGAINST PUNTA TORO VIRUS
PRESCREEN PROTOCOL

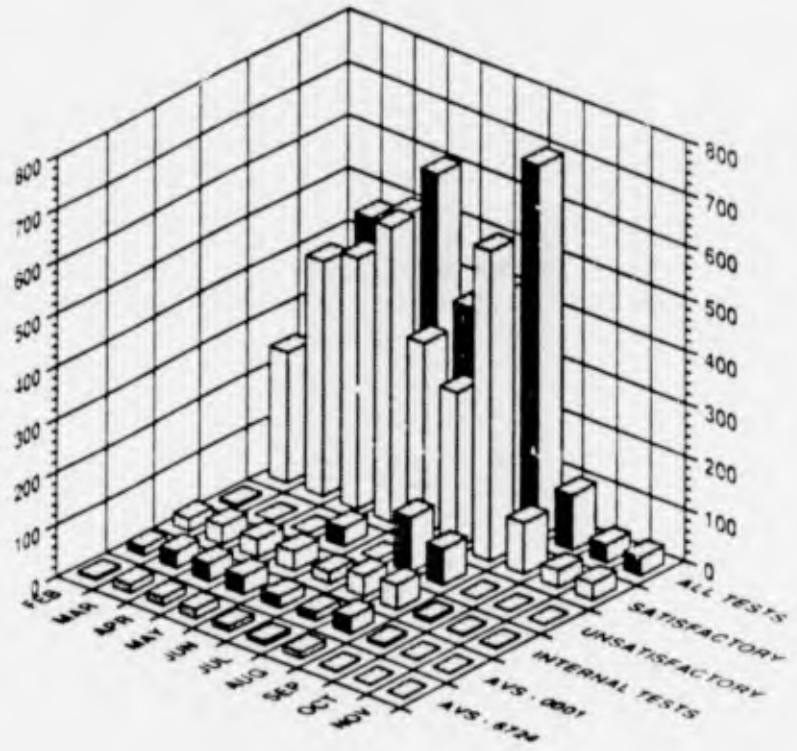


Figure 20

	KPC (Positive Control - 2-Thio-6-Azauridine) =	90
Quality Control Tests =	KPC (Positive Control - Ribavirin) =	196
	(+ + + +) (Internal Virus and Cell Load Controls) =	254
	(UT) Unsatisfactory Test (QC rejects) =	215
Accepted Single Drug Tests =		2845
Total number of PT Tests =		3600

4.2.3.1 Prescreen PT-Quality Controls:

4.2.3.1.1 Antiviral Activity of Ribavirin vs PT Virus:

Control Compound-Antiviral Performance: Ribavirin (AVS-0001) has been the primary control compound against PT in these MTT-assay prescreens. The mean and median antiviral inhibition and cytotoxicity patterns of the positive control drug (Ribavirin) are illustrated in **Figure 21-A.**

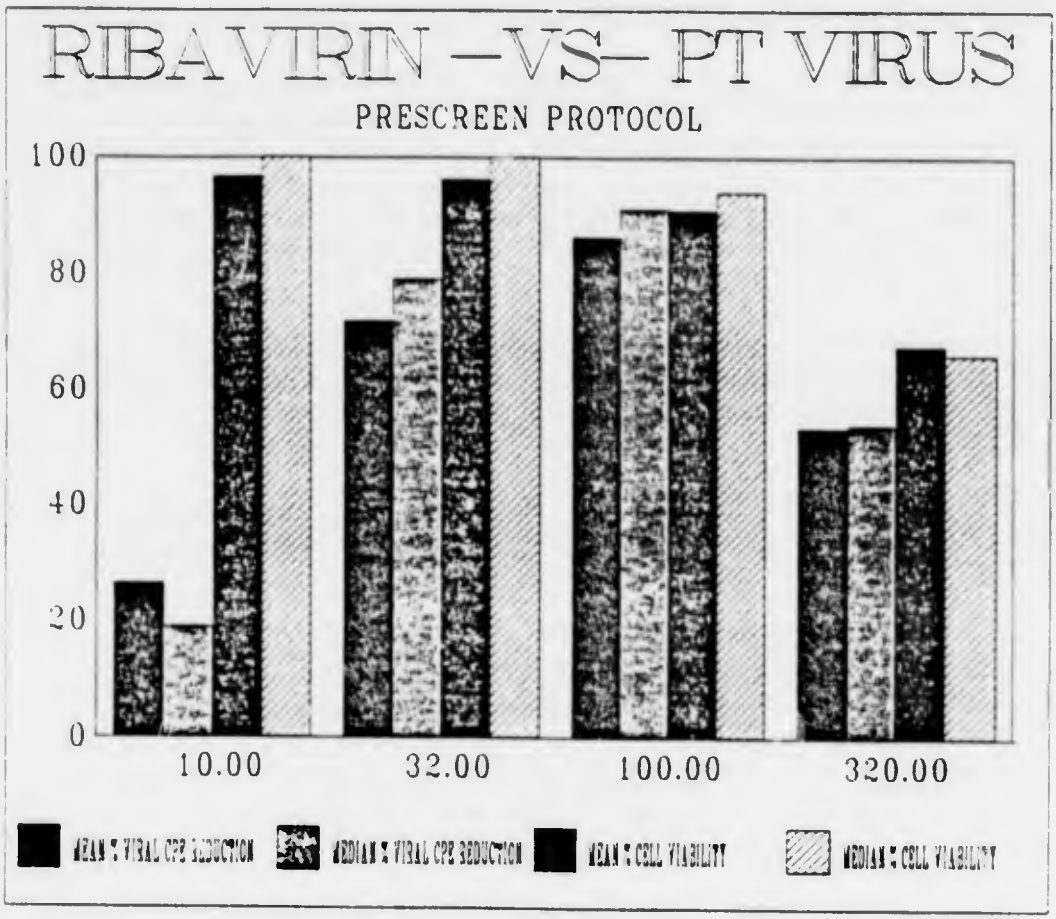
The 196 control tests performed with Ribavirin gave a mean **Selectivity Index** of 13.79 (SD ± 9.66) and the median value was 11.94 (range = 0 - >32.00), indicating moderate antiviral selectivity for Ribavirin. SI is calculated by dividing the TC_{25} by the IC_{50}.

The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 29.05 μg/ml (SD ± 43.73). The median IC_{50} value was 17.7 μg/ml (range = 0 - 320). This indicates that Ribavirin does reach 50% antiviral reduction levels. The TC_{25} and IC_{50} values can be measured relatively consistent at the 10 - 320 μg/ml (0.5 Log_{10}) drug concentration scale.

The average maximum antiviral inhibitory level of 196 Ribavirin tests **(Figure 21-A)** was reached at 100 μg/ml of the compound with 88% antiviral effect. Maximum antiviral effect (~89%) was found with a simultaneous ~9% cytotoxic suppression. Above this concentration (100 μg/ml) Ribavirin starts to lose its antiviral potency with increasing cytotoxicity. An increase of the concentration of Ribavirin to 320 μg/ml, does not improve the antiviral activity **(Figure 21-A).** The antiviral activity decreases from 86% (at 100 μg/ml) to 53% (at 320 μg/ml). The highest concentration (320) of Ribavirin is needed to properly evaluate the Cellular Toxicity 25% (TC_{25}) value.

As reported previously, a different maximum antiviral value is obtained depending upon which concentration scale is used. In those tests, the log_{10} scale of 0.1 - 100 measured more accurately the **antiviral effect** of the control compound, Ribavirin, whereas the log_{10} scale of 0.32 - 320 measured more accurately the **cytotoxicity effect.**

In the present 196 control assays, we tested Ribavirin at 0.5 log_{10} scale from 10 - 320 μg/ml concentrations to maximize the correct measurements of both the antiviral and cytotoxicity effects. This enables us to monitor our quality control parameters more accurately.



CONCENTRATION (μg/ml)

	% Viral CPE Reduction				% Cell Viability			
Conc.(μg/ml)	10	32	100	320	10	32	100	320
Mean	27	72	86	53	97	96	91	68
Median	20	80	91	54	100	100	94	66
Std. Dev.	0.21	0.27	0.17	0.22	0.05	0.07	0.11	0.17

Figure 21-A
Average Antiviral and Cytotoxicity Values for 196 Positive Control Compounds Assays

4.2.3.1.2 **Maximum Antiviral Effect of Ribavirin vs PT Virus:**

A bar graph scatter plot (**Figure 22-A**) depicts the distribution of the maximum antiviral reduction values of all 196 control compound prescreen assays for Ribavirin. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 89% (SD ± 13.37) reduction levels. The maximum reduction levels vary from 49 - 100% but remain quite consistently around the median of 95%. The assay control values give a shifted half-bell-shaped distribution curve toward the maximum 100% reduction level. This indicates quite a consistent day-to-day performance of the control compound in the PT prescreen-MTT assay.

During this period the positive control compound performance criteria for Ribavirin versus the PT virus in the pre-screen format has not been set to a definite endpoint We have collected data in order to find out what would constitute a reasonable quality control endpoint and the data indicates that it could be set at the 50% reduction level.

In order to measure the maximum antiviral endpoints of Ribavirin correctly, the concentration scale and the highest concentration to be used, must be evaluated at the proper (semi-log) scale as seen in **Figure 21-A**.

Ribavirin is active *in vitro* against PT virus and functions as a reasonable quality control compound. On the other hand, regardless of the performance of the PT-quality control drug Ribavirin, around 216 other compounds have equal or better antiviral activity against PT virus than AVS-0001.

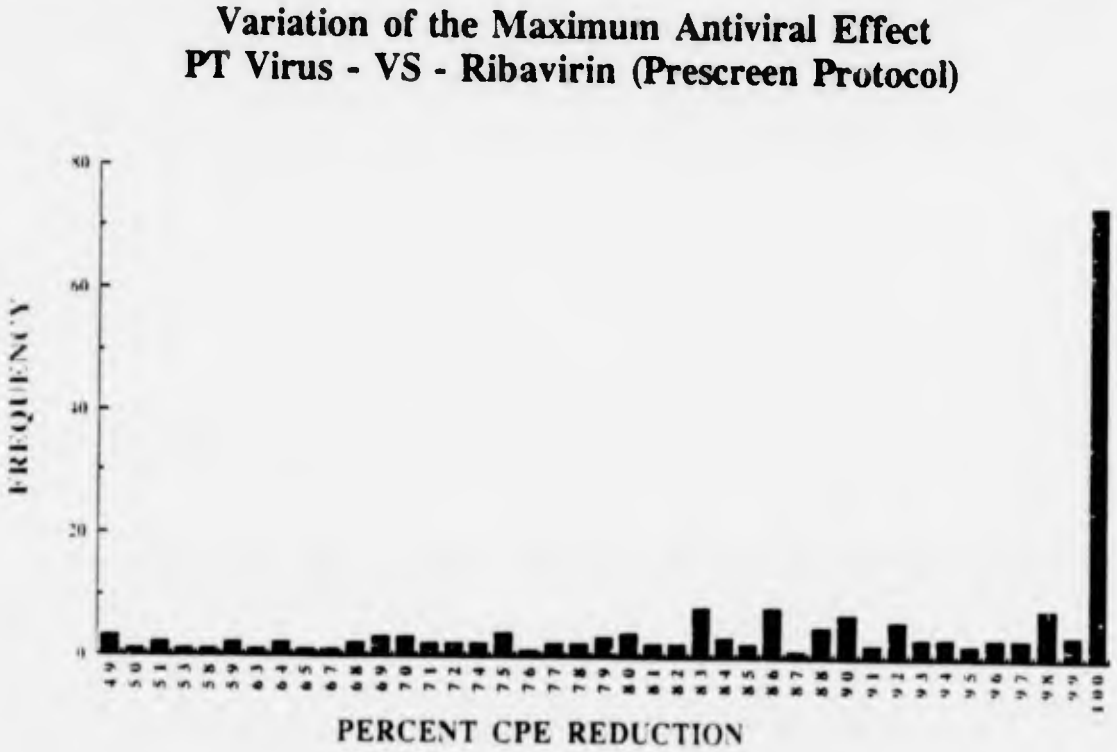


Figure 22-A
Maximum Antiviral CPE Reduction (%).
Summary of 196 Control Tests.

4.2.3.1.3 **Cellular Cytotoxicity of Ribavirin vs PT Virus:**

PT-Control Compound-Cytotoxicity Performance: The 196 cytotoxicity values of the positive control compound Ribavirin are also very consistent. The mean cell **Toxic Concentration 25% (TC_{25})** was 235 μg/ml (SD $\pm$ 85) and the median was 254 μg/ml (range of 12.3 to >320 μg/ml). The reason for this discrepancy is that at 10 - 320 μg/ml scale, the TC_{25} cytotoxicity cannot always be measured consistently.

As can be seen from **Figure 21-A**, a definite TC_{25} toxicity value can be measured with a 10 - 320 μg/ml $\log_{10}$ scale. Further increase in the concentration of Ribavirin would be needed to consistently evaluate the maximum cytotoxicity of Ribavirin.

Also **Figure 21-A**, indicates that when the cytotoxicity reaches ~9% at 100 μg/ml, the control compound (Ribavirin) has reached simultaneously its maximum antiviral effect. The cytotoxic effect of Ribavirin is insignificant between 1 and 100 μg/ml. The average maximum cytotoxicity reached 32% at 320 μg/ml, which was the highest Ribavirin concentration tested.

Ribavirin has a definite cytotoxic suppression on cellular metabolism and growth. However, the TC_{25} and TC_{50} toxicity could not be consistently achieved with the 100 μg/ml concentration of Ribavirin. Therefore, a readjustment to 320 μg/ml (as being the highest Ribavirin concentration tested) was done. However, at this concentration (320 μg/ml) the TC_{50} and TC_{95} cannot yet be measured consistently.

4.2.3.1.4 **PT-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (Ribavirin):**

PT-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 196 control assays is plotted in **Figure 23-A**. The results indicate that the cell O.D. readings reached a mean of 1.212 (SD ± 0.168) with a median of 1.245 (range of 0.516 - 1.538). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

PT-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 196 control assays is presented in **Figure 24-A**. The results indicate that the average virus load O.D. reading is 0.237 (SD ± 0.188) with a median of 0.168 (range of -0.02 - 0.763). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

PT-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 196 control assays is provided in **Figure 25-A**. The results indicate that the average differential O.D. reading is 0.974 (SD ± 0.231) with a median of 0.987 (range 0.498 - 1.421). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 97% measurement accuracy.

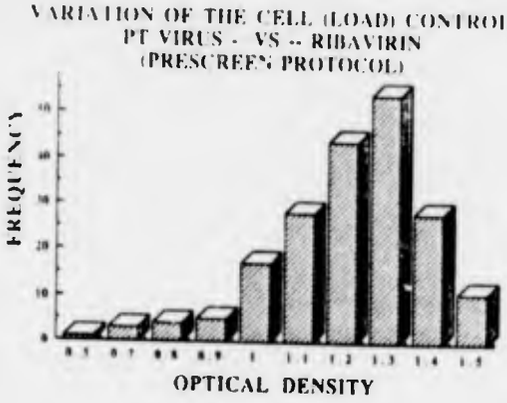


Figure 23-A

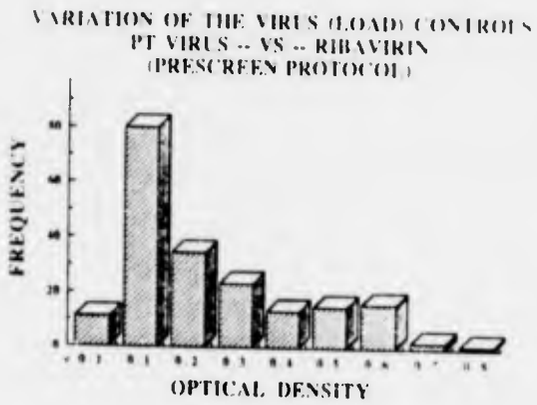


Figure 24-A

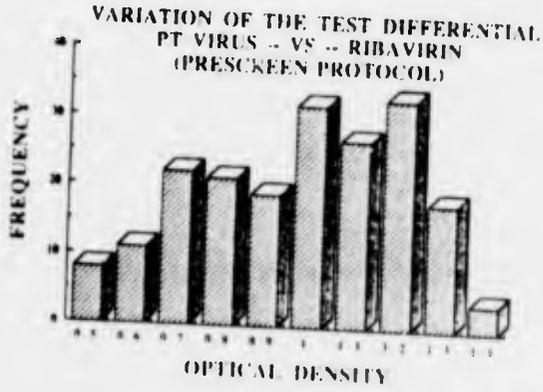


Figure 25-A

4.2.3.1 **Prescreen PT-Quality Controls:**

4.2.3.1.1 **Antiviral Activity of 2-Thio-6-Azauridine vs PT Virus:**

Second Control Compound-Antiviral Performance: 2-Thio-6-Azauridine (AVS-6724) has been tested as a second control compound against PT in these MTT-assay prescreens. The mean and median antiviral inhibition and cytotoxicity patterns of this second positive control drug are illustrated in **Figure 21-B**.

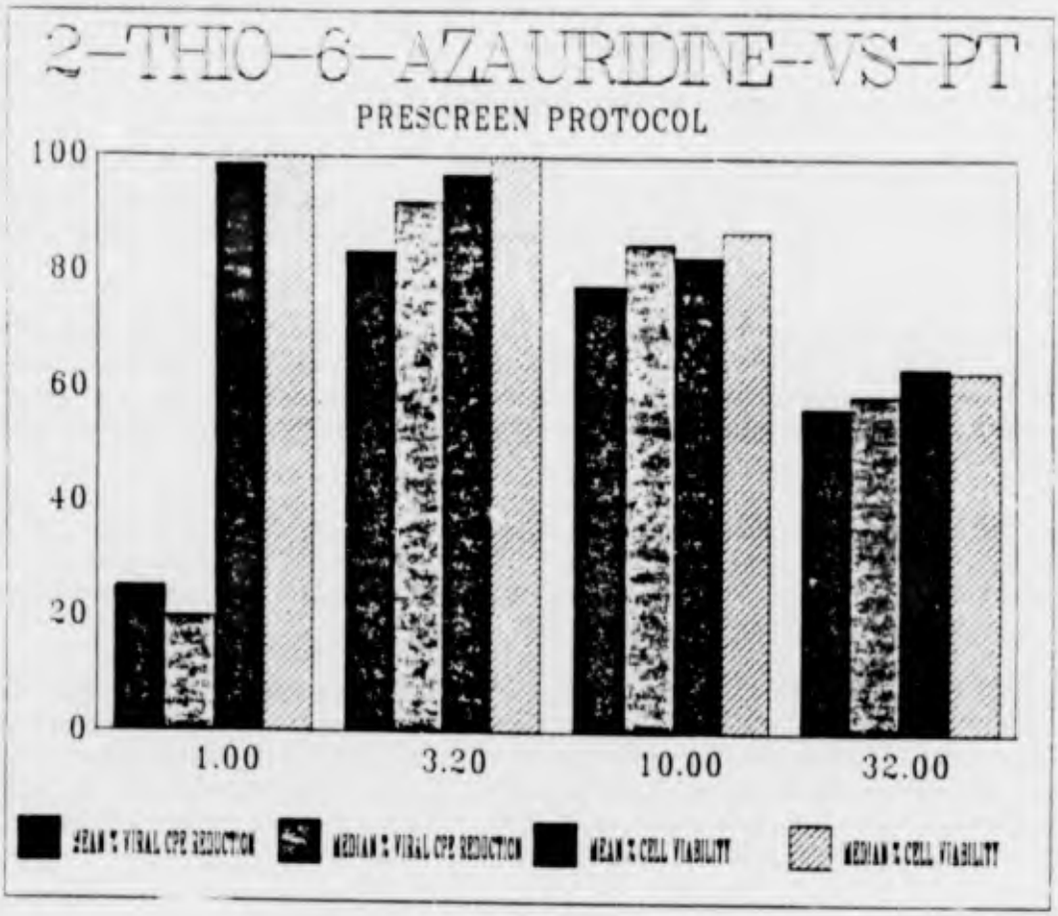
The 90 control tests performed with 2-Thio-6-Azauridine gave a mean **Selectivity Index** (SI) of 13.16 (SD ± 8.97) and the median value was 12.52 (range = 0 - >32.00), indicating good antiviral selectivity for 2-Thio-6-Azauridine. The reason for this occasional discrepancy is that at 32 μg/ml the 25% cytotoxicity cannot always be measured properly to execute the SI calculations. SI is calculated by dividing the TC_{25} by the IC_{50}.

The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 2.52 μg/ml (SD ± 4.50). The median IC_{50} value was 1.66 μg/ml (range = 0 - 32). This indicates that 2-Thio-6-Azauridine does reach 50% antiviral reduction levels. The TC_{25} and IC_{50} values can be measured with relative consistency at the 1 - 32 μg/ml (0.5 Log_{10}) drug concentration scale.

The average maximum antiviral inhibitory level of 90 2-Thio-6-Azauridine tests **(Figure 21-B)** was reached at 3.2 μg/ml of the compound with 88% antiviral effect. Maximum antiviral effect (~88%) was found with a simultaneous ~3% cytotoxic suppression. Above this concentration (10 μg/ml) 2-Thio-6-Azauridine starts to lose its antiviral potency with increasing cytotoxicity **(Figure 21-B)**. An increase of the concentration of 2-Thio-6-Azauridine to 32 μg/ml, does not improve the antiviral activity **(Figure 21-B)**. The antiviral activity decreases from 85% (at 10 μg/ml) to 59% (at 32 μg/ml).

A different maximum antiviral value is obtained depending upon which concentration scale is used. In these tests **(Figure 21-B)**, the log_{10} scale of 1 - 32 measures more accurately the **antiviral effect** of the control compound, 2-Thio-6-Azauridine. As previously determined, the log_{10} scale of 0.32 - 320 μg/ml measures more accurately the **cytotoxicity effect.**

In the present 90 positive control assays, we tested 2-Thio-6-Azauridine at 0.5 log_{10} scale concentrations (1 - 32 μg/ml) to maximize the correct measurements of both the antiviral and cytotoxicity effects. This enables us to monitor our quality control parameters more accurately.



CONCENTRATION (μg/ml)

	% Viral CPE Reduction				% Cell Viability			
Conc.(μg/ml)	1	3.2	10	32	1	3.2	10	32
Mean	25	83	78	57	99	97	83	64
Median	20	92	85	59	100	100	87	63
Std. Dev.	0.21	0.22	0.25	0.24	0.04	0.08	0.18	0.18

Figure 21-B
Average Antiviral and Cytotoxicity Values for 90 Positive Control Compound Tests

4.2.3.1.2 Maximum Antiviral Effect of 2-Thio-6-Azauridine vs PT Virus:

A bar graph scatter plot (**Figure 22-B**) depicts the distribution of the maximum antiviral reduction values of all 90 control compound prescreen assays for 2-Thio-6-Azauridine. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 89% (SD ± 16.46) reduction levels. The maximum reduction levels vary from 24 - 100% but remain quite consistently around the median of 100%. The assay control values give a shifted half-bell-shaped distribution curve toward the maximum 100% reduction level. This indicates quite a consistent day-to-day performance of the second control compound in the PT prescreen-MTT assay.

During this period the positive control compound performance criteria for 2-Thio-6-Azauridine versus the PT virus in the pre-screen format has not been set to a definite endpoint. We have collected data in order to find out what would constitute a reasonable quality control endpoint and the data indicates that it could be set at the 50% reduction level.

In order to measure the maximum antiviral endpoints of 2-Thio-6-Azauridine correctly, the concentration scale and the highest concentration to be used, must be evaluated at the proper semi-log scale as seen in **Figure 21-B**.

2-Thio-6-Azauridine is active *in vitro* against PT virus and functions as a reasonable candidate for a second quality control compound.

Variation of the Maximum Antiviral Effect PT Virus - VS - 2-Thio-6-Azauridine (Prescreen Protocol)

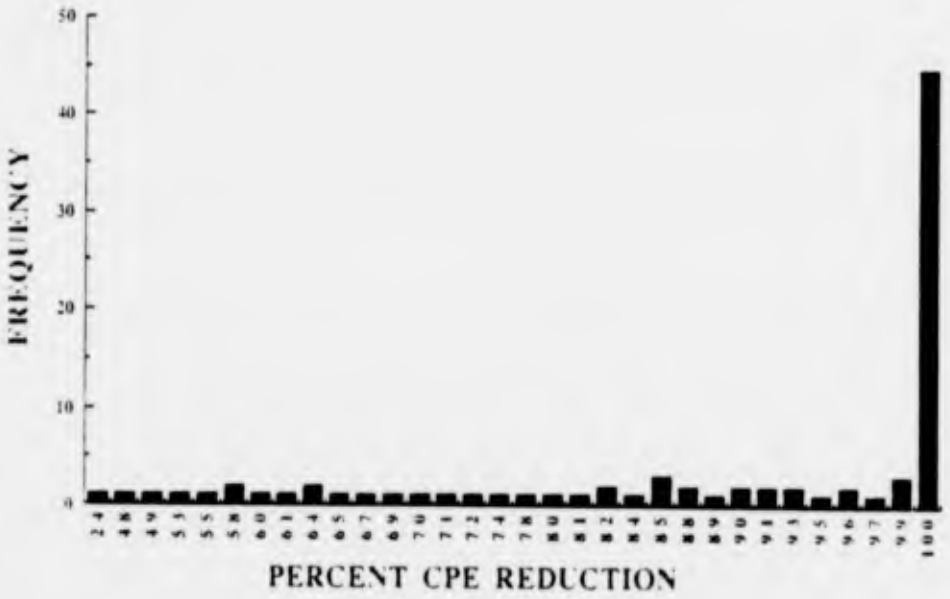


Figure 22-B
Maximum Antiviral CPE Reduction (%).
Summary of 90 Control Tests.

4.2.3.1.3 Cellular Cytotoxicity of 2-Thio-6-Azauridine vs PT Virus:

PT-Control Compound-Cytotoxicity Performance: The 90 cytotoxicity values of the positive control compound 2-Thio-6-Azauridine are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 21.63 μg/ml (SD $\pm$ 14.83) and the median was 22.30 μg/ml (range of 2.43 - 100 μg/ml). The reason for this discrepancy is that at 1 - 32 μg/ml scale the TC_{25} cytotoxicity cannot always be measured accurately.

As can be seen from **Figure 21-B**, a definite TC_{25} toxicity value can be measured with a 1 - 32 μg/ml 0.5 $\log_{10}$ scale. Further increase in the concentration of 2-Thio-6-Azauridine would be needed to properly evaluate the maximum cytotoxicity of 2-Thio-6-Azauridine.

Also **Figure 21-B**, indicates that when the cytotoxicity reaches ~0 - 3% at 3.2 μg/ml, the control compound (2-Thio-6-Azauridine) has reached simultaneously its maximum antiviral effect. The cytotoxic effect of 2-Thio-6-Azauridine is insignificant below 3.2 μg/ml. The average cytotoxicity reached 36% at 32 μg/ml, which was the highest 2-Thio-6-Azauridine concentration in most tests.

2-Thio-6-Azauridine has a definite cytotoxic suppression on cellular metabolism and growth. However, the TC_{25} toxicity could not be consistently achieved with the 32 μg/ml concentration of 2-Thio-6-Azauridine. Therefore, a readjustment to 100 μg/ml (as being the highest 2-Thio-6-Azauridine concentration tested) would be needed to properly evaluate the TC_{25} endpoint. However, at this concentration (100 μg/ml) the IC_{50} cannot be measured consistently.

4.2.3.1.4 PT-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (2-Thio-6-Azauridine):

PT-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 90 control assays is plotted in **Figure 23-B**. The results indicate that the cell O.D. readings reached a mean of 1.199 (SD ± 0.191) with a median of 1.220 (range of 0.459 - 1.538). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

PT-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 90 control assays is presented in **Figure 24-B**. The results indicate that the average virus load O.D. reading is 0.216 (SD ± 0.181) with a median of 0.152 (range of -0.025 - 0.763). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

PT-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 90 control assays is provided in **Figure 25-B**. The results indicate that the average differential O.D. reading is 0.983 (SD ± 0.245) with a median of 1.014 (range 0.465 - 1.421). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 98% measurement accuracy.

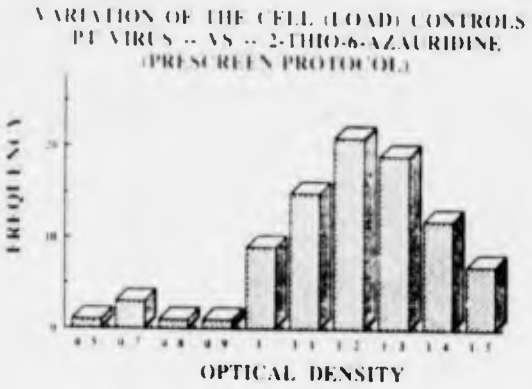


Figure 23-B

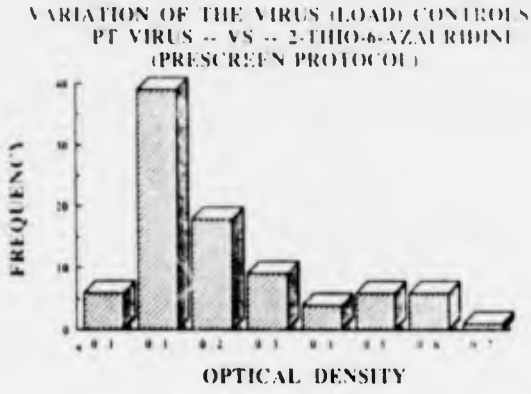


Figure 24-B

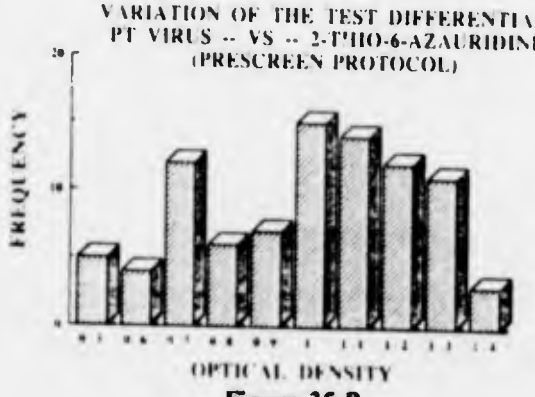


Figure 25-B

4.2.3.1.5 Overall PT-Assay Plate Quality Controls:

PT-Overall-Cell Load Performance: A bar graph scatter plot of the overall mean cell control (O.D. reading) of 90 accepted assays is plotted in **Figure 23-C**. The results indicate that the overall cell O.D. readings reached a mean of 1.191 (SD ± 0.159) with a median of 1.211 (range of 0.419 - 1.538). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

PT-Overall-Virus Load Performance: A bar graph scatter plot of the overall mean virus load O.D. readings of the 90 control assays is presented in **Figure 24-C**. The results indicate that the overall average virus load O.D. reading is 0.248 (SD ± 0.186) with a median of 0.182 (range of -0.006 - 0.848). This demonstrates that a reasonable cell destruction was taking place and a uniform load of virus (32 $TCID_{50}$) was administered on the cell monolayer with consistent viral CPE results.

PT-Overall-Assay Differential Performance: A bar graph scatter plot of the overall mean O.D. differential values of the 90 control assays is provided in **Figure 25-C**. The results indicate that the average overall differential O.D. reading is 0.944 (SD ± 0.224) with a median of 0.959 (range 0.418 - 1.421). The single bell-shaped curve is reasonably sharp and uniform. **This reflects that overall the assay plates were executed consistently and were repeatable during day-to-day operation with close to 95% measurement accuracy.**

GRAPHIC ILLUSTRATIONS OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ALL ACCEPTED PT PLATE DATA

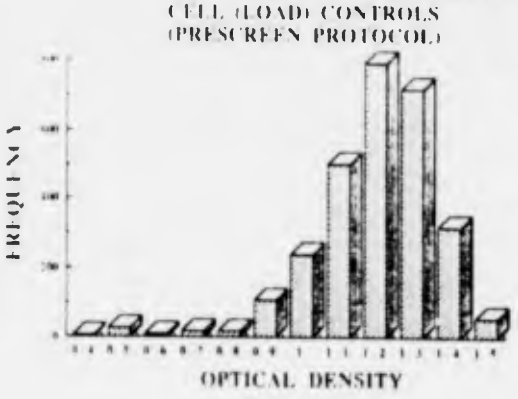


Figure 23-C

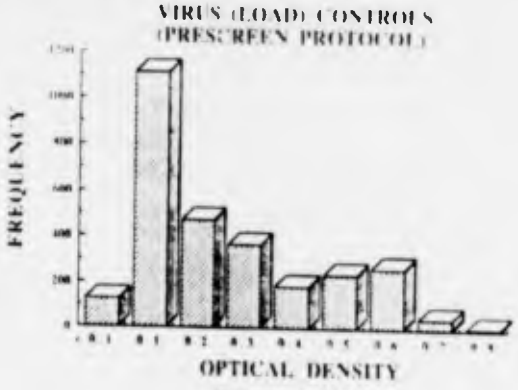


Figure 24-C

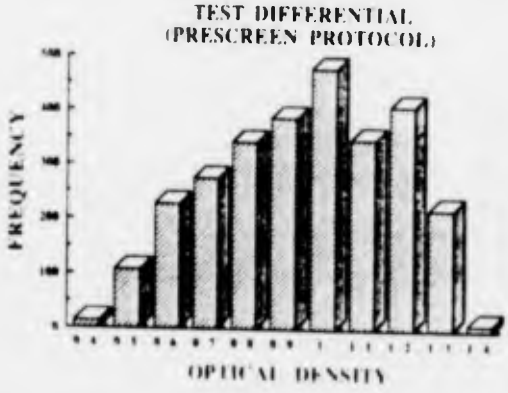


Figure 25-C

4.2.3.2 Prescreen PT-Antiviral Activity Results:

New Drugs with 50% Antiviral Reduction Levels: Out of the 2845 accepted single drug tests, 216 new compounds demonstrated antiviral activity, having antiviral reduction values equal to or better than 50%. This represents around 8% of the test compounds being active at this antiviral reduction level. These compounds are summarized in **Table 5** according to the highest **Selectivity Index (SI)**. Five compounds, GRP21287, GRP21320, GRP21706, B678018/F244 and B849297, demonstrated the best antiviral promise having SI's of > 15. Fifteen other compounds demonstrated moderate antiviral activity, having SI's that ranged from 5 - 9.5. The rest (196 compounds) showed marginal to moderate antiviral activity with SI's that ranged from 0.01 to 4.7.

Table 5

New Prescreen Drugs that Produced 50% Antiviral Reduction Against PT Virus

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP21287	PT	3R2	16P	03/07/91	423.00	6.11	69.18
GRP21320	PT	3Y6	16P	03/12/91	325.00	9.13	35.63
GRP21706	PT	4CC	18P	03/29/91	> 1000.0	42.00 >	23.80
B678018/F244	PT	3IZ	15P	02/26/91	183.00	9.28	19.76
B849297	PT	4BN	17P	03/27/91	32.80	2.12	15.45
GRP23594	PT	6LO	24P	06/18/91	487.00	51.10	9.52
GRP23607	PT	6MS	24P	06/19/91	> 1000.0	108.00 >	9.29
B723954	PT	5UE	22P	05/24/91	464.00	52.20	8.89
GRP23728	PT	681	23P	06/04/91	252.00	29.70	8.48
GRP23482	PT	6G8	23P	06/12/91	352.00	41.70	8.43
GRP23461	PT	6DX	23P	06/11/91	293.00	38.80	7.55
B849192	PT	610	22P	05/31/91	307.00	43.90	7.01
DN181399P	PT	5Q8	21P	05/22/91	492.00	71.80	6.85
GRP23476	PT	6DY	23P	06/11/91	335.00	50.70	6.60
GRP23610	PT	6MT	24P	06/19/91	313.00	50.90	6.15
GRP21689	PT	4BV	18P	03/28/91	344.00	58.00	5.93
GRP23729	PT	682	23P	06/04/91	165.00	29.80	5.55
GRP23525	PT	6HQ	23P	06/13/91	> 1000.0	188.00 >	5.33
DN181387A	PT	5ND	21P	05/17/91	389.00	75.30	5.16
GRP21705	PT	4CB	18P	03/29/91	228.00	45.10	5.04
B723989	PT	5ZE	22P	05/29/91	140.00	29.60	4.73
GRP23453	PT	6DV	23P	06/11/91	328.00	70.10	4.68
GRP23511	PT	6HN	23P	06/13/91	253.00	55.00	4.60
GRP21778	PT	69B	23P	06/07/91	265.00	59.10	4.47
B849289	PT	4AZ	17P	03/26/91	417.00	94.00	4.44
B723835	PT	5GO	20P	05/10/91	325.00	76.30	4.26
GRP23516	PT	6HO	23P	06/13/91	338.00	80.30	4.21
GRP21752	PT	695	23P	06/06/91	190.00	46.40	4.09
B723949	PT	5UD	22P	05/24/91	31.10	7.66	4.06
B849299	PT	4BP	17P	03/27/91	349.00	89.70	3.89
DN181182F	PT	5NB	21P	05/17/91	215.00	55.20	3.89
GRP23597	PT	6LO	24P	06/18/91	317.00	83.70	3.78
GRP23589	PT	6LN	24P	06/18/91	> 1000.0	272.00 >	3.67
DN181515Q	PT	6F4	21P	06/11/91	214.00	58.40	3.66
GRP21291	PT	3R3	16P	03/07/91	> 1000.0	276.00 >	3.62
B849271	PT	436	17P	03/22/91	> 1000.0	280.00 >	3.57
GRP23590	PT	6LN	24P	06/18/91	> 1000.0	288.00 >	3.48
B724230	PT	56Z	19P	04/30/91	> 1000.0	292.00 >	3.43
GRP23190	PT	4CF	18P	03/29/91	> 1000.0	295.00 >	3.39
GRP23284	PT	4I6	18P	04/05/91	> 1000.0	296.00 >	3.38
B724187	PT	52W	19P	04/26/91	17.50	5.26	3.33
GRP23816	PT	8J1	27P	08/15/91	> 1000.0	310.00 >	3.23
DN181122O	PT	5MJ	21P	05/15/91	> 1000.0	311.00 >	3.21
GRP23604	PT	6MS	24P	06/19/91	> 1000.0	316.00 >	3.17
GRP23608	PT	6MS	24P	06/19/91	> 1000.0	316.00 >	3.17
B724266	PT	59R	20P	05/02/91	> 1000.0	316.00 >	3.16
GRP21743	PT	68A	23P	06/05/91	> 1000.0	318.00 >	3.15

Table 5 (Cont'd)

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP23632	PT	6OZ	24P	07/02/91	921.00	299.00	3.08
GRP23643	PT	6P1	24P	07/02/91	> 1000.0	328.00 >	3.05
GRP19743	PT	6P9	24P	07/03/91	> 1000.0	329.00 >	3.04
GRP23287	PT	4I7	18P	04/05/91	> 1000.0	330.00 >	3.03
GRP19539	PT	3YR	17P	03/13/91	> 1000.0	336.00 >	2.98
GRP23519	PT	6HP	23P	06/13/91	> 1000.0	336.00 >	2.98
GRP23567	PT	6HZ	24P	06/14/91	915.00	314.00	2.91
B724140	PT	4Y3	19P	04/24/91	18.20	6.31	2.88
GRP23676	PT	8LA	26P	08/22/91	> 1000.0	348.00 >	2.87
GRP23562	PT	6HY	24P	06/14/91	> 1000.0	352.00 >	2.84
GRP23429	PT	4TQ	18P	04/17/91	> 1000.0	356.00 >	2.81
GRP21550	PT	93X	28P	08/29/91	> 1000.0	363.00 >	2.75
GRP23537	PT	6HS	23P	06/13/91	> 1000.0	366.00 >	2.73
B720445	PT	36C	14P	02/15/91	> 1000.0	371.00 >	2.70
GRP23408	PT	4TL	18P	04/16/91	142.00	52.50	2.70
GRP23569	PT	6IO	24P	06/14/91	> 1000.0	374.00 >	2.67
DN181143M	PT	5N2	21P	05/16/91	> 1000.0	377.00 >	2.65
GRP21708	PT	4CC	18P	03/29/91	> 1000.0	383.00 >	2.61
GRP23363	PT	4MK	18P	04/11/91	> 1000.0	386.00 >	2.59
GRP19459	PT	3K7	16P	02/27/91	> 1000.0	399.00 >	2.51
GRP23474	PT	6DY	23P	06/11/91	> 1000.0	407.00 >	2.46
GRP23222	PT	4GM	18P	04/03/91	> 1000.0	410.00 >	2.44
B724240	PT	57K	19P	05/01/91	> 1000.0	410.00 >	2.44
B723833	PT	5GO	20P	05/10/91	> 1000.0	409.00 >	2.44
GRP23722	PT	7N4	26P	07/30/91	> 1000.0	409.00 >	2.44
GRP23779	PT	8IE	27P	08/14/91	243.00	100.00	2.43
GRP21511	PT	934	28P	08/28/91	> 1000.0	415.00 >	2.41
B724291	PT	59X	20P	05/03/91	> 1000.0	417.00 >	2.40
GRP23931	PT	AJ8	26P	10/09/91	> 1000.0	424.00 >	2.36
GRP23227	PT	4GN	18P	04/03/91	> 1000.0	427.00 >	2.34
GRP23538	PT	6HS	23P	06/13/91	> 1000.0	428.00 >	2.33
GRP19778	PT	6WC	24P	07/09/91	> 1000.0	429.00 >	2.33
GRP23226	PT	4GN	18P	04/03/91	> 1000.0	430.00 >	2.32
GRP23602	PT	6MR	24P	06/19/91	> 1000.0	431.00 >	2.32
DN181499X	PT	6FO	21P	06/11/91	> 1000.0	436.00 >	2.30
GRP23570	PT	6LJ	24P	06/18/91	> 1000.0	439.00 >	2.28
GRP19798	PT	6X9	25P	07/11/91	> 1000.0	438.00 >	2.28
GRP23634	PT	6PO	24P	07/02/91	> 1000.0	441.00 >	2.27
GRP23265	PT	4HQ	18P	04/04/91	981.00	436.00	2.25
GRP23372	PT	4MM	18P	04/11/91	> 1000.0	447.00 >	2.24
B724301	PT	59Z	20P	05/03/91	142.00	63.60	2.23
GRP23344	PT	4KR	18P	04/10/91	> 1000.0	452.00 >	2.21
GRP23293	PT	4JU	18P	04/09/91	> 1000.0	463.00 >	2.16
B723958	PT	5Z7	22P	05/29/91	> 1000.0	465.00 >	2.15
DN181170Q	PT	5N7	21P	05/16/91	> 1000.0	472.00 >	2.12
GRP23274	PT	4I4	18P	04/05/91	> 1000.0	474.00 >	2.11
GRP23629	PT	6MX	24P	06/19/91	> 1000.0	475.00 >	2.10
GRP23564	PT	6HZ	24P	06/14/91	> 1000.0	482.00 >	2.07
GRP23004	PT	7QW	26P	08/01/91	> 1000.0	490.00 >	2.04
GRP23515	PT	6HO	23P	06/13/91	886.00	436.00	2.03
GRP23510	PT	6HN	23P	06/13/91	174.00	86.80	2.01
GRP19462	PT	3K8	16P	02/27/91	764.00	386.00	1.98
B724136	PT	4Y2	19P	04/24/91	197.00	100.00	1.97
GRP19717	PT	6P5	24P	07/02/91	> 1000.0	516.00 >	1.94
B849199	PT	611	22P	05/31/91	> 1000.0	520.00 >	1.92
GRP19700	PT	435	17P	03/22/91	> 1000.0	531.00 >	1.88
DN181178Y	PT	5NA	21P	05/17/91	880.00	468.00	1.88
GRP19516	PT	3YA	17P	03/12/91	> 1000.0	544.00 >	1.84
GRP23591	PT	6LN	24P	06/18/91	> 1000.0	550.00 >	1.82
B849268	PT	436	17P	03/22/91	> 1000.0	557.00 >	1.80
GRP21325	PT	3Y7	16P	03/12/91	> 1000.0	564.00 >	1.77
GRP23238	PT	4GP	18P	04/03/91	> 1000.0	568.00 >	1.76
B724289	PT	59W	20P	05/03/91	> 1000.0	573.00 >	1.75
GRP21223	PT	3OU	16P	03/05/91	> 1000.0	576.00 >	1.74

Table 5 (Cont'd)

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
GRP19685	PT	432	17P	03/22/91	157.00	90.30	1.74
B723879	PT	5LN	20P	05/14/91	965.00	555.00	1.74
GRP23543	PT	6HU	23P	06/14/91	967.00	562.00	1.72
GRP23470	PT	6DX	23P	06/11/91	> 1000.0	584.00 >	1.71
B724001	PT	5ZH	22P	05/29/91	> 1000.0	593.00 >	1.69
GRP19545	PT	3YS	17P	03/13/91	> 1000.0	603.00 >	1.66
B724275	PT	59S	20P	05/02/91	> 1000.0	606.00 >	1.65
GRP19691	PT	433	17P	03/22/91	989.00	611.00	1.62
B678018/F243	PT	3IZ	15P	02/26/91	> 1000.0	628.00 >	1.59
GRP21267	PT	3QY	16P	03/06/91	> 1000.0	639.00 >	1.57
B724131	PT	4YO	19P	04/23/91	129.00	82.40	1.57
GRP23186	PT	4CF	18P	03/29/91	> 1000.0	651.00 >	1.54
GRP23338	PT	4KQ	18P	04/10/91	775.00	510.00	1.52
GRP23624	PT	6MW	24P	06/19/91	> 1000.0	656.00 >	1.52
GRP19511	PT	3LY	16P	03/01/91	> 1000.0	682.00 >	1.47
GRP19602	PT	3ZU	17P	03/15/91	825.00	560.00	1.47
GRP23459	PT	6DW	23P	06/11/91	> 1000.0	686.00 >	1.46
GRP23544	PT	6HV	23P	06/14/91	> 1000.0	691.00 >	1.45
GRP19710	PT	6P3	24P	07/02/91	> 1000.0	694.00 >	1.44
GRP23568	PT	6HZ	24P	06/14/91	> 1000.0	709.00 >	1.41
GRP23906	PT	8RW	27P	08/21/91	> 1000.0	710.00 >	1.41
B718656	PT	2ZW	14P	02/01/91	> 1000.0	716.00 >	1.40
GRP23730	PT	682	23P	06/04/91	140.00	100.00	1.40
GRP21536	PT	93V	28P	08/29/91	> 1000.0	713.00 >	1.40
GRP21222	PT	3OU	16P	03/05/91	> 1000.0	733.00 >	1.37
B604736/F046	PT	3IU	15P	02/26/91	> 1000.0	736.00 >	1.36
B724340	PT	5FZ	20P	05/08/91	553.00	417.00	1.33
GRP19676	PT	42Z	17P	03/20/91	128.00	96.90	1.32
B724210	PT	530	19P	04/26/91	> 1000.0	763.00 >	1.31
DN181494S	PT	5QC	21P	05/22/91	> 1000.0	764.00 >	1.31
GRP23540	PT	6HU	23P	06/14/91	882.00	679.00	1.30
B724268	PT	59R	20P	05/02/91	> 1000.0	806.00 >	1.24
GRP23377	PT	4MN	18P	04/11/91	841.00	684.00	1.23
B849284	PT	4AZ	17P	03/26/91	986.00	808.00	1.22
GRP23534	PT	6HS	23P	06/13/91	795.00	650.00	1.22
GRP23532	PT	6HR	23P	06/13/91	865.00	713.00	1.21
GRP23245	PT	4HM	18P	04/04/91	> 1000.0	831.00 >	1.20
B723937	PT	5UB	22P	05/24/91	759.00	639.00	1.19
DN181491P	PT	5QC	21P	05/22/91	816.00	693.00	1.18
GRP19728	PT	6P7	24P	07/03/91	966.00	818.00	1.18
GRP23412	PT	4TM	18P	04/16/91	779.00	669.00	1.17
B849272	PT	436	17P	03/22/91	745.00	654.00	1.14
GRP23446	PT	6DU	23P	06/11/91	> 1000.0	877.00 >	1.14
B719007	PT	310	14P	02/08/91	> 1000.0	888.00 >	1.13
GRP19701	PT	435	17P	03/22/91	> 1000.0	887.00 >	1.13
B724108	PT	4UI	19P	04/19/91	> 1000.0	883.00 >	1.13
GRP23925	PT	8UW	27P	08/22/91	> 1000.0	882.00 >	1.13
GRP23233	PT	4GO	18P	04/03/91	708.00	632.00	1.12
DN181402S	PT	5Q8	21P	05/22/91	758.00	676.00	1.12
GRP19675	PT	42Z	17P	03/20/91	718.00	649.00	1.11
GRP19955	PT	7NW	26P	07/31/91	> 1000.0	911.00 >	1.10
GRP19635	PT	42S	17P	03/19/91	> 1000.0	928.00 >	1.08
GRP19985	PT	7QT	26P	08/01/91	> 1000.0	931.00 >	1.07
B723998	PT	52G	22P	05/29/91	872.00	823.00	1.06
GRP23641	PT	6P1	24P	07/02/91	705.00	666.00	1.06
GRP23460	PT	6DW	23P	06/11/91	760.00	726.00	1.05
GRP23566	PT	6HZ	24P	06/14/91	80.40	79.50	1.01
GRP19610	PT	3ZW	17P	03/15/91	792.00	790.00	1.00
B724219	PT	56X	19P	04/30/91	> 1000.0	1000.0 >	1.00
GRP21344	PT	7EQ	25P	07/17/91	928.00	963.00	0.96
GRP23524	PT	6HQ	23P	06/13/91	446.00	472.00	0.94
GRP21744	PT	68A	23P	06/05/91	538.00	580.00	0.93
GRP23331	PT	4KO	18P	04/10/91	605.00	666.00	0.91
GRP23618	PT	6MU	24P	06/19/91	885.00	973.00	0.91

Table 5 (Cont'd)

DRUG #	VIR	PLT #	SHIP #	TEST DATE	TC 25	IC 50	SI
B604736/F057	PT	3IV	15P	02/26/91	641.00	714.00	0.90
B724004	PT	5ZH	22P	05/29/91	753.00	836.00	0.90
GRP23330	PT	4KO	18P	04/10/91	767.00	859.00	0.89
B723957	PT	5Z7	22P	05/29/91	302.00	345.00	0.88
GRP21806	PT	69G	23P	06/07/91	46.10	53.20	0.87
B723908	PT	5U4	22P	05/23/91	590.00	685.00	0.86
GRP23620	PT	6MV	24P	06/19/91	694.00	819.00	0.85
GRP19463	PT	3K8	16P	02/27/91	768.00	913.00	0.84
GRP19649	PT	42U	17P	03/19/91	784.00	942.00	0.83
B723910	PT	5U5	22P	05/23/91	695.00	845.00	0.82
B724323	PT	5F7	20P	05/07/91	622.00	779.00	0.80
GRP23927	PT	8UX	27P	08/22/91	713.00	904.00	0.79
B724202	PT	52Z	19P	04/26/91	633.00	814.00	0.78
GRP21321	PT	3Y6	16P	03/12/91	686.00	924.00	0.74
GRP23339	PT	4KQ	18P	04/10/91	624.00	872.00	0.72
B724244	PT	57L	19P	05/01/91	566.00	832.00	0.68
GRP19780	PT	6WC	24P	07/09/91	624.00	931.00	0.67
GRP19490	PT	3KE	16P	02/28/91	599.00	930.00	0.64
GRP21264	PT	3QX	16P	03/06/91	583.00	938.00	0.62
B604736/F058	PT	3IV	15P	02/26/91	553.00	897.00	0.62
B724113	PT	4XW	19P	04/23/91	452.00	737.00	0.61
GRP23575	PT	6LK	24P	06/18/91	613.00	1000.0	0.61
GRP23530	PT	6HR	23P	06/13/91	393.00	660.00	0.60
GRP21195	PT	3M0	16P	03/01/91	576.00	1000.0	0.58
B720430	PT	368	14P	02/12/91	535.00	943.00	0.57
GRP21737	PT	689	23P	06/05/91	522.00	909.00	0.57
GRP21278	PT	3R1	16P	03/07/91	558.00	1000.0	0.56
GRP23252	PT	4HN	18P	04/04/91	555.00	1000.0	0.56
GRP21680	PT	4BT	18P	03/28/91	512.00	1000.0	0.51
B849298	PT	4BN	17P	03/27/91	3.52	7.29	0.48
GRP23439	PT	4TS	18P	04/17/91	441.00	961.00	0.46
GRP23628	PT	6MW	24P	06/19/91	404.00	949.00	0.43
GRP21233	PT	3OW	16P	03/05/91	327.00	820.00	0.40
B724045	PT	60J	22P	05/30/91	3.67	9.21	0.40
GRP21745	PT	68A	23P	06/05/91	219.00	618.00	0.35
GRP19492	PT	3KE	16P	02/28/91	319.00	1000.0	0.32
GRP19601	PT	3ZU	17P	03/15/91	213.00	734.00	0.29
GRP21847	PT	84V	27P	08/08/91	257.00	896.00	0.29
GRP19612	PT	3ZW	17P	03/15/91	99.30	600.00	0.17
GRP21720	PT	685	23P	06/05/91	6.52	372.00	0.02
GRP23549	PT	6HW	23P	06/14/91	0.83	74.10	0.01

The *in vitro* antiviral activity of the compounds in **Table 5** should be further confirmed. Verification of the antiviral activity of these prescreen actives were scheduled to be tested using the primary screening (confirmatory) protocol. Because of the discontinuation of this antiviral program by the Sponsor, some of the active compounds in **Table 5** have not been confirmed in the primary screening protocol due to lack of funding.

4.2.4 Confirmatory Assays (Compounds Selected from Prescreen Testing):

During this reporting period 1802 compounds were advanced (from prescreen testing) for confirmatory testing. If a compound had an SI of ≥ 1 then it was considered as a candidate for confirmatory primary screen testing. The SI of ≥ 1 was only a preliminary endpoint that was being used and was subject to change as more data was generated. Data from the confirmatory assays are summarized in **Table 6**. Out of 1802 confirmatory tests, 1252 natural products (~75%) were confirmed active in the primary screening protocol during this reporting period. The criteria for activity is that the confirmatory test has to show $\geq 25\%$ reduction in CPE in one or more of the viruses tested. Failure to confirm the activity in these compounds was probably due to differences during the assay conditions:

1) In confirmatory assays the concentration range is adjusted to a more accurate semilog scale to maximize the SI and calculate the TAI and it should indicate more accurately the antiviral potential of the test compound.

2) Differences in the "differential" of the two runs can cause the compound to read positive or negative, falsely. The variability in the differential can cause false positives or false negative bias in day-to-day testing calculations, reflecting the variability in the maximum activity of the compound.

3) The metabolic rate, age, and passage number, etc., of the cells may cause the above observed variability in test compound activity.

4) Problems associated with stability and storage of the compound (i.e., different lot numbers, solubility, light sensitivity, hygroscopic, etc.).

5) Problems associated with technical execution of large numbers of plates by different technicians.

6) During the beginning of the prescreen testing (Shipment 1P - 5P), the assays were performed with confluent and stationary cellular monolayers. This procedure has a tendency to create false positive results as compared to the confirmatory assay results. In confirmatory testing actively metabolizing subconfluent monolayers have been used.

7) Two different MTT protocols are being compared. The prescreen protocol was prophylactic when we were testing drug shipments 1P - 5P. The drug was delivered to the cells before final addition of 62 $TCID_{50}$ of virus to total volume of 200 μl. The confirmatory protocol is therapeutic. The virus 32 $TCID_{50}$ is delivered onto the cells before the addition of the drug in total volume of 100 μl. In later drug shipments the same protocol was used for both prescreen and confirmatory assays.

The results seem to indicate that prophylactic treatment with confluent stationary monolayers causes inconsistent cell infections and therefore causes numerous drugs to read as false positive. Later prescreen testing (starting with shipments 5P) was done with the same therapeutic protocol in order to evaluate more properly the correlation of actives from the prescreen to those of the confirmatory (primary screen) results. The conflicting results should be retested based upon the availability of the compound.

Recommendations of Prescreen Confirmed-Actives Based Upon the *In Vitro* Results with MTT Assay (Vero Cells).

Based upon the confirmatory *in vitro* results with the MTT assay (Vero cells) we recommend the following:

1) Compounds with the highest TAI in the 95% activity category that have confirmed results with the exception of "colored" compounds should receive the highest priority for further profile studies and *in vivo* animal testing.

2) Compounds with the highest TAI in the 50% activity category that have confirmed results with the exception of "colored" compounds should receive the next highest priority for further profile studies and *in vivo* animal testing.

Table 6

Confirmatory Testing of Compounds Selected From Prescreen Testing

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
15P	B604736-F046	2	3	56	11029	0	0	1	0	0	NR
15P	B604736-F047	14	5	38	11030	1	0	0	2	2	NR
15P	B604736-F057	11	4	59	11031	1	0	0	1	0	NR
15P	B631963-F015	8	6	17	11032	0	0	0	0	0	NR
15P	B631963-F016	3	23	17	11033	0	0	0	0	0	NR
15P	B634131-F031	27	40	5	11034	0,0	0,0	0,0	0,0	0,0	NR
15P	B634131-F032	18	34	1	11035	0	0	0	0	0	NR
15P	B634131-F065	33	32	22	11036	0,0	0,0	0,0	0,0	0,0	NR
15P	B634131-F068	33	46	20	11037	0	0	0	0	0	NR
15P	B642761-F016	13	12	28	11038	0	0	0	0	0	NR
15P	B642761-F017	7	9	36	11039	0	0	0	0	0	NR
15P	B642761-F018	5	10	26	11040	0	0	0	0	0	NR
15P	B644263-F106	2	1	17	11041	0	0	1	0	0	NR
15P	B644263-F107	1	27	22	11042	0	0	0	0	0	NR
15P	B676018-F245	43	10	42	11045	2,2	2,2	1,1	1,1	0,1	NR
15P	B678018-F243	2	2	62	11043	1,1	2,1	2,1	0,0	0,2	NR
15P	B678018-F244	54	13	57	11044	2,2	2,2	0,1	0,1	0,1	NR
15P	B678018-F246	5	17	2	11046	0	0	0	0	0	NR
4P	B708143-D032	9	–	3	7322	0	0	0	0	0	1

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
23P	B712496-F725	27	13	45	11988	1	1	0	0	0	NT
12P	B714994	1	1	25	9121	1,1	0	0	0	2	0
12P	B714997	0	3	26	9122	0	0	0	0	0	0
12P	B715001	40	4	11	9123	2,1	0,0	0,0	0,0	0,0	0
12P	B715010	0	10	1	9124	0	0	1	0	0	0
12P	B715011	0	11	6	9125	0	0	0	0	0	0
12P	B715012	1	22	14	9126	0	0	0	0	0	0
12P	B715022	1	3	18	9127	1,2	0,0	1,1	0,0	1,0	0
12P	B715023	0	3	16	9128	2,2	0,0	1,0	0,0	1,0	0
12P	B715026	0	4	25	9129	0	0	1	0	0	0
12P	B715060	7	21	26	9130	1	0	1	0	0	0
13P	B715141	15	14	52	11047	1,1	0,0	1,0	2,2	1,2	NR
4P	B716493-D021	14	--	33	7323	1,0	0,0	0,0	1,0	2,0	0
14P	B718574	0	70	32	11048	0,0	0,0	2,2	0,0	2,0	NR
14P	B718577	21	2	15	11049	0	0	0	0	1	NR
14P	B718580	1	2	29	11050	0	0	0	0	0	NR
14P	B718582	10	13	52	11051	0	0	0	1	0	NR
14P	B718583	0	0	28	11052	0	0	0	0	0	NR
14P	B718586	2	1	35	11053	0	0	1	0	1	NR
14P	B718587	0	3	24	11054	0	0	0	0	0	NR
14P	B718595	3	12	22	11055	0	0	1	0	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
14P	B718598	1	12	23	11056	0	0	1	0	0	NR
14P	B718599	0	21	24	11057	0	0	1	0	0	NR
14P	B718603	1	1	21	11058	0	0	1	0	0	NR
14P	B718636	22	30	4	11059	0	0	0	0	1	NR
14P	B718648	25	16	27	11060	1	0,0	2,1	1,0	2,1	NR
14P	B718649	18	7	3	11061	0,1	0	0	0	0	NR
14P	B718656	22	7	58	11062	1,1	0,0	0,0	0,0	2,1	NR
14P	B718789	30	19	0	11063	0	0	0	0	0	NR
14P	B718799	17	32	5	11064	0,2	0,1	2,2	0,0	0,0	NR
14P	B718807	7	19	36	11065	0	0	1	0	1	NR
14P	B718813	0	46	6	11066	0	0	1	0	0	NR
14P	B718819	0	38	23	11067	0	0	1	0	0	NR
14P	B718821	0	8	24	11068	0	0	1	0	0	NR
14P	B718838	6	32	31	11069	0,0	0,0	1,0	0,0	0,0	NR
14P	B718840	1	20	13	11070	0	0	0	0	1	NR
14P	B718841	7	44	33	11071	0	0	0	0	0	NR
14P	B718842	10	14	21	11072	0	0	1	0	0	NR
14P	B718843	39	1	0	11073	1	0	1	0	0	NR
14P	B718849	7	18	25	11074	0,1	0,0	0,0	2,0	0,0	NR
14P	B718860	38	13	0	11075	1,1	0,0	1,2	1,0	0,0	NR
14P	B718862	4	7	16	11076	1,0	0,2	0,0	2,1	1,3	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
14P	B718876	53	6	0	11077	0	0	0	0	0	NR
14P	B718882	1	41	3	11078	0,0	0,0	2,2	0,0	0,0	NR
14P	B718889	1	27	0	11079	0	0	1	0	0	NR
14P	B718917	5	39	13	11080	0	0	1	0	0	NR
14P	B718924	6	31	8	11081	0	0	1	0	0	NR
14P	B718934	27	28	16	11082	0	0	1	0	0	NR
14P	B718935	10	16	14	11083	0	0	1	0	0	NR
14P	B718942	1	21	10	11084	0	0	1	0	0	NR
14P	B718954	2	25	8	11085	0	0	1	0	0	NR
14P	B718969	4	62	7	11086	0,2	0,0	3,2	2,0	1,0	NR
14P	B718978	4	17	1	11087	0	0	1	1	0	NR
14P	B718979	6	26	48	11088	0	0	1	1	0	NR
14P	B718982	0	45	21	11089	0	0	1	0	0	NR
14P	B718983	5	16	13	11090	1	0	1	1	0	NR
14P	B718990	4	13	20	11091	0	0	0	1	0	NR
14P	B718996	2	7	13	11092	0	0	0	0	0	NR
14P	B719004	6	23	19	11093	0,0	0,0	2,2	0,0	0,0	NR
14P	B719006	3	47	38	11094	0	0	1	1	0	NR
14P	B719007	25	25	53	11095	1,2	0,0	2,3	1,1	1,2	NR
14P	B719008	22	13	14	11096	1	0	1	1	0	NR
12P	B719215	26	1	8	9131	0	0	0	0	0	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
12P	B719216	16	10	15	9132	1	0	1	0	1	0
12P	B719217	16	1	2	9133	0	0	0	0	0	0
12P	B719218	33	64	11	9134	1,0	0,0	2,2,1	0,0	1,0	0
12P	B719219	33	7	6	9135	1	0	1	0	0	0
12P	B719220	22	5	13	9136	0,0	0,0	0,0	0,0	0,0	0
12P	B719221	16	27	10	9137	0,0	0,0	1,0	0,0	0,0	0,0
12P	B719222	19	9	32	9138	0	0	1	0	1	0
12P	B719224	28	1	13	9139	0	0	0	0	0	0
12P	B719225	27	1	5	9140	0	0	0	0	0	0
12P	B719226	13	3	7	9141	0	0	0	0	0	0
12P	B719228	13	6	3	9142	0	0	0	0	1	0
12P	B719229	11	23	9	9143	0	0	0	0	0	0
12P	B719230	9	6	11	9144	0	0	0	0	0	0
12P	B719232	7	10	36	9145	0	0	1	0	0	0
12P	B719238	10	4	28	9146	0	0	0	0	0	0
12P	B719240	14	15	8	9147	0	0	0	0	0	0
12P	B719241	14	2	15	9148	0	0	1	0	0	0
12P	B719242	20	15	37	9149	1,2	0,0	1,1	2,0	0,1	1
12P	B719244	13	15	3	9150	1	0	1	1	1	0
12P	B719245	16	6	16	9151	0	0	0	1	0	0
12P	B719246	20	3	16	9152	1	0	1	1	1	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
12P	B719247	16	6	18	9153	0,1	0,0	1,0	2,0	0,0	0
12P	B719255	10	1	14	9154	0	0	0	0	0	0
12P	B719256	5	6	17	9155	0	0	0	0	0	0
12P	B719257	13	3	4	9156	0	0	0	0	0	0
12P	B719258	16	2	2	9157	1	0	0	0	0	0
12P	B719259	23	5	5	9158	0	0	0	0	0	0
12P	B719260	15	6	19	9159	0	0	0	0	0	0
12P	B719265	9	16	4	9160	1	0	1	1	0	0
12P	B719266	7	36	11	9161	1	0	1	0	0	0
12P	B719267	10	30	19	9162	0,1	0,0	2,1	0,0	0,0	0
12P	B719272	8	20	5	9163	1,0	0,0	2,1	0,0	0,0	0
12P	B719274	18	1	5	9164	2,0	0,0	0,0	1,0	0,1	0
12P	B719278	0	10	10	9165	0	0	1	0	0	0
14P	B720392	5	72	30	11097	0,1	0,0	2,1	0,0	0,1	NR
14P	B720394	21	1	0	11098	0	0	1	0	1	NR
14P	B720396	0	19	0	11099	0	0	0	0	1	NR
14P	B720398	2	72	24	11100	0,0	0,0	3,0	0,1	0,0	NR
14P	B720399	3	52	11	11101	0,0	2,0	2,1	0,0	0,0	NR
14P	B720400	23	4	0	11102	0,1	0,0	2,1	1,0	0,0	NR
14P	B720405	0	22	12	11103	0,0	0,0	2,1	0,0	0,0	NR
14P	B720409	33	5	21	11104	0	0	0	1	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
14P	B720410	20	4	0	11105	0	0	0	0	0	NR
14P	B720412	42	5	13	11106	0	0	0	1	0	NR
14P	B720415	32	3	1	11107	0,1	0,0	1,0	2,0	0,0	NR
14P	B720418	4	8	22	11108	0,0	0,0	0,2	2,0	0,0	NR
14P	B720419	21	12	0	11109	0,0	0,0	2,0	0,0	1,0	NR
14P	B720424	9	5	27	11110	0,0	0,0	0,1	2,0	0,0	NR
14P	B720425	41	32	1	11111	0,1	0,0	2,1	0,0	0,0	NR
14P	B720425	–	–	–	11112	–	–	–	–	–	–
14P	B720427	25	28	1	11113	0	0	1	0	0	NR
14P	B720427	–	–	–	11114	–	–	–	–	–	–
14P	B720430	13	12	51	11115	0,1	0,0	1,1	2,1	0,0	NR
14P	B720430	–	–	–	11116	–	–	–	–	–	–
14P	B720431	14	11	8	11117	0,1	0,1	2,1	1,0	0,0	NR
14P	B720433	28	6	3	11118	1,2	0,0	1,1	2,0	1,0	NR
14P	B720434	8	19	0	11119	0,0	0,0	2,1	0,0	0,0	NR
14P	B720435	5	20	10	11120	0	0	0	0	0	NR
14P	B720436	28	4	0	11121	0,0	0,0	2,2	3,0	1,0	NR
14P	B720443	25	1	5	11122	1,1	2,1	0,0	2,0	2,2	NR
14P	B720445	42	4	82	11123	2,2	1,1	1,1	3,2	2,2	NR
8P	B720490	19	3	3	8507	0	0	0	0	0	0
8P	B720492	20	0	2	8508	0	0	0	0	0	1

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
8P	B720493	26	4	1	8509	0	0	0	0	0	0
8P	B720494	18	20	3	8273	0	0	0	0	0	0
8P	B720498	17	8	2	8510	0	0	1	0	0	0
12P	B720820	7	12	5	9166	1,0	0,0	2,1	0,0	0,0	1
12P	B720821	16	1	4	9167	1,2	0,0	1,1	2,0	0,1	0
12P	B720825	14	18	50	9168	1,1	0,0	1,0	1,0	0,0	0
12P	B720828	23	9	1	9169	1	0	1	0	0	0
12P	B720832	0	13	27	9170	0	0	1	0	0	0
12P	B720834	7	31	25	9171	0,0	0,0	2,1	0,0	0,0	0
4P	B720892	6	–	27	7324	0	0	0	0	0	0
4P	B720894	18	–	29	7325	0	0	0	0	0	0
4P	B720895	18	–	35	7326	0	0	0	0	0	0
4P	B720896	22	–	30	7327	0	0	0	0	0	0
4P	B720900	28	–	41	7328	1,1	0,0	0,0	0,0	0,0	0
4P	B720903	5	–	29	7329	0	0	0	0	0	0
4P	B720905	6	–	45	7330	0	0	0	0	0	0
8P	B720910	23	11	22	8511	2,2	1,0	2,1	2,2	2,2	0
8P	B720911	14	14	55	8272	1,0	0,0	1,0	2,0	1,0	0
4P	B720912	6	–	43	7331	0,0	0,0	1,0	0,0	1,1	0
4P	B720914	33	–	37	7332	2,1	0,0	1,1	0,1	2,2	0
4P	B720915	4	–	31	7333	0,0	0,0	0,0	0,0	2,2	0

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
4P	B720916	9	–	55	7099	0	0	0	0	0	0
8P	B720931	0	25	46	8274	0	0	0	1	1	0
8P	B720933	0	100	47	8513	0,1	0,0	3,3	0,1	1,1	1
8P	B720935	18	10	2	8325	0	0	0	0	0	0
4P	B720937	11	–	55	7100	0	0	0	0	0	0
4P	B720940	2	–	60	7101	0	0	0	0	0	0
4P	B720941	0	–	51	7102	0	0	0	0	0	0
4P	B720946	6	–	32	7336	0	0	1	0	1	0
4P	B720951	11	–	62	7103	0	0	0	0	0	2
4P	B720951	–	–	–	7337	–	–	–	–	–	–
4P	B720952	11	–	56	7104	0	0	0	0	0	0
4P	B720952	–	–	–	7338	–	–	–	–	–	–
4P	B720958	1	–	60	7105	0	0	0	0	0	0
4P	B720963	0	–	59	7106	0	0	0	0	1	1
8P	B720968	0	26	40	8514	1	0	1	1	0	0
4P	B720972	10	–	24	7339	1,0	1,0	1,0	0,0	1,0	0
4P	B720979	10	–	63	7107	0	0	0	0	1	0
4P	B720979	–	–	–	7340	–	–	–	–	–	–
4P	B720985	7	–	0	7341	1,1	0,0	0,1	1,0	0,0	0
4P	B720986	7	–	0	7342	0	0	0	0	0	0
4P	B720987	12	–	17	7343	0	0	1	0	1	0

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
4P	B720988	4	–	4	7344	1,0	0,0	0,1	0,0	2,0	0
4P	B720989	28	–	1	7345	0	0	0	0	0	0
4P	B720990	7	–	23	7346	0	0	0	0	0	0
4P	B720994	4	–	0	7347	0,0	0,0	1,0	0,0	1,0	0
4P	B720995	7	–	0	7348	0	0	0	0	0	0
4P	B720998	8	–	1	7349	0,0	0,0	0,0	2,1	0,0	0
4P	B721000	12	–	24	7350	1,1	0,1	0,0	2,0	1,0	0
4P	B721001	3	–	3	7351	1,1	0,0	0,0	0,0	0,0	0
4P	B721002	2	–	27	7352	0	0	0	0	0	0
4P	B721005	10	–	12	7353	0	0	0	0	0	0
4P	B721006	32	–	2	7354	2,2	0,1	0,0	0,0	0,0	0
4P	B721007	36	–	8	7355	0,0	0,0	0,0	1,0	0,0	0
4P	B721011	37	–	60	7298	0,0	0,0	1,0	0,0	0,0	1
4P	B721012	32	–	36	7356	1,0	0,0	0,0	1,0	2,0	0
4P	B721013	0	–	10	7357	0	0	0	0	0	0
4P	B721015	7	–	0	7358	1,0	0,0	0,0	0,0	1,1	0
4P	B721016	0	–	34	7359	0	0	0	0	0	0
4P	B721017	0	–	39	7360	0	0	0	0	0	0
4P	B721018	3	–	32	7361	0,0	0,0	1,0	0,0	0,0	0
4P	B721019	0	–	28	7362	0,0	0,0	0,0	1,0	0,0	0
4P	B721020	4	–	10	7363	1,0	1,0	1,0	0,0	1,0	0

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
4P	B721021	0	–	61	7299	0	0	0	0	0	1
4P	B721022	0	–	32	7364	0	0	0	1	0	0
4P	B721026	3	–	37	7365	1,0	0,0	0,0	0,0	2,0	0
4P	B721029	0	–	29	7366	0	0	0	0	0	0
4P	B721030	12	–	25	7367	0	0	0	1	0	0
4P	B721031	0	–	43	7368	0,0	0,0	0,0	2,0	0,0	0
4P	B721033	10	–	2	7369	0,0	0,0	0,0	0,0	2,0	0
4P	B721034	11	–	10	7370	0	0	0	0	0	0
8P	B721035	0	28	22	8515	1	0	0	0	0	1
4P	B721040	7	–	5	7371	0	0	0	0	0	0
4P	B721042	8	–	0	7372	1,0	1,1	0,0	0,0	1,0	0
4P	B721045	13	–	17	7373	2,1	0,0	0,0	2,2	2,1	0
4P	B721050	10	–	5	7374	0,0	1,0	0,0	1,1	0,0	1
4P	B721051	47	–	15	7375	2,0	2,0	0,0	2,1	1,0	2
4P	B721052	12	–	4	7376	0	0	0	0	0	0
4P	B721053	8	–	0	7377	2,0	1,0	0,0	0,0	1,0	0
4P	B721054	35	–	15	7378	2,1	0,0	1,0	0,0	2,0	1
4P	B721055	12	–	7	7379	0,0	0,0	0,0	1,1	0,0	0
4P	B721056	10	–	20	7380	1,0	1,0	0,0	0,0	1,0	0
4P	B721058	14	–	0	7381	0,0	1,0	0,1	0,1	0,0	1
4P	B721059	19	–	4	7382	0,1	0,0	0,0	1,0	2,1	0

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
4P	B721060	7	–	72	7300	0	0	0	0	0	0
4P	B721061	1	–	81	7301	0,0	0,0	0,0	1,2	0,1	0
4P	B721062	18	–	86	7302	0,0	0,0	0,0	0,1	1,1	2,1
4P	B721063	15	–	36	7383	1,2	0,0	0,0	2,0	1,1	0
4P	B721064	8	–	80	7303	0,0	0,0	0,1	1,2	0,0	0
4P	B721173	62	–	20	7108	1,1	1,0	0,0	0,0	0,0	0
4P	B721177	10	–	9	7385	1,0	0,0	0,1	2,0	1,0	0
4P	B721178	3	–	10	7386	0,1	0,0	0,2	2,1	0,0	0
8P	B721181	20	10	4	8326	2,1	0,0	0,0	1,1	2,2	0
8P	B721192	21	5	1	8327	2,1	0,0	0,1	1,0	0,0	0
4P	B721193	0	–	23	7387	0,0	0,0	0,0	1,0	0,0	0
4P	B721196	–	–	31	7388	0	0	0	0	0	0
4P	B721211	27	–	0	8334	0	0	0	0	0	0
8P	B721213	15	3	2	8504	0	0	0	0	0	0
8P	B721220	24	8	[illegible]	8505	NT	NT	1	0	0	0
8P	B721234	18	8	0	8506	NT	NT	1	1	1	1
8P	B721236	15	22	17	8328	0	0	1	2	1	0
4P	B721256	14	–	36	7389	0	0	0	0	0	0
4P	B721257	12	–	10	7390	2,0	0,0	0,0	0,1	1,0	0
8P	B721258	18	3	11	8329	0	0	0	0	0	0
4P	B721260	10	–	21	7391	0,0	0,0	0,0	2,1	1,1	2,1

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
4P	B721261	8	–	19	7392	0	0	0	0	1	0
4P	B721264	6	–	6	7393	0	0	0	0	0	0
4P	B721266	3	–	12	7394	0	0	0	1	0	0
8P	B721274	27	4	2	8330	1	0	0	2	0	0
8P	B721295	12	6	60	8309	0,0	0,0	1,1	2,3	0,0	0,0
8P	B721303	0	12	40	8310	0	0	0	0	0	0
4P	B721319	1	–	39	8230	0	0	1	2	0	0
4P	B721326	9	–	38	8231	0	0	1	1	1	0
8P	B721339	18	3	24	8331	1	0	0	1	1	0
8P	B721348	8	4	56	8311	2,1	1,0	0,0	2,2	2,1	0
4P	B721374	0	–	61	7304	0	0	0	0	0	0
4P	B721377	9	–	64	7305	0	0	0	0	0	0
4P	B721378	4	–	51	7306	0	0	0	0	0	0
4P	B721392	6	–	52	7307	0	0	0	0	0	1
8P	B721413	36	2	48	8312	1,1	0,0	0,1	2,2	1,1	1
8P	B721420	7	2	37	8249	0	0	0	0	0	0
8P	B721474	4	2	41	8313	0	0	1	0	2	0
8P	B721477	3	2	42	8314	0	0	0	0	0	0
8P	B721498	0	23	56	8315	0,1	0,0	2,2	1,1	1,1	0
8P	B721520	0	0	35	8316	0	0	0	0	0	0
8P	B721543	0	27	29	8317	0	0	1	0	0	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
8P	B721579	4	43	46	8318	1,1	0,0	2,2	2,1	1,0	0
8P	B721582	3	2	30	8319	1	0	0	1	0	0
4P	B721584	40	–	8	8335	1	1	0	0	0	0
4P	B721586	32	–	0	8336	1	0	0	0	1	0
4P	B721588	35	–	2	8337	1	1	1	0	0	2
4P	B721592	28	–	59	7308	0,0	0,0	1,1	0,1	0,0	0
8P	B721594	1	6	35	8320	0	0	0	0	0	0
4P	B721595	14	–	55	7309	0	0	0	0	0	0
4P	B721596	11	–	52	7310	0,0	0,0	1,0	0,0	0,0	1
8P	B721598	6	1	27	8321	0	0	0	1	0	0
8P	B721601	0	1	38	8322	0	0	0	1	0	0
4P	B721602	12	–	44	7395	1,1	0,0	0,0	1,1	1,0	0
8P	B721604	18	2	72	8323	0	0	0	0	1	0
8P	B721605	0	14	52	8500	0	0	1	1	1	1
8P	B721607	0	0	33	8501	0	0	0	0	0	0
4P	B721610	5	–	22	7396	0	0	0	0	0	2
4P	B721611	10	–	50	7311	1,0	0,0	0,0	1,0	0,0	1
8P	B721612	0	9	37	8502	1	0	1	0	0	0
4P	B721614	7	–	44	7397	0	0	0	0	0	0
4P	B721615	5	–	24	7398	0	0	0	0	0	0
4P	B721616	34	–	32	7399	1,1	0,0	0,1	2,2	1,1	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
4P	B721617	9	–	45	7400	0	0	0	0	0	0
8P	B721618	0	3	32	8503	1	0	0	0	0	1
4P	B721619	5	–	45	7401	0	0	0	0	0	0
8P	B721621	0	1	30	8324	0	0	0	0	0	0
4P	B721625	6	–	40	7402	0	0	0	0	0	0
4P	B721628	3	–	39	7403	0,1	0,0	0,2	1,1	0,0	0
4P	B721630	6	–	41	7404	0	0	0	0	0	0
12P	B721643	15	12	17	9172	1,1	1,0	2,0	0,0	1,0	0
4P	B721645	22	–	24	8338	1	0	0	0	0	0
1P	B721690	16	–	90	6241	0	0	0	0	0	NR
1P	B721693	19	-	62	6242	0	0	0	0	0	NR
1P	B721702	54	–	69	6243	0,1	0,0	0,0	3,0	0,0	NR
1P	B721714	52	–	70	6244	0	0	0	0	0	NR
1P	B721728	74	–	78	6245	0	0	0	1	0	NR
1P	B721729	22	–	49	6246	0,1	0,0	1,1	1,0	0,1	NR
10P	B721746	11	6	0	9173	2,2,1	0,0	2,2	2	2,2	0
1P	B721749	5	67	100	6584	0,1	0,0	1,0	1	0,0	NR
1P	B721754	51	–	77	6247	0	0	0	1	0	NR
10P	B721777	0	31	0	9174	0,0,0	0,0	1,0	0	0,0	0
10P	B721778	16	6	0	8379	0	0	1	0	0	0
10P	B721781	12	5	46	8380	1	0	0	1	0	0

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
10P	B721786	0	18	14	9175	0,2	0	2,0	0,0	0	0
3P	B721787	10	–	76	6585	0	0	0	0	0	0
3P	B721818	23	–	67	6586	0	0	0	0	0	0
1P	B721823	82,59	–	100,100	6248	0,0	0,0	1,0	2,0	0,0	0
3P	B721826	31	–	66	6587	0	0	0	0	0	0
3P	B721838	19	–	66	6588	1,1	0,0	0,0	1,0	0,0	0
9P	B721860	0	17	24	9176	1	0	1	0	0	1
9P	B721863	0	23	38	8516	1	0	1	1	1	1
9P	B721877	10	1	15	9177	0	0	0	0	0	0
3P	B721880	50	–	76	6589	2,0	0,0	0,0	1,0	1,0	0
9P	B721881	0	–	2	8517	1	0	1	0	0	0
9P	B721883	0	2	23	8518	0	0	0	0	0	0
3P	B721892	12	–	50	6590	0	0	0	0,0	0	0
3P	B721899	100	–	61	6591	1,1	0,0	0,0	1,0	1,0	0
3P	B721905	15	65	28	6592	1,1	0,0	0,0	1,0	1,0	0
3P	B721908	1	–	50	6593	0	0	0	0	0	0
3P	B721910	32	–	98	6594	1,0	0,0	0,0	1,0	0,0	0
3P	B721917	0	–	76	6595	0	0	0	0	0	0
3P	B721925	6	–	67	6596	0	0	0	0	0	0
3P	B721953	5,99	–	38,84	6597	0	0	0	0	0	0
3P	B721958	0	–	58	6598	0	0	0	0	0	0

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
3P	B721979	49	–	94	6599	0	0	0	0	0	0
3P	B722006	29	–	56	6600	0	0	0	0	0	0
9P	B722033	2	21	34	8519	3	0	0	0	0	0
9P	B722038	11	16	4	9178	0	0	1	0	0	0
3P	B722048	–	–	79	6601	1,0	0,0	0,0	2,0	0,0	0
3P	B722052	3	–	68	6602	0	0	0	0	0	0
3P	B722054	32	–	87	6603	1,0	0,0	0,0	2,0	0,0	0
9P	B722055	28	13	25	9179	0	0	1	0	0	0
9P	B722060	26	2	55	9180	1	0	0	0	0	0
9P	B722072	13	1	0	9181	0	0	0	0	0	0
3P	B722076	23	–	71	6604	1,1	0,0	0,0	1,0	1,0	0
3P	B722077	41	–	66	6605	1,1	1,0	1,1	1,0	1,0	0
3P	B722078	36	–	68	6606	1,1	0,0	0,0	0,0	0,0	0
3P	B722080	47	–	57	6607	1,1	0,0	0,0	2,0	1,0	0
3P	B722081	30	–	55	6608	1,1	0,0	0,0	1,0	0,0	0
3P	B722087	59	–	71	6609	0	0	0	0	0	0
3P	B722089	3	–	50	6610	0	0	0	0	0	0
3P	B722091	8	–	98	6611	0	0	0	1	0	0
3P	B722094	10	–	63	6612	0	0	0	1	0	0
9P	B722098	9	2	5	9182	0	0	0	0	0	0
3P	B722109	52	–	51	6613	1,0	0,0	0,0	0,0	0,0	0

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
3P	B722111	76	–	47	6614	1	0	0	0	0	0
9P	B722116	73	3	55	9183	2	1,1	0	0	1,0	1,0
9P	B722117	46	27	58	9184	2,2	2,2	0,0	1,0	2,0	0
3P	B722141	17	–	63	6615	0	0,0	0	0	0	0
9P	B722161	2,5	1,5	31,34	9185	1	0	0	0	1	1
3P	B722162	12	–	55	6616	1	0	0	0	0	0
3P	B722165	79	–	58	6617	2,0	2,0	0,0	0,0	0,0	0
3P	B722168	23	–	82	6618	2,0	0,0	0,0	0,0	0,0	0
9P	B722172	2	25	14	9186	0	0	1	0	0	0
9P	B722174	14	10	30	8366	0	0	0	2	0	0
9P	B722174	14	10	30	9187	0	0	0	0	0	0
9P	B722179	25	1	17	9188	1	1	0	0	1	1
3P	B722181	51	–	51	6619	1	0	0	0	0	0
3P	B722182	88	–	58	6620	2	0	0	0	0	0
3P	B722183	90	–	76	6621	1	0	0	0	0	0
9P	B722184	0	22	49	8367	1	0	1	1	1	1
9P	B722186	23	1	17	9189	1	0	0	0	0	0
9P	B722190	22	0	4	9190	0	0	0	0	0	0
9P	B722194	20	1	4	9191	0	0	0	0	0	0
9P	B722198	0	30	12	9192	1	0	1	0	0	0
9P	B722199	0	28	28	9193	1	0	1	0	0	0

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
9P	B722210	1	2	13	8363	0	0	0	0	0	0
9P	B722215	15	1	10	9194	0	0	0	0	0	0
3P	B722222	46	–	90	6622	0,0	0,0	0,0	1,0	0,0	0
3P	B722224	4	–	79	6623	1,0	0,0	0	1,0	0,0	0
3P	B722228	49	–	91	6624	0	0	0	0	0	0
9P	B722229	2,30	12	25,5	9195	1	0	1	0	0	0
3P	B722230	23,1	35	72,35	6625	0,0	0,0	0	1,0	0,0	0
9P	B722231	0,13	17	40,38	8368	1	0	1	2	1	1
9P	B722233	26	0	0	9196	0	0	0	0	0	0
9P	B722234	11	23	21	9197	0	0	1	0	1	0
9P	B722236	19	0	6	9198	0	0	0	0	0	0
3P	B722239	78,9	2	59,32	6626	0	0	0	0	0	0
9P	B722240	16	4	29	8364	3,1	2,0	0,0	1,1	1,0	0
3P	B722241	100	–	78	6627	0	0	0	0	0	0
9P	B722242	6,47	2	45,6	9199	1,2	2,1	0,0	0,0	0,0	0
9P	B722244	0,0	25	17,9	9200	0	1	0	0	0	0
9P	B722245	0,14	0	17,0	9201	0	0	0	0	0	0
3P	B722246	63,10	4	56,36	6628	2	0	0	0	0	0
3P	B722247	61,0	2	56,2	6629	1	0	0	0	0	1
9P	B722248	0,64	5	29,3	9202	1,2	2,1	0,0	0,0	0,0	0
9P	B722255	0	26	0	9203	1	1	0	0	1	0

Results of Prescreen Testing[a]					Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
9P	B722256	0	13	0	9204	1	1	1	0	1	0
9P	B722278	13	2	0	8365	0	0	0	0	0	0
7P	B722279	13	1	60	8238	0	0	0	1	0	1
7P	B722280	5	0	63	8239	0	0	0	1	0	2,2
10P	B722504	5	32	8	9205	1	0	1	0	0	0
10P	B722506	4	22	1	8381	0	0	0	0	0	0
10P	B722508	9	20	41	8382	1	0	1	1	0	0
10P	B722510	0	37	5	8383	1	0	1	0	0	0
3P	B722518	64	–	0	7109	1,0	0,0	0,0	0,0	0,0	0
3P	B722525	0	–	79	7110	0,0	0,0	0,1	1,0	0,2	3,2
7P	B722559	28	14	56	8240	2,2	0,0	1,2	2,2	1,2	1
7P	B722566	29	6	14	8270	1,1	0,0	2,2	2,1	0,1	1
7P	B722591	15	3	51	8241	1,1	0,0	2,1	1,1	1,0	1
7P	B722607	0	2	54	8242	0	0	1	0	0	0
7P	B722614	1	4	50	8243	1	0	0	0	0	0
7P	B722628	1	3	61	8244	1	0	1	0	0	0
7P	B722632	0	1	57	8245	0	0	0	0	0	0
7P	B722634	4	1	65	8246	0	0	0	0	0	0
7P	B722635	20	1	12	8512	1	0	1	0	0	0
7P	B722666	0	1	28	8247	1	0	1	0	0	0
7P	B722668	1	5	42	8248	1,1	0,0	1,1	0,1	1,0	1

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
7P	B722689	2	15	67	8228	1,2	0,0	0,1	2,2	1,2	0
7P	B722733	25	–	0	8339	0,0	0,0	0,0	0,2	0,0	0,0
7P	B722743	4	10	68	8250	0,0	0,0	0,0	2,1	2,1	0
7P	B722745	1	11	65	8251	2,0	0,0	1,1	2,2	2,2	0
3P	B722752	19	–	0	7405	0,0	0,0	0,0	2,0	1,0	0
7P	B722757	0	17	13	8229	1,0	0,0	1,1	2,1	0,1	1
7P	B722778	0	8	33	8252	0	0	1	0	0	1
7P	B722785	0	9	49	8253	1	0	1	1	1	0
3P	B722805	1	–	57	7111	0	0	0	0	0	0
3P	B722808	5	–	61	7112	0	0	0	0	0	1
3P	B722811	3	–	50	7113	0	0	0	0	0	0
7P	B722823	3	1	73	8271	2,2	0,0	0,0	2,2	3,2	0
3P	B722824	100	–	100	7114	0	0	0	0	0	0
3P	B722849	7	–	55	7115	0	0	0	0	0	0
3P	B722854	11,18	–	19,28	7406	0,1	0,0	0,0	2,2	1,0	0
3P	B722858	9,18	–	13,30	7407	1,1	1,1	0,0	0,0	1,0	1
7P	B722866	0,20	–	22,36	8340	0,0	0,0	0,0	0,0	0,0	0
3P	B722867	0,0	–	49,54	7312	0	0	0	0	0	0
3P	B722871	24,9	–	71,0	7313	0	0	0	0	0	0
10P	B722872	8	18	25	8413	0	0	0	0	0	0
10P	B722872	8	18	25	9208	0	0	1	0	0	0

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
10P	B722873	36	7	64	8373	2,1	0,0	1,1	1,0	0,0	0
10P	B722873	36	7	64	9209	1,1	0,0	1,0	0,0	0,0	0
3P	B722874	3,2	–	76,30	7314	0,0	0,0	0,0	1,0	0,0	0
10P	B722876	15	0	2	8388	0	0	0	0	0	0
10P	B722876	15	0	2	9210	0	0	0	0	0	0
10P	B722883	9,5,2	15,2	16,55	7315	0	0	0	1	0	0
3P	B722883	9,5,2	15,2	16,55	7315	0	0	0	1	0	0,0
10P	B722884	12	0	10	8389	0	0	0	0	0	0
10P	B722884	12	0	10	9212	1	0	0	0	0	0
10P	B722886	0,0,8	3	21,61,40	7316	0	0	0	1	0	0,0
3P	B722886	0,0,8	28	21,61,40	7316	0	0	0	1	0	0,0
3P	B722889	4,3,8	28	19,51,17	7317	0	0	0	0	0	0,0
10P	B722889	4,3,8	28	19,51,17	7317	0	0	0	0	0	0,0
10P	B722890	13	31	19	8390	0	0	0	0	0	0
10P	B722890	13	31	19	9215	0	0	0	0	0	0
10P	B722899	0	26	34	9216	0	0	1	0	0	0
10P	B722902	8	5	22	8374	3,2	2,1	2,2	2,1	3,2,2	0
10P	B722902	8	5	22	9217	2,2	1,1	1,3	1,0	3,2	0
3P	B722904	6,13	–	33,52	7318	0	0	0	1	0	0
10P	B722905	8	17	3	9218	0	0	0	0	0	0
10P	B722908	0	19	0	9219	1	0	1	0	0	0

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
10P	B722909	22	8	4	8391	0,0,0	0,0,0,0	0,1,0	0,0,1	0,0,0	0
10P	B722911	0	23	5	8220	1	0	0	0	0	0
10P	B722914	1	39	0	9221	1,0	0,2	2,1	0,0	0,0	0
10P	B722915	4	29	37	8375	0,0	0,0	1,0	2,1	0,0,0	0
10P	B722915	4	29	37	9222	1	0	1	0	0	0
10P	B722917	3	25	4	9223	1	0	0	0	0	0
10P	B722921	25	14	25	8376	1,1	0,1	1,0	2,2	2,1	1
10P	B722921	–	–	–	8392	–	–	–	–	–	–
10P	B722922	4	12	6	8337	1	1	1	0	0	2
10P	B722922	4	12	6	9224	0	0	0	0	0	0
10P	B722926	6	27	40	9225	1,1	2,2	1,0	2,0	0,0	0
10P	B722929	1	15	64	8414	0	0	1	1	0	0
10P	B722931	12	20	4	9226	0	0	1	1	0	0
10P	B722932	7	30	15	9227	0,2	2,1	1,0	2,0	0,0	0
10P	B722933	20	8	3	8364	3,1	2,0	0,0	1,1	1,0	0
10P	B722935	4	21	19	9228	0	0	1	1	0	0
10P	B722936	6	42	62	9229	1,2	0,0	1,0	2,0	0,0	0
10P	B722937	21	10	17	8369	1	0	1	0	0	0
10P	B722938	6	30	13	9230	0	0	1	0	0	0
10P	B722942	13	33	11	8386	0	0	1	0	0	0
10P	B722944	6	29	9	9231	0	0	0	0	0	0

Results of Prescreen Testing[a]					Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
10P	B722950	6	27	10	9232	1	0	1	0	0	0
10P	B722965	10	32	21	8370	2,0	0,0	1,0	1,1	3,2	1
10P	B722965	10	32	21	9233	0,1	0,0	0,0	0,0	3,0	0
10P	B722976	8	20	4	9234	0	0	0	1	0	0
10P	B722987	3	30	3	9235	1,0	2,1	0,0	2,1	1,2	0
12P	B722992	4	20	0	9236	0,0	0,0	2,1	0,0	0,0	0
3P	B722997	27	–	9	8341	0,0	0,0	0,0	0,0	0,0	0,0
10P	B723035	7	39	27	8371	2,1	0,0	1,1	1,1	0,0	0
3P	B723037	9	–	56	7116	0,0	0,0	1,2	1,2	0,0	0
3P	B723044	15	–	52	7117	0	0	0	0	0	0
10P	B723047	12	19	29	8372	2,2	2,2	1,0	1,2	0,0	0
5P	B723061	0	63	0	7443	0	0	0	0	0	NR
5P	B723062	43	28	54	7445	0,2	2,3	0,0	0,2	0,0	NR
5P	B723064	0	49	7	8196	0,0	0,0	0,0	0,0	0,0	0
5P	B723089	5	22	8	8197	0,0	0,0	0,0	0,0	0,0	0
5P	B723096	2	84	10	7439	1,0	0,0	1,0	0,2	1,1	NR
3P	B723106	14	–	0	7408	0,0	0,0	0,0	2,0	0,0	0
3P	B723107	13	–	1	7409	0,0	0,0	0,0	1,0	1,0	0
3P	B723110	7	–	14	7410	0,0	0,0	0,0	2,0	1,0	0
5P	B723123	72	5	9	7438	2,2	1,1	0,0	0,1	0,0	NR
3P	B723126	27	–	10	8342	1	1	0	0	1	0

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
5P	B723136	7	53	3	7446	0	0	0	0	0	NR
5P	B723139	49	8	5	3262	2,1	0,0	0,2	2,1	1,0	0
5P	B723141	100	69	3	7440	0	0	0	0	0	NR
5P	B723143	5	6	0	7442	0	0	0	0	0	NR
5P	B723148	3	73	0	7441	0	0	0	0	0	NR
5P	B723159	0	49	0	8198	0	0	0,1	1,0	1,0	1
5P	B723160	0	28	0	8199	0	0	0,1	1,0	1,0	0
5P	B723169	48	14	27	9238	1,2	1,1	0,0	2,0	0,0	0
5P	B723172	4	1	26	9239	0	0	0	0	0	0
5P	B723199	35	16	14	8263	2,1	2,2	0,1	2,2	1,0	0
5P	B723201	28	15	0	8264	1	0	0	0	0	0
5P	B723203	0	60	6	7444	0,0	0,0	1,2	0,0	0,0	NR
5P	B723223	0	45	4	8332	2,1	0,0	1,1	2,1	2,1	1
5P	B723230	21	4	2	8265	1	0	0	0	0	0
5P	B723233	0	38	3	8200	0	0	1,1	0,0	0,0	1
5P	B723240	0	59	0	8333	1	0	1	0	0	1
5P	B723245	3	6	48	8232	0	0	0	0	0	0
5P	B723247	0	71	34	8201	0	0	1,1	0,0	1,0	0
6P	B723250	–	64	–	8212	0,2	0,0	2,2	1,2	1,1	1
6P	B723256	0	–	27	8213	0	0	1	1	0	0
6P	B723261	0	–	29	8214	0	0	1	0	0	0

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
6P	B723262	12	–	44	8215	0	0	1	0	0	0
6P	B723266	19	–	36	8267	1	0	0	1	1	0
6P	B723268	11	–	51	8216	1	0	0,0	0,0	0,1	0
6P	B723275	49	–	49	8217	1	0	0,0	1,0	1,0	0
6P	B723278	9	–	57	8218	1,1	0,0	1,1	2,1	1,0	1
6P	B723279	45	–	42	8268	2,1	1,0	0,0	1,1	0,0	0
6P	B723285	3	–	17	8219	1,0	0,0	0,1	2,1	0,1	1
6P	B723286	23	–	39	8220	0	0	1	1	0	0
6P	B723289	21	–	39	8221	1,1	2,2	2,3	1,1	0,1	0
6P	B723290	28	–	59	8222	1,1	0,0	1,1	1,1	1,1	0
6P	B723297	3	–	73	8223	0	0	1	1	0	1
6P	B723298	35	–	39	8224	1,1	0,0	1,1	2,1	0,0	0
6P	B723300	30	–	29	8269	2,1	0,0	1,1	1,1	0,2	0
6P	B723306	48	28	7	8225	2,2	0,0	0,0	1,1	0,0	0
6P	B723315	0	74	14	8226	0	0	1	1	0	0
6P	B723318	7	50	0	8227	0	0	1	1	1	1
6P	B723322	0	78	33	8210	0	0	0,0	0,0	0,0	0
6P	B723364	38	57	42	8211	1	0,0	1,1	0,0	1,0	0
6P	B723398	21	10	32	8266	1	0	0	0	0	1
6P	B723400	1	38	37	8202	0	0	1,0	0,0	0,0	0
6P	B723401	1	27	25	8203	0	0	0,0	0,0	0,0	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
6P	B723409	0	59	32	8204	0	0	1,1	0,0	1,1	0
6P	B723410	22	43	35	8205	1	1	0,1	1,1	0,0	0
6P	B723412	53	22	46	8206	1	1	0,0	1,0	0,0	0
6P	B723413	22	36	35	8207	0	0	0,0	0,0	0,0	0
6P	B723414	0	75	26	8208	0	0	1,1	1,1	1,0	1
12P	B723420	5	14	5	9240	0	0	0	0	0	0
10P	B723424	3	24	2	9241	1	0	0	0	0	0
10P	B723425	0	22	2	9242	0	0	0	1	0	0
10P	B723426	1	27	4	9243	0	0	0	0	0	0
10P	B723427	51	21	16	8393	0	0	0	0	0	0
10P	B723428	42	9	24	8394	1	0	0	0	1	0
10P	B723429	0	18	0	9244	0	0	0	0	0	0
10P	B723430	0	30	2	9245	0	0	0	1	0	0
10P	B723431	15	65	56	8378	2,1	0,0	1,1	3,2	2,2	0
10P	B723432	6	45	18	9246	0,0	0,0	1,0	2,0	0,0	0
10P	B723435	26	5	0	9247	2,0	0,0	0,0	0,0	0,2	0
10P	B723436	50	4	11	9248	2,2	2,2	1,0	2,0	3,3	0
10P	B723438	19	2	8	9249	0	0	1	0	1	0
10P	B723441	55	2	3	9250	1	0	0	0	0	0
10P	B723444	15	5	0	9251	1,1	0,0	0,0	0,0	0,0	0
10P	B723448	0	26	4	8403	1	0	1	0	0	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
10P	B723449	0	31	4	9252	0	0	1	0	0	0
10P	B723452	18	2	7	9253	0,0	1,1	2,1	2,0	0,1	0
10P	B723455	0	20	17	9254	0	0	1	0	1	0
10P	B723457	5	17	26	8404	2	0	1	1	0	0
10P	B723459	46	1	40	9255	0,2	1,2	0,0	1,1	1,2	0
10P	B723460	24	24	32	8395	2,1	0,0	2,1	0,0	0,0	0
10P	B723463	10	46	39	8396	1,1,1	0,0	2,1	1,1	0,0	1
10P	B723465	0	65	2	8397	1,1	0,0	1	0,1	1,0	1
10P	B723466	2	6	35	9256	0,0	0,0	1,0	1,0	0,0	0
10P	B723467	7	37	19	9257	0	0	0	0	1	0
10P	B723468	6	24	11	8398	1,1	0,0	2,2,1	0,0	0,0	1
10P	B723471	4	4	62	9258	0,2	0,0	1,0	2,1	0,0	0
10P	B723472	2	18	22	8399	1,0	0,0	1,1	0,0	0,0	2
10P	B723473	5	25	14	9259	0,0	0,0	0,1	2,0	0,0	1
10P	B723474	4	23	2	8400	1	0	2	0	0	2
10P	B723475	3	24	1	8401	0	0	0	0	0	0
10P	B723477	8	3	15	9260	0	0	0	0	0	0
10P	B723482	3	23	14	8402	0	0	0	0	0	0
10P	B723483	6	7	16	9261	0	0	1	1	0	0
10P	B723484	19	4	20	9262	0	1	1	0	1	0
10P	B723485	44	4	15	9263	0	1	1	2	1	0

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
10P	B723486	1	34	7	9264	0	0	2	0	2	1
10P	B723487	4	27	24	8265	0	0	1	1	1	0
10P	B723493	0	4	19	9266	0	0	0	1	0	0
10P	B723801	14	16	16	9267	2	0	2	0	0	1
10P	B723802	4	79	7	9268	0	0	2	0	0	0
10P	B723807	5	62	48	9269	0	0	1	2	1	0
20P	B723816	37	8	14	11785	0	0	0	0	0	NR
20P	B723817	0	14	21	11786	0	0	0	0	1	NR
20P	B723821	9	34	1	11787	1	0	1	1	0	NR
20P	B723822	22	0	0	11788	0	0	0	0	0	NR
20P	B723826	59	65	9	11789	0	0	0	0	0	NR
20P	B723827	0	21	0	11790	0	0	1	0	0	NR
20P	B723828	35	28	38	11791	1,2	1,1	1,0	0,1	0,0	NR
20P	B723829	0	19	29	11792	0	0	1	0	1	NR
20P	B723830	0	6	40	11793	0	0	0	0	1	NR
20P	B723831	0	100	0	11794	0	0	0	0	0	NR
20P	B723833	37	92	78	11795	1,1	0,0	2,0	2,2	2,2	NR
20P	B723835	37	11	56	11796	2,2	2,2	0,0	0,2	0,1	NR
20P	B723838	0	0	39	11797	1	0	1	0	0	NR
20P	B723839	8	33	30	11798	1	0	0	0	0	NR
20P	B723840	0	36	13	11799	0	0	0	0	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
20P	B723842	4	49	9	11800	0	0	0	0	0	NR
20P	B723846	4	1	45	11801	0	0	0	0	0	NR
20P	B723849	8	17	7	11802	0	0	1	0	0	NR
20P	B723857	8	45	1	11803	0,1	0,0	1,1	0,0	1,2	NR
20P	B723863	3	48	32	11804	0,0	0,0	2,1	0,0	0,1	NR
20P	B723864	48	63	29	11805	2,2	2,2	0,0	0,0	0,1	NR
20P	B723865	17	73	1	11806	0,0	0,0	2,2	0,0	0,0	NR
20P	B723868	32	6	12	11807	1,1	1,0	1,1	0,0	2,1	NR
20P	B723871	46	3	9	11808	2,1	0,2	0,0	0,0	0,2	NR
20P	B723872	4	40	38	11809	0	0	1	0	1	NR
20P	B723873	0	34	24	11810	0	0	0	0	0	NR
20P	B723874	11	21	7	11811	0	0	0	0	0	NR
20P	B723875	32	0	27	11812	1,1	1,0	0,0	0,1	2,1	NR
20P	B723876	11	8	35	11813	0	0	1	0	1	NR
20P	B723877	0	35	36	11814	0,0	0,0	0,1	0,1	2,2	NR
20P	B723879	21	43	66	11815	1	0	1	0	0	NR
20P	B723881	81	17	22	11816	2,0	0,0	0,0	2,2	2,2	NR
20P	B723882	8	29	49	11817	0	0	0	0	1	NR
20P	B723883	31	32	33	11818	0	0	0	0	1	NR
20P	B723887	21	16	15	11819	1	0	0	0	0	NR
22P	B723888	59	4	21	11820	2,2	1,0	0 0	0,0	1,0	NR

Results of Prescreen Testing[a]					Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
22P	B723893	48	5	15	11821	2,2	1,1	0,0	0,1	1,0	NR
22P	B723898	3	19	35	11822	0,1	1,1	0,1	0,1	2,0	NR
22P	B723900	26	16	46	11823	1	0	0	0	1	NR
22P	B723903	28	0	30	11824	1,0	1,1	0,0	0,1	2,2	NR
22P	B723904	7	18	26	11825	1,1	0,0	0,1	1,2	2,1	NR
22P	B723908	10	34	57	11826	1	0	1	0	1	NR
22P	B723909	22	62	43	11827	1,0	2,2	2,2	0,2	3,2	NR
22P	B723910	2	46	53	11828	0,0	0,0	2,1	0,1	2,1	NR
22P	B723911	56	17	23	11829	1,1	1,0	0,0	0,0	3,2	NR
22P	B723912	71	26	12	11830	2,1	2,1	0,0	0	3,3	NR
22P	B723915	4	37	10	11831	0	0	1	0	1	NR
22P	B723917	71	21	20	11832	2,2	2,2	0,0	2,2	2,3	NR
22P	B723918	59	3	20	11833	2,2	1,1	0,0	1,1	0,0	NR
22P	B723922	0	72	10	11834	2,2	1,1	0,0	0,0	0,0	NR
22P	B723932	28	0	15	11835	0	0	0	0	0	NR
22P	B723933	8	0	42	11836	0	0	0	0	0	NR
22P	B723934	6	11	33	11837	1	0	0	0	0	NR
22P	B723937	3	42	62	11838	1,2	0,1	2,2	0,2	1,2	NR
22P	B723939	1	27	36	11839	1,1	1,1	2,2	1,1	1,1	NR
22P	B723940	1	19	29	11840	0	0	1	0	0	NR
22P	B723944	45	3	19	11841	0	0	0	1	0	NR

Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	[illegible]	SF	VV
		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
22P	B723946	5	43	42	11842	0,1	0,1	2,2	0,0	1,1	NR
22P	B723948	16	42	35	11843	0,0	0,0	2,1	1,0	0,1	NR
22P	B723949	48	0	56	11844	2,2	2,2	0,0	0,2	1,0	NR
22P	B723950	22	0	2	11845	0	0	0	1	0	NR
22P	B723951	0	28	11	11846	1	0	0	1	0	NR
22P	B723954	50	1	67	11847	2,2	2,2	0,0	0,2	0,0	NR
22P	B723957	64	100	92	11848	2,2	0,2	2,2	1,2	2,1	NR
22P	B723958	42	18	63	11849	2,2	1,0	1,0	0,2	0,0	NR
22P	B723959	4	25	12	11850	0	0	1	0	0	NR
22P	B723960	2	24	33	11851	0	0	0	0	0	NR
22P	B723962	3	21	5	11852	1	0	1	0	0	NR
22P	B723963	9	47	4	11853	0	0	0	0	0	NR
22P	B723964	7	31	6	11854	1,1	1,1	2,2	0,1	1,0	NR
22P	B723965	5	64	9	11855	0	0	0	0	0	NR
22P	B723970	9	71	18	11856	0	0	0	0	2	NR
22P	B723972	36	0	20	11857	1,1	1,1	1,0	0,2	2,0	NR
22P	B723973	3	9	47	11858	0	0	0	0	1	NR
22P	B723975	2	41	42	11859	0	0	1	0	0	NR
22P	B723977	17	1	42	11860	1	0	0	0	2	NR
22P	B723979	5	1	43	11861	1	0	0	1	1	NR
22P	B723980	0	24	27	11862	0	0	0	0	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
22P	B723983	3	30	21	11863	0	0	1	0	0	NR
22P	B723985	12	48	3	11864	0	0	2	0	0	NR
22P	B723989	0	2	65	11865	1	0	0	0	2	NR
22P	B723992	0	35	32	11866	0,0	0,0	2,0	0,1	2,1	NR
22P	B723993	40	1	6	11867	1	0	1	0	0	NR
22P	B723994	4	27	28	11868	0	0	1	0	0	NR
22P	B723995	6	21	19	11869	0	0	1	0	0	NR
22P	B723996	0	42	27	11870	0	0	1	0	0	NR
22P	B723997	3	48	9	11871	0	0	0	0	0	NR
22P	B723998	12	8	53	11872	0	0	0	0	0	NR
22P	B723999	8	22	13	11873	0	0	2	0	1	NR
22P	B724001	1	49	62	11874	0	0	1	0	2	NR
22P	B724004	8	8	54	11875	0	0	0	1	1	NR
22P	B724005	3	35	11	11876	1,1	0,0	1,2	0,2	2,1	NR
22P	B724009	2	20	37	11877	0	0	0	0	1	NR
22P	B724010	7	66	29	11878	0	1	1	0	2	NR
22P	B724011	1	26	15	11879	1	0	1	0	1	NR
22P	B724012	15	41	1	11880	1	0	1	0	1	NR
22P	B724015	11	72	35	11881	1	0	2	UT	1	NR
22P	B724019	4	26	5	11882	0	0	0	0	1	NR
22P	B724021	11	27	28	11883	0	0	0	0	1	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
22P	B724025	36	52	37	11884	2,2	0,0	0,1	0,2	2,1	NR
22P	B724029	9	39	15	11885	0,1	0,0	1,2	0,0	2,2	NR
22P	B724030	2	33	1	11886	0	0	0	0	0	NR
22P	B724033	10	19	0	11887	0	0	1	0	0	NR
22P	B724035	1	25	10	11888	0	0	0	0	0	NR
22P	B724040	7	53	1	11889	2,0	1,2	0,0	0,0	0,0	NR
22P	B724042	22	0	0	11890	0	0	0	1	2	NR
22P	B724045	5	10	51	11891	0	1	0	0	0	NR
22P	B724046	0	28	3	11892	0	0	0	0	0	NR
19P	B724055	6	2	33	11589	1	0	0	0	0	NR
19P	B724058	11	10	37	11590	0	0	0	0	0	NR
19P	B724060	18	1	34	11591	0	0	0	0	1	NR
19P	B724062	75	27	18	11592	1	1	1	0	0	NR
19P	B724063	77	10	11	11593	1	1	1	0	0	NR
19P	B724067	24	2	4	11594	0	0	0	0	1	NR
19P	B724069	0	17	4	11595	0	0	0	0	0	NR
19P	B724070	0	33	4	11596	1	0	0	0	1	NR
19P	B724073	45	1	40	11597	2	2	0	0	2	NR
19P	B724075	6	3	39	11598	0	0	0	0	1	NR
19P	B724076	0	1	34	11599	0	0	0	0	0	NR
19P	B724078	0	53	24	11600	0	0	1	0	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
19P	B724081	0	13	40	11601	0	0	0	0	1	NR
19P	B724086	23	0	12	11602	0	0	0	0	0	NR
19P	B724087	1	40	22	11603	1	0	1	0	0	NR
19P	B724091	45	0	6	11604	1	2	0	0	1	NR
19P	B724093	0	20	11	11605	0	0	1	0	0	NR
19P	B724100	22	1	27	11606	0	1	0	0	0	NR
19P	B724108	32	4	53	11607	1	0	0	1	1	NR
19P	B724109	20	0	0	11608	0	0	0	0	0	NR
19P	B724110	17	0	46	11609	0	0	0	0	0	NR
19P	B724111	28	100	12	11610	0	0	2	0	1	NR
19P	B724112	19	16	0	11611	1	0	2	0	0	NR
19P	B724113	17	81	58	11612	0	0	2	0	0	NR
19P	B724114	7	96	3	11613	0	0	2	0	1	NR
19P	B724124	48	15	14	11614	0	1	0	1	1	NR
19P	B724127	28	2	7	11615	0	0	2	0	0	NR
19P	B724130	65	0	8	11616	2	0	0	0	1	NR
19P	B724131	14	0	55	11617	1	1	0	0	1	NR
19P	B724135	2	21	14	11618	0	0	2	0	0	NR
19P	B724136	0	0	50	11619	0	1	0	0	0	NR
19P	B724137	57	0	2	11620	0	1	1	0	1	NR
19P	B724139	0	0	34	11621	2	2	0	0	1	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
19P	B724140	67	1	60	11622	2	2	0	0	1	NR
19P	B724141	0	9	27	11623	0	0	2	0	1	NR
19P	B724143	8	9	43	11624	0	0	0	0	0	NR
19P	B724145	52	1	12	11625	0	0	1	0	2	NR
19P	B724148	16	23	26	11626	0	0	1	0	0	NR
19P	B724151	1	0	46	11627	0	0	0	0	0	NR
19P	B724155	0	0	37	11628	1	0	0	0	0	NR
19P	B724163	29	17	0	11629	0	0	0	0	1	NR
19P	B724164	9	0	20	11630	0	0	2	0	0	NR
19P	B724165	5	26	18	11631	0	0	1	0	0	NR
19P	B724170	4	29	23	11632	0	0	1	0	0	NR
19P	B724172	0	1	20	11633	0	0	0	0	0	NR
19P	B724173	0	16	0	11634	0	0	0	0	0	NR
19P	B724181	4	61	32	11635	0	0	2	0	2	NR
19P	B724182	0	12	24	11636	0	0	0	0	0	NR
19P	B724184	2	48	36	11637	0	1	1	0	2	NR
19P	B724186	38	2	20	11638	0	0	0	0	4	NR
19P	B724187	41	1	61	11639	1	2	0	0	3	NR
19P	B724192	4	14	34	11640	1	0	0	0	0	NR
19P	B724194	5	37	19	11641	0	0	0	0	0	NR
19P	B724198	0	28	15	11642	0	0	0	0	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
19P	B724199	7	21	41	11643	0	0	0	0	0	NR
19P	B724201	0	14	30	11644	0	0	0	0	0	NR
19P	B724202	0	20	55	11645	0	0	0	0	1	NR
19P	B724203	0	22	26	11646	0	0	1	0	0	NR
19P	B724205	2	17	15	11647	0	0	0	0	1	NR
19P	B724207	11	18	20	11648	0	0	0	0	0	NR
19P	B724209	1	21	29	11649	0	0	0	0	0	NR
19P	B724210	0	64	57	11650	0	0	2	0	2	NR
19P	B724212	6	32	18	11651	0	0	0	0	0	NR
19P	B724214	23	35	31	11652	0	0	0	0	0	NR
19P	B724215	48	24	1	11653	0	0	0	0	0	NR
19P	B724216	5	0	35	11654	0	0	0	0	0	NR
19P	B724217	0	32	11	11655	0	0	1	0	0	NR
19P	B724219	11	88	50	11656	0	0	2	0	1	NR
19P	B724220	28	21	4	11657	1	0	0	0	0	NR
19P	B724221	0	54	13	11658	0	0	2	0	0	NR
19P	B724223	14	26	0	11659	0	0	1	0	0	NR
19P	B724224	6	59	16	11660	0	0	1	0	0	NR
19P	B724225	16	32	5	11661	0	0	1	0	0	NR
19P	B724226	23	0	24	11662	0	0	0	0	0	NR
19P	B724227	0	17	33	11663	0	0	1	0	0	NR

Results of Prescreen Testing[a]					Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
19P	B724228	0	24	6	11664	0	0	0	0	0	NR
19P	B724230	77	81	100	11665	2	0	0	0	0	NR
19P	B724231	0	30	2	11666	0	0	1	0	0	NR
19P	B724232	4	36	4	11667	0	0	0	0	0	NR
19P	B724233	0	28	0	11668	0	0	1	0	0	NR
19P	B724238	22	1	4	11718	0	0	0	0	0	NR
19P	B724240	0	35	82	11719	2,0	0,0	2,0	1,0	2,0	NR
19P	B724242	0	33	22	11720	0,0	0,0	2,0	0,0	0,0	NR
19P	B724243	20	47	2	11721	0	0	0	0	1	NR
19P	B724244	0	6	54	11722	2,0	2,2	0,0	0,0	0,2	NR
19P	B724247	1	4	38	11723	0	0	0	0	0	NR
19P	B724248	26	10	0	11724	0	0	1	0	0	NR
19P	B724249	0	62	21	11725	0	0	1	0	0	NR
20P	B724251	63	18	2	11726	0	0	1	0	1	NR
20P	B724252	0	100	44	11727	0	0	2	0	0	NR
20P	B724253	0	40	31	11728	0	0	1	0	0	NR
20P	B724254	10	47	20	11729	0	0	1	0	0	NR
20P	B724256	23	0	2	11730	0	0	0	0	0	NR
20P	B724257	0	62	3	11731	0,0	0,0	2,0	0,0	0,0	NR
20P	B724260	0	24	6	11732	1	0	1	0	0	NR
20P	B724261	20	17	2	11733	0	0	0	0	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
20P	B724266	14	29	100	11734	1,0	0,0	2,0	0,2	1,2	NR
20P	B724268	100	1	55	11735	2,2	0,2	1,0	0,2	2,2	NR
20P	B724270	25	6	15	11736	0	0	0	0	0	NR
20P	B724271	73	10	2	11737	0,0	0,0	2,2	1,0	1,0	NR
20P	B724275	50	7	63	11738	1,1	0,0	2,0	2,2	2,2	NR
20P	B724279	45	15	2	11739	0,0	0,0	0,0	0,0	2,1	NR
20P	B724280	7	3	48	11740	2,1	1,0	0,0	0,1	2,2	NR
20P	B724281	36	3	9	11741	0	0	1	0	0	NR
20P	B724283	6	13	37	11742	1	0	0	0	0	NR
20P	B724287	87	4	7	11743	2,2	0,0	1,0	0,0	0,0	NR
20P	B724288	3	18	15	11744	0	0	1	0	0	NR
20P	B724289	6	17	64	11745	2,1	0,0	0,0	0,2	2,2	NR
20P	B724290	32	9	19	11746	1	0	1	0	0	NR
20P	B724291	0	17	80	11747	0	0	1	0	1	NR
20P	B724293	0	2	46	11748	2,1	0,0	1,0	1,2	0,1	NR
20P	B724294	37	5	6	11749	0	0	0	0	0	NR
20P	B724296	0	19	45	11750	0	0	0	0	0	NR
20P	B724297	29	11	4	11751	0	0	0	0	0	NR
20P	B724298	63	10	9	11752	1,2	0	0	0	0	NR
20P	B724299	60	12	11	11753	2	0,1	0,0	0,0	0,0	NR
20P	B724300	42	18	19	11754	1	0	1	0	0	NR

Results of Prescreen Testing[a]					Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
20P	B724301	14	0	62	11755	1	1	1	0	0	NR
20P	B724303	7	12	44	11756	2,1	0,0	0,0	0,1	0,1	NR
20P	B724308	45	10	37	11757	0	0	0	0	0	NR
20P	B724310	10	0	38	11758	0	0	0	0	0	NR
20P	B724312	37	10	0	11759	0	0	0	0	0	NR
20P	B724313	51	0	27	11760	0	1	0	0	0	NR
20P	B724316	28	0	5	11761	1	0	1	0	0	NR
20P	B724320	0	22	7	11762	0	0	1	0	1	NR
20P	B724323	25	4	55	11763	1	0	0	0	1	NR
20P	B724324	23	38	2	11764	0	0	0	0	0	NR
20P	B724327	22	21	17	11765	0	0	1	0	0	NR
20P	B724335	1	34	38	11766	1	0	1	0	0	NR
20P	B724337	0	19	40	11767	0	0	0	0	0	NR
20P	B724338	68	4	5	11768	0,0	1,1	2,0	1,0	2,3	NR
20P	B724339	0	32	1	11769	0,0	0,0	2,1	0,0	0,0	NR
20P	B724340	0	50	81	11770	1	0	1	0	1	NR
20P	B724348	0	1	37	11771	0	0	0	0	0	NR
20P	B724354	61	6	36	11772	2,2	1,1	1,1	0,1	0,0	NR
20P	B724355	21	0	2	11773	1	1	1	0	0	NR
20P	B724356	24	0	46	11774	1	1	1	0	0	NR
20P	B724358	82	2	0	11775	2,1	0,0	0,1	0,2	0,0	NR

Results of Prescreen Testing[a]					Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
20P	B724361	4	17	15	11776	0	0	1	0	0	NR
20P	B724363	0	36	2	11777	0	0	0	0	0	NR
20P	B724365	0	32	3	11778	1	0	0	0	1	NR
20P	B724366	0	52	26	11779	1	0	1	0	0	NR
20P	B724367	0	37	3	11780	0,0	0,0	2,1	0,0	0,0	NR
20P	B724368	0	44	0	11781	0,0	0,0	2,0	0,0	0,0	NR
1P	B724373	2	50	100	6630	0	0	0	0	0	0
1P	B724379	19	–	66	6631	0	0	0	0	0	0
1P	B724382	15	–	70	6632	2,0	0,0	0,0	0	0,0	0
1P	B724384	8	–	70	6633	1,0	0,0	0,0	0	0,0	0
1P	B724385	6	–	53	6634	0	0	0	0	0	0
1P	B724387	20	–	65	6635	0	0	0	0	0	0
1P	B724394	9	–	60	6636	1,0	0,0	0,0	0	0,0	0
1P	B724396	9	–	51	6637	0	0	0	0	0	0
1P	B724405	4	–	66	6638	0	0	0	0	0	0
1P	B724406	3	–	20	6639	0,1	0,0	0,0	0,0	1,0	0
1P	B724411	1	–	50	6640	0	0	0	0	0	0
1P	B724413	5	–	74	6641	0	0	0	0	0	0
1P	B724415	11	–	72	6642	0	0	0	0	0	0
1P	B724416	1	–	75	6643	1,0	0,0	0,0	1,0	0,0	0
1P	B724417	0	–	84	6644	1,0	0,0	0,0	0,0	1,0	0

Results of Prescreen Testing[a]					Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
1P	B724418	5	–	80	6645	1,0	0,0	0,0	0,0	0,0	0
1P	B724420	0	–	0	6646	1,0	0,0	0,0	1,0	0,0	0
1P	B724423	25	–	77	6647	0	0	0	0	0	0
1P	B724433	7	–	77	6648	1,0	0,0	0,0	0,0	0,0	0
1P	B724434	9	–	61	6649	0	0	0	0	0	0
1P	B724436	28	–	55	6650	0	0	0	0	0	0
1P	B724439	0	–	56	6651	1,0	0,0	0,0	0,0	0,0	0
1P	B724442	59	–	64	6652	0	0	0	0	0	0
1P	B724447	10	–	64	6653	1,0	0,0	0,0	1,0	1,0	0
1P	B724453	7	–	67	6654	1,0	0,0	0,0	0,0	1,0	0
1P	B724455	12	–	78	6655	1,0	0,0	0,1	0,0	0,0	0
1P	B724456	30	–	96	6656	0,0	0,0	0,0	1,1	1,0	0
1P	B724457	17	–	86	6657	1,0	0,0	0,0	1,1	0,0	0
1P	B724458	12	–	88	6658	0	0	0	0	0	0
1P	B724466	75	–	73	6659	0	0	2,0	0	1	0
1P	B724468	50	–	36	6660	0,0	0,0	1,1	0,1	0,0	1
1P	B724508	9	–	65	6661	0	0	0	0	0	0
1P	B724509	30	–	52	6662	0	0	0	0	0	0
1P	B724512	11	–	62	6663	0	0	0	1	0	0
1P	B724517	23	–	57	6664	1	0	0	0	0	0
1P	B724519	19	–	76	6665	0	0	0	0	0	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
IP	B724521	4	–	97	6666	0	0	0	0	0	0
IP	B724525	20	–	61	6667	0	0	0	1	0	0
IP	B724526	37	–	66	6668	1	0	0	0	0	0
IP	B724527	0	–	76	6669	0	0	0	0	0	0
IP	B724530	60	–	51	6670	0	0	0	0	0	0
IP	B724535	12	–	57	6671	0	0	0	0	0	0
IP	B724544	16	–	63	6672	0	0	0	0	0	0
IP	B724546	14	–	42	6249	0,0	0,0	0,0	2,1	0,0	NR
IP	B724549	0	–	50	6673	0	0	0	0	0	0
IP	B724553	44	–	75	6674	0	0	0	0	0	0
IP	B724558	1	–	59	6675	1,1	0,1	1,1	1,0	2,0	1
IP	B724566	25	–	85	6676	1,1	0,0	0,0	0,1	0,0	0
IP	B724586	0	–	76	6677	0	0	0	1	0	0
IP	B724590	2	–	63	6678	0	0	0	0	0	0
IP	B724592	81	–	56	6679	1	1	0	0	0	0
IP	B724596	11	–	51	6680	0	0	0	1	0	NR
IP	B724607	0	–	70	6681	1	0	0	1	0	0
IP	B724610	76	–	21	6682	0	0	0	1	0	0
IP	B724618	88	–	24	6683	2	1	0	0	0	0
IP	B724627	18	–	68	6684	0	0	0	0	0	0
IP	B724633	3	–	54	6685	0	0	0	0	0	0

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
1P	B724642	5	–	62	6686	0	0	0	0	0	0
1P	B724644	12	–	61	6687	0,0	0,0	0,0	0,0	0,0	0
1P	B724652	13	–	64	6688	0	0	0	0	0	0
1P	B724654	54	–	63	6689	0	0	0	0	0	0
1P	B724657	56	–	68	6690	0	0	0	0	0	0
1P	B724661	15	–	60	6691	0	0	0	0	0	0
1P	B724664	32	–	72	6692	0	0	0	0	0	0
1P	B724667	16	–	55	6693	0	0	0	0	0	0
1P	B724670	29	–	60	6249	1,1	0,0	0,0	1,1	0,0	NR
1P	B724697	40	–	72	6251	0,0	0,0	0,0	1,0	0,0	NR
1P	B724698	29	–	75	6252	0	0	0	1	0	NR
1P	B724701	80	–	33	6253	0,0	0,0	0,0	0,0	0,0	NR
1P	B724712	13	–	79	6254	1	0	0	0	0	NR
1P	B724714	15	–	61	6255	0,0	0,0	0,0	1,0	0,0	NR
1P	B724716	22	–	61	6256	2,1	1,1	0,0	1,0	0,0	NR
1P	B724719	17	–	70	6257	0	0	0	0	0	NR
1P	B724720	5	–	62	6258	1,1	0,0	0,0	1,0	0,0	NR
1P	B724722	21	–	83	6259	0	0	0	0	0	NR
1P	B724724	14	–	54	6694	0	0	0	0	0	0
1P	B724728	22	–	76	6695	0	0	0	0	0	0
1P	B724729	28	–	71	6696	0	0	0	0	0	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
1P	B724731	60	–	41	6260	0	0	0	0	0	NR
1P	B724732	0	–	51	6697	1,0	0,0	1,0	0,0	0,0	1
1P	B724740	47	–	85	6261	0,0	0,0	1,0	0,0	0,0	NR
1P	B724762	7	–	79	6262	1	0	0	0	0	NR
1P	B724764	28	–	82	6263	1	0	0	0	0	NR
1P	B724769	4	–	63	6264	0	0	0	0	0	NR
1P	B724772	58	–	0	6698	0	0	0	0	0	0
1P	B724781	13	–	84	6265	1,1	0,0	0,0	0,0	0,0	NR
1P	B724783	17	–	79	6699	0,0	0,0	1,0	0,0	0,0	0
1P	B724785	8	–	60	6700	0	0	0	0	0	0
1P	B724797	0	–	52	6266	0	0	0	0	0	NR
1P	B724812	55	–	33	6701	0	0	0	0	0	NR
1P	B724819	3	–	5	6267	0	0	0	0	0	NR
1P	B724820	24	–	90	6268	1,1	1,0	0,0	0,0	0,0	NR
1P	B724825	38	–	74	6269	1	0	0	0	0	NR
1P	B724832	4	–	61	6702	1,0	0,0	0,0	0,0	0,0	0
1P	B724844	71	–	32	6270	0	0	0	0	0	NR
1P	B724847	26	–	56	6271	0	0	0	1	0	NR
1P	B724852	60	–	36	6272	0	0	0	0	0	NR
1P	B724853	4	–	55	6273	0	0	0	0	0	NR
1P	B724855	10	–	69	6703	0	0	0	0	0	0

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
1P	B724860	1	–	61	6274	1,1	0,0	0,0	0,0	0,0	NR
1P	B724863	59	–	64	6275	1	0	0	0	0	NR
1P	B724866	16	–	58	6704	0	0	0	0	0	0
1P	B724871	0	–	51	6276	0	0	0	0	0	NR
1P	B724885	24	–	65	6705	0	0	0	0	0	0
1P	B724886	25	–	61	6277	0	0	0	0	0	NR
1P	B724898	88	–	39	6706	1	0	0	0	0	0
4P	B724983	28	–	8	7411	0	0	0	0	0	0
4P	B724984	21	–	0	7412	0	0	0	0	0	0
4P	B724990	12	–	6	7413	0	0	0	0	0	1,2
16P	B725006	32	29	3	11323	1	0	0	0	0	NR
16P	B725007	32	23	0	11324	0	0	1	0	0	NR
16P	B725008	40	5	6	11325	0	0	1	0	0	NR
27P	B725014-K110	7	0	16	11981	0	0	0	0	0	NR
27P	B725024	12	1	27	11982	0	0	0	0	0	NR
7P	B848622	3	1	29	8254	1	0	0	0	0	0
7P	B848628	0	2	23	8255	1	0	0	0	1	0
11P	B848631	1,5	1,1	13,4	9270	0	0	1	2	0	0
11P	B848633	0	5	22	9271	0	0	0	2	0	0
11P	B848634	7	1	13	9272	0	0	0	0	0	0
11P	B848635	0	0	17	9273	0	0	0	0	0	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
11P	B848649	6	40	19	9274	1	0	6	0	1	2
11P	B848650	4	8	13	9275	0	0	0	0	0	0
11P	B848653	0,41	9,79	61,76	9276	0	1	2	2	2	0
11P	B848654	0,9	2,9	22,21	9277	0	0	0	0	0	0
11P	B848656	0,5	1,9	11,28	9278	0	0	1	0	0	0
11P	B848657	0	0	15	9279	0	0	0	0	0	0
11P	B848659	0	1	15	9280	0	0	0	0	0	0
11P	B848660	0	2	11	9281	0	0	0	0	0	0
11P	B848662	0	1	26	9282	0	0	0	0	0	0
11P	B848663	0	2	18	9283	0	0	0	0	0	0
11P	B848669	0,3	2,2	82,36	9284	0	0	0	0	1	3,3
11P	B848670	0,8	3,10	19,19	9285	0	0	0	0	0	0
11P	B848671	1,7	1,2	4,51	9286	0	0	0	0	0	0
11P	B848673	0	4	21	9287	0	0	0	0	0	0
11P	B848676	0	1	27	9288	0	0	0	0	0	0
11P	B848678	0	3	16	9289	1	0	0	0	0	0
11P	B848679	0	1	15	9290	1	0	0	0	0	0
11P	B848681	0	2	22	9291	0	0	0	0	0	0
11P	B848691	2,7	0,0	2,61	9292	1	0	0	0	0	0
11P	B848699	0,18	0,9	35,11	9293	1	0	1	0	0	1
11P	B848700	5	1	39	9294	0	0	1	0	0	0

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
11P	B848703	5	2	25	9295	0	0	0	0	0	0
11P	B848706	0	2	30	9296	0	0	1	0	0	0
11P	B848709	4	18	22	9297	0	0	1	0	0	0
11P	B848711	0	12	23	9298	0	1	2	0	0	0
11P	B848716	1	1	26	9299	0	0	0	0	0	1
11P	B848717	0	3	36	9300	0	0	1	0	0	0
11P	B848720	1	4	24	9301	1	0	1	1	0	0
11P	B848722	3,2	1,1	3,11	9302	1	0	0	1	0	0
11P	B848724	2	0	31	9303	2	1	0	1	0	0
11P	B848725	5,5	1,1	3,23	9304	0	1	0	1	0	0
11P	B848727	2,19	3,73	5,18	9305	1	0	1	1	0	0
11P	B848728	0,0	1,1	3,18	9306	1	0	0	1	0	0
11P	B848729	0	1	35	9307	1	0	0	1	0	0
11P	B848730	6,0	4,6	1,4	9308	0	0	1	1	0	0
11P	B848731	4,0	5,6	4,6	9309	0	0	0	1	0	0
11P	B848732	0	5	34	9310	1	0	1	1	0	0
11P	B848733	0,0	1,0	8,12	9311	0	0	0	1	0	0
11P	B848734	0,0	1,0	3,23	9312	0	0	0	0	0	0
11P	B848735	3,1	0,0	11,16	9313	0	0	0	1	0	0
11P	B848736	2,1	6,18	9,19	9314	0	0	1	1	0	0
11P	B848737	6,5	0,1	1,8	9315	1	0	0	1	0	0

Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
11P	B848738	1,0	1,3	9,26	9316	1	0	0	1	0	0
11P	B848739	1	14	35	9317	2	0	1	2	0	0
11P	B848740	5,0	2,9	7,26	9318	1	0	1	1	0	0
11P	B848741	8	6	36	9319	1	0	0	2	1	0
11P	B848742	3,1	0,3	0,5	9320	1	0	0	0	1	0
11P	B848745	0	2	25	9321	0	0	1	0	0	0
11P	B848747	0,0	4,9	43,46	9322	1	1	1	2	0	0
11P	B848748	0	4	35	9323	0	0	0	1	2	0
11P	B848749	0	1	42	9324	1	0	0	2	0	0
11P	B848750	0	5	88	9325	0	0	0	2	0	0
11P	B848751	0	4	23	9326	0	0	0	0	0	0
11P	B848752	5,50	2,22	74,86	9327	1	0	1	2	2	0
11P	B848753	0,0	9,18	22,54	9328	0	0	0	0	2	0
11P	B848755	3	3	20	9329	1	0	0	0	0	0
11P	B848757	0,0	0,0	8,17	9330	0	0	0	0	0	0
11P	B848767	4	1	28	9331	0	0	0	0	0	0
11P	B848771	7,5	2,0	0,9	9332	0	0	0	0	1	0
11P	B848774	0,0	1,1	0,4	9333	0	0	0	0	0	0
11P	B848782	1	1	17	9334	0	0	0	0	0	0
11P	B848788	1	3	18	9335	0	0	0	0	0	0
11P	B848790	3	1	27	9336	0	0	0	0	0	0

Shipment No.	Prescreen No.	Results of Prescreen Testing[a]			AVS No.	Results of Primary Screen Testing[b]					
		YF	VE	PT		YF	JE	VE	PT	SF	VV
11P	B848792	0	1	31	9337	0	0	0	1	1	0
11P	B848793	0,0	4,21	5,41	9338	0	0	0	0	2	0
11P	B848794	16	7	39	9339	0	0	1	2	0	0
11P	B848795	41,0	0,6	0,43	9340	0	0	0	0	0	0,0
11P	B848796	22,0	0,27	0,7	9341	0	0	0	0	1	0
11P	B848797	0,11	2,40	21,16	9342	0	0	1	2	1	0
11P	B848798	0,4	1,6	0,17	9343	0	0	0	0	0	0
11P	B848800	24	3	2	9344	0	0	1	0	0	0
11P	B848801	5	21	9	9345	0	0	0	0	0	0
11P	B848804	0,0	0,12	0,66	9346	0	0	0	0	0	0
11P	B848805	0,0	1,9	4,16	9347	0	0	0	0	0	0
11P	B848808	0,0	1,14	1,2	9348	0	0	1	0	0	0
11P	B848809	9,17	3,44	0,17	9349	0	0	1	0	0	0
11P	B848810	0,0	2,12	1,19	9350	1	0	2	1	0	0
11P	B848811	0,1	1,42	3,55	9351	1	0	1	0	0	0
11P	B848814	0,20	0,32	2,40	9352	1	0	1	0	0	0
11P	B848816	0	1	25	9353	0	0	1	0	0	0
9P	B848820	2	11	7	9354	0	0	0	1	2	0
11P	B848838	0	10	32	9355	0	0	0	0	0	0
11P	B848839	0,5	4,21	3,43	9356	1	0	1	0	0	0
11P	B848841	0,0	8,25	8,55	9357	1	0	1	0	0	1

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
11P	B848843	2,0	2,12	0,27	9358	1	0	1	0	0	0
11P	B848845	0,0	2,24	19,21	9359	1	0	1	0	0	0
11P	B848848	1,0	1,10	0,26	9360	0	0	2	0	0	0
11P	B848861	0	2	25	9361	1	0	0	0	0	0
12P	B848864	3	25	23	9362	1	0	1	0	1	1
12P	B848866	0	2	2	9363	0	0	1	0	0	0
12P	B848869	6	20	15	9364	2	0	0	0	2	0
12P	B848870	5	32	32	9365	1	0	1	0	1	0
12P	B848873	5	2	34	9366	0	0	2	0	0	0
12P	B848874	3	0	39	9367	0	0	0	0	0	1
12P	B848876	0	1	23	9368	0	0	0	0	0	0
12P	B848879	4	9	40	9369	0	0	0	0	0	1
12P	B848880	2	1	56	9370	1	0	1	0	0	1
12P	B848881	0	0	53	9371	1	0	0	0	1	1
12P	B848882	0	0	57	9372	1	0	1	0	0	1
12P	B848883	0	2	45	9373	1	0	0	0	0	1
12P	B848892	0	12	4	9374	1	0	1	0	0	1
12P	B848893	0	15	28	9375	0	0	1	0	0	1
12P	B848895	1	16	23	9376	0	0	1	1	0	0
12P	B848896	1	9	35	9377	0	0	0	1	0	0
12P	B848897	0	12	30	9378	0	0	1	1	0	0

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
12P	B848899	10	4	29	9379	0	0	1	1	0	0
12P	B848900	0	6	48	9380	0	0	0	1	0	0
12P	B848901	0	7	45	9381	0	0	2	1	0	2
12P	B848903	0	6	47	9382	0	0	0	1	0	0
12P	B848904	1	11	44	9383	0	0	0	1	0	0
12P	B848905	18	8	19	9384	0	0	1	1	0	0
12P	B848906	0	12	35	9385	0	0	0	1	1	0
12P	B848907	0	30	52	9386	0	0	0	1	0	0
12P	B848909	4	6	31	9387	0	0	1	1	0	0
12P	B848911	0	54	79	9388	1	0	1	2	1	1
12P	B848913	0	3	44	9389	0	0	2	2	0	1
12P	B848914	19	0	23	9390	0	0	0	0	0	0
12P	B848916	18	0	7	9391	1	0	0	2	1	0
12P	B848917	22	0	0	9392	0	0	0	0	1	0
12P	B848920	1	5	31	9393	0	0	1	2	1	1
12P	B848921	1	3	48	9394	0	0	3	2	3	0
12P	B848924	13	1	3	9395	1	0	0	0	0	0
12P	B848926	1	2	56	9396	1	0	0	2	0	0
7P	B848985	0	1	47	8256	0	0	0	1	0	0
7P	B848986	9	3	58	8257	1	0	0	1	0	0
7P	B848989	0	0	64	8258	0	0	1	1	0	0

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
7P	B848990	0	1	53	8259	1,1	0,0	0,0	2,2	0,0	0
7P	B848991	0	1	40	8260	1,1	0,0	1,1	2,2	0,0	0
7P	B848992	12	3	44	8261	2,2	0,0	1,1	1,2	1,0	0
6P	B849022	13	18	58	8233	1,0	0,0	0,0	2,2	0,0	0
6P	B849035	16	9	60	8234	1,1	0,0	1,1	2,2	0,0	0
6P	B849037	11	6	51	8235	0	0	1	1	0	0
6P	B849177	4	57	30	8209	0	0	0,0	0,0	0,0	0
6P	B849179	8	7	64	8236	1	0	0	2	0	0
6P	B849180	0	8	56	8237	1,2	0,0	1,0	2,2	0,1	0
22P	B849188	20	22	9	11893	0	0	0	0	0	NR
22P	B849190	0	5	42	11894	0	0	0	0	0	NR
22P	B849192	34	61	78	11895	2,0	2,3	0,1	0,2	2,2	NR
22P	B849195	14	24	10	11896	0	0	0	0	0	NR
22P	B849196	46	27	13	11897	2,0	2,1	0,1	0,1	2,1	NR
22P	B849197	0	46	11	11898	0	0	0	0	0	NR
22P	B849199	4	1	67	11899	0	0	0	0	0	NR
22P	B849200	0	36	2	11900	0	0	1	0	0	NR
22P	B849201	1	21	34	11901	0	0	0	0	0	NR
22P	B849202	0	38	4	11902	0	0	0	0	0	NR
22P	B849204	6	41	5	11903	0	0	0	0	0	NR
22P	B849206	10	37	30	11904	0	0	2	0	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
22P	B849208	0	21	48	11983	0	0	0	0	0	NR
20P	B849216	15	34	9	11782	2,2	0,0	0,1	0,2	2,1	NR
20P	B849219	0	33	29	11783	0	0	1	0	0	NR
20P	B849220	1	21	1	11784	0	0	1	0	0	NR
10P	B849239	9	21	48	9397	0	0	0	0	0	0
10P	B849240	0	40	0	9398	0	0	1	0	0	1
10P	B849259	27	9	18	8387	1	0	0	0	0	0
17P	B849268	1	2	67	11387	0	0	0	0	0	NR
17P	B849269	1	23	15	11388	0	0	0	0	0	NR
17P	B849271	3	39	98	11389	0	0	1	0	1	NR
17P	B849272	3	24	61	11390	0	0	1	0	0	NR
17P	B849275	0	4	38	11391	0	0	1	0	0	NR
17P	B849277	0	0	27	11392	0	0	0	0	0	NR
17P	B849278	0	10	21	11393	0	0	0	0	0	NR
17P	B849280	0	1	26	11394	0	0	1	0	0	NR
17P	B849281	0	1	30	11395	0	0	2	0	0	NR
17P	B849283	15	5	34	11396	0	0	0	0	0	NR
17P	B849284	0	8	55	11397	0	0	0	0	0	NR
17P	B849285	0	2	20	11398	0	0	0	0	0	NR
17P	B849289	0	1	51	11399	0	0	0	0	1	NR
17P	B849292	2	2	46	11400	0	0	2	0	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
17P	B849295	0	20	8	11401	0	0	1	0	0	NR
17P	B849297	19	28	83	11402	0	0	0	0	0	NR
17P	B849298	27	31	54	11403	2	2	1	0	1	NR
17P	B849299	2	10	52	11404	0	0	0	0	0	NR
17P	B849300	15	20	15	11405	0	0	1	0	1	NR
17P	B849302	0	34	22	11406	0	0	1	0	0	NR
17P	B849307	13	33	29	11407	0	0	1	1	2	NR
17P	B849308	3	35	18	11408	0	0	0	0	0	NR
24P	B849324	14	21	20	11984	0	0	0	0	0	NR
24P	B849337	32	5	0	11985	0	0	0	0	0	NR
27P	B849342	47	67	27	11986	3	0	0	0	1	NR
27P	B849346	11	19	35	11987	0	0	0	0	0	NR
21P	D-N181116-I	7	3	0	9911	0	0	0	0	0	NR
21P	D-N181117-J	14	5	10	9912	0	0	0	0	0	NR
21P	D-N181118-K	8	3	0	9913	1	0	0	1	0	NR
21P	D-N181119-L	29	0	1	9914	0	0	0	0	0	NR
21P	D-N181121-N	10	0	0	9915	0	1	0	0	1	NR
21P	D-N181122-O	100	20	100	9916	2,2	3,1	1,0	2,2	3,2	NR
21P	D-N181123-P	19	17	16	9917	0,0	1,0	2,0	1,0	0,0	NR
21P	D-N181124-Q	18	0	18	9918	0	0	0	1	0	NR
21P	D-N181125-R	10	0	1	9919	0	0	0	0	0	NR

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
21P	D-N181126-S	20	17	18	9920	0	0	0	0	0	NR
21P	D-N181127-T	33	23	5	9921	0	0	0	0	0	NR
21P	D-N181128-U	28	13	7	9922	0	1	0	0	0	NR
21P	D-N181129-V	6	0	3	9923	0	0	0	0	0	NR
21P	D-N181130-W	9	3	4	9924	0	0	0	1	0	NR
21P	D-N181131-X	5	0	5	9925	0	0	0	0	0	NR
21P	D-N181132-Y	14	11	2	9926	1	0	0	0	0	NR
21P	D-N181133-Z	14	1	2	9927	0	0	0	0	0	NR
21P	D-N181134-A	11	0	2	9928	0	0	0	1	0	NR
21P	D-N181135-C	6	0	0	9929	0	0	0	0	0	NR
21P	D-N181136-F	10	3	4	9930	1,0	0,0	0,0	1,0	2,0	NR
21P	D-N181137-G	8	19	25	9931	2,0	0,0	0,0	2,0	0,0	NR
21P	D-N181138-H	15	10	38	9932	0	0	0	1	0	NR
21P	D-N181139-I	11	2	2	9933	0	0	0	1	0	NR
21P	D-N181140-J	8	0	3	9934	1	0	0	1	1	NR
21P	D-N181141-K	2	4	18	9935	1	0	0	2	2	NR
21P	D-N181142-L	14	20	5	9936	0	0	0	0	0	NR
21P	D-N181143-M	40	5	86	9937	2,0	1,0	0,0	2,0	2,1	NR
21P	D-N181144-N	2	2	9	9938	0	0	0	1	0	NR
21P	D-N181145-O	0	1	8	9939	0	0	0	1	0	NR
21P	D-N181146-P	0	6	6	9940	0	0	0	1	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
21P	D-N181147-Q	1	7	15	9941	1	0	0	0	0	NR
21P	D-N181148-R	4	0	1	9942	0	0	0	1	0	NR
21P	D-N181149-S	2	2	5	9943	0	0	0	1	0	NR
21P	D-N181150-T	5	0	18	9944	1,1	0,0	0,0	1,1	0,0	NR
21P	D-N181151-U	0	0	22	9945	1	0	0	0	0	NR
21P	D-N181152-V	7	9	16	9946	1,0	0,0	0,0	1,0	0,0	NR
21P	D-N181153-W	3	0	1	9947	1	0	0	1	0	NR
21P	D-N181154-X	0	26	12	9948	0	0	1	1	0	NR
21P	D-N181155-Y	0	12	21	9949	1	0	1	1	0	NR
21P	D-N181156-Z	0	22	6	9950	0	0	0	1	0	NR
21P	D-N181157-A	1	8	7	9951	0	0	0	0	0	NR
21P	D-N181160-G	0	27	5	9952	0	0	1	0	0	NR
21P	D-N181161-H	4	5	11	9953	0	0	0	0	0	NR
21P	D-N181162-I	12	35	3	9954	1,0	1,0	0,0	1,0	2,0	NR
21P	D-N181163-J	17	8	0	9955	0	1	0	0	0	NR
21P	D-N181164-K	5	23	1	9956	0	0	0	0	0	NR
21P	D-N181165-L	7	4	22	9957	0	0	0	0	1	NR
21P	D-N181166-M	4	47	32	9958	0	1	1	1	0	NR
21P	D-N181167-N	14	15	7	9959	0	1	0	1	0	NR
21P	D-N181168-O	0	1	11	9960	0	0	0	0	0	NR
21P	D-N181169-P	7	13	27	9961	0	1	0	1	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
21P	D-N181170-Q	35	4	74	9962	2,2	0,0	0,0	2,2	2,0	NR
21P	D-N181171-R	3	19	8	9963	0	0	0	0	0	NR
21P	D-N181172-S	2	74	28	9964	0	0	0	0	0	NR
21P	D-N181173-T	0	2	2	9965	0	0	0	0	0	NR
21P	D-N181174-U	0	0	25	9966	0	0	0	0	0	NR
21P	D-N181175-V	5	16	47	9967	0,0	1,0	0,0	1,0	1,0	NR
21P	D-N181176-W	8	9	12	9968	0,0	0,0	0,0	1,0	0,0	NR
21P	D-N181177-X	18	8	36	9969	0,0	0,0	0,3	2,0	2,3	NR
21P	D-N181178-Y	4	60	67	9970	3,1	2,2	2,1	3,3	3,3	NR
21P	D-N181179-Z	17	21	19	9971	1,0	0,0	0,0	2,0	2,1	NR
21P	D-N181180-A	7	41	29	9972	0	0	0	0	0	NR
21P	D-N181181-C	5	49	35	9973	0	0	0	0	0	NR
21P	D-N181182-F	14	15	67	9974	0,0	0,0	0,0	2,0	0,0	NR
21P	D-N181183-G	0	1	32	9975	0	0	0	0	0	NR
21P	D-N181184-H	2	33	42	9976	0	0	0	0	0	NR
21P	D-N181185-I	7	24	16	9977	0	1	0	0	0	NR
21P	D-N181186-J	10	5	20	9978	1	0	0	0	0	NR
21P	D-N181187-K	3	4	1	9979	0	0	0	1	0	NR
21P	D-N181190-N	19	1	3	9980	0	0	0	0	0	NR
21P	D-N181191-O	9	1	0	9981	2	0	0	2	1	NR
21P	D-N181192-P	6	2	3	9982	0	0	0	1	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
21P	D-N181195-S	8	5	19	9983	1	0	0	1	0	NR
21P	D-N181196-T	4	24	17	9984	0	0	0	1	0	NR
21P	D-N181197-U	6	4	19	9985	0	0	0	1	0	NR
21P	D-N181198-V	0	29	46	9986	0	0	1	1	0	NR
21P	D-N181384-X	22	2	13	9987	0	0	0	0	0	NR
21P	D-N181385-Y	NT	NT	NT	9988	NT	NT	NT	NT	NT	NR
21P	D-N181386-Z	NT	NT	NT	9989	NT	NT	NT	NT	NT	NR
21P	D-N181387-A	27	27	56	9990	2,0	2,0	0,0	2,2	1,1	NR
21P	D-N181388-C	10	18	14	9991	0	0	0	0	0	NR
21P	D-N181389-F	3	1	45	9992	0,0	0,0	0,0	2,1	0,0	NR
21P	D-N181390-G	NT	NT	NT	9993	NT	NT	NT	NT	NT	NR
21P	D-N181391-H	2	4	4	9994	0	0	0	0	0	NR
21P	D-N181392-I	10	1.	25	9995	0	0	0	0	0	NR
21P	D-N181393-J	3	1	14	9996	0	0	0	0	0	NR
21P	D-N181394-K	9	10	30	9997	0	0	0	0	0	NR
21P	D-N181395-L	18	2	9	9998	0,2	1,1	0,0	0,0	1,0	NR
21P	D-N181396-M	2	1	0	9999	0	0	0	0	0	NR
21P	D-N181397-N	2	1	22	10000	1	0	1	1	0	NR
21P	D-N181398-O	2	36	46	10001	0,0	0,0	0,0	2,0	0,0	NR
21P	D-N181399-P	2	11	58	10002	2,0	0,0	0,0	1,0	1,0	NR
21P	D-N181400-Q	0	2	15	10003	0	0	0	0	0	NR

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
21P	D-N181401-R	5	0	3	10004	0	0	0	0	0	NR
21P	D-N181402-S	2	0	60	10005	0	0	0	0	0	NR
21P	D-N181403-T	0	0	32	10006	0	0	0	2	0	NR
21P	D-N181404-U	7	27	42	10007	0	0	0	1	0	NR
21P	D-N181405-V	0	0	23	10008	0	0	0	0	0	NR
21P	D-N181406-W	2	29	0	10009	0	0	0	0	0	NR
21P	D-N181407-X	3	15	15	10010	0	0	1	0	0	NR
21P	D-N181408-Y	0	10	5	10011	2,0	0,0	1,1	1,0	2,3	NR
21P	D-N181409-Z	2	1	32	10012	0	0	0	0	0	NR
21P	D-N181410-A	0	1	8	10013	0	0	0	0	0	NR
21P	D-N181411-C	6	4	23	10014	0	0	0	0	0	NR
21P	D-N181412-F	1	1	11	10015	0	0	0	0	2	NR
21P	D-N181413-G	14	6	6	10016	0	0	0	0	0	NR
21P	D-N181485-J	1	1	10	10017	0	0	0	0	0	NR
21P	D-N181486-K	1	7	11	10018	0	0	0	0	0	NR
21P	D-N181487-L	2	1	0	10019	0	0	0	0	0	NR
21P	D-N181488-M	2	5	22	10020	0	0	0	0	0	NR
21P	D-N181489-N	0	1	1	10021	0	0	0	0	0	NR
21P	D-N181490-O	8	4	16	10022	1,0	0,0	0,0	0,0	1,0	NR
21P	D-N181491-P	4	20	58	10023	0	0	0	1	0	NR
21P	D-N181492-Q	2	3	12	10024	0	0	0	0	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
21P	D-N181493-R	4	4	12	10025	0	0	1	0	0	NR
21P	D-N181494-S	0	6	56	10026	0	0	0	1	0	NR
21P	D-N181495-T	12	39	38	10027	0	0	1	1	0	NR
21P	D-N181496-U	10	2	7	10028	0	0	0	0	0	NR
21P	D-N181497-V	0	0	33	10029	0	0	0	1	1	NR
21P	D-N181498-W	44	15	4	10030	3,2	2,2	1,0	3,0	3,2	NR
21P	D-N181499-X	17	52	74	10031	3,0	2,0	0,2	3,1	3,1	NR
21P	D-N181500-Y	5	4	0	10032	0	0	0	0	0	NR
21P	D-N181501-Z	0	1	4	10033	0	0	0	0	0	NR
21P	D-N181502-A	0	1	41	10034	0	0	0	0	1	NR
21P	D-N181503-C	13	2	0	10035	0	0	0	0	0	NR
21P	D-N181504-F	0	1	0	10036	1	0	0	0	0	NR
21P	D-N181505-G	3	4	0	10037	0	0	0	0	1	NR
21P	D-N181506-H	1	2	0	10038	0	0	0	0	0	NR
21P	D-N181507-I	2	1	29	10039	0	0	0	0	0	NR
21P	D-N181508-J	0	20	0	10040	0	0	0	0	0	NR
21P	D-N181509-K	6	7	0	10041	0	0	0	1	0	NR
21P	D-N181510-L	19	7	0	10042	0	0	0	0	0	NR
21P	D-N181511-M	1	2	0	10043	0	0	0	0	0	NR
21P	D-N181512-N	7	6	11	10044	0	0	0	0	0	NR
21P	D-N181513-O	5	13	15	10045	1	0	0	0	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
21P	D-NI81514-P	2	1	14	10046	0	0	0	0	0	NR
21P	D-NI81515-Q	9	1	63	10047	2,0	0,0	0,0	2,0	2,2	NR
21P	D-NI81516-R	0	1	23	10048	0	0	0	0	1	NR
21P	D-NI81517-S	15	13	9	10049	1	0	0	0	1	NR
14P	GRP18058	0	1	29	11124	0,0	0	0	0,0	0	NR
14P	GRP18060	0	1	34	11125	0,0	0	0,1	0,0	0	NR
14P	GRP18061	0	4	34	11126	0,0	0	0	0,0	0	NR
14P	GRP18062	32	1	0	11127	0,0	0	0	0,0	0	NR
14P	GRP18063	3	17	48	11128	0,0	0	0	0,0	0	NR
14P	GRP18066	84	5	3	11129	0,0	0	0	0,3	0	NR
14P	GRP18068	20	3	1	11130	0,0	0	0	0,0	0	NR
4P	GRP19380	78	–	16	7319	2,0	2,0	0,0	1,0	0,0	NR
4P	GRP19381	35	–	15	4280	2,2,2	3,2,1	0,0,0	0,1,2	0,0,1	0
4P	GRP19386	3	–	77	7320	1,1	0,0	1,0	1,2	2,1	1
4P	GRP19396	61	–	9	7321	2,0	2,0	0,0	0,0	1,0	0
4P	GRP19416	9	–	7	7414	0,1	0,0	0,0	2,1	0,0	1
4P	GRP19418	0	–	28	7415	0,0	0,0	0,0	2,0	1,0	0
4P	GRP19422	8	–	31	7416	0,1	0,0	0,1	1,1	1,0	0
4P	GRP19423	3	–	14	7417	0,0	0,0	0,0	2,0	1,0	0
4P	GRP19424	3	–	27	7418	0,0	0,0	0,0	3,0	3,2	2
4P	GRP19425	0	–	13	7419	0	0	0	0	1	2

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
4P	GRP19427	0	–	12	7420	0	0	0	0	0	0
4P	GRP19432	0	–	10	7421	0	0	0	1	0	0
4P	GRP19433	0	–	18	7422	0	0	0	0	0	0
4P	GRP19434	15	–	14	7423	0	0	0	0	1	0
4P	GRP19435	6	–	25	7424	0,2	0,0	0,0	2,2	1,0	2
4P	GRP19437	20	–	5	7425	0	0	0	0	0	0
4P	GRP19438	0	–	14	7426	0	0	0	0	0	0
4P	GRP19439	23	–	6	7427	1,0	0,0	0,0	0,1	2,1	1
4P	GRP19440	15	–	6	7428	1,1	0,0	0,0	0,0	0,1	0
4P	GRP19441	11	–	27	7429	0,0	0,0	0,0	0,1	2,0	0
4P	GRP19442	20	–	20	7430	1,0	0,0	0,0	0,2	3,1	0
4P	GRP19446	7	–	9	7431	0	0	0	0	1	0
4P	GRP19447	9	–	20	7432	0	0	0	0	1	0
4P	GRP19448	6	–	8	7433	2,2	2,2	0,0	0,0	0,0	0
4P	GRP19449	5	–	6	7434	0,2	0,0	0,0	1,2	2,2	0
4P	GRP19450	–	–	8	7435	0,1	0,0	0,0	0,1	1,1	0
16P	GRP19457	10	23	32	11131	2,1	1,0	2,2	2,0	2,2	NR
16P	GRP19458	14	26	31	11132	1	0	0	1	0	NR
16P	GRP19459	13	8	83	11133	2,2	0,0	0,0	3,0	2,0	NR
16P	GRP19462	0	30	72	11134	0,1	0,0	1,1	0,3	3,3	NR
16P	GRP19463	11	3	52	11135	1	0	0	2	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
16P	GRP19464	13	19	17	11136	0	0	1	0	0	NR
16P	GRP19465	0	19	16	11137	1	0	0	0	1	NR
16P	GRP19467	27	1	5	11138	0	0	1	1	1	NR
16P	GRP19469	7	5	48	11139	1,0	0,0	1,1	2,0	1,0	NR
16P	GRP19470	7	15	23	11140	0,1	0,1	1,1	2,0	1,1	NR
16P	GRP19471	0	15	41	11141	0	0	0	0	1	NR
16P	GRP19472	0	2	46	11142	0	0	0	0	0	NR
16P	GRP19473	42	27	23	11143	1	0	1	0	0	NR
16P	GRP19474	1	12	35	11144	1,0	0,1	2,2	2,0	1,0	NR
16P	GRP19479	21	13	2	11145	0	0	0	0	0	NR
16P	GRP19480	2	12	39	11146	0	0	0	0	0	NR
16P	GRP19481	4	30	46	11147	0	0	0	0	1	NR
16P	GRP19481	–	–	–	11148	–	–	–	–	–	–
16P	GRP19486	0	17	35	11149	1	0	1	0	0	NR
16P	GRP19490	0	63	52	11150	0,0	0,0	2,1	2,0	1,0	NR
16P	GRP19491	0	49	45	11151	0	0	1	0	0	NR
16P	GRP19492	0	2	50	11152	0	0	0	1	0	NR
16P	GRP19493	0	2	28	11153	1	1	1	0	0	NR
16P	GRP19501	1	21	31	11154	0	0	0	1	0	NR
16P	GRP19502	27	41	28	11155	0,2	1,3	2,1	2,1	0,1	NR
16P	GRP19509	0	2	35	11156	0	0	1	1	0	NR

		Results of P escreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
16P	GRP19510	7	8	33	11157	0	0	1	0	0	NR
16P	GRP19511	0	12	57	11158	0	0	1	0	0	NR
16P	GRP19512	2	4	29	11159	0,0	0,0	0,0	0,0	0,0	NR
16P	GRP19514	15	1	12	11160	0	0	1	0	0	NR
17P	GRP19516	3	2	68	11326	0	0	1	0	1	NR
17P	GRP19521	0	3	38	11327	0	0	1	0	2	NR
17P	GRP19522	4	5	33	11328	0	0	1	0	0	NR
17P	GRP19523	4	16	31	11329	0	1	2	0	0	NR
17P	GRP19525	4	22	6	11330	0	0	0	0	0	NR
17P	GRP19529	6	6	43	11331	0	0	2	1	0	NR
17P	GRP19533	3	2	32	11332	0	0	0	0	2	NR
17P	GRP19538	0	1	41	11333	0	0	1	0	1	NR
17P	GRP19539	3	8	79	11334	0	1	0	1	1	NR
17P	GRP19541	1	6	46	11335	0	0	0	0	1	NR
17P	GRP19544	1	3	46	11336	0	0	0	0	1	NR
17P	GRP19545	0	9	63	11337	0	0	0	0	0	NR
17P	GRP19550	0	0	31	11338	0	0	0	1	1	NR
17P	GRP19573	4	3	25	11339	0	0	0	0	0	NR
17P	GRP19574	0	2	30	11340	0	0	0	0	0	NR
17P	GRP19576	0	1	43	11341	0	0	0	0	0	NR
17P	GRP19578	11	10	32	11342	0	1	0	0	0	NR

		Results of Prescreen Testing[a]			Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
17P	GRP19579	0	9	48	11343	0	0	0	0	0	NR
17P	GRP19583	1	3	48	11344	2	1	0	1	2	NR
17P	GRP19584	0	23	0	11345	0	0	0	0	0	NR
17P	GRP19594	11	21	27	11346	1	0	0	0	1	NR
17P	GRP19598	0	23	16	11347	0	0	1	0	0	NR
17P	GRP19599	0	5	31	11348	0	0	0	0	0	NR
17P	GRP19601	0	2	58	11349	0	2	0	0	1	NR
17P	GRP19602	6	19	65	11350	0	0	0	0	1	NR
17P	GRP19603	1	2	30	11351	0	1	1	0	0	NR
17P	GRP19605	5	3	49	11352	1	0	0	0	0	NR
17P	GRP19608	4	4	27	11353	1	1	1	0	1	NR
17P	GRP19610	12	56	45	11354	0	0	1	0	0	NR
17P	GRP19611	1	3	46	11355	0	0	0	0	0	NR
17P	GRP19612	1	2	61	11356	0	1	0	1	1	NR
17P	GRP19619	6	1	31	11357	0	0	0	0	0	NR
17P	GRP19621	32	6	44	11358	0	1	0	0	1	NR
17P	GRP19623	0	22	15	11359	0	0	1	0	0	NR
17P	GRP19631	20	70	24	11360	0	0	2	0	0	NR
17P	GRP19634	17	50	17	11361	0	0	1	0	0	NR
17P	GRP19635	2	32	52	11362	0	0	0	1	0	NR
17P	GRP19636	1	27	26	11363	0	0	0	0	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
17P	GRP19640	2	34	45	11364	0	0	1	2	1	NR
17P	GRP19643	1	20	9	11365	0	0	0	0	0	NR
17P	GRP19647	24	54	25	11366	0	0	2	0	1	NR
17P	GRP19649	2	7	51	11367	1	0	0	0	0	NR
17P	GRP19653	0	12	23	11368	0	0	2	0	0	NR
17P	GRP19655	7	5	68	11369	0	0	1	0	0	NR
17P	GRP19657	0	11	42	11370	0	0	0	0	1	NR
17P	GRP19664	22	0	47	11371	1	0	0	2	0	NR
17P	GRP19670	9	37	41	11372	1	0	2	0	1	NR
17P	GRP19671	5	56	27	11373	0	0	0	0	1	NR
17P	GRP19672	0	28	5	11374	0	0	0	0	0	NR
17P	GRP19674	15	4	48	11375	0	0	0	3	1	NR
17P	GRP19675	40	12	61	11376	1	3	0	3	2	NR
17P	GRP19676	12	6	51	11377	0	0	0	0	1	NR
17P	GRP19679	1	15	35	11378	0	0	0	0	0	NR
17P	GRP19680	0	4	28	11379	0	0	1	0	0	NR
17P	GRP19681	4	18	39	11380	0	0	0	0	0	NR
17P	GRP19685	0	17	52	11381	0	0	0	0	0	NR
17P	GRP19686	0	81	40	11382	0	0	0	0	0	NR
17P	GRP19691	0	29	64	11383	0	0	2	1	2	NR
17P	GRP19696	27	13	14	11384	0	0	1	0	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
17P	GRP19700	1	14	62	11385	0	0	1	0	0	NR
17P	GRP19701	8	4	52	11386	0	0	1	0	1	NR
24P	GRP19743	10	74	97	11989	0	0	2	0	0	NR
24P	GRP19757	5	55	19	11990	0	0	2	0	0	NR
24P	GRP19778	7	19	79	11991	0	1	1	1	0	NR
24P	GRP19783	8	64	36	11992	0	0	0	0	0	NR
25P	GRP19798	13	19	78	11993	0	0	0	1	0	NR
25P	GRP19804	9	77	5	11994	0	2	1	0	0	NR
25P	GRP19806	10	70	3	11995	0	0	0	0	0	NR
25P	GRP19809	15	100	0	11996	0	1	0	0	0	NR
25P	GRP19816	9	72	0	11997	0	0	0	0	0	NR
25P	GRP19862	0	–	–	11998	0	0	0	0	0	NR
25P	GRP19873	0	–	–	11999	0	0	1	0	0	NR
25P	GRP19879	0	–	–	12000	1	0	0	0	0	NR
25P	GRP19881	0	–	–	12001	0	0	0	0	0	NR
25P	GRP19894	13	–	11	12002	0	0	1	0	1	NR
25P	GRP19896	8	–	42	12003	0	0	0	0	1	NR
25P	GRP19913	0	–	–	12004	2	0	0	0	1	NR
25P	GRP19914	4	–	–	12005	0	0	0	0	0	NR
25P	GRP19916	3	–	–	12006	0	0	0	0	0	NR
25P	GRP19920	0	–	–	12007	0	0	0	0	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	[illegible] No.	[illegible]	JE	VE	PT	SF	VV
25P	GRP19923	7	–	–	12008	0	0	[illegible]	[illegible]	[illegible]	NR
25P	GRP19925	0	–	–	12009	0	[illegible]	[illegible]	0	[illegible]	NR
25P	GRP19926	0	–	–	12010	0	0	[illegible]	0	0	NR
26P	GRP19955	0	55	52	12011	0	0	[illegible]	0	1	NR
26P	GRP19962	0	55	0	12012	0	0	[illegible]	0	0	NR
26P	GRP19967	0	54	1	12013	0	0	0	0	0	NR
26P	GRP19970	6	71	7	12014	[illegible]	0	1	0	0	NR
26P	GRP19971	3	41	37	12015	0	0	0	0	0	NR
26P	GRP19979	0	53	16	12016	0	0	1	0	0	NR
26P	GRP19982	0	54	24	12017	1	0	0	0	0	NR
26P	GRP19984	1	57	21	12018	0	0	0	0	0	NR
26P	GRP19985	0	62	52	12019	2	1	0	0	1	NR
26P	GRP19993	9	62	40	12020	0	0	0	0	1	NR
26P	GRP19994	1	84	19	12021	0	1	0	0	0	NR
4P	GRP21185	2	–	43	7436	0	0	0	0	0	0
4P	GRP21186	4	–	11	7437	0	0	0	0	0	0
16P	GRP21188	11	43	34	11161	0	0	0	1	0	NR
16P	GRP21189	13	0	32	11162	0	0	0	1	0	NR
16P	GRP21190	13	0	9	11163	0	0	0	0	0	NR
16P	GRP21191	14	0	21	11164	0	0	0	0	0	NR
16P	GRP21194	11	4	45	11165	0	0	0	0	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
16P	GRP21195	5	3	50	11166	1,1	0,0	0,0	2,1	0,0	NR
16P	GRP21197	1	30	47	11167	0	0	0	0	0	NR
16P	GRP21199	8	23	2	11168	0,1	0,0	2,0	1,1	1,1	NR
16P	GRP21201	11	0	20	11169	0	0	0	0	0	NR
16P	GRP21205	3	70	25	11170	0	0	1	0	1	NR
16P	GRP21207	30	24	47	11171	1	0	0	0	0	NR
16P	GRP21214	0	36	16	11172	0	0	1	0	0	NR
16P	GRP21220	3	20	24	11268	0	0	0	0	0	NR
16P	GRP21221	2	1	32	11269	0	0	0	0	0	NR
16P	GRP21222	1	2	56	11270	0	0	0	0	0	NR
16P	GRP21223	10	4	64	11271	0	0	0	0	0	NR
16P	GRP21224	2	29	45	11272	0	0	1	0	0	NR
16P	GRP21225	31	15	46	11273	1	0	0	0	1	NR
16P	GRP21226	27	42	47	11274	0	0	1	0	0	NR
16P	GRP21228	2	45	19	11275	0		1	0	0	NR
16P	GRP21229	6	5	42	11276	0	0	1	0	0	NR
16P	GRP21232	14	23	26	11277	0	1	0	0	0	NR
16P	GRP21233	2	34	54	11278	0	0	2	0	0	NR
16P	GRP21234	37	77	24	11279	1	1	3	0	1	NR
16P	GRP21236	22	29	31	11280	0	0	0	0	0	NR
16P	GRP21238	15	2	27	11281	0	0	0	0	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
16P	GRP21242	0	17	19	11282	0	0	1	0	0	NR
16P	GRP21247	24	12	30	11283	0	0	0	0	1	NR
16P	GRP21248	29	50	27	11284	1	0	1	0	0	NR
16P	GRP21251	20	11	19	11285	0	0	0	0	0	NR
16P	GRP21253	13	94	41	11286	0	0	2	0	1	NR
16P	GRP21255	39	66	23	11287	0	0	2	0	0	NR
16P	GRP21261	24	12	0	11288	0	0	1	0	0	NR
16P	GRP21263	15	3	49	11289	0	0	0	0	1	NR
16P	GRP21264	37	82	51	11290	1	1	2	0	1	NR
16P	GRP21266	6	9	36	11291	0	0	1	0	0	NR
16P	GRP21267	0	39	59	11292	0	0	1	0	0	NR
16P	GRP21268	0	2	46	11293	0	0	0	0	0	NR
16P	GRP21272	21	24	41	11294	0	0	0	0	1	NR
16P	GRP21275	22	3	24	11295	0	0	0	0	1	NR
16P	GRP21278	21	1	50	11296	0	0	2	0	0	NR
16P	GRP21281	0,39	0,'	13,2	11431	0	1	0	0	1	NR
16P	GRP21282	22	2	10	11297	0	0	0	0	0	NR
16P	GRP21285	0	1	33	11298	0	0	1	0	0	NR
16P	GRP21286	0	2	44	11299	0	0	0	0	0	NR
16P	GRP21287	15	0	62	11300	0	0	0	0	0	NR
16P	GRP21288	26	2	22	11301	1	0	0	0	0	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
16P	GRP21289	12	7	46	11302	0	1	2	1	2	NR
16P	GRP21291	40	2	69	11303	0	0	1	0	2	NR
16P	GRP21292	20	3	28	11304	0	0	1	0	0	NR
16P	GRP21293	10	0	34	11305	0	0	0	0	0	NR
16P	GRP21294	15	28	21	11306	0	0	2	0	2	NR
16P	GRP21297	2	0	25	11307	0	0	1	0	0	NR
16P	GRP21301	32	1	29	11308	0	1	2	0	1	NR
16P	GRP21303	44	25	0	11309	0	0	1	0	0	NR
16P	GRP21306	22	3	27	11310	0	1	1	0	0	NR
16P	GRP21307	1	4	27	11311	0	0	1	0	0	NR
16P	GRP21314	23	1	36	11312	0	0	0	0	0	NR
16P	GRP21315	47	2	36	11313	1	1	2	0	0	NR
16P	GRP21317	0	3	39	11314	0	0	1	0	0	NR
16P	GRP21320	12	10	52	11315	0	0	0	0	0	NR
16P	GRP21321	31	11	52	11316	0	0	1	0	1	NR
16P	GRP21323	19	5	27	11317	0	0	1	0	0	NR
16P	GRP21325	21	17	64	11318	1	0	1	0	1	NR
16P	GRP21326	8	19	11	11319	0	0	1	0	0	NR
16P	GRP21328	2	7	34	11320	0	0	1	0	0	NR
16P	GRP21329	4	6	27	11321	0	1	0	0	0	NR
16P	GRP21330	0	6	42	11322	0	0	0	0	1	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
25P	GRP21344	4	–	51	12022	2	0	0	0	2	NR
25P	GRP21351	10	–	12	12023	2	1	0	1	1	NR
25P	GRP21354	9	–	2	12024	2	2	2	1	2	NR
25P	GRP21366	45	–	8	12025	2	2	2	0	1	NR
25P	GRP21367	9	–	0	12026	1	0	2	0	0	NR
25P	GRP21376	54	–	4	12027	1	0	1	0	0	NR
25P	GRP21395	48	–	–	12028	2	0	0	0	0	NR
25P	GRP21400	13	–	–	12029	2	2	0	0	0	NR
28P	GRP21459	27	90	0	12030	2	4	2	2	2	NR
28P	GRP21478	10	65	33	12031	1	2	2	0	1	NR
28P	GRP21479	60	17	15	12032	1	0	0	0	0	NR
28P	GRP21503	12	89	33	12033	2	1	2	0	1	NR
28P	GRP21506	47	18	0	12034	2	0	0	0	0	NR
28P	GRP21511	67	19	81	12035	3	0	2	2	2	NR
28P	GRP21519	63	100	14	12036	2	1	0	0	0	NR
28P	GRP21526	35	0	0	12037	1	0	0	0	0	NR
28P	GRP21536	45	56	59	12038	1	1	0	1	1	NR
28P	GRP21550	65	34	89	12039	3	0	1	2	0	NR
28P	GRP21555	68	22	28	12040	2	1	1	0	0	NR
28P	GRP21558	28	0	0	12041	1	1	0	0	0	NR
28P	GRP21560	32	29	20	12042	0	0	0	0	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
28P	GRP21564	6	88	0	12043	0	0	2	0	1	NR
18P	GRP21668	60	4	25	11409	1	0	2	0	1	NR
18P	GRP21671	44	0	22	11410	0	0	0	0	0	NR
18P	GRP21680	38	47	50	11411	1	1	2	0	1	NR
18P	GRP21687	22	2	19	11412	0	0	1	0	0	NR
18P	GRP21689	1	19	57	11413	0	0	1	0	2	NR
18P	GRP21690	3	4	46	11414	0	0	0	0	0	NR
18P	GRP21691	0	22	21	11415	0	0	0	0	0	NR
18P	GRP21698	0	21	19	11416	0	0	1	0	0	NR
18P	GRP21703	38	10	15	11417	0	0	0	0	0	NR
18P	GRP21704	1	32	13	11418	1	0	2	0	0	NR
18P	GRP21705	24	23	63	11419	0	0	1	0	1	NR
18P	GRP21706	2	34	80	11420	1	0	2	0	2	NR
18P	GRP21708	0	39	72	11421	0	0	0	0	0	NR
23P	GRP21720	16	69	88	12044	2	2	2	0	2	NR
23P	GRP21737	3	53	52	12045	1	0	1	0	0	NR
23P	GRP21743	53	3	86	12046	3	3	2	0	0	NR
23P	GRP21757	0	76	8	12047	0	0	2	0	0	NR
23P	GRP21758	0	0	0	12048	0	0	1	0	0	NR
23P	GRP21790	47	87	17	12049	2	1	1	0	1	NR
23P	GRP21807	51	6	22	12050	0	0	0	0	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
27P	GRP21812	42	8	4	12051	1	0	0	0	0	NR
27P	GRP21821	57	80	26	12052	2	1	2	0	1	NR
27P	GRP21835	41	4	26	12053	2	0	2	0	0	NR
27P	GRP21840	59	41	33	12054	1	1	0	0	0	NR
27P	GRP21844	60	11	30	12055	2	0	1	0	0	NR
27P	GRP21846	27	73	47	12056	2	1	0	0	0	NR
27P	GRP21860	68	4	21	12057	1	1	0	0	0	NR
27P	GRP21870	43	3	8	12058	0	0	0	0	0	NR
27P	GRP21879	8	74	14	12059	2	1	0	0	0	NR
27P	GRP21883	37	2	16	12060	1	1	0	0	0	NR
27P	GRP21889	51	1	20	12061	0	0	0	0	0	NR
27P	GRP21895	40	0	21	12062	0	0	0	0	0	NR
27P	GRP21896	49	16	0	12063	2	2	0	0	0	NR
27P	GRP21906	68	7	10	12064	1	1	0	0	0	NR
26P	GRP23004	6	86	70	12065	0	0	1	0	1	NR
26P	GRP23010	5	38	49	12066	1	0	0	0	0	NR
26P	GRP23015	9	70	100	12067	1	2	2	0	1	NR
26P	GRP23020	9	59	0	12068	0	0	1	0	0	NR
26P	GRP23035	0	99	58	12069	1	2	1	0	0	NR
26P	GRP23039	2	85	57	12070	1	1	1	0	0	NR
26P	GRP23043	0	99	10	12071	0	0	0	0	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
28P	GRP23078	98	32	11	12072	2	2	0	0	0	NR
28P	GRP23079	97	36	13	12073	2	2	1	0	0	NR
18P	GRP23174	5	26	35	11422	0	0	1	0	1	NR
18P	GRP23175	2	47	48	11423	0	0	2	0	0	NR
18P	GRP23184	7	63	23	11424	0	0	2	0	0	NR
18P	GRP23185	0	29	40	11425	0	0	1	0	0	NR
18P	GRP23186	3	19	58	11426	0	0	1	0	0	NR
18P	GRP23188	0	9	40	11427	0	0	1	0	1	NR
18P	GRP23189	10	4	32	11428	0	1	0	0	0	NR
18P	GRP23190	20	32	100	11429	0	0	1	1	0	NR
18P	GRP23191	7	13	48	11430	0	0	1	0	0	NR
18P	GRP23192	26	5	25	11432	0	0	0	0	0	NR
18P	GRP23193	68	0	17	11433	0	0	0	UT	0	NR
18P	GRP23194	50	7	30	11434	1	1	0	UT	1	NR
18P	GRP23195	21	34	4	11435	0	0	0	UT	0	NR
18P	GRP23198	30	6	8	11436	0	0	0	UT	0	NR
18P	GRP23199	22	17	2	11437	0	0	0	UT	0	NR
18P	GRP23200	21	10	26	11438	0	0	1	UT	1	NR
18P	GRP23202	23	39	52	11439	0	0	0	UT	0	NR
18P	GRP23203	9	32	32	11440	0	0	0	UT	1	NR
18P	GRP23204	3	1	22	11441	0	0	0	UT	1	NR

	Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]						
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
18P	GRP23206	4	28	32	11442	0	0	1	UT	1	NR
18P	GRP23207	5	17	28	11443	0	0	0	UT	0	NR
18P	GRP23210	31	18	45	11444	0	0	0	UT	1	NR
18P	GRP23211	17	31	32	11445	0	0	1	UT	0	NR
18P	GRP23213	29	18	47	11446	0	0	0	UT	0	NR
18P	GRP23214	19,35	7	33	11447	0,2	0,0	0,2	UT,UT	2,1	NR
18P	GRP23216	3	19	32	11448	0	0	1	UT	0	NR
18P	GRP23218	2	51	26	11449	0	0	1	UT	0	NR
18P	GRP23221	78	0	24	11450	0	0	1	UT	0	NR
18P	GRP23222	59	1	78	11451	2	2	0	UT	3	NR
18P	GRP23225	3	18	30	11452	0	0	0	UT	0	NR
18P	GRP23226	14	2	79	11453	2	0	1	UT	2	NR
18P	GRP23227	17	2	79	11454	2	0	0	UT	2	NR
18P	GRP23229	0	21	36	11455	0	0	0	UT	1	NR
18P	GRP23230	19	39	21	11456	0	0	1	UT	1	NR
18P	GRP23233	17	34	62	11457	0	0	0	UT	1	NR
18P	GRP23234	0	39	8	11458	0	0	1	0	0	NR
18P	GRP23235	87	100	11	11459	2	0	0	0	2	NR
18P	GRP23236	0	77	11	11460	0	0	0	0	0	NR
18P	GRP23237	2	35	18	11461	0	0	0	0	0	NR
18P	GRP23238	4	35	66	11462	0	0	0	0	1	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
18P	GRP23240	12	35	39	11463	3	2	1	0	3	NR
18P	GRP23242	12	19	40	11464	0	0	0	0	0	NR
18P	GRP23243	22	77	41	11465	1	0	0	0	0	NR
18P	GRP23244	5	41	21	11466	0	0	1	0	0	NR
18P	GRP23245	82	37	54	11467	2	2	0	2	2	NR
18P	GRP23247	0	48	46	11468	0	0	0	0	1	NR
18P	GRP23248	21	58	18	11469	0	0	1	0	0	NR
18P	GRP23249	14	42	42	11470	0	0	0	0	1	NR
18P	GRP23250	8	53	26	11471	0	0	0	0	0	NR
18P	GRP23252	0	53	50	11472	2	0	1	0	2	NR
18P	GRP23253	20	43	11	11473	0	0	0	0	0	NR
18P	GRP23254	10	19	38	11474	0	0	0	0	0	NR
18P	GRP23255	29	70	33	11475	0	0	1	0	1	NR
18P	GRP23256	19	13	38	11476	0	0	0	0	0	NR
18P	GRP23257	0	3	37	11477	0	0	0	0	0	NR
18P	GRP23258	21	88	14	11478	0	0	1	0	0	NR
18P	GRP23260	24	9	4	11479	0	0	0	0	0	NR
18P	GRP23261	22	41	20	11480	0	0	0	0	0	NR
18P	GRP23262	24	37	11	11481	0	0	0	0	0	NR
18P	GRP23263	32	5	28	11482	0	0	0	0	0	NR
18P	GRP23264	32	14	0	11483	0	0	0	0	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
18P	GRP23265	11	65	68	11484	0	0	0	1	1	NR
18P	GRP23267	1	58	13	11485	1	0	1	1	1	NR
18P	GRP23269	13	39	12	11486	1	0	0	2	2	NR
18P	GRP23270	81	22	27	11487	0	0	1	2	1	NR
18P	GRP23271	1	53	29	11488	0	0	1	1	0	NR
18P	GRP23272	14	21	4	11489	1	2	2	0	2	NR
18P	GRP23274	0	15	72	11490	0	1	2	2	0	NR
18P	GRP23275	9	49	25	11491	0	0	1	1	0	NR
18P	GRP23276	27	6	2	11492	0	0	0	0	1	NR
18P	GRP23278	4	20	3	11493	0	0	0	0	0	NR
18P	GRP23279	0	21	27	11494	0	0	1	0	0	NR
18P	GRP23280	49	14	0	11495	1	2	1	2	2	NR
18P	GRP23282	6	29	47	11496	1	0	1	2	2	NR
18P	GRP23283	10	6	7	11497	0	0	0	1	0	NR
18P	GRP23284	26	25	100	11498	2	1	1	3	2	NR
18P	GRP23285	7	30	24	11499	0	0	1	0	0	NR
18P	GRP23286	17	1	47	11500	2	2	1	1	2	NR
18P	GRP23287	21	11	87	11501	0	0	1	3	1	NR
18P	GRP23288	5	39	25	11502	0	0	1	0	0	NR
18P	GRP23290	21	2	13	11503	0	0	0	0	0	NR
18P	GRP23291	6	20	18	11504	1	0	0	1	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
18P	GRP23292	11	22	33	11505	0	0	1	0	0	NR
18P	GRP23293	100	1	75	11506	3	3	3	3	3	NR
18P	GRP23306	4	67	14	11507	1	0	2	0	1	NR
18P	GRP23308	1	45	40	11508	0	0	0	UT	1	NR
18P	GRP23311	8	4	37	11509	1	0	0	NT	2	NR
18P	GRP23316	0	29	4	11510	0	0	0	NT	0	NR
18P	GRP23317	8	21	17	11511	0	0	0	UT	0	NR
18P	GRP23318	2	25	33	11512	0	0	1	UT	2	NR
18P	GRP23319	0	7	47	11513	0	1	1	UT	1	NR
18P	GRP23322	10	25	27	11514	0	0	0	UT	1	NR
18P	GRP23323	0	34	35	11515	0	0	0	NT	1	NR
18P	GRP23325	2	26	20	11516	0	0	1	UT	0	NR
18P	GRP23326	48	4	24	11517	1	0	0	UT	1	NR
18P	GRP23327	1	7	10	11518	0	1	0	UT	1	NR
18P	GRP23328	0	0	13	11519	0	0	0	UT	0	NR
18P	GRP23329	0	33	13	11520	0	0	1	UT	0	NR
18P	GRP23330	7	19	54	11521	0	0	0	UT	0	NR
18P	GRP23331	0	64	60	11522	0	0	0	UT	1	NR
18P	GRP23333	0	7	43	11523	0	0	0	UT	0	NR
18P	GRP23334	0	21	24	11524	0	0	0	UT	1	NR
18P	GRP23335	0	40	47	11525	0	0	0	UT	1	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
18P	GRP23337	6	14	46	11526	0	0	0	UT	1	NR
18P	GRP23338	0	39	70	11527	1	2	1	UT	1	NR
18P	GRP23339	30	4	53	11528	1	0	0	UT	2	NR
18P	GRP23342	0	20	19	11529	0	0	1	UT	0	NR
18P	GRP23343	0	38	32	11530	0	0	1	UT	1	NR
18P	GRP23344	0	40	76	11531	0	0	0	UT	0	NR
18P	GRP23346	26	4	41	11532	1	0	1	UT	1	NR
18P	GRP23347	0	8	33	11533	0	0	0	0	1	NR
18P	GRP23348	27	4	2	11534	0	0	0	0	0	NR
18P	GRP23349	28	7	36	11535	0	2	1	0	2	NR
18P	GRP23350	21	0	29	11536	1	2	2	0	1	NR
18P	GRP23353	8	13	5	11537	0	0	0	0	0	NR
18P	GRP23354	1	41	34	11538	0	0	0	0	0	NR
18P	GRP23355	13	8	41	11539	0	0	1	0	0	NR
18P	GRP23356	0	27	22	11540	0	0	1	0	0	NR
18P	GRP23357	6	33	20	11541	0	0	0	0	0	NR
18P	GRP23359	19	15	0	11542	0	0	0	0	2	NR
18P	GRP23361	0	45	13	11543	0	0	1	0	0	NR
18P	GRP23363	5	17	85	11544	1	0	0	0	1	NR
18P	GRP23364	17	11	5	11545	0	0	1	0	0	NR
18P	GRP23365	7	27	16	11546	0	0	0	0	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
18P	GRP23366	0	25	5	11547	0	0	1	0	0	NR
18P	GRP23369	3	31	0	11548	0	0	0	0	0	NR
18P	GRP23370	15	84	38	11549	1	1	1	0	1	NR
18P	GRP23371	19	73	29	11550	1	1	2	0	1	NR
18P	GRP23372	20	7	77	11551	1	0	0	1	2	NR
18P	GRP23374	0	71	14	11552	0	0	1	0	0	NR
18P	GRP23375	2	42	18	11553	0	0	1	0	1	NR
18P	GRP23376	59	0	63	11554	0	0	1	0	1	NR
18P	GRP23377	12	25	59	11555	2	1	0	0	1	NR
18P	GRP23378	0	10	31	11556	0	0	0	0	0	NR
18P	GRP23379	0	38	9	11557	0	0	0	0	0	NR
18P	GRP23380	2	8	77	11558	0	0	1	0	0	NR
18P	GRP23382	0	25	17	11559	0	0	0	0	0	NR
18P	GRP23383	0	67	16	11560	0	0	2	0	1	NR
18P	GRP23386	0	72	10	11561	1	0	1	0	0	NR
18P	GRP23387	1	25	16	11562	0	0	0	0	0	NR
18P	GRP23388	0	73	30	11563	0	0	1	0	1	NR
18P	GRP23389	0	23	13	11564	0	0	0	0	0	NR
18P	GRP23391	0	55	23	11565	0	0	0	0	2	NR
18P	GRP23392	13	28	29	11566	0	0	2	0	1	NR
18P	GRP23394	0	22	5	11567	0	0	0	0	0	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
18P	GRP23396	0	69	20	11568	0	0	2	0	1	NR
18P	GRP23397	11	63	18	11569	0	0	1	0	0	NR
18P	GRP23398	0	67	22	11570	0	0	2	0	0	NR
18P	GRP23400	8	55	34	11571	0	0	0	0	0	NR
18P	GRP23401	0	9	33	11572	0	0	1	0	0	NR
18P	GRP23402	9	49	5	11573	0	0	0	0	0	NR
18P	GRP23404	0	32	18	11574	0	0	2	0	1	NR
18P	GRP23405	0	0	45	11575	2	2	1	0	2	NR
18P	GRP23407	0	22	26	11576	0	0	0	0	0	NR
18P	GRP23408	55	33	68	11577	3	2	1	0	1	NR
18P	GRP23410	6	35	19	11578	0	0	1	0	0	NR
18P	GRP23412	52	20	60	11579	1	1	1	0	0	NR
18P	GRP23418	2	27	30	11580	3	2	1	0	0	NR
18P	GRP23426	6	11	27	11581	0	0	0	0	1	NR
18P	GRP23428	0	58	44	11582	0	0	2	0	2	NR
18P	GRP23429	2	34	90	11583	0	2	0	0	0	NR
18P	GRP23430	17	29	15	11584	1	2	1	0	1	NR
18P	GRP23433	0	19	9	11585	0	0	0	0	0	NR
18P	GRP23434	0	20	3	11586	0	0	0	0	0	NR
18P	GRP23437	1	13	48	11587	0	1	0	0	1	NR
18P	GRP23439	0	3	51	11588	0	0	0	0	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
23P	GRP23446	13	24	52	12074	0	0	0	0	0	NR
23P	GRP23459	6	12	57	12075	0	0	0	0	0	NR
23P	GRP23474	7	7	81	12076	0	0	0	0	0	NR
23P	GRP23482	10	37	81	12077	2	1	1	0	0	NR
23P	GRP23487	1	78	12	12078	0	0	1	0	0	NR
23P	GRP23507	20	50	25	12079	2	1	0	0	2	NR
23P	GRP23515	6	100	73	12080	0	0	0	0	0	NR
23P	GRP23519	6	29	77	12081	1	0	0	2	1	NR
23P	GRP23525	3	47	64	12082	1	0	1	0	0	NR
23P	GRP23528	0	66	41	12083	0	0	1	0	0	NR
23P	GRP23537	30	17	86	12084	3	1	0	3	2	NR
23P	GRP23538	1	62	73	12085	0	0	0	0	1	NR
23P	GRP23545	0	68	11	12086	0	0	0	0	0	NR
23P	GRP23549	11	65	57	12087	0	0	1	0	1	NR
24P	GRP23562	16	10	87	12088	3	0	0	3	3	NR
24P	GRP23563	0	93	6	12089	0	0	1	0,0	0	NR
24P	GRP23567	27	44	88	12090	2	0	0	0,0	1	NR
24P	GRP23569	30	40	82	12091	2	0	0	1	1	NR
24P	GRP23570	8	24	78	12092	2	1	0	2	1	NR
24P	GRP23589	62	59	78	12093	2	1	0	2	3	NR
24P	GRP23590	6	2	100	12094	0	0	1	2	3	NR

		Results of Prescreen Testing[a]				Results of Primary Screen Testing[b]					
Shipment No.	Prescreen No.	YF	VE	PT	AVS No.	YF	JE	VE	PT	SF	VV
24P	GRP23591	36	19	68	12095	2	1	0	0	0	NR
24P	GRP23602	5	68	79	12096	2	2	0	3	2	NR
24P	GRP23604	5	14	71	12097	0	0	0	0	1	NR
24P	GRP23607	30	14	96	12098	0	0	0	2	3	NR
24P	GRP23608	81	56	100	12099	3	3	0	3	2	NR
24P	GRP23624	16	6	53	12100	1	0	0	0	1	NR
24P	GRP23629	0	16	64	12101	0	0	1	0	1	NR
24P	GRP23632	9	65	100	12102	3	2	0	0	2	NR
24P	GRP23643	9	30	97	12103	2	2	0	2	2	NR
24P	GRP23648	0,0	23,10	37,0	12104	1	0	0	0	0	NR
26P	GRP23663	0	52	0	12105	0	0	1	0	0	NR
26P	GRP23675	2	72	7	12106	0	0	0	0	0	NR
26P	GRP23676	41	12	92	12107	3	2	0	3	2	NR
26P	GRP23708	0	21	46	12108	1	0	1	0	1	NR
26P	GRP23717	10	14	42	12109	0	0	0	0	1	NR
26P	GRP23722	1	49	82	12110	2	0	0	0	2	NR
27P	GRP23770	12	29	41	12111	0	0	0	0	0	NR
27P	GRP23777	0	56	0	12112	0	0	0	0	0	NR
27P	GRP23779	13	76	49	12113	0	0	1	2	0	NR
27P	GRP23800	8	96	3	12114	0	0	0	0	0	NR
27P	GRP23804	1	100	2	12115	1	0	0	0	0	NR

Shipment No.	Prescreen No.	Results of Prescreen Testing[a] YF	VE	PT	AVS No.	Results of Primary Screen Testing[b] YF	JE	VE	PT	SF	VV
27P	GRP23805	2	70	4	12116	0	0	0	0	0	NR
27P	GRP23810	0	100	42	12117	0	0	0	0	0	NR
27P	GRP23814	5	88	8	12118	0	0	1	0	0	NR
27P	GRP23816	67	45	100	12119	3	2	0	3	3	NR
27P	GRP23817	3	100	17	12120	1	2	2	2	1	NR
27P	GRP23854	2	61	10	12121	0	0	0	0	0	NR
27P	GRP23856	7	100	7	12122	0	0	1	0	0	NR
27P	GRP23860	4	66	13	12123	0	0	0	0	0	NR
27P	GRP23867	0	88	4	12124	3	3	0	0	3	NR
27P	GRP23885	15	59	6	12125	3	2	0	2	2	NR
27P	GRP23887	27	4	8	12126	0	0	0	0	0	NR
27P	GRP23906	14	74	59	12127	0	1	1	2	1	NR
27P	GRP23922	0	56	0	12128	0	0	1	0	0	NR
27P	GRP23925	3	42	53	12129	2	2	0	0	1	NR
27P	GRP23927	16	33	52	12130	2	2	0	1	1	NR
26P	GRP23931	11	24	80	12131	3	2	0	0	3	NR
27P	GRP23935	0	50	9	12132	1	0	0	0	0	NR
27P	GRP23950	36	7	28	12133	0	0	0	0	0	NR
27P	GRP23961	8	53	40	12134	0	0	1	0	0	NR

KEY FOR ABBREVIATIONS:

a	=	Maximum percent reduction in CPE observed in the assay.
b	=	Explanation for Abbreviations/Numbers (Each number represents one test):
3	=	Active at the 95% level (% reduction in viral CPE of ≥95% at one or more concentrations).
2	=	Active at the 50% level (% reduction in viral CPE of ≥50% to ≤94% at one or more concentrations).
1	=	Active at the 25% level (% reduction in viral CPE at ≥25% to ≤49% at one or more concentrations).
0	=	Inactive (<25% reduction in viral CPE at all concentrations).
NT	=	Not Tested.
NR	=	Not Required.
Note:		Symbols are separated by commas where more than one test was performed (i.e. "1,0" designates two tests, one was active at the 25% level and the second test was inactive).

4.3 Antiviral Screening *in Vitro*: DNA, Exotic RNA Viruses and Retroviruses (Primary Protocol):

During this reporting period from February 1, 1991 through December 31, 1991, a total of 2713 primary screen test compounds were received at SRI for evaluation in the *in vitro* antiviral screen (**Table 2-B**). The compounds were evaluated *in vitro* against Vaccinia Virus (VV), Yellow Fever Virus (YF), Japanese Encephalitis Virus (JE), Venezuelan Equine Encephalomyelitis Virus (VE), Punta Toro Virus (PT), Sandfly Virus (SF) and Human Immunodeficiency Virus (HIV) as they were received. A cumulative summary of the compound shipments received, and the number of compounds in each shipment, are presented in **Table 2-B**.

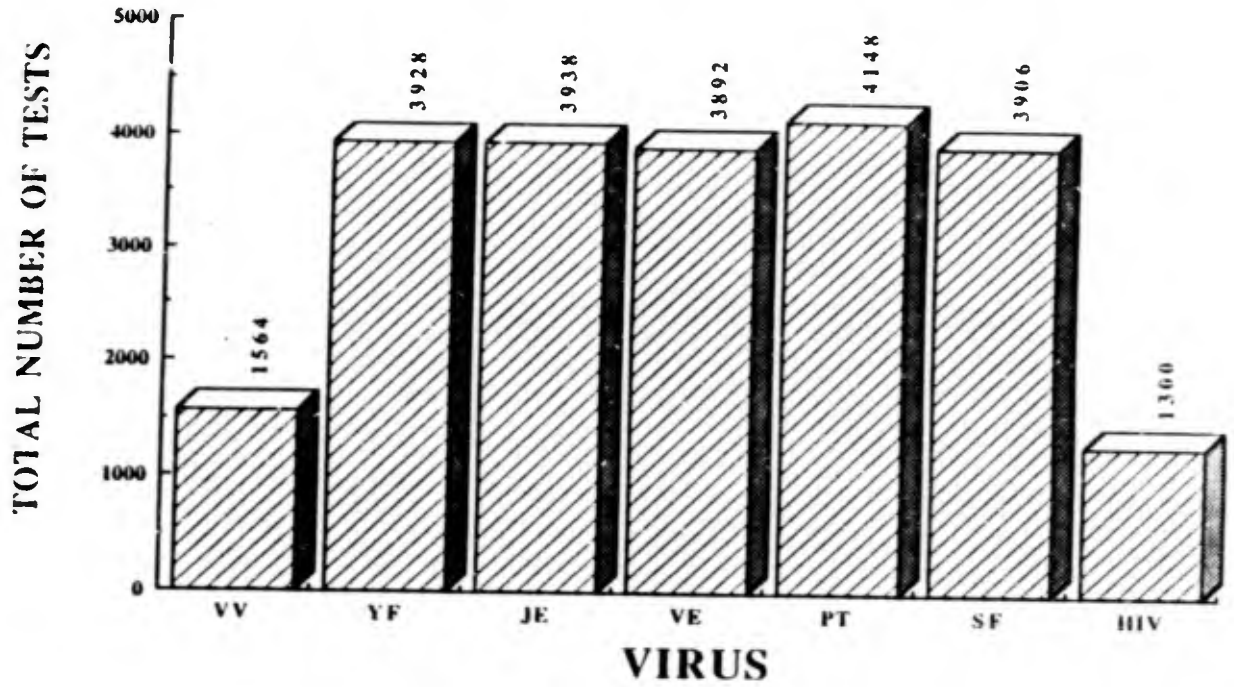
Approximately 21,376 *in vitro* antiviral assays were performed during this reporting period (**Figure 26**) against the DNA and exotic RNA viruses. Around 847 compounds out of the 15,965 single drug tests (5%) were found to be active against one or more of these viruses in the **primary screen** (VV, YF, JE, VE, PT and SF) at an AI_{95} activity level. **Figure 27** illustrates the number of compounds found active per virus at three antiviral activity levels (moderate, good and excellent).

Thirty-five compounds were considered active (SI of >1) against HIV during this reporting period. This represents around 3% of the compounds showing anti-HIV activity at the $\geq$50% reduction level.

The results for compounds found to be active are summarized in the following sections for each individual virus along with the proper quality control data (control compound data and plate variation data). Also the results of the confirmatory testing of compounds with greatest activity in the primary screen are summarized at the end of each individual virus section.

A cumulative summary presenting all of the *in vitro* antiviral tests results (both positive and negative) from assays performed during this reporting period is included in **Volume II, Appendix B**.

TOTAL NUMBER OF *IN VITRO* ANTIVIRAL ASSAYS TESTED IN THE PRIMARY SYSTEM DURING THIS REPORTING PERIOD

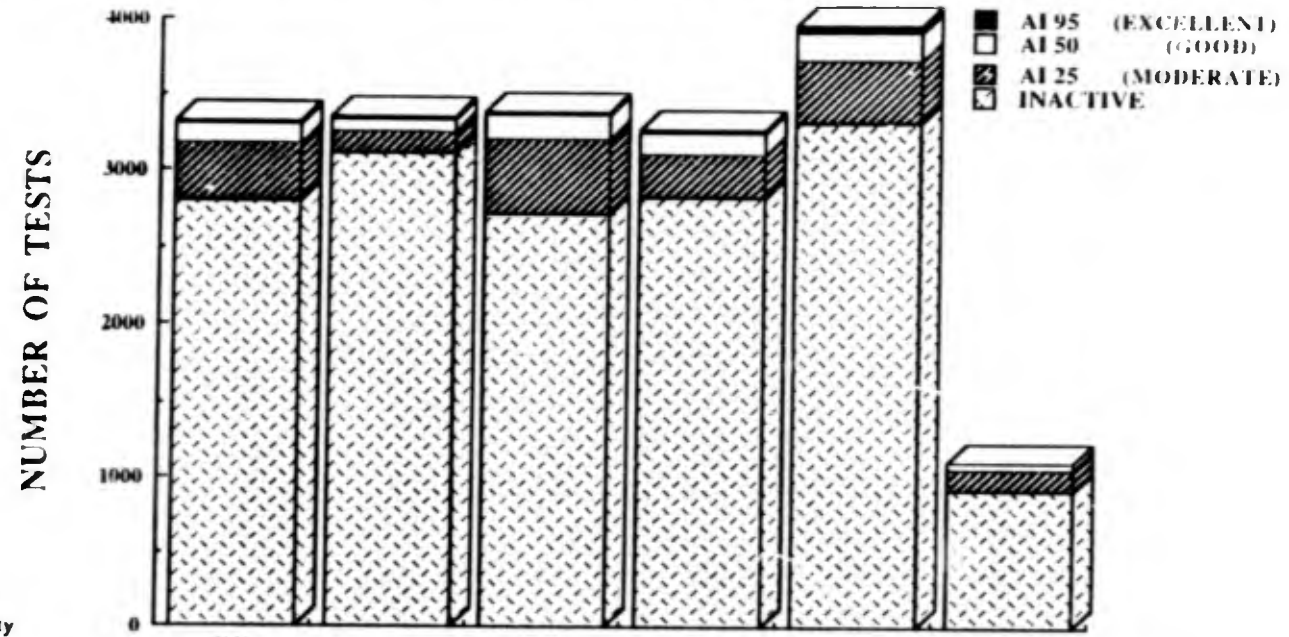


Total No Virus	FEB	MARCH	APR	MAY	JUNE	JULY	AUG	SEPT	OCT	NOV	DEC	Tests/ Virus
Primary Screen												
VV	160	126	120	188	110	100	160	158	300	142	0	1564
YF	222	356	256	300	240	450	450	410	638	512	94	3928
JE	270	232	242	372	240	450	450	444	632	510	96	3938
VE	180	326	242	300	240	360	544	442	504	640	114	3892
PT	270	234	272	270	240	424	654	570	638	512	64	4148
SF	240	262	312	270	240	450	450	474	664	544	0	3906
Subtotal	1342	1536	1444	1700	1310	2234	2708	2498	3376	2860	368	21376
AIDS												
HIV	247	0	0	128	45	42	320	74	0	0	0	856

Grand Total = 22232

Figure 26

ACTIVE COMPOUNDS FROM THE PRIMARY SCREEN



Activity Status	VF	JE	VE	PT	SF	VV	[illegible]
AI_{95}	19	12	15	20	38	8	11.
AI_{50}	125	70	159	141	192	48	735
AI_{25}	378	143	492	290	420	137	1860
Inactive	2800	3117	2720	2820	3313	926	13157
Total	**3322**	**3342**	**3386**	**3271**	**2663**	**1221**	**15965** •

• Grand Total of Accepted Single Drug Tests (Excluding Positive Control Tests)

Figure 27

4.3.1 Vaccinia Virus (VV):

During the time period covered by this report, compounds were screened for activity against the Lederle CA strain of VV in Vero cell monolayer cultures using an MTT-inhibition assay procedure.

A total of 1564 assays were reported (1221 excluding positive control drug testing); **Figure 28** shows the number of compounds evaluated each month for this 10-month period. Numerous compounds were submitted to SRI in quantities insufficient to permit evaluation against the lower priority virus VV.

TOTAL NUMBER OF TESTS AGAINST VACCINIA VIRUS (VV)

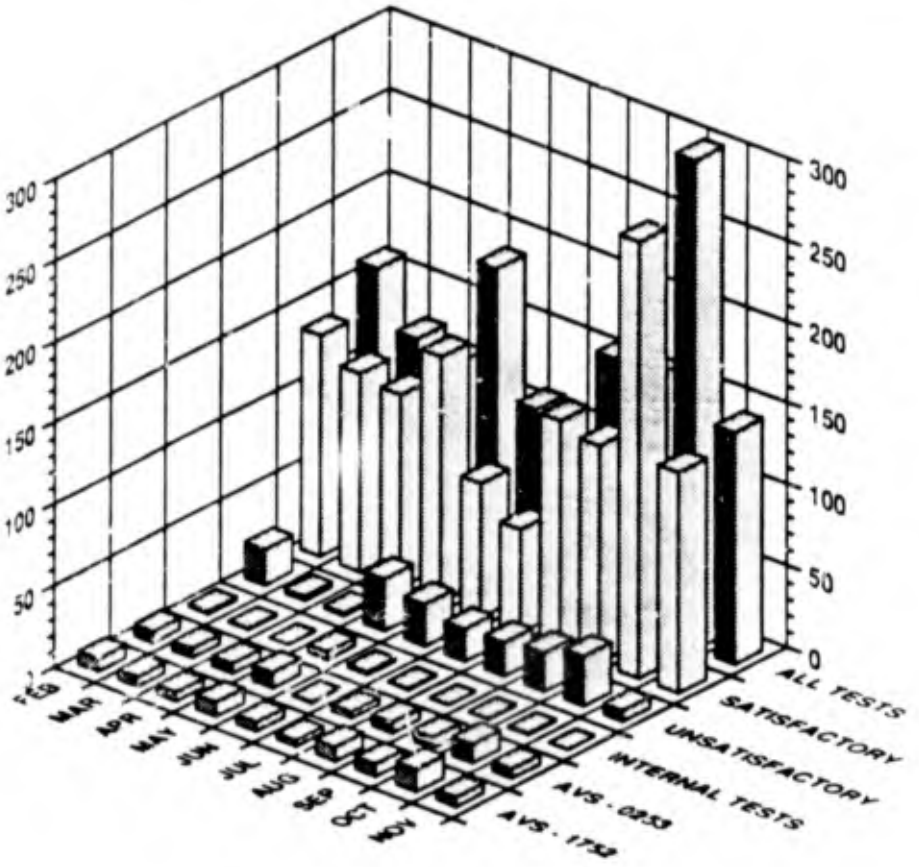


Figure 28

Quality Control Tests =	KPC1 = Positive Control, Ara-A =	76
	KPC2 = Positive Control, Selenazofurin =	66
	Internal Controls =	13
Acceptable Single Drug Tests =	Unsatisfactory, Compounds Toxic =	188
		1221
Total Number of VV Tests =		1564

4.3.1.1 Quality Control:

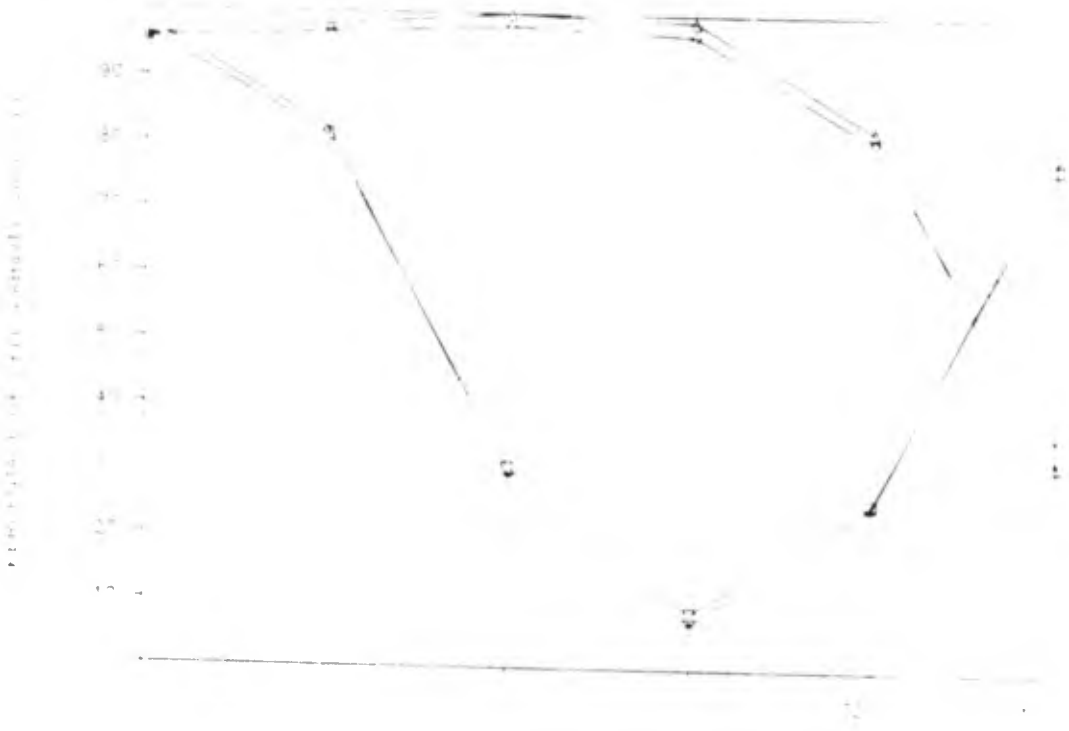
Two compounds were utilized as positive control drugs, Ara-A (AVS-1752) and Selenazofurin (AVS-0253).

Ara-A continues to be a reliable positive control drug. The following indices summarize the Ara-A data.

	IC_{50} µg/ml	TC_{25} µg/ml	SI	TAI
Mean	2.26	43.89	21.47	44
S.D.	0.82	19.00	12.62	9.4
Median	2.07	42.00	18.20	44

The closeness of the mean and median values indicate the reproducibility of the effect of Ara-A on VV and on host cells. The average antiviral and cytotoxicity values for 76 Positive Control Tests at each concentration of Ara-A are listed and plotted in **Figure 29-A**. Excellent reproducibility was achieved in the 76 assays.

ARA-A -VS- VV VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability ΔMedian % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	0.32	1.0	3.2	10	32	100	0.32	1.0	3.2	10	32	100
Mean	96	81	31	9	26	77	96	97	98	97	81	35
Median	98	82	30	7	25	79	96	98	100	99	83	32
Std. Dev.	0.04	0.09	0.15	0.08	0.15	0.13	0.04	0.04	0.04	0.05	0.14	0.13

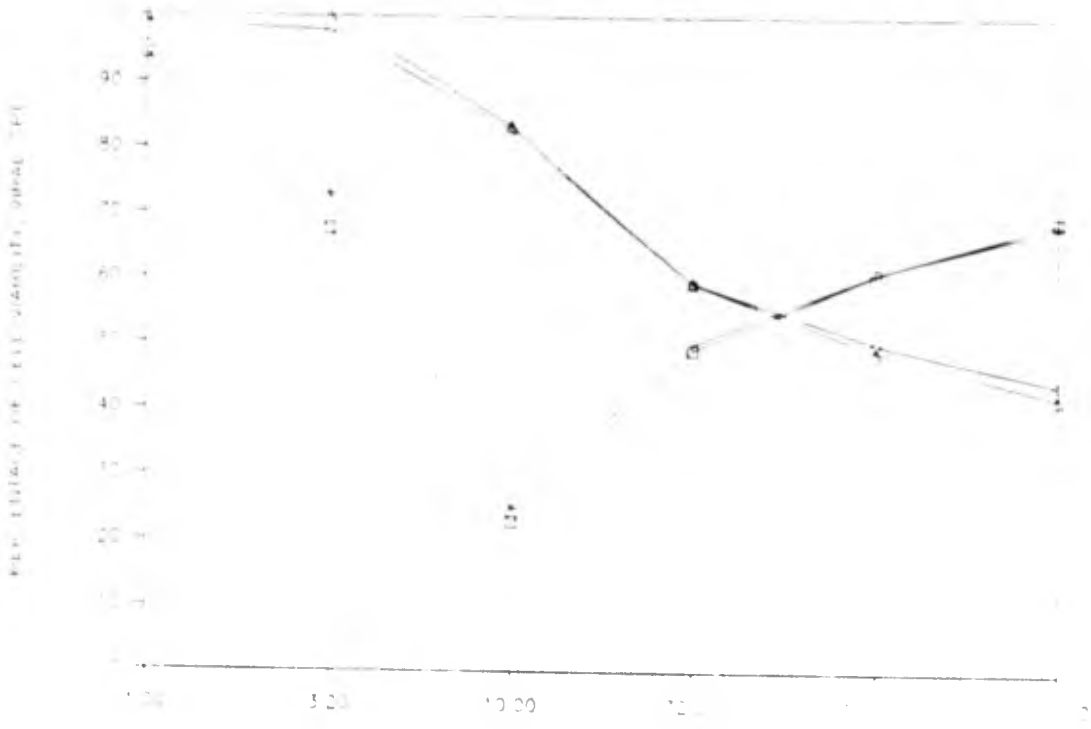
Figure 29-A
Average Antiviral and Cytotoxicity Values for 76 Positive Control Compound Tests

The following indices summarize the data for Selenazofurin:

	IC_{50} µg/ml	TC_{25} µg/ml	SI	TAI
Mean	4.93	17.93	3.86	20.76
S.D.	1.43	9.83	2.02	6.51
Median	5.26	17.35	3.83	20.33

The mean and medium values of the IC_{50}, TC_{25}, SI, and TAI are very close indicating better than normal reproducibility. The average antiviral and cytotoxicity values for 66 Positive Control Tests at each concentration of Selenazofurin are listed and plotted in **Figure 29-B**. Excellent reproducibility was achieved in the 66 assays.

SELENAZOFURIN -VS- VV VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability ΔMedian % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	1	3.2	10	32	100	320	1	3.2	10	32	100	320
Mean	94	68	23	49	61	69	99	98	83	60	50	44
Median	96	73	25	50	61	69	100	100	84	60	49	42
Std. Dev.	0.06	0.18	0.10	0.07	0.07	0.07	0.02	0.04	0.10	0.07	0.08	0.09

Figure 29-B
Average Antiviral and Cytotoxicity Values for 25 Positive Control Compound Tests

The distribution of the maximum VV CPE inhibition values (% CPE reduction) recorded from the 142 positive control assays (76 tests were Ara-A and 66 tests were Selenazofurin) conducted during the report period is depicted in **Figure 30**. As expected, Ara-A has a much greater inhibitory effect than Selenazofurin. The maximum percent reduction in CPE observed with Ara-A ranged from 71 - 100% whereas the maximum percent reduction in CPE for Selenazofurin ranged from 51 - 100%.

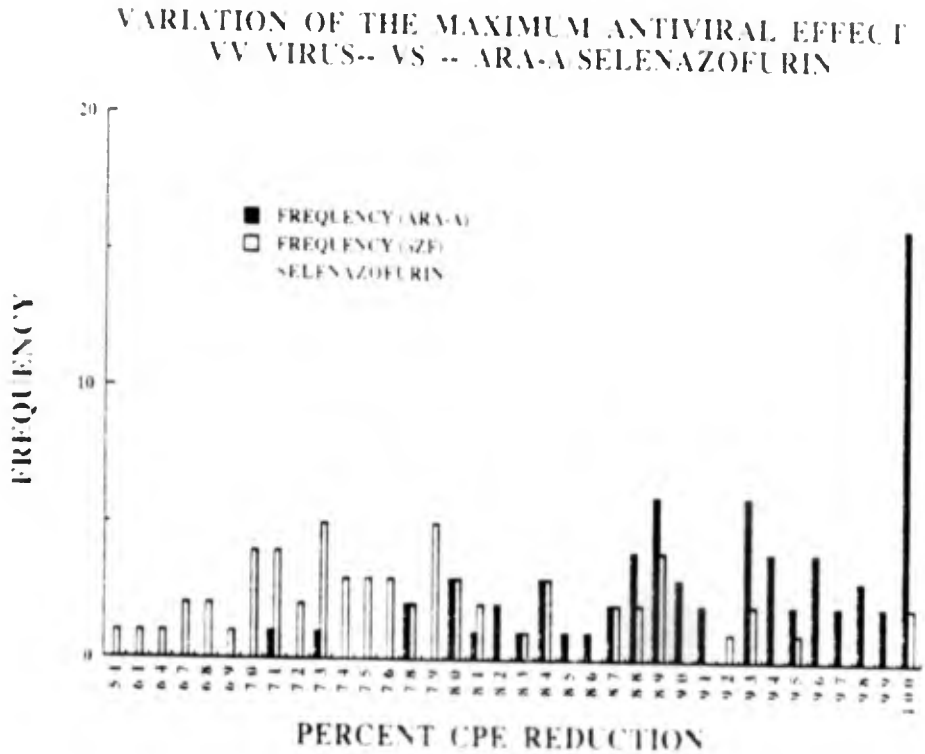


Figure 30

Scatter plots of the mean cell control O.D. and the mean virus control O.D. from all assays tested during this reporting period are presented in **Figures 31 and 32**, respectively. The cell control data indicates that the cell load was satisfactory, but somewhat less consistent than the virus load. The plot of the virus control O.D.'s indicates good host cell destruction and a uniform load of virus among the day-to-day experiments. **Figure 33** gives the frequency and variation of the mean values (O.D.) of the differential from each assay. Each differential value represents the difference between the mean cell control O.D. and the mean O.D. of the virus controls of each of the 1221 single drug assays. The variation of the cell load is reflected in the differential figures.

GRAPHIC ILLUSTRATION OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ALL ACCEPTED VV PLATE DATA

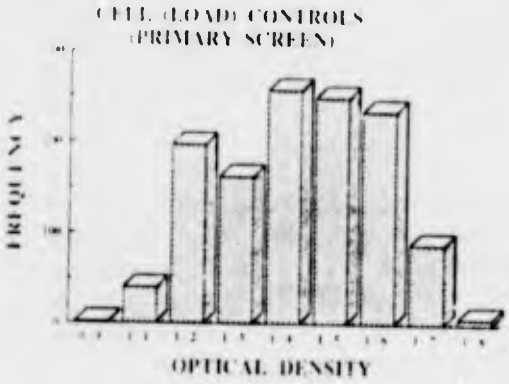


Figure 31

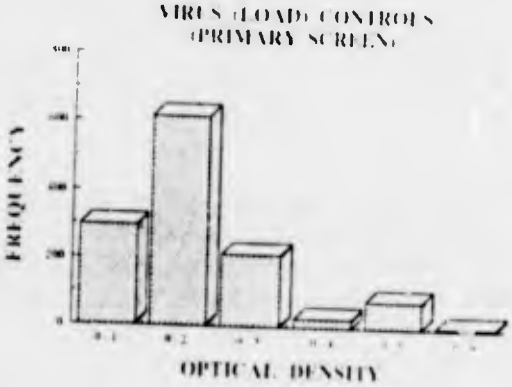


Figure 32

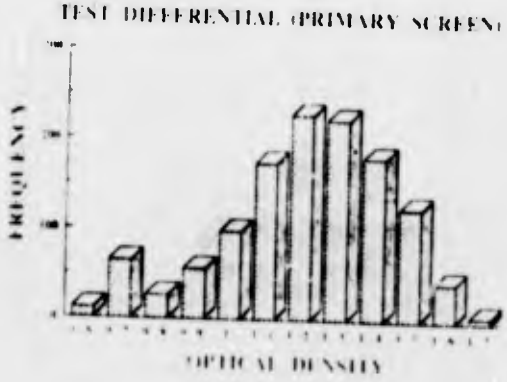


Figure 33

4.3.1.2 **VV-Antiviral Activity Results:**

New Drugs with 95 % Antiviral Reduction Levels: Out of the 1221 actual single drug tests, eight new compounds demonstrated marked antiviral activity, having antiviral reduction values equal to or better than 95%. This represents around 1% of the test compounds being active at this maximum reduction level. The data from the evaluations of the eight compounds are summarized in **Table 7**. These drugs showed significant inhibition of VV as determined by TAI's that ranged from 19 - 51% and SI values that ranged from 2 - 26.

Table 7

AVS Compounds Active Against Vaccinia (VV) at AI_{95} Level

AVS No.	Shipment#	Test Date	Differntl.	IC 95	TC 95	AI 95	SI	TAI
VV 9480	80	09/19/91	1.085	29.10 >	320.00 >	11.00 >	25.95 >	51.02
VV 9589	79	07/25/91	0.997	79.60	309.00	3.88	10.86 >	42.28
VV 9607	79	05/30/91	1.145	7.89	155.00	19.65	11.89 >	36.30
VV 9590	79	06/13/91	1.436	181.00 >	100.00 >	0.55 >	8.80 >	31.76
VV 9795	79	09/04/91	1.260	9.20 >	100.00 >	10.87	6.55	29.09
VV 9842	79	09/04/91	1.309	89.00	311.00	3.49	4.88	25.96
VV 9423	80	10/03/91	0.604	94.30 >	320.00 >	3.39	3.10 >	23.00
VV 9284	77	04/18/91	1.226	297.00 >	320.00 >	1.08 >	2.13 >	18.97

New Drugs with 50% Antiviral Reduction Levels: Out of the 1221 actual single drug tests, 33 new compounds caused antiviral reduction values equal to or better than 50%. This represents around 2.7% of the test compounds being active at this antiviral reduction level. Test results are summarized in **Table 8** according to the highest **Total Antiviral Index (TAI)**. Compound 2318 appeared to be the most active in AI $\geq$ 50% category with an SI of 154. Compounds 8586, 9416 and 9672 were moderately active with **Selectivity Indices** $\geq$ 10. All other compounds had SI's that ranged from 1 - 6.4.

Table 8

AVS Compounds Active Against Vaccinia (VV) at AI_{50} Level

AVS No.	Shipment#	Test Date	Differntl.	IC 50	TC 50	AI 50	SI	TAI
VV 2318	82	10/24/91	0.714	0.31	186.00	595.79	154.29	A
VV 8586	76	06/13/91	1.306	18.30 >	320.00 >	17.44 >	17.44 >	36.48
VV 9776	79	08/15/91	0.929	36.40	210.00	5.77	4.26	32.97
VV 9672	79	07/25/91	0.963	1.34	19.50	14.55	9.77 >	32.41
VV 9416	82	10/24/91	0.744	6.11 >	100.00 >	16.36	10.14	29.78
VV 9512	80	09/26/91	1.359	19.30	177.00	9.69	5.78	28.69
VV 9422	80	10/03/91	0.604	29.60	280.00	9.47	6.42	27.17
VV 9634	79	06/06/91	1.051	2.76	18.30	6.61	4.12	19.63
VV 9489	80	10/24/91	0.792	22.80	247.00	10.82	4.63	18.81
VV 9819	79	08/22/91	1.162	47.70	211.00	4.42	3.26	18.76
VV 9426	80	10/03/91	0.803	64.10 >	320.00 >	4.99	4.16 >	17.67
VV 10138	82	11/07/91	1.335	8.41	50.50	6.01	3.07	17.59
VV 10136	82	10/10/91	1.582	7.40	54.70	7.39	1.89	14.57
VV 9640	79	06/06/91	1.044	3.95	16.70	4.23	2.31 >	13.79
VV 9756	79	03/08/91	1.091	17.30	62.00	3.59	2.45	13.16
VV 11914	82	09/19/91	1.039	155.00 >	320.00 >	2.07 >	2.07 >	12.45
VV 9604	79	05/30/91	1.031	198.00 >	320.00 >	1.61	1.22 >	12.39
VV 9561	79	05/23/91	1.239	1.97	8.16	4.15	2.35	11.90

Table 8 (Cont'd)

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
VV 9424	80	10/03/91	0.670	68.40	293.00	4.28	2.87	11.70
VV 8532	76	02/14/91	1.139	17.90	54.10	3.03	1.41	11.07
VV 9603	79	05/30/91	0.841	1.42	6.29	4.44	3.08 >	10.87
VV 9421	80	10/03/91	0.623	22.90	60.70	2.66	1.76	10.68
VV 9735	79	08/01/91	1.057	8.32	20.30	2.44	1.74	10.19
VV 10164	82	10/16/91	1.417	58.90	173.00	2.94	1.23	9.94
VV 10186	82	11/07/91	1.392	18.60	58.00	3.11	1.77	9.28
VV 8674	76	03/14/91	1.122	227.00 >	320.00 >	1.41 >	1.41 >	7.15
VV 10134	82	10/10/91	1.599	26.20	75.40	2.88	1.12	6.95
VV 9274	77	04/18/91	1.248	276.00 >	320.00 >	1.16 >	1.16 >	5.03
VV 9834	79	08/29/91	1.330	3.02	7.43	2.46	1.21	4.14
VV 10252	82	10/24/91	0.646	294.00 >	320.00 >	1.09 >	1.09 >	3.73
VV 10258	82	10/24/91	0.668	283.00 >	320.00 >	1.13 >	1.13 >	3.73
VV 10253	82	10/24/91	0.646	238.00 >	320.00 >	1.34 >	1.34 >	3.65
VV 9670	79	09/19/91	1.054	2.57	5.14	2.00	1.07	a

a = A TAI value is not calculated for 12-Drug Concentration tests.

DIFFRNTL = The differential is the difference in the cell control and the virus control optical densities. A differential value of >0.5 is required for the assay to be considered satisfactory.

IC_{50} = (Viral) inhibitory concentration = The drug concentration (μg/ml) that inhibited viral CPE by 50% -- calculated by using a regression analysis for semilog curve fitting.

TC_{50} = (Cell) toxicity concentration = The drug concentration (μg/ml) that reduced cell viability by 50%.

AI_{50} = Antiviral Index = A single point ratio of the antiviral and anticellular effect of the compound, calculated with 50% reduction values (calculated by dividing the TC_{50} by the IC_{50}).

SI = Selectivity Index = A ratio calculated by dividing the TC_{25} by the IC_{50}.

TAI = Total Antiviral Index = The area between the cytotoxicity and the antiviral curves (based upon 6 one-half log_{10} dilutions (μg/ml) on a scale of 0-100%).

4.3.1.3 **Confirmatory Assays:**

The CPE-inhibition assay procedure using MTT was employed to confirm the inhibitory effects of compounds shown to be active in the primary screen. The results of the confirmatory evaluations on 30 compounds are summarized on **Table 9**. The antiviral effects were confirmed on all but nine of the compounds, several of which showed only equivocal activity on the primary assay. The only disappointment was the result of the second assay on AVS-2318. Because of a wide range of slight toxicity, it was necessary to use 12 concentrations per test. The cytotoxicity varied on the two tests. Clearly, this compound needs to be retested against VV. The significant activity demonstrated by AVS-9480 was confirmed.

Table 9

Confirmatory Assays for Compounds Active Against Vaccinia Virus (VV)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	SI	TAI	ACT
2318	82	10/24/91	1YN	0.714	0.17	48.20	282.77	0.31	186.00	595.79	0.00 >	320.0	154.29	a	+
2318	82	11/14/91	20N	1.542	0.20	0.0076	0.004	0.62	4.56	7.37	0.00 >	320.0	0.00	a	+
8586	76	02/21/91	1F1	1.158	15.00	180.00	11.62	82.70	259.00	3.14	0.00 >	320.0	2.17	15.40	+
8586	76	06/13/91	1N1	1.306	6.77 >	320.00 >	47.25	18.30 >	320.00 >	17.44	0.00 >	320.0 >	17.44 >	36.48	+
8674	76	02/21/91	1F6	1.085	159.00 >	320.00 >	2.02	252.00 >	320.00 >	1.27	0.00 >	320.0 >	1.27 >	6.35	+
8674	76	03/14/91	1GP	1.122	151.00 >	320.00 >	2.12	227.00 >	320.00 >	1.41	0.00 >	320.0 >	1.41 >	7.15	+
9284	77	04/18/91	1J3	1.226	103.00 >	320.00 >	3.10	150.00 >	320.00 >	2.13	297.00 >	320.0 >	2.13 >	18.97	+
9284	77	05/09/91	1KF	1.158	123.00 >	320.00 >	2.61	169.00 >	320.00 >	1.89	300.00 >	320.0 >	1.89 >	14.47	+
9416	82	10/09/91	1NA	1.420	5.27	17.60	3.34	8.69	57.90	6.66	0.00 >	320.0	2.03	8.16	+
9416	82	10/24/91	1YN	0.744	4.42	62.00	14.02	6.11 >	100.00 >	16.36	0.00 >	100.0	10.14	29.78	+
9422	80	10/03/91	1V3	0.604	15.50	190.00	12.25	29.60	280.00	9.47	0.00 >	320.0	6.42	27.17	+
9422	80	10/24/91	1YJ	0.716	45.00	196.00	4.36	67.70	293.00	4.33	0.00 >	320.0	2.90	13.76	+
9480	80	09/19/91	1U4	1.085	4.32 >	320.00 >	74.09	12.30 >	320.00 >	25.95	29.10 >	320.0 >	25.95 >	51.02	+
9480	80	11/21/91	21J	1.078	6.52	249.00	38.12	17.60 >	320.00 >	18.21	84.10 >	320.0	14.15	38.09	+
9489	80	09/19/91	1U7	1.018	9.09	14.90	1.64	20.60	192.00	9.30	0.00 >	320.0	0.72	11.96	+
9489	80	10/24/91	1YL	0.792	2.30	106.00	46.03	22.80	247.00	10.82	0.00 >	320.0	4.63	18.81	+
9489	80	11/14/91	20K	1.497	14.20	19.60	1.38	54.90	142.00	2.58	0.00 >	320.0	0.36 >	3.80	+
9512	80	09/26/91	1UJ	1.359	12.80	106.00	8.28	18.30	177.00	9.69	0.00	306.0	5.78	28.69	+
9512	80	10/10/91	1WV	1.710	39.00	156.00	3.99	57.00	211.00	3.70	0.00	311.0	2.73	15.20	+
9561	79	05/23/91	1LH	1.239	1.40	4.52	3.29	1.97	8.16	4.15	0.00 >	320.0	2.35	11.90	+
9561	79	06/13/91	1N9	1.464	0.83	1.14	1.38	0.00	2.86	0.00	0.00 >	32.0	0.00 >	1.06	+
9589	79	05/23/91	1N1	1.225	14.10	155.00	10.98	40.20	210.00	5.23	0.00	309.0	3.86	20.98	+
9589	79	06/13/91	1N0	1.294	70.40 >	320.00 >	4.54	170.00 >	320.00 >	1.88	0.00 >	320.0 >	1.88 >	15.05	+
9589	79	07/25/91	1PR	0.997	5.00	155.00	31.01	14.30	210.00	14.72	79.60	320.0	10.86 >	42.28	+
9590	79	05/23/91	1N1	1.225	14.20	25.30	1.78	33.40	112.00	3.36	89.60	299.0	0.76	13.00	+
9590	79	06/13/91	1N6	1.436	2.44 >	100.00 >	41.00	11.40 >	100.00 >	8.80	181.00 >	100.0 >	8.80 >	31.76	+
9603	79	05/30/91	1N9	0.841 <	1.00	4.36 >	4.36	1.42	6.29	4.44	0.00	9.7	3.08 >	10.87	+
9603	79	06/20/91	1NZ	1.379	1.36	3.63	2.66	0.00	6.33	0.00	0.00	25.1	0.00	3.34	+
9604	79	05/30/91	1NA	1.031	49.10	241.00	4.91	198.00 >	320.00 >	1.61	0.00 >	320.0	1.22 >	12.39	+
9604	79	06/20/91	1NZ	1.379	93.60	173.00	1.85	0.00	288.00	0.00	0.00 >	320.0	0.00	3.27	+

Table 9 (Cont'd)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	SI	TAI	ACT
9607	79	05/30/91	1MB	1.145 <	1.00	20.00 >	20.00	1.68	30.00	17.84	7.89	155.0	11.89 >	36.30	+
9607	79	06/20/91	1Q1	1.431	1.74	17.90	10.24	4.45	25.70	5.78	0.00 >	100.0	4.02 >	25.37	+
9634	79	06/06/91	1MQ	1.051	1.63	11.40	6.99	2.76	18.30	6.61	0.00	30.	4.12	19.63	+
9634	79	07/11/91	1P1	1.084	6.31	28.50	4.52	14.30	53.70	3.75	0.00	98.0	1.99	11.39	+
9640	79	06/06/91	1MT	1.044	1.84	9.11	4.96	3.95	16.70	4.23	0.00	30.5	2.31 >	13.79	+
9640	79	07/11/91	1P2	1.138	11.70	47.20	4.02	20.70	65.30	3.15	0.00	97.8	2.27	12.82	+
9670	79	09/19/91	1UF	1.054	1.17	2.76	2.36	2.57	5.14	2.00	0.00	9.5	1.07	-3.07	+
9670	79	10/03/91	1VK	0.721	0.51	3.29	6.48	2.01	5.62	2.80	0.00	9.8	1.64 >	14.82	+
9672	79	06/20/91	1NX	1.429	1.98	7.88	3.97	6.34	16.30	2.57	0.00	30.4	1.24 >	8.75	+
9672	79	07/25/91	1PT	0.963	0.51	13.10	25.55	1.34	19.50	14.55	0.00	31.0	9.77 >	32.41	+
9756	79	08/08/91	1QS	1.091	11.30	42.30	3.75	17.30	62.00	3.59	0.00	97.6	2.45	13.16	+
9756	79	08/29/91	1SJ	1.278	23.10	18.50	0.80	0.00	42.40	0.00	0.00	94.2	0.00	0.00	+
9776	79	08/15/91	1RA	0.929	4.06	155.00	38.20	36.40	210.00	5.77	0.00	309.0	4.26	32.97	+
9776	79	09/04/91	1SR	1.158	4.87	25.40	5.22	0.00	48.50	0.00	0.00	94.8	0.00	10.20	+
9795	79	08/15/91	1RK	0.943	5.66	53.80	9.51	13.50	75.60	5.60	21.40	270.0	3.98 >	26.30	+
9795	79	09/04/91	1ST	1.260	1.29	28.30	21.88	4.33	55.40	12.82	9.20 >	100.0	6.55	29.09	+
9795	79	09/26/91	1UU	1.448	5.66	32.40	5.90	17.10	68.10	3.99	0.00	279.0	1.96 >	16.94	+
9819	79	08/22/91	1RY	1.162	25.80	156.00	6.03	47.70	211.00	4.42	0.00	311.0	3.26	18.78	+
9819	79	09/12/91	1TU	1.425	38.00	130.00	3.43	58.60	194.00	3.31	0.00	307.0	2.23	10.02	+
9834	79	06/29/91	1SC	1.330	1.46	3.65	2.50	3.02	7.43	2.46	0.00	28.7	1.21	4.14	+
9834	79	09/19/91	1UE	1.081	3.02	5.57	1.85	0.00	8.27	0.00	0.00 >	10.0	0.00	3.51	+
9842	79	09/04/91	1SY	1.309	16.10	152.00	9.46	31.20	209.00	6.70	89.00	311.0	4.88	25.96	+
9842	79	09/26/91	1UV	1.531	19.90	131.00	6.62	38.60	197.00	5.11	97.90	315.0	3.41	18.53	+
10130	82	10/10/91	1WQ	1.562	5.47	19.90	3.64	28.10	112.00	4.00	0.00	299.0	0.71	7.18	+
10130	82	11/07/91	1ZW	1.485	4.02	23.40	5.83	23.90	64.20	2.68	0.00 >	100.0	0.98 >	9.29	+
10134	82	10/10/91	1WS	1.599	9.42	29.30	3.11	26.20	75.40	2.88	0.00 >	320.0	1.12	6.95	+
10134	82	11/07/91	1ZW	1.485	2.01	14.40	7.17	0.00	30.10	0.00	0.00 >	100.0	0.00	7.26	+
10136	82	10/10/91	1WT	1.582	3.20	14.00	4.38	7.40	54.70	7.39	0.00 >	320.0	1.89	14.67	+
10136	82	11/07/91	1ZX	1.335	4.29	20.60	4.79	11.20	31.60	2.81	0.00 >	100.0	1.33	14.28	+
10138	82	10/16/91	1WY	1.277	3.05	21.60	7.10	6.57	46.60	7.09	0.00	255.0	3.29	17.31	+
10138	82	11/07/91	1ZX	1.335	3.55	25.80	7.28	8.41	50.50	6.01	0.00	96.9	3.07	17.59	+

Table 9 (Cont'd)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	SI	TAI	ACT
10164	82	10/16/91	1XB	1.417	13.10	72.40	5.53	58.90	173.00	2.94	0.00 >	320.0	1.23	9.94	+
10164	82	11/07/91	120	1.322	20.70	134.00	6.49	0.00	206.00	0.00	0.00 >	320.0	0.00	4.95	+
11914	82	09/19/91	1U9	1.039	82.00 >	320.00 >	3.90	155.00 >	320.00 >	2.07	0.00 >	320.0 >	2.07 >	12.45	+
11914	82	10/03/91	1VK	0.721	46.80	131.00	2.81	97.10 >	320.00 >	3.29	0.00 >	320.0	1.35 >	4.89	+

a = A TAI value is not calculated for 12-Drug Concentration tests.

DIFFRNTL = The differential is the difference in the cell control and the virus control optical densities.

$IC_{25,50,95}$ = (Viral) inhibitory concentration 25%, 50% and 95% = The drug concentration (µg/ml) that inhibited viral CPE by 25%, 50% or 95% calculated by using a regression analysis for semilog curve fitting.

$TC_{25,50,95}$ = (Cell) toxicity concentration 25%, 50% and 95% = The drug concentration (µg/ml) that reduced cell viability by 25%, 50% or 95%.

$AI_{25,50,95}$ = Antiviral Index = A single point ratio of the antiviral and anticellular effect of the compound, calculated with 25%, 50% or 95% reduction values (calculated by dividing the $TC_{25,50,95}$ by the $IC_{25,50,95}$).

SI = Selectivity Index = A ratio calculated by dividing the TC_{25} by the IC_{50} (based upon 6 one-half-log_{10} dilutions, µg/ml, the maximum scale is 0-320).

TAI = Total Antiviral Index = The area between the cytotoxicity and the antiviral curves (based upon a scale of 0-100%).

ACT = Activity = A "+" denotes a rest that produced ≥25% reduction in CPE. A "-" denotes an inactive test (i.e. ≤25% reduction in CPE).

4.3.2 Yellow Fever Virus (YF):

The total output of YF-testing during this reporting period is summarized in monthly increments in Figure 34. During this period 3928 tests were performed against YF-virus with MTT-assay format. Out of these, 191 were control compound assays - Selenazofurin (AVS-0253) and 121 were control compound assays - 2-Thio-6-Azauridine (AVS-6724). Two hundred two tests were internal (+ + +) virus load, cell load, and other quality control tests. 92 tests were considered unsatisfactory based on the criteria of the quality controls set during this reporting period. The rest, totaling 3322 were actual single drug assays. The total number of assays represents approximately 66% above our yearly contractual obligation (i.e. 3322/2000).

Out of the 3322 test compounds, 144 demonstrated antiviral activity at greater than 50% reduction levels. This represents around 4% of the tested compounds having *in vitro* antiviral activity against YF-virus. The remainder, 3178 compounds (96%), are to be considered inactive with this assay protocol

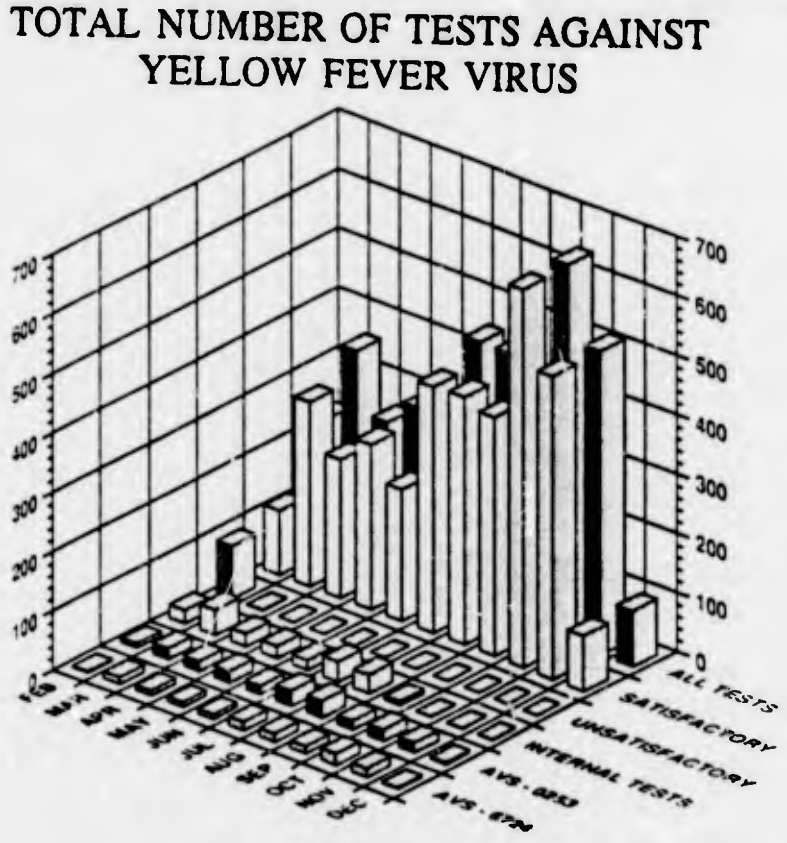


Figure 34

Quality Control Tests =	KPC (Positive Control - 2-Thio-6-Azauridine) =	121
	KPC (Positive Control - Selenazofurin) =	191
	(+ + + +) (Internal Virus and Cell Load Controls) =	202
	(UT) Unsatisfactory Test (QC rejects) =	92
Accepted Single Drug Tests =		3322
Total Number of YF Tests =		3928

4.3.2.1 **YF-Quality Controls:**

4.3.2.1.1 **Antiviral Activity of Selenazofurin vs YF Virus:**

Control Compound-Antiviral Performance: Selenazofurin (AVS-0253) has been the primary control compound against YF in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of the positive control drug (Selenazofurin) are illustrated in **Figure 35-A.**

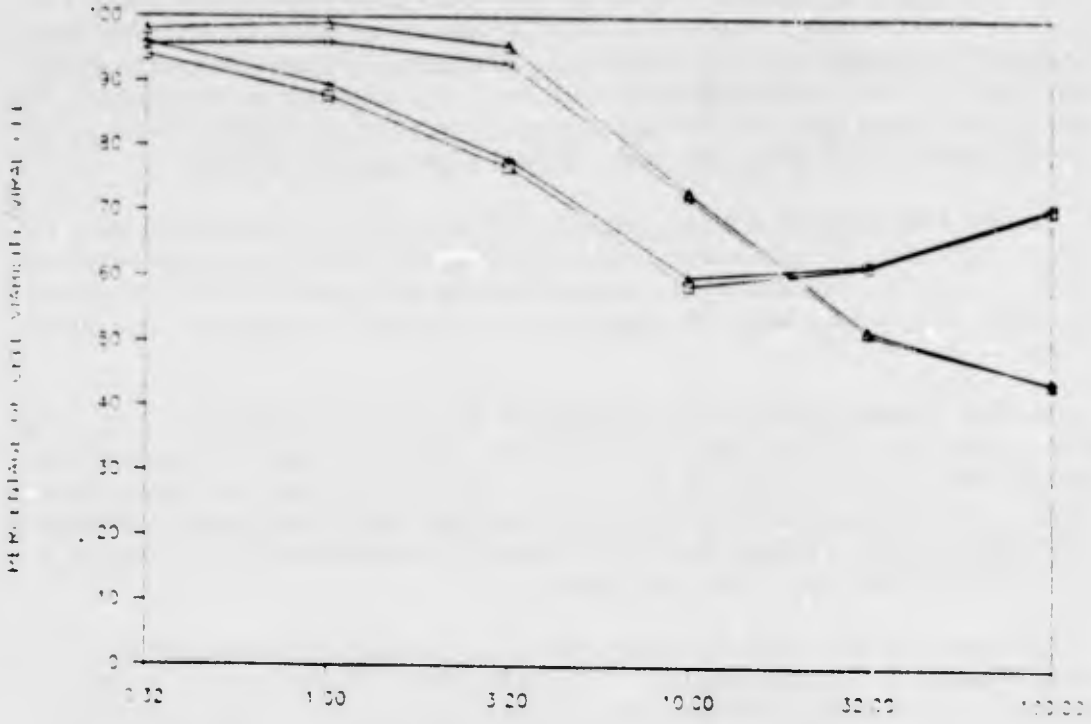
The 191 control tests performed with Selenazofurin gave a mean **Total Antiviral Index** (TAI) of 9.1% (SD ± 7) and the median value was 8%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from ~0 - >29% during this period. The mean **Selectivity Index (SI)** was only 0.46 (SD ± 0.98) and the median SI value was 0, indicating poor antiviral selectivity for Selenazofurin and it ranged from ~0 - 3.97 during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25% (AI_{25})** value was 5.7 (SD ± 8.36). The median AI_{25} value was 2.75 (range <0.08 - 54). The mean **Antiviral Index 50% (AI_{50})** was 2.59 (SD ± 5.82) with a median of 0 (range 0 >34.26). This indicates that Selenazofurin does not consistently reach 50% antiviral reduction levels. The **Antiviral Index 95% (AI_{95})** was not attainable with Selenazofurin versus Yellow Fever Virus.

The mean **Antiviral Inhibitory Concentration 25% (IC_{25})** was 4.11 μg/ml (SD ± 2.88). The median IC_{25} value was 3.93 μg/ml (range = <0.32 - 23.9 μg/ml). The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 1.86 μg/ml (SD ± 4.28). The median IC_{50} value was 0 μg/ml (range 0 - 29 μg/ml). This discrepancy indicates that the control compound Selenazofurin does not consistently reach 50% reduction levels. The mean **Antiviral Inhibitory Concentration 95% (IC_{95})** could not be attained with Selenazofurin versus Yellow Fever Virus.

The average maximum antiviral inhibitory level of 191 Selenazofurin tests (**Figure 35-A**) was reached at 10 μg/ml of the compound with 40% antiviral effect. Further increase of the drug concentration does not improve its antiviral activity. Maximum antiviral effect (~40%) was found with a simultaneous ~30% cytotoxic suppression. Above 10 μg/ml concentration, the antiviral protection levels off to 3·% at 100 μg/ml while simultaneously Selenazofurin becomes maximally toxic (~55%)

SELENAZOFURIN -VS- YF VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability Δ Median % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	0.32	1	3.2	10	32	100	0.32	1	3.2	10	32	100
Mean	94	88	77	59	62	71	96	96	93	72	51	44
Median	96	90	78	60	62	71	98	99	96	73	52	44
Std. Dev.	0.07	0.08	0.10	0.09	0.09	0.08	0.06	0.06	0.08	0.12	0.09	0.08

Figure 35-A
Average Antiviral and Cytotoxicity Values for 191 Positive Control Compound Tests

4.3.2.1.2 Maximum Antiviral Effect of Selenazofurin vs YF Virus:

A bar graph scatter plot (**Figure 36-A**) depicts the distribution of the maximum antiviral reduction values of all 191 control compound assays for Selenazofurin. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 43% (SD ± 8.8) reduction levels. The maximum reduction levels vary from 27 - 66% but remain quite consistently around the median of 42%. The assay control values give a relatively broad bell-shaped distribution curve. This indicates quite a consistent day-to-day performance of the control compound in the YF-MTT assay.

The positive control compound performance criteria for Selenazofurin versus the YF Virus is set at 25% reduction level. All assays in which Selenazofurin did not meet this accepted quality control level (≥25%) were rejected (i.e., 92 unsatisfactory tests).

Since Selenazofurin is only marginally active against YF virus,better quality control compounds are needed. However, regardless of the poor performance of the YF quality control drug Selenazofurin, around 144 different compounds have equal or better antiviral activity against YF virus than AVS-0253. Some of these could certainly be used as a better *in vitro* antiviral control compound in this large-scale antiviral screening program.

Variation of the Maximum Antiviral Effect
YF Virus - VS - Selenazofurin

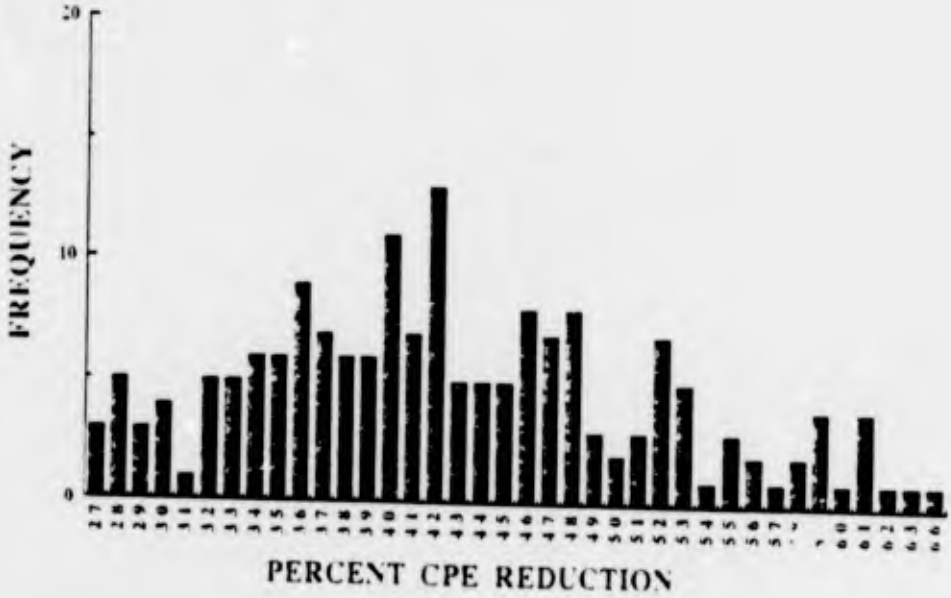


Figure 36-A
Maximum Antiviral CPE Reduction (%).
Summary of 191 Control Tests.

4.3.2.1.3 Cellular Cytotoxicity of Selenazofurin vs YF Virus:

YF-Control Compound-Cytotoxicity Performance: The 191 cytotoxicity values of the positive control compound Selenazofurin are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 11.66 μg/ml (SD ± 8.88) and the median was 9.26 μg/ml (range of < 0.32 - 100 μg/ml). The mean cell **Toxic Concentration 50%** (TC_{50}) value was 55.48 μg/ml (SD ± 32.5) and the median was 51.45 μg/ml (range of 4.82 - > 100 μg/ml). The mean cell **Toxic Concentration 95%** (TC_{95}) value was 99.23 μg/ml (SD ± 7.23) and the median was 100 μg/ml (range of > 32 - > 100).

As can be seen from **Figure 35-A**, the toxicity starts to become measurable above the concentration of 3.2 μg/ml and the maximum toxicity has not been reached at 100 μg/ml.

When the cytotoxicity reaches around 30% (10 μg/ml), the control compound (Selenazofurin) loses its maximum antiviral effect (~40%). Above 10 μg/ml the antiviral protection of Selenazofurin starts to decrease (down to ~30%). Selenazofurin becomes maximally toxic (55%) at 100 μg/ml concentration. The highest Selenazofurin concentration tested in these assays was 100 μg/ml.

Selenazofurin has a definite cytotoxic suppression on cellular metabolism and growth. The TC_{25} and TC_{50} toxicity can be achieved with relative consistency at 100 μg/ml.

4.3.2.1.4 **YF-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (Selenazofurin):**

YF-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 191 control assays is plotted in **Figure 37-A**. The results indicate that the cell O.D. readings reached a mean of 1.233 (SD ± 0.163) with a median of 1.228 (range of 0.751 - 1.698). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

YF-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 191 control assays is presented in **Figure 38-A**. The results indicate that the average virus load O.D. reading is 0.214 (SD ± 0.085) with a median of 0.210 (range of 0.031 - 0.573). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

YF-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 191 control assays is provided in **Figure 39-A**. The results indicate that the average differential O.D. reading is 1.019 (SD ± 0.148) with a median of 1.019 (range 0.665 - 1.521). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 98% measurement accuracy.

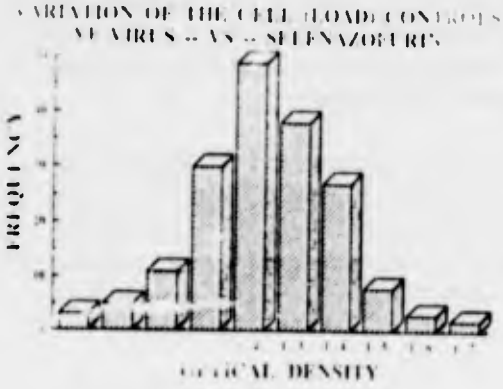


Figure 37-A

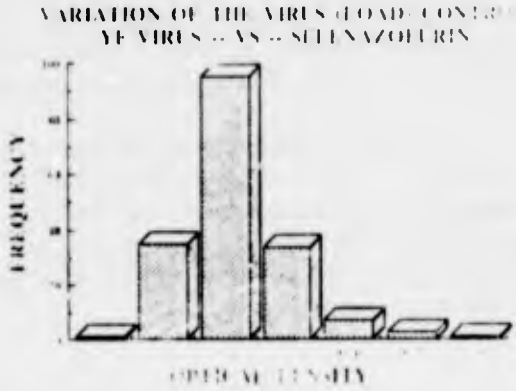


Figure 38-A

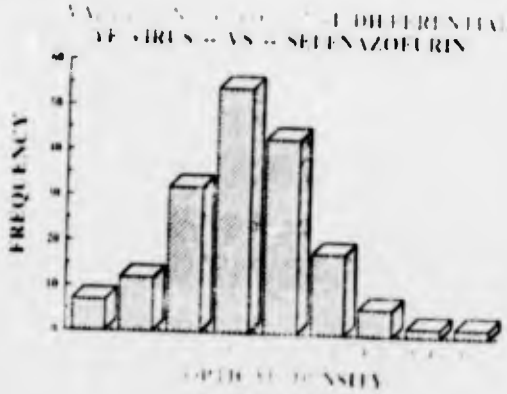


Figure 39-A

4.3.2.1 YF-Quality Controls:

4.3.2.1.1 Antiviral Activity of 2-Thio-6-Azauridine vs YF Virus:

Second Control Compound-Antiviral Performance: 2-Thio-6-Azauridine (AVS-6724) has been tested as a second control compound against YF in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of this second positive control drug are illustrated in **Figure 35-B**.

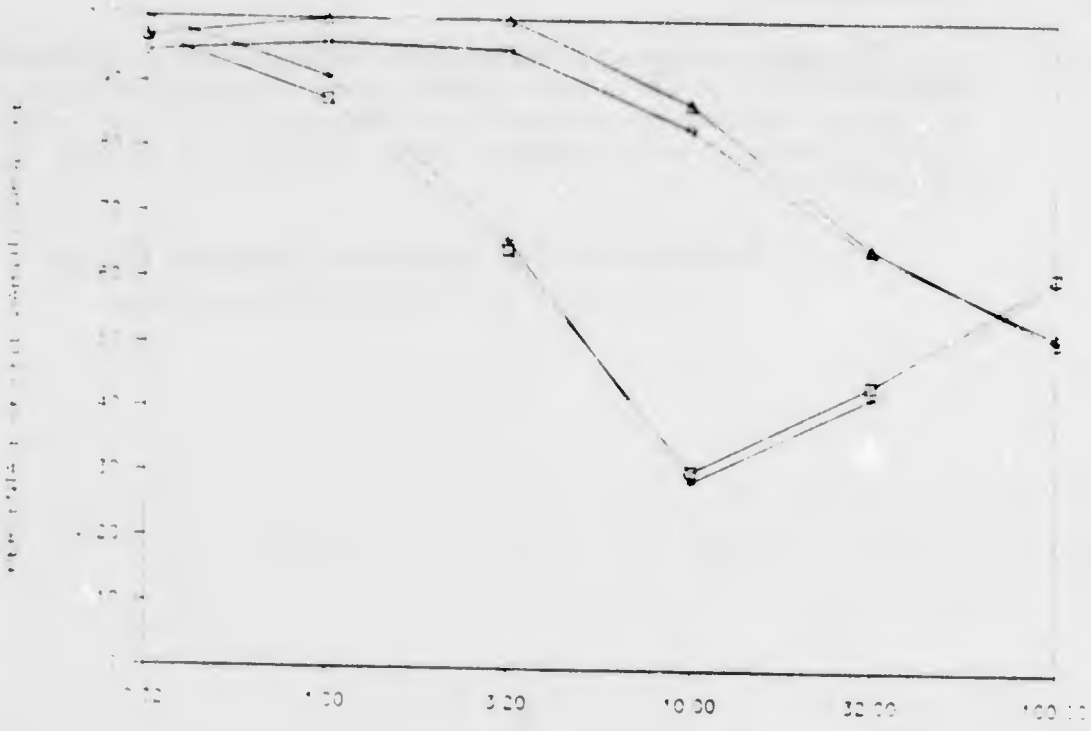
The 121 control tests performed with 2-Thio-6-Azauridine gave a mean **Total Antiviral Index** (TAI) of 24.48% (SD ± 15) and the median value was 23%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from 0 - >53% during this period. The mean **Selectivity Index (SI)** was 7.6 (SD ± 9.7) and the median SI value was 4.1, indicating moderate antiviral selectivity for 2-Thio-6-Azauridine and it ranged from 0 - 58.33 during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25%** (AI_{25}) value was 21.18 (SD ± 26.57). The median AI_{25} value was 10 (range <0.02 - 135.24). The mean **Antiviral Index 50%** (AI_{50}) was 19.11 (SD ± 18.81) with a median of 18.31 (range 0 - >146.2). This indicates that 2-Thio-6-Azauridine does not consistently reach 50% antiviral reduction levels. The **Antiviral Index 95%** (AI_{95}) was 1.04 (SD ± 3.17) with a median of 0 (range 0 - >11.53).

The mean **Antiviral Inhibitory Concentration 25%** (IC_{25}) was 2.27 μg/ml (SD ± 1.57). The median IC_{25} value was 2.06 μg/ml (range = 2.32 - 14.7 μg/ml). The mean **Antiviral Inhibitory Concentration 50%** (IC_{50}) was 4.59 μg/ml (SD ± 2.84). The median IC_{50} value was 4.72 μg/ml (range 0 - 18.7 μg/ml). This discrepancy indicates that the control compound 2-Thio-6-Azauridine does not consistently reach 50% reduction levels. The mean **Antiviral Inhibitory Concentration 95%** (IC_{95}) was 1.02 μg/ml (SD ± 3.19). The median IC_{95} value was 0 (range 0 - 18.9 μg/ml).

The average maximum antiviral inhibitory level of 121 2-Thio-6-Azauridine tests (**Figure 35-B**) was reached at 10 μg/ml of the compound with 70% antiviral effect. Further increase of the drug concentration does not improve its antiviral activity. Maximum antiviral effect (~70%) was found with a simultaneous ~15% cytotoxic suppression. Above 10 μg/ml concentration the antiviral protection levels off to 40% reduction level at 100 μg/ml while simultaneously 2-Thio-6-Azauridine become maximally toxic (~50%).

2-THIO-6-AZAURIDINE -VS- YF VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability ΔMedian % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	0.32	1	3.2	10	32	100	0.32	1	3.2	10	32	100
Mean	97	99	65	31	44	61	95	96	95	83	64	52
Median	100	91	66	29	42	60	97	100	100	87	65	51
Std. Dev.	0.05	0.11	0.13	0.19	0.17	0.14	0.07	0.06	0.08	0.17	0.15	0.1

Figure 35-B
Average Antiviral and Cytotoxicity Values for 121 Positive Control Compound Tests

4.3.2.1.2 Maximum Antiviral Effect of 2-Thio-6-Azauridine vs YF Virus:

A bar graph scatter plot (**Figure 36-B**) depicts the distribution of the maximum antiviral reduction values of all 121 control compound assays for 2-Thio-6-Azauridine. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 70% (SD ± 17.98) reduction levels. The maximum reduction levels vary from 27 - 100% but remain quite consistently around the median of 70%. The assay control values give a relatively shifted bell-shaped distribution curve. This indicates quite a consistent day-to-day performance of the control compound in the YF-MTT assay.

Recommendations:

Based upon the data obtained in parallel studies with Selenazofurin, we recommend that 2-Thio-6-Azauridine (AVS #6724) will be used as a second control compound against YF virus. It's overall performance is much better than the present control, Selenazofurin. It is readily available from Sigma Chemical Company, it is inexpensive and works as effectively at low drug concentrations as Selenazofurin.

Variation of the Maximum Antiviral Effect
YF Virus - VS - 2-Thio-6-Azauridine

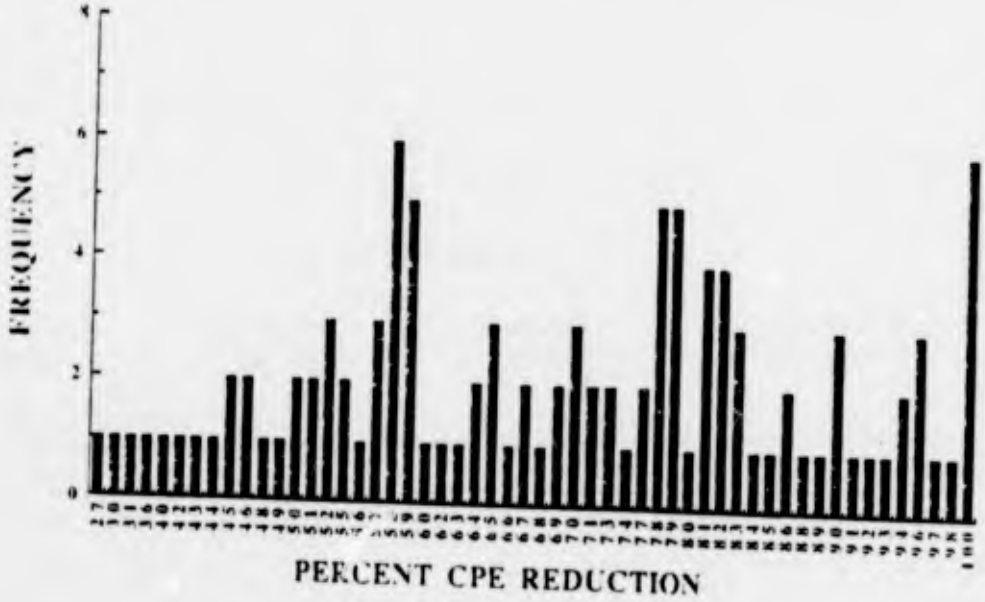


Figure 36-B
Maximum Antiviral CPE Reduction (%).
Summary of 121 Control Tests.

4.3.2.1.3 Cellular Cytotoxicity of 2-Thio-6-Azauridine vs YF Virus:

YF-Control Compound-Cytotoxicity Performance: The 121 cytotoxicity values of the positive control compound 2-Thio-6-Azauridine are also very consistent. The mean cell **Toxic Concentration 25% (TC_{25})** was 28 μg/ml (SD ± 23.62) and the median was 22.5 μg/ml (range of 0.29 - > 100 μg/ml). The mean cell **Toxic Concentration 50% (TC_{50})** value was 78.56 μg/ml (SD ± 32.36) and the median was 100 μg/ml (range of 6.74 - > 100 μg/ml). The mean cell **Toxic Concentration 95% (TC_{95})** was 99.38 μg/ml (SD ± 6.51) and the median was 100 μg/ml (range of > 32 - > 100 μg/ml).

As can be seen from **Figure 35-B**, the toxicity starts to become measurable above the concentration of 3.2 μg/ml and the maximum toxicity has not been reached at 100 μg/ml.

When the cytotoxicity reaches around 20% (10 μg/ml), the control compound (2-Thio-6-Azauridine) loses its maximum antiviral effect (~70%). Above 10 μg/ml the antiviral protection of 2-Thio-6-Azauridine starts to decrease (down to ~30%). 2-Thio-6-Azauridine becomes maximally toxic at 100 μg/ml concentration (50%). The highest 2-Thio-6-Azauridine concentration tested in these assays was 100 μg/ml.

2-Thio-6-Azauridine has a definite cytotoxic suppression on cellular metabolism and growth. The TC_{25} and TC_{50} toxicity can be achieved with relative consistency at 100 μg/ml.

4.3.2.1.4 YF-Assay Plate Quality Controls; Cell Load and Virus Load Parameters (2-Thio-6-Azauridine):

YF-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 121 control assays is plotted in **Figure 37-B**. The results indicate that the cell O.D. readings reached a mean 1.201 (SD ± 0.138) with a median of 1.21 (range of 0.845 - 1.631). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

YF-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O D. readings of the 121 control assays is presented in **Figure 38-B**. The results indicate that the average virus load O.D. reading is 0.22 (SD ± 0.117) with a median of 0.198 (range of 0.031 - 0.793). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

YF-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 121 control assays is provided in **Figure 39-B**. The results indicate that the average differential O.D. reading is 0.985 (SD ± 0.129) with a median of 0.98 (range 0.546 - 1.476). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 98% measurement accuracy.

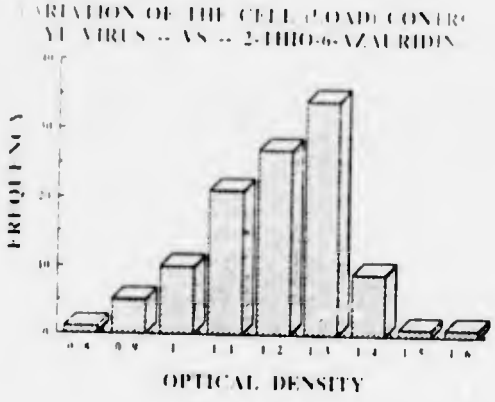


Figure 37-B

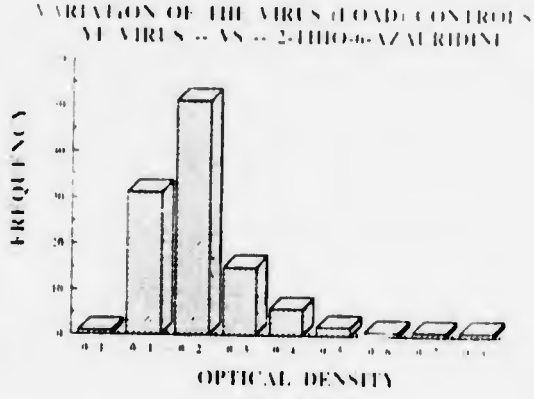


Figure 38-B

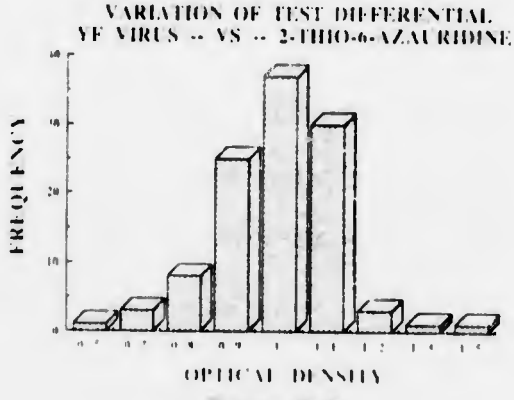


Figure 39-B

4.3.2.1.5 Overall YF-Assay Plate Quality Controls:

YF-Overall-Cell Load Performance: A bar graph scatter plot of the overall mean cell control (O.D. reading) of 3322 accepted assays is plotted in **Figure 37-C**. The results indicate that overall the cell O.D. readings reached a mean 1.196 (SD ± 0.148) with a median of 1.199 (range of 0.719 - 1.698). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

YF-Overall-Virus Load Performance: A bar graph scatter plot of the overall mean virus load O.D. readings of the 3322 accepted assays is presented in **Figure 38-C**. The results indicate that the overall average virus load O.D. reading is 0.21 (SD ± 0.102) with a median of 0.200 (range of 0.020 - 0.880). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

YF-Overall-Assay Differential Performance: A bar graph scatter plot of the overall mean O.D. differential values of the 3322 accepted assays is provided in **Figure 39-C**. The results indicate that the overall average differential O.D. reading is 0.987 (SD ± 0.141) with a median of 0.983 (range 0.393 - 1.521). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 98% measurement accuracy.

GRAPHIC ILLUSTRATION OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ACCEPTED YF PLATE DATA

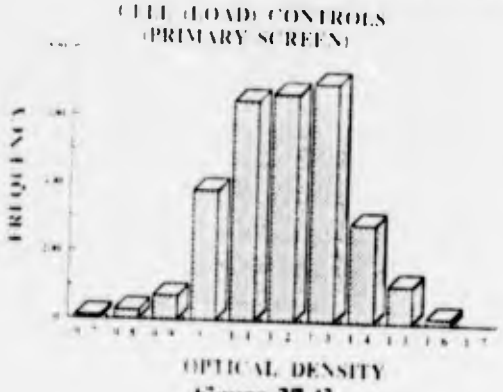


Figure 37-C

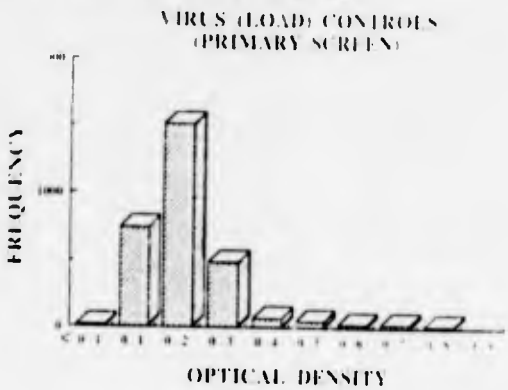


Figure 38-C

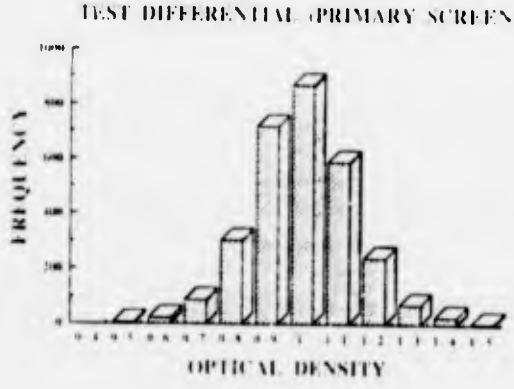


Figure 39-C

4.3.2.2 YF-Antiviral Activity Results:

Drugs with 95% Antiviral Reduction Levels: Out of the 3322 actual single drug tests, 19 new compound demonstrated excellent antiviral activity, having antiviral reduction values of equal to or better than 95%. This represents around 0.6% of the test compounds being active at this excellent reduction level. These data are summarized in **Table 10**. Compounds AVS-9590, 9589 and 11506 demonstrated the greatest *in vitro* promise, having TAI's that ranged from 51 through 43% and SI values that ranged from 15 - 133. Twelve compounds demonstrated moderate antiviral activity with TAI's that ranged from 22 - 38 and SI values that ranged from 3.7 - 9.0.

Table 10
AVS Compounds Active Against Yellow Fever (YF) at AI_{95} Level

AVS No.	Shipment#	Test Date	Differntl.	IC 95	TC 95	AI 95	SI	TAI
YF 9590	79	10/01/91	0.741	445.00 >	1000.00 >	2.25 >	18.83 >	51.60
YF 9589	79	05/02/91	0.958	92.30 >	320.00 >	3.47 >	132.57 >	45.61
YF 11506	81	07/31/91	0.875	861.00	9530.00	11.12	14.60	43.82
YF 10031	79	07/03/91	0.849	715.00	3200.00	4.47	8.99 >	37.91
YF 9460	79	10/01/91	0.809	29.10	957.00	32.83	7.08 >	35.92
YF 11717	82	09/26/91	0.825	32.00 >	320.00 >	10.00	8.05 >	32.87
YF 10030	79	07/03/91	0.849	92.40	952.00	10.31	6.39 >	31.75
YF 9458	79	10/01/91	0.809	90.20	928.00	10.29	5.74 >	29.64
YF 9970	79	06/18/91	0.680	291.00	2950.00	10.14	5.06 >	29.27
YF 9416	82	09/04/91	0.908	30.00 >	320.00 >	10.68	4.72 >	28.45
YF 11577	81	08/07/91	0.854	29.90 >	1000.00 >	33.46	7.31 >	28.36
YF 11224	80	07/10/91	0.934	9.65	270.00	27.98	4.54 >	24.56
YF 11223	80	07/10/91	0.908	29.40 >	320.00 >	10.88	4.39	24.35
YF 11580	81	08/07/91	0.886	29.70 >	1000.00 >	33.67	4.11 >	22.50
YF 11463	81	07/30/91	0.967	298.00	2910.00	9.78	3.71	21.66
YF 9530	79	10/01/91	0.835	30.00	100.00	3.33	2.98	18.71
YF 11185	80	10/08/91	0.888	935.00 >	1000.00 >	1.07 >	1.95 >	17.23
YF 9414	81	10/08/91	1.121	930.00 >	1000.00 >	1.07 >	2.06 >	13.25
YF 11238	80	09/24/91	0.968	302.00 >	320.00 >	1.06 >	1.79 >	10.18

Drugs with 50% Antiviral Reduction Levels: Out of the 3322 actual single drug tests, 125 new compounds demonstrated good antiviral activity, having antiviral reduction values equal to or better than 50%. This represents around 3.8% of the test compounds being active at this good antiviral reduction levels. These compounds are summarized in **Table 11** according to the highest **Total Antiviral Index (TAI)**. AVS-11924 and 9255 demonstrated the best antiviral activity with TAI's of 52 and 50% respectively and SI's of 43 and 27. The next sixteen compounds demonstrated moderate antiviral activity, having TAI's that ranged from 30 - 48 and SI's from 6 - 133. The rest (107 compounds) showed marginal antiviral activity with TAI's that ranged from 0 to 29% and SI's from 0.1 to 10.

Table 11
AVS Compounds Active Against Yellow Fever (YF) at AI_{50} Level

AVS No	Shipment#	Test Date	Differntl.	IC 50	TC 50	AI 50	SI	TAI
YF 11924	82	10/01/91	0.913	7.52 >	320.00 >	42.55 >	42.55 >	51.75
YF 9255	77	10/10/91	0.778	183.00	6780.00	36.95	27.20 >	50.00
YF 9454	79	10/01/91	0.848	5.66 >	1000.00 >	176.78	106.31 >	47.86
YF 11907	82	09/26/91	0.841	4.82 >	320.00 >	66.40	19.57 >	46.33
YF 11847	82	08/22/91	0.963	20.70	803.00	38.79	13.87 >	41.61
YF 11820	82	08/21/91	0.850	25.90	779.00	30.09	21.22 >	39.53
YF 9455	79	10/01/91	0.848	11.20	779.00	69.39	48.94 >	39.49
YF 11772	82	08/20/91	1.007 <	10.00	2190.00 >	219.02 >	128.85 >	38.50
YF 11821	82	08/21/91	0.917	6.84	749.00	109.57	65.97 >	38.34
YF 11844	82	08/22/91	0.871	4.59	66.00	14.39	10.53	37.09
YF 9916	79	06/12/91	1.444	167.00	3040.00	18.26	10.40 >	36.08
YF 11833	82	08/22/91	1.003	6.52 >	1000.00 >	153.31	132.46 >	36.04
YF 9238	77	10/10/91	0.851	3.20	202.00	63.04	44.56 >	34.54
YF 9990	79	06/19/91	1.210	37.30	701.00	18.81	9.69 >	33.07
YF 11849	82	08/22/91	0.877 <	32.00	1650.00 >	51.58 >	27.39 >	32.57
YF 11044	78	10/10/91	1.077	10.90	320.00	29.33	6.49 >	30.04
YF 11743	82	08/15/91	1.092	15.10	919.00	60.66	33.95 >	29.71
YF 11931	82	10/01/91	0.926	50.00 >	320.00 >	6.40 >	6.40 >	29.62
YF 9445	79	10/01/91	0.932	5.18	66.70	12.87	9.52 >	29.19
YF 11175	80	07/10/91	0.823	119.00 >	320.00 >	2.69 >	2.69 >	27.70
YF 11123	78	10/15/91	0.917	1630.00 >	10000.0 >	6.14 >	6.14 >	27.68
YF 11133	78	04/17/91	0.904	629.00	5640.00	8.97	5.50 >	26.52
YF 9317	77	03/05/91	0.978	752.00 >	3200.00 >	4.25	2.39 >	26.29
YF 11218	80	09/24/91	1.049	9.09 >	320.00 >	35.19	2.95 >	25.89
YF 9184	77	10/09/91	0.820	62.30	624.00	10.02	5.95	25.72
YF 11775	82	08/20/91	1.039	239.00	2590.00	10.84	6.87 >	25.19
YF 10047	79	07/03/91	0.795	33.90	213.00	6.29	4.62 >	25.11
YF 9607	79	10/01/91	0.837	5.13	27.70	5.41	3.68 >	24.32
YF 11622	81	08/08/91	0.954	2.12	19.40	9.14	5.89 >	23.22
YF 11796	82	08/21/91	0.981	53.70	415.00	7.73	4.30 >	23.06
YF 9937	79	06/13/91	1.188	274.00	1950.00	7.12	4.13 >	22.63
YF 9962	79	06/18/91	0.722	320.00	2080.00	6.49	4.74	21.72
YF 11045	78	10/10/91	1.042	100.00	686.00	6.86	4.25	21.32
YF 9149	77	10/09/91	0.660	5940.00 >	10000.0 >	1.68	1.11 >	21.13
YF 9909	81	07/10/91	0.831	62.60 >	320.00 >	5.11	3.97 >	20.58
YF 11095	78	10/08/91	0.941	6550.00 >	10000.0 >	1.53 >	1.53 >	20.10
YF 9248	77	02/14/91	0.614	708.00 >	3200.00 >	4.52 >	4.52 >	20.10
YF 11467	81	07/30/91	0.885	288.00	1960.00	6.82	4.68	19.89
YF 9183	77	02/07/91	0.717 <	10.00	100.00 >	10.00 >	6.60 >	19.18
YF 9217	77	02/13/91	0.919	305.00	1950.00	6.40	4.35	17.01
YF 9849	79	06/06/91	1.030	8.81	41.40	4.70	2.26 >	16.60
YF 11453	81	07/31/91	0.822	615.00	3460.00	5.63	3.49	16.51
YF 11830	82	08/21/91	0.945	835.00	2670.00	3.19	2.19 >	16.49
YF 11616	81	08/08/91	1.026	614.00 >	3200.00 >	5.21	1.81	16.09
YF 10002	79	06/19/91	1.047	71.30	874.00	12.26	4.16 >	15.64
YF 9874	79	06/11/91	1.056	6.84	65.60	9.60	7.04	15.45
YF 9475	79	10/01/91	0.853	8.85	46.30	5.23	2.57 >	15.26

Table 11 (Cont'd)

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
YF 11679	81	07/10/91	0.772	6.31	19.10	3.02	2.00 >	14.84
YF 11575	81	08/07/91	0.791	545.00	2140.00	3.93	2.62	14.79
YF 9433	80	10/08/91	0.931	516.00	1730.00	3.36	1.94	14.40
YF 11454	81	07/31/91	0.867	1800.00	6340.00	3.52	2.50	14.12
YF 11403	81	07/23/91	1.061	14.00	68.90	4.91	2.85	14.08
YF 11805	82	08/21/91	0.980	171.00	618.00	3.61	2.50	13.98
YF 11678	81	10/08/91	0.914	205.00 >	1000.00 >	4.87	3.09	13.43
YF 11722	82	08/14/91	0.865	162.00	600.00	3.70	2.46	13.18
YF 11447	82	08/14/91	0.796	212.00	762.00	3.58	2.55	12.84
YF 9842	79	10/03/91	1.041	62.30	216.00	3.47	2.44	12.66
YF 11735	82	08/15/91	1.143	641.00	3700.00	5.78	3.32	12.57
YF 11816	82	08/21/91	0.942	642.00	2100.00	3.27	2.41	12.40
YF 11941	82	09/24/91	0.904	181.00 >	320.00 >	1.77 >	1.77 >	12.35
YF 11832	82	08/22/91	1.003	601.00	2170.00	3.62	2.09	12.15
YF 9267	77	03/12/91	0.572	183.00	626.00	3.41	2.25	12.11
YF 11191	80	10/08/91	0.862	850.00 >	1000.00 >	1.18 >	1.18 >	11.57
YF 10543	83	10/24/91	0.883	273.00 >	320.00 >	1.17	1.14 >	11.51
YF 11064	78	10/10/91	0.841	3200.00	4040.00	1.26	0.65 >	11.47
YF 9680	79	10/02/91	0.941	0.74	2.07	2.78	2.02 >	11.47
YF 9173	77	10/09/91	0.748	2450.00	8770.00	3.59	2.45	11.33
YF 11804	82	08/29/91	0.897	704.00	1730.00	2.46	1.42	11.31
YF 9541	79	10/01/91	0.835	0.29	0.95	3.28	2.10	11.29
YF 11498	81	07/31/91	0.865	676.00	2020.00	2.98	2.11	11.04
YF 9981	79	06/19/91	1.144	7760.00 >	10000.0 >	1.29 >	1.29 >	10.59
YF 11889	82	08/29/91	0.873	201.00	605.00	3.01	2.03	10.43
YF 11756	82	08/20/91	0.925	259.00	703.00	2.72	1.52 >	10.29
YF 11665	81	08/14/91	0.878	203.00	630.00	3.10	2.19	9.88
YF 11895	82	08/29/91	1.006	96.70	205.00	2.12	1.50	9.50
YF 9364	77	03/06/91	1.047	232.00	564.00	2.44	1.49	9.36
YF 9247	77	02/14/91	0.614	249.00	901.00	3.61	2.19	9.17
YF 11753	82	08/15/91	1.098	224.00	928.00	4.14	2.15	9.16
YF 9908	79/C2	08/14/91	0.880	69.40	177.00	2.55	1.52	8.70
YF 11748	82	08/15/91	1.051	694.00	1990.00	2.87	2.00	8.56
YF 11131	78	04/17/91	1.033	2090.00	4140.00	1.98	1.11 >	8.47
YF 11897	82	08/29/91	0.952	26.20	56.20	2.15	1.29	8.37
YF 9446	79	04/24/91	1.187	81.00	193.00	2.39	1.59	7.75
YF 11911	82	09/26/91	1.022	202.00 >	320.00 >	1.58 >	1.58 >	7.72
YF 11451	81	07/31/91	0.914	850.00	2020.00	2.38	1.63	7.56
YF 11740	82	08/15/91	1.142	19.00	49.50	2.61	1.07 >	7.53
YF 11459	81	07/30/91	0.985	238.00	541.00	2.27	1.27 >	7.27
YF 9931	79	06/13/91	1.270	74.50	471.00	6.32	1.34	7.25
YF 9303	77	03/05/91	0.973	298.00	500.00	1.68	0.80 >	6.94
YF 11745	82	08/15/91	1.074	937.00	2090.00	2.23	1.46	6.64
YF 9886	79	06/11/91	1.036	8.08	14.50	1.80	0.86 >	6.49
YF 9449	79	10/01/91	0.979	74.00	189.00	2.56	1.55	6.40
YF 11834	82	08/22/91	0.975	212.00	576.00	2.72	1.56	6.15
YF 9436	80	07/10/91	0.937	312.00 >	320.00 >	1.03 >	1.03 >	6.14
YF 9258	77	10/10/91	0.758	909.00	1650.00	1.81	0.35 >	6.08
YF 9439	79	10/01/91	0.866	2520.00 >	3200.00 >	1.27 >	1.27 >	5.88
YF 9227	77	10/09/91	0.738	277.00	320.00	1.16	0.76	5.81
YF 11472	81	07/30/91	1.087	873.00	2740.00	3.14	1.67	5.68
YF 11848	82	08/22/91	0.877	902.00	1760.00	1.96	1.14	5.66
YF 11155	78	10/10/91	0.850	238.00 >	320.00 >	1.34 >	1.34 >	5.64
YF 11555	81	08/06/91	1.092	801.00	1400.00	1.74	0.94	5.48
YF 9167	77	10/09/91	0.732	1820.00	3820.00	2.10	0.75	4.19
YF 9767	79	10/03/91	1.039	0.03	0.04	1.60	0.71	4.10
YF 11621	81	08/08/91	0.954	217.00	670.00	3.09	0.44	4.07
YF 9511	80	07/10/91	0.956	71.70	161.00	2.27	0.80 >	3.82
YF 9445	79	04/24/91	1.204	224.00 >	320.00 >	1.43	1.14 >	3.55
YF 11344	81	07/17/91	1.113	2120.00 >	3200.00 >	1.51	0.31 >	3.39
YF 10672	83	10/31/91	0.818	24.20	78.80	3.25	0.09	3.15

Table 11 (Cont'd)

AVS No.	Shipment#	Test Date	Differntl.	IC 50	TC 50	AI 50	SI	TAI
YF 9202	77	10/09/91	0.844	902.00	1690.00	1.88	1.04	3.04
YF 11086	78	10/08/91	0.917	2820.00	4950.00	1.76	0.89	2.56
YF 11687	82	09/25/91	0.955	249.00 >	320.00 >	1.28	0.93 >	2.37
YF 9545	79	04/25/91	1.047 <	1.00	2.10 >	2.10 >	1.55 >	2.27
YF 11782	82	08/21/91	0.957	833.00	1340.00	1.60	0.82	2.13
YF 9199	77	10/09/91	0.844	918.00	1650.00	1.80	0.86	2.05
YF 11500	81	07/31/91	0.910	1000.00	1760.00	1.76	0.30	2.00
YF 10850	84	11/12/91	0.944	25.90	147.00	5.66	0.06 >	1.92
YF 10684	83	10/31/91	0.904	28.10	9.28	0.33	0.09 >	1.52
YF 10334	82	09/25/91	1.032	32.00	125.00	3.91	0.07	1.42
YF 9543	79	04/25/91	1.224 <	1.00	1.96 >	1.96 >	1.35 >	1.25
YF 10671	83	10/31/91	0.904	32.00	79.60	2.49	0.09 >	1.17
YF 9164	77	02/06/91	0.964	3200.00 >	3200.00 >	1.00	0.10 >	1.01
YF 11597	81	08/07/91	0.831	757.00	71.70	0.09 <	0.01	0.78
YF 11719	82	08/14/91	0.992	977.00	1270.00	1.30	0.68	0.55
YF 11118	78	10/15/91	1.037	8370.00 >	10000.0 >	1.19	0.41 >	0.51
YF 11808	82	08/21/91	0.958	671.00	660.00	0.98	0.73	0.00

Drugs with 25% Antiviral Reduction Levels: Of the 3322 actual single drug tests, 378 new compounds demonstrated minimal antiviral activity, having antiviral reduction values equal to or better than 25%. This represents around 11% of the test compounds being active at this marginal antiviral reduction level.

In general, when compared to the 95% and 50% antiviral activity categories, the compounds in this (25%) category do not appear to have any significant antiviral promise and probably do not need to be presently confirmed any further.

4.3.2.3 Confirmatory Assays:

Some of the compounds were sent to us in more than one separate drug shipment. These compounds were tested more than once. Data from the confirmatory assays are summarized in Table 12. If a compound showed $\geq$50% reduction in CPE during this contract period, then it was considered a candidate for confirmatory testing. The confirmatory tests are from active compounds taken from the prescreen and primary MTT assays. Out of 96 confirmatory tests, 71 compounds were confirmed active during this reporting period and the remaining 25 compounds gave conflicting results. The criteria for activity is that the confirmatory test has to show $\geq$25% reduction in CPE. Failure to confirm the activity in these compounds was probably due to differences during the assay conditions:

1) In confirmatory assays the concentration range is adjusted to a more accurate semilog scale to maximize the TAI window.

2) Differences in the "differential" of the two runs can cause the compound to read positive or negative, falsely. The variability in the differential can cause false positive or negative bias to be introduced into the calculations, thus reflecting the variability in the maximum activity of the compound.

3) The metabolic rate, cell and virus load/well, age, and passage number of the cells may cause the above observed variability in the confirmatory results.

4) Problems associated with stability and storage of the compound (i.e., different lot numbers, solubility, light sensitivity, hygroscopic, etc.).

5) Problems associated with technical execution of large numbers of plates by different technicians.

During this reporting period the overall confirmatory rate against YF was 74%. The conflicting results should be retested at a later date based on the availability of the compound.

Some of the compounds have not been confirmed due to the discontinuation of this project by the sponsor.

4.3.2.4 Recommendations of YF-Actives Based Upon the *In Vitro* Results with MTT Assay (Vero Cells).

Based upon the *in vitro* results with the MTT assay (Vero cells) we recommend the following:

1) Compounds with the highest TAI in the 95% activity category that have confirmed results with the exception of "colored" compounds should receive the highest priority for further profile studies and *in vivo* animal testing.

2) Compounds with the highest TAI in the 50% activity category that have confirmed results with the exception of "colored" compounds should receive the next highest priority for further profile studies and *in vivo* animal testing.

Table 12

Confirmatory Assays for Compounds Active Against Yellow Fever Virus (YF)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
0360	48	02/06/89	OJU	1.193	0.05	0.21	4.38	0.08	0.51	6.47	0.00	2.93	0.00	2.72 >	11.70	+
0360	2	08/22/89	RA3	0.842	0.03	0.20	7.39	0.05	0.29	6.23	0.00 >	1.00	0.00	4.19	22.90	+
0360	2	11/01/90	ZX8	0.915	0.14	0.71	5.25	0.32 >	1.00 >	3.13	0.00 >	1.00	0.00	2.23	10.18	+
0360	2/48	03/14/91	3VG	0.701	0.48 >	1.00 >	2.07	0.75 >	1.00 >	1.33	0.00 >	1.00	0.00 >	1.33 >	5.75	+
2318	53	04/10/89	PER	0.955 <	1.00 <	1.00 ~	1.00	0.00	2.51	0.00	0.00 >	320.00	0.00	0.00 >	0.40	+
2318	13	08/29/89	RDQ	0.872	0.27	0.26	0.97	0.52 >	10.00 >	19.12	0.00 >	10.00	0.0	0.50	14.62	+
2318	67	05/31/90	WGA	0.989	0.25	2.83	11.17	0.46 >	10.00 >	21.98	0.97 >	10.00 >	10.34	6.23	31.89	+
2318	67	07/12/90	XFS	0.666	0.19	2.80	15.00	0.40 >	10.00 >	25.07	0.00 >	10.00	0.00	7.02 >	28.34	+
2318	82	10/01/91	A38	0.997 <	1.00	5.47 >	5.47 <	1.00	66.00 >	66.00 <	1.00 >	320.00 >	320.00 >	5.47 >	27.04	+
2811	48	02/06/89	OJZ	1.184	0.01	0.09	6.08	0.03	0.23	9.00	0.00	0.94	0.00	3.55 >	18.20	+
2811	RS-1	03/14/91	3VI	0.701	0.05	0.20	3.99	0.08	0.36	4.52	0.00 >	1.00	0.00	2.46 >	9.33	+
2812	48	02/06/89	OKO	1.135	0.00	0.01	4.57	0.00 >	0.01 >	3.28	0.00 >	0.01	0.00	2.49 >	12.11	+
2812	61	12/06/89	SPI	0.813 <	0.00	0.02 >	7.12	0.01	0.04	8.66	0.00	0.17	0.00	4.75 >	20.52	+
2812	61	12/06/90	1S9	1.033	0.00 <	0.32	0.00	0.00 <	0.32	0.00	0.00 <	0.32	0.00	0.00	0.00	-
2812	RS-1	03/14/91	3VJ	0.772	0.01	0.02	3.79	0.01	0.03	3.57	0.00	0.10	0.00	1.96 >	8.18	+
2979	48	02/07/89	OL3	1.083	0.68	7.31	10.80	1.93	14.90	7.73	0.00	28.60	0.00	3.79 >	17.57	+
2979	48	03/10/89	PO2	1.067	4.36	13.20	3.03	6.55	24.70	3.77	0.00	95.60	0.00	2.02	8.93	+
2979	27	04/17/89	PLZ	1.045	1.77	12.90	7.27	0.00	23.00	0.00	0.00	89.90	0.00	0.00	11.98	+
2979	25	09/07/89	RHE	0.754	0.00	1.64	0.00	0.00	2.53	0.00	0.00	9.09	0.00	0.00	0.00	-
2979	25/27	03/14/91	3VK	0.674	2.07	12.90	6.21	5.76	20.00	3.47	0.00	72.80	0.00	2.23 >	13.25	+
4074	48	02/07/89	OL7	1.156	0.00 >	0.03	0.00	0.00 >	0.03	0.00	0.00 >	0.03	0.00	0.00	0.00	-
4074	48	03/10/89	PO4	0.984	0.02 >	0.03 >	1.72	0.00 >	0.03	0.00	0.00 >	0.03	0.00	0.00 >	2.81	+
4074	48	12/07/89	STB	1.211 <	1.00	1.60 >	1.60 <	1.00	2.68 >	2.68 <	1.00	26.80 >	26.82 >	1.60 >	6.33	+
4074	48	02/13/90	U13	0.798	0.04	1.27	29.84	0.15	2.39	16.02	0.00 >	10.00	0.00	8.50 >	31.63	+
4074	RS-1	03/14/91	3VL	0.638	0.52	1.63	3.14	0.84	2.75	3.27	0.00	27.60	0.00	1.94	6.79	+
4113	48	02/07/89	OL8	1.184	0.23 >	0.32 >	1.39	0.00 >	0.32	0.00	0.00 >	0.32	0.00	0.00 >	2.43	+
4113	48	03/10/89	PO5	0.961	0.31 >	0.32 >	1.05	0.00 >	0.32	0.00	0.00 >	0.32	0.00	0.00 >	1.48	+
4113	39	05/23/89	Q68	1.252	0.47 <	0.03 <	0.07	0.00	0.74	0.00	0.00	5.75	0.00	0.00	0.00	+
4113	39	09/11/89	RJ7	0.624	0.11 >	1.00 >	8.93	0.17 >	1.00 >	5.96	0.57 >	1.00 >	1.77 >	5.96 >	30.37	+
4113	39	11/01/90	ZXF	0.945	0.13 >	0.32 >	2.43	0.22 >	0.32 >	1.43	0.00 >	0.32	0.00 >	1.43 >	6.96	+
4113	39/48	03/14/91	3VM	0.610	0.29	1.45	4.94	0.66	2.57	3.88	0.00	9.22	0.00	2.19 >	12.45	+
4280	42	09/12/89	RL7	0.742	3.53 >	10.00 >	2.83	6.56 >	10.00 >	1.52	0.00 >	10.00	0.00 >	1.52 >	8.97	+
4280	42	11/01/90	ZXG	0.956	2.25	11.40	5.08	6.84	20.30	2.97	0.00	90.30	0.00	1.67 >	12.25	+
4280	77	03/13/91	3SY	1.172	1.73	16.80	9.73	6.14	44.60	7.27	0.00	281.00	0.00	2.74 >	13.28	+
4855	48	02/21/89	OOL	1.222	10.00	80.80	8.07	20.00	171.00	8.58	89.60 >	320.00 >	3.57	4.04 >	34.86	+
4855	48	09/12/89	RLG	0.751	7.22 >	320.00 >	44.31	13.40 >	320.00 >	23.82	29.30 >	320.00 >	10.93 >	23.82 >	55.28	+
4855	61	12/06/89	SPO	0.765	7.83 >	320.00 >	40.85	14.80 >	320.00 >	21.59	0.00 >	320.00	0.00 >	21.59 >	45.81	+
4855	48	12/06/90	1SA	0.986	5.76 >	100.00 >	17.38	11.90 >	100.00 >	8.37	29.00 >	100.00 >	3.45 >	8.37 >	39.69	+
4855	48	03/13/91	3T1	1.273	9.39	187.00	19.95	20.50	275.00	13.42	87.50 >	320.00 >	3.66	9.16	34.16	+

Table 12 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
7940	74	10/16/90	2**	0.908	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00	1.07	-
7940	75	11/08/9	1**	0.983	49.10	274.00	*****	0.00	514.00	0.00	0.00	1.00	0.00	0.00 >	11.47	+
7940	75	03/28/91	488	1.065	46.40	320.00	1.90	86.20	1670.00	19.40	0.00	3050.00	0.00	3.71	25.69	+
7949	75	12/12/90	1TS	1.153	0.00	66.00	0.00	0.00	290.00	0.00	0.00 >	3200.00	0.00	0.00	0.00	-
7949	75	03/28/91	488	1.023	466.00	3.08	0.01	677.00	85.50	0.13	0.00	913.00	0.00	0.00	0.00	+
8212	75	11/29/90	1HO	0.867	0.00	660.00	0.00	0.00	1560.00	0.00	0.00	3040.00	0.00	0.00	0.00	-
8212	75	03/20/91	3ZZ	1.220	225.00	802.00	3.56	722.00	1580.00	2.19	0.00	3040.00	0.00	1.11	8.16	+
8225	75	12/06/90	1RD	1.265	1.18	27.60	23.37	32.00	202.00	6.30	0.00 >	320.00	0.00	0.86 >	19.75	+
8225	75	03/20/91	402	1.274	2.42	77.30	31.95	10.00	302.00	30.17	0.00 >	320.00	0.00	7.73 >	25.78	+
8228	75	12/06/90	1RE	1.258	150.00	79.20	0.53	0.00	627.00	0.00	0.00	2870.00	0.00	0.00 >	1.40	+
8228	75	03/20/91	403	1.300	167.00	446.00	2.67	298.00	630.00	2.11	0.00	963.00	0.00	1.49	6.37	+
8237	75	12/06/90	1RJ	1.197	1030.0	320.00	0.31	0.00	2540.00	0.00	0.00 >	3200.00	0.00	0.00	0.00	+
8237	75	03/20/91	405	1.241	231.00	94.30	0.41	2550.00 >	3200.00 >	1.26	0.00 >	3200.00	0.00	0.04 >	2.83	+
8240	75	12/06/90	1RK	1.097	374.00	1130.0	3.03	714.00	2230.00	3.13	0.00 >	3200.00	0.00	1.59 >	6.17	+
8240	75	03/20/91	405	1.241	225.00	260.00	1.16	684.00	1650.00	2.41	0.00	3050.00	0.00	0.38 >	4.46	+
8261	76	12/12/90	1U2	0.826	64.00	622.00	9.72	170.00	2940.00	17.28	0.00 >	3200.00	0.00	3.66	24.07	+
8261	76	03/20/91	409	1.263	142.00	247.00	1.74	293.00	1440.00	4.91	0.00 >	3200.00	0.00	0.84 >	4.51	+
8271	76	12/12/90	1VI	0.679	77.30	476.00	6.15	189.00	830.00	4.40	0.00 >	1000.00	0.00	2.52 >	11.88	+
8271	76	03/21/91	44W	1.320	73.30	400.00	5.46	273.00	600.00	2.20	0.00	960.00	0.00	1.46	6.66	+
8352	75	11/30/90	1L7	0.941 <	1.00	2.43 >	2.43	0.00	5.24	0.00	0.00	27.00	0.00	0.00 >	5.07	+
8352	75	03/28/91	48G	0.924	2.49	8.20	3.30	6.14	20.10	3.27	0.00 >	32.00	0.0	1.34	11.57	+
8353	75	11/30/90	1L7	0.941	32.80	163.00	4.97	58.60 >	320.00 >	5.46	0.00 >	320.00	0.00	2.78 >	18.13	+
8353	75	03/27/91	46D	1.063	15.60	76.70	4.93	31.10	377.00	12.10	0.00 >	1000.00	0.00	2.47 >	16.30	+
8372	76	01/17/91	2Q3	0.595	498.00 >	3200.0 >	6.43	890.00 >	3200.00 >	3.59	0.00 >	3200.00	0.00 >	3.59 >	15.24	+
8372	76	03/21/91	453	1.270	1090.0	505.00	0.46	3200.00	4300.00	1.34	0.00 >	10000.0	0.00	0.16	0.21	+
8374	76	01/17/91	2Q4	0.640	108.00	1700.0	15.73	210.00	2470.00	11.77	929.00 >	3200.00 >	3.44	8.12 >	35.14	+
8374	76	03/21/91	454	1.131	131.00	1000.0	7.65	827.00	1730.00	2.10	0.00	3050.00	0.00	1.21 >	10.36	+
8511	76	12/20/90	24R	0.741	249.00 >	3200.0 >	12.83	599.00 >	3200.00 >	5.34	0.00 >	3200.00	0.00 >	5.34 >	27.10	+
8511	76	03/21/91	457	1.161	166.00	1000.0	6.01	1570.00	5070.00	3.23	0.00	9510.00	0.00	0.64	11.32	+
9121	77	01/31/91	2XI	0.874	805.00 >	3200.0 >	3.98	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	2.86	+
9121	77	10/09/91	AMU	0.674	1040.0 >	10000 >	9.61	2840.00 >	10000.0 >	3.52	0.00 >	10000.0	0.00 >	3.52 >	23.48	+
9127	77	01/31/91	2XL	0.871	168.00	149.00	0.89	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00	0.00	+
9127	77	10/09/91	ANV	0.657	468.00	4140.0	8.84	1430.00	6090.00	4.25	0.00	9610.00	0.00	2.88 >	21.55	+
9128	77	01/31/91	2XN	0.858	66.10	505.00	7.65	2990.00 >	3200.00 >	1.07	0.00 >	3200.00	0.00	0.17 >	5.41	+
9128	77	10/09/91	ANW	0.647	505.00	3930.0	7.78	1830.00	5950.00	3.25	0.00	9600.00	0.00	2.15	13.71	+

Table 12 (Cont'd)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9149	77	02/06/91	31K	0.991	563.00	2680.0	4.75	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	9.65	+
9149	77	10/09/91	AHY	0.660	453.00	6600.0	14.58	5940.00 >	10000.0 >	1.68	0.00 >	10000.0	0.00	1.11 >	21.13	+
9164	77	02/06/91	31R	0.96[illegible]	1360.0	320.00	0.24	3200.00 >	3200.00 >	1.00	0.00 >	3200.00	0.00	0.10 >	1.01	+
9164	77	10/09/9[illegible]	AI0	0.715	0.00	1550.0	0.00	0.00	2100.00	0.00	0.00	3090.00	0.00	0.00	2.21	-
9167	77	02/06/91	31T	0.961	909.00	773.00	0.85	0.00	3100.00	0.00	0.00 >	3200.00	0.00	0.00	0.00	+
9167	77	10/09/91	AI1	0.732	722.00	1370.0	1.89	1820.00	3820.00	2.10	0.00	9380.00	0.00	0.75	4.19	+
9173	77	02/07/91	347	0.990	1410.0	163.00	0.12	2860.00 >	3200.00 >	1.12	0.00 >	3200.00	0.00	0.06 >	3.11	+
9173	77	10/09/91	AI3	0.748	1160.0	5990.0	5.16	2450.00	8770.00	3.59	0.00 >	10000.0	0.00	2.45	11.33	+
9183	77	02/07/91	34C	0.717 <	10.00	66.00 >	6.60 <	10.00	100.00 >	10.00	0.00	879.00	0.00 >	6.60 >	19.18	+
9183	77	10/09/91	AI4	0.820	14.70	100.00	6.79	26.10	210.00	8.06	0.00 >	320.00	0.00	3.84	18.68	+
9184	77	02/07/91	34D	0.802	10.30	106.00	10.34	21.30	268.00	12.59	0.00	982.00	0.00	5.00 >	19.38	+
9184	77	10/09/91	AI4	0.820	40.20	371.00	9.22	62.30	624.00	10.02	0.00 >	1000.00	0.00	5.95	25.72	+
9199	77	02/13/91	36T	0.881	496.00	254.00	0.51	0.00	1200.00	0.00	0.00	3000.00	0.00	0.00 >	0.02	+
9199	77	10/09/91	AI5	0.844	536.00	791.00	1.48	918.00	1650.00	1.80	0.00	3050.00	0.00	0.86	2.05	+
9202	77	02/13/91	36U	0.914	545.00	569.00	1.05	0.00	1240.00	0.00	0.00	3000.00	0.00	0.00	0.00	+
9202	77	10/09/9[illegible]	AI5	0.844	537.00	941.00	1.75	902.00	1690.00	1.88	0.00	3050.00	0.00	1.04	3.04	+
9217	77	02/13/91	371	0.919	164.00	1330.0	8.08	305.00	1950.00	6.40	0.00	3080.00	0.00	4.35	17.01	+
9217	77	10/09/91	AI7	0.701	443.00	2380.0	5.36	873.00 >	3200.00 >	3.66	0.00 >	3200.00	0.00	2.72 >	15.08	+
9227	77	02/14/91	39Q	0.698	0.00	151.00	0.00	0.00	346.00	0.00	0.00	935.00	[illegible].00	0.00	[illegible].00	-
9227	77	10/09/91	AI8	0.738	112.00	210.00	1.88	277.00	320.00	1.16	0.00	932.00	0.00	0.76	5.81	+
9238	77	02/14/91	39V	0.744 <	3.20 <	3.20 -	1.00	0.00	238.00	0.00	0.00	923.00	0.00	0.00 >	2.86	+
9238	77	10/10/91	AJV	0.851 <	3.20	143.00 >	44.56	3.20	202.00	63.04	0.00	308.00	0.00	44.56 >	34.54	+
9247	77	02/14/91	3A0	0.614	148.00	547.00	3.68	249.00	901.00	3.61	0.00	3090.00	0.00	2.19	9.17	+
9247	77	10/10/91	AJW	0.745	0.00	550.00	0.00	0.00	779.00	0.00	0.00	2780.00	0.00	0.00 >	7.59	-
9248	77	02/14/91	3A0	0.614	230.00 >	3200.0 >	13.94	708.00 >	3200.00 >	4.52	0.00 >	3200.00	0.00 >	4.52 >	20.10	+
9248	77	10/10/91	AJW	0.745	124.00	490.00	3.94	309.00	660.00	2.14	0.00	966.00	0.00	1.59	9.99	+
9255	77	03/12/91	3XH	0.773	0.00	616.00	0.00	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00	0.72	-
9255	77	10/10/91	AJY	0.778	62.00	4990.0	80.46	183.00	6780.00	36.95	0.00	10000.0	0.00	27.20 >	50.00	+
9258	77	03/12/91	3XJ	0.723	0.00	95.10	0.00	0.00	1160.00	0.00	0.00	3000.00	0.00	0.00	0.00	-
9258	77	10/10/91	AJZ	0.758	280.00	320.00	1.14	909.00	1650.00	1.81	0.00	3100.00	0.00	0.35 >	6.08	+
9414	81	07/10/91	73N	0.887	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00 >	0.40	-
9414	81	10/08/91	ACA	1.121	339.00 >	1000.0 >	2.95	486.00 >	1000.00 >	2.06	930.00 >	1000.00 >	1.07 >	2.06 >	13.25	+
9433	80	07/10/91	73N	0.788	100.00 >	320.00 >	3.20	264.00 >	320.00 >	1.21	0.00 >	320.00	0.00 >	1.21 >	10.94	+
9433	80	10/08/91	ACG	0.931	231.00	1000.0	4.33	516.00	1730.00	3.36	0.00	3050.00	0.00	1.94	14.40	+

Table 12 (Cont'd)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9436	80	07/10/91	73J	0.937	163.00 >	320.00 >	1.96	312.00 >	320.00 >	1.03	0.00 >	320.00	0.00 >	1.03 >	6.14	+
9436	80	10/08/91	ACH	0.874	247.00	496.00	2.01	0.00	706.00	0.00	0.00 >	1000.00	0.00	0.00 >	6.40	+
9439	79	04/24/91	4VE	1.373	201.00 >	320.00 >	1.59	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	1.81	+
9439	79	10/01/91	A3N	0.866	1590.0 >	3200.0 >	2.02	2520.00 >	3200.00 >	1.27	0.00 >	3200.00	0.00 >	1.27 >	5.88	+
9445	79	04/24/91	4VH	1.197	11.70	30.40	2.60	30.80	54.70	1.78	0.00	99.00	0.00	0.99	7.88	+
9445	79	10/01/91	A3O	0.932	1.42	49.30	34.81	5.18	66.70	12.87	0.00	97.90	0.00	9.52 >	29.19	+
9446	79	04/24/91	4VI	1.187	47.80	128.00	2.69	81.00	193.00	2.39	0.00	310.00	0.00	1.59	7.75	+
9446	79	10/01/91	A3O	0.932	0.00	159.00	0.00	0.00	217.00	0.00	0.00	433.00	0.00	0.00	0.14	-
9448	79	04/24/91	4VJ	1.204	143.00	256.00	1.79	224.00 >	320.00 >	1.43	0.00 >	320.00	0.00	1.14 >	3.55	+
9448	79	10/01/91	A3P	0.889	224.00	492.00	2.19	0.00	663.00	0.00	0.00	973.00	0.00	0.00 >	5.18	+
9449	79	04/24/9	4.'	1.204	0.00	51.20	. 3	0.00	73.00	0.00	3.00	31.00	0.0	0.00	0.00	-
9449	79	10/01/9.	A3Q	0.979	48.60	115.00	2.36	74.00	189.00	2.56	0.00	433.00	0.00	1.55	6.40	+
9454	79	04/24/91	4VM	1.252 <	1.00 >	320.00 >	320.00	2.45 >	320.00 >	130.79	0.00 >	320.00	0.00 >	130.79 >	46.75	+
9454	79	10/01/91	A5L	0.848 <	3.20	601.00 >	187.93	5.66 >	1000.00 >	176.78	0.00 >	1000.00	0.00	106.31 >	47.86	+
9455	79	04/24/91	4VM	1.252	1.20	227.00	188.39	4.68 >	320.00 >	68.40	0.00 >	320.00	0.00	48.50 >	34.36	+
9455	79	10/01/91	A5L	0.848	4.58	550.00	120.08	11.20	779.00	69.39	0.00 >	1000.00	0.00	48.94 >	39.49	+
9458	79	04/24/91	4VO	1.231	39.20	145.00	3.70	57.90	211.00	3.65	0.00 >	320.00	0.00	2.50 >	13.51	+
9458	79	10/01/91	A5M	0.809	16.80	204.00	12.15	35.50	308.00	8.67	90.20	928.00	10.29	5.74 >	29.64	+
9460	79	04/24/91	4VP	1.206	10.90	87.00	7.95	16.00	196.00	12.29	31.50 >	320.00 >	10.15	5.45 >	26.62	+
9460	79	10/01/91	A5M	0.809	6.29	88.70	14.09	12.50	310.00	24.71	29.10	957.00	32.83	7.08 >	35.92	+
9475	79	04/25/91	50A	1.006	4.75	26.20	5.51	19.40	50.40	2.59	0.00	247.00	0.00	1.35 >	14.51	+
9475	79	10/01/91	A5N	0.853	3.20	22.80	7.12	8.85	46.30	5.23	0.00	100.00	0.00	2.57 >	15.26	+
9511	80	07/10/91	72M	0.956	39.60	57.50	1.45	71.70	163.00	2.27	0.00	304.00	0.00	0.80 >	3.82	+
9511	80	10/08/91	AEC	1.000	64.50	38.60	0.60	0.00	93.40	0.00	0.00	297.00	0.00	0.00	0.00	+
9530	79	04/25/91	50E	1.045	0.00	21.50	0.00	0.00	34.80	0.00	0.00	98.60	0.00	0.00 >	0.41	-
9530	79	10/01/91	A5P	0.835	12.10	49.90	4.11	16.80	67.80	4.04	30.00	100.00	3.33	2.98	18.71	+
9541	79	04/25/91	50J	0.852 <	1.00 <	1.00 -	1.00	0.00 <	1.00	0.00	0.00	2.91	0.00	0.00	0.00	+
9541	79	10/01/91	A5P	0.835	0.15	0.61	4.00	0.29	0.95	3.28	0.00 >	1.00	0.00	2.10	11.29	+
9543	79	04/25/91	50K	1.224 <	1.00	1.35 >	1.35 <	1.00	1.96 >	1.96	0.00	3.08	0.00 >	1.35 >	1.25	+
9543	79	10/01/91	A5Q	0.788	0.12	0.54	4.58	0.00	0.77	0.00	0.00	2.85	0.00	0.00 >	12.87	+
9545	79	04/25/91	50L	1.047 <	1.00	1.55 >	1.55 <	1.00	2.10 >	2.10	0.00	3.09	0.00 >	1.55 >	2.27	+
9545	79	10/01/91	A5R	1.010	0.20	0.71	3.52	0.00	1.27	0.00	0.00	3.01	0.00	0.00	7.86	+
9589	79	05/02/91	55E	0.958	1.55 >	320.00 >	205.97	2.41 >	320.00 >	132.57	92.30 >	320.00 >	3.47 >	132.57 >	45.61	+
9589	79	10/01/91	A5S	0.810	19.90	490.00	24.56	39.90	660.00	16.54	91.20	966.00	10.59	12.28	42.07	+

Table 12 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9590	79	05/02/91	55F	1.047	45.80	68.90	1.51	0.00	232.00	0.00	0.00 >	320.00	0.00	0.00	2.36	+
9590	79	10/01/91	A5T	0.741	16.30 >	1000.0 >	61.33	53.10 >	1000.00 >	18.83	445.00 >	1000.00 >	2.25 >	18.83 >	51.60	+
9607	79	05/08/91	5AE	1.176	2.64	16.10	6.11	0.00	26.30	0.00	0.00	95.90	0.00	0.00 >	9.81	+
9607	79	10/01/91	A5W	0.837	1.09	18.90	17.37	5.13	27.70	5.41	0.00	92.60	0.00	3.68 >	24.32	+
9680	79	05/22/91	5JC	1.079 <	1.00	1.30 >	1.30	0.00	1.94	0.00	0.00	3.07	0.00	0.00 >	0.50	+
9680	79	10/02/91	A8A	0.941	0.40	1.50	3.75	0.74	2.07	2.78	0.00	3.09	0.00	2.02 >	11.47	+
9767	79	05/29/91	5UX	0.830	0.00 <	1.00	0.00	0.00 <	1.00	0.00	0.00 <	1.00	0.00	0.00	0.00	-
9767	79	10/03/91	A9W	1.039	0.01	0.02	2.48	0.03	0.04	1.60	0.00	0.10	0.00	0.71	4.10	+
9842	79	06/05/91	630	1.242	32.00	188.00	5.88	0.00	310.00	0.00	0.00 >	320.00	0.00	0.00	4.94	+
9842	79	10/03/91	AA6	1.041	36.40	152.00	4.17	62.30	216.00	3.47	0.00	660.00	0.00	2.44	12.66	+
9849	79	06/06/91	65X	1.030 <	1.00	19.90 >	19.94	8.81	41.40	4.70	0.00	94.10	0.00	2.26 >	16.60	+
9849	79	10/03/91	AA8	1.102	2.39	13.00	5.42	0.00	23.50	0.00	0.00	88.70	0.00	0.00 >	8.02	+
9874	79	06/11/91	6A4	1.056	1.79	48.10	26.90	6.84	65.60	9.60	0.00	97.20	0.00	7.04	15.45	+
9874	79	10/08/91	AC6	0.892	0.00	46.30	0.00	0.00	64.20	0.00	0.00	96.40	0.00	0.00 >	7.09	-
9886	79	06/11/91	6AA	1.036	3.32	6.98	2.10	8.08	14.50	1.80	0.00	30.30	0.00	0.86 >	6.49	+
9886	79	10/08/91	AC7	0.895	7.22	10.60	1.46	0.00	17.70	0.00	0.00	30.60	0.00	0.00 >	5.24	+
9916	79	06/12/91	6CE	1.444	51.40	1730.0	33.69	167.00	3040.00	18.26	0.00 >	3200.00	0.00	10.40 >	36.08	+
9916	79/C2	08/14/91	8C0	0.749	417.00	1670.0	4.01	630.00	2340.00	3.72	0.00 >	3200.00	0.00	2.65	13.33	+
9931	79	06/13/91	6GI	1.270	28.80	100.00	3.47	74.50	471.00	6.32	0.00 >	3200.00	0.00	1.34	7.25	+
9931	79/C2	08/14/91	8C2	0.746	0.00	586.00	0.00	0.00	851.00	0.00	0.00 >	3200.00	0.00	0.00 >	0.20	-
9937	79	06/13/91	6GL	1.188	60.30	1130.0	18.77	274.00	1950.00	7.12	0.00 >	3200.00	0.00	4.13 >	22.63	+
9937	79/C2	08/14/91	8C2	0.746	0.00	550.00	0.00	0.00	779.00	0.00	0.00	2850.00	0.00	0.00	0.00	-
9962	79	06/18/91	6JF	0.722	146.00	1520.0	10.40	320.00	2080.00	6.19	0.00	3090.00	0.00	4.74	21.72	+
9962	79/C2	08/14/91	8C4	0.774	537.00	1560.0	2.90	902.00	2110.00	2.34	0.00	3110.00	0.00	1.73	7.41	+
9970	79	06/18/91	6JJ	0.680	56.60	624.00	11.02	123.00	927.00	7.52	291.00	2950.00	10.14	5.06 >	29.27	+
9970	79/C2	08/14/91	8C6	0.773	203.00	509.00	2.50	0.00	698.00	0.00	0.00 >	3200.00	0.00	0.00	5.40	+
9990	79	06/19/91	6LW	1.210	12.50	361.00	28.85	37.30	701.00	18.81	0.00 >	1000.00	0.00	9.69 >	33.07	+
9990	79/C2	08/14/91	8C8	0.878	0.00	192.00	0.00	0.00	283.00	0.00	0.00	915.00	0.00	0.00	0.31	-
9998	79	06/19/91	6M0	1.169	0.00	128.00	0.00	0.00	192.00	0.00	0.00	307.00	0.00	0.00 >	4.36	-
9998	79/C2	08/14/91	8C9	0.880	40.50	106.00	2.61	69.40	177.00	2.55	0.00	306.00	0.00	1.52	8.70	+
10002	79	06/19/91	6M2	1.067	33.00	296.00	8.98	71.30	874.00	12.26	0.00	2960.00	0.00	4.16 >	15.64	+
10002	79/C2	08/14/91	8CA	0.808	0.00	570.00	0.00	0.00	820.00	0.00	0.00 >	1000.00	0.00	0.00	0.00	-
10030	79	07/03/91	6U2	0.849	24.50	290.00	11.84	45.30	521.00	11.50	92.40	952.00	10.31	6.39 >	31.75	+
10030	79/C2	08/14/91	8C8	0.872	46.10	169.00	3.66	66.40	238.00	3.58	0.00	830.00	0.00	2.54	11.63	+

Table 12 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
10031	79	07/03/91	6U2	0.849	112.00	1580.0	14.16	176.00	2160.00	12.28	715.00	3200.00	4.47	8.99 >	37.91	+
10031	79/C2	08/14/91	8CC	0.759	0.00	490.00	0.00	0.00	660.00	0.00	0.00	966.00	0.00	0.00	1.22	-
10047	79	07/03/91	6UA	0.795	15.50	157.00	10.09	33.90	213.00	6.29	0.00	315.00	0.00	4.62 >	25.11	+
10047	79/C2	08/14/91	8CC	0.759	0.00	157.00	0.00	0.00	215.00	0.00	.00	318.00	0.00	0.00	0.00	-
11044	78	04/04/91	419	1.146	12.30	37.90	3.09	21.70	74.90	3.45	0.00 >	320.00	0.00	1.75	8.77	+
11044	78	10/10/91	AK0	1.077	4.45	70.90	15.91	10.90	320.00	29.33	0.00 >	320.00	0.00	5.49 >	30.04	+
11045	78	04/04/91	41A	1.110	121.00	320.00	2.64	197.00	682.00	3.46	0.00 >	3200.00	0.00	1.62	7.58	+
11045	78	10/10/91	AK1	1.042	38.70	425.00	10.97	100.00	686.00	6.86	0.00	2650.00	0.00	4.25	21.32	+
11064	78	04/04/91	41J	1.095	0.00	887.00	0.00	0.00	2180.00	0.00	0.00	8870.00	0.00	0.00	0.00	-
11064	78	10/10/91	AK3	0.841	514.00	2070.0	4.02	3200.00	4040.00	1.26	0.00	9400.00	0.00	0.65 >	11.47	+
11086	78	04/10/91	41X	1.213	0.00	649.00	0.00	0.00	1570.00	0.00	0.00 >	3200.00	0.00	0.00	0.00	-
11086	78	10/08/91	AEN	0.917	1660.0	2520.0	1.52	2820.00	4950.00	1.76	0.00	9890.00	0.00	2.89	2.56	+
11095	78	04/11/91	4N7	0.986	1900.0	5610.0	2.95	0.00	8350.00	0.00	0.00 >	10000.0	0.00	0.00 >	11.45	+
11095	78	10/08/91	AEO	0.941	603.00 >	10000 >	16.59	6550.00 >	10000.0 >	1.53	0.00 >	10000.0	0.00 >	1.53 >	20.10	+
11118	78	04/11/91	4NN	0.915	3200.0 >	10000 >	3.13	0.00 >	10000.0	0.00	0.00 >	10000.0	0.00	0.00 >	11.61	+
11118	78	10/15/91	ALO	1.037	3440.0	3470.0	1.01	8370.00 >	10000.0 >	1.19	0.00 >	10000.0	0.00	0.41 >	0.51	+
11123	78	04/17/91	40G	0.988	566.00	7030.0	12.42	1920.00 >	10000.0 >	5.20	0.00 >	10000.0	0.00	3.65 >	22.93	+
11123	78	10/15/91	ALQ	0.917	765.00 >	10000 >	13.07	1630.00 >	10000.0 >	6.14	0.00 >	10000.0	0.00 >	6.14 >	27.68	+
11131	78	04/17/91	40K	1.033	1150.0	2310.0	2.00	2090.00	4140.00	1.98	0.00	9410.00	0.00	1.11 >	8.47	+
11131	78	10/15/91	ALU	0.957	5370.0	5590.0	1.04	0.00	8740.00	0.00	0.00 >	10000.0	0.00	0.00	0.00	+
11133	78	04/17/91	40L	0.904	388.00	3460.0	8.92	629.00	5640.00	8.97	0.00	9560.00	0.00	5.50 >	26.52	+
11133	78	10/15/91	ALU	0.957	1160.0	2800.0	2.42	2870.00	5840.00	2.04	0.00 >	10000.0	0.00	0.98 >	8.67	+
11155	78	04/18/91	4R2	0.865	0.00	103.00	0.00	0.00	175.00	0.00	0.00	306.00	0.00	0.00	2.12	-
11155	78	10/10/91	AK6	0.850	154.00 >	320.00 >	2.07	238.00 >	320.00 >	1.34	0.00 >	320.00	0.00 >	1.34 >	5.64	+
11185	80	07/09/91	6Y3	1.018	0.00	492.00	0.00	0.00	663.00	0.00	0.00	973.00	0.00	0.00 >	5.90	-
11185	80	10/08/91	AEF	0.888	366.00 >	1000.0 >	2.73	512.00 >	1000.00 >	1.95	935.00 >	1000.00 >	1.07 >	1.95 >	17.23	+
11191	80	07/09/91	6Y6	1.078	449.00	1290.0	2.88	1440.00	3130.00	2.17	0.00 >	3200.00	0.00	0.90 >	10.59	+
11191	80	10/08/91	AEM	0.862	377.00 >	1000.0 >	2.66	850.00 >	1000.00 >	1.18	0.00 >	1000.00	0.00 >	1.18 >	11.57	+
11223	80	07/10/91	72R	0.908	7.52	60.40	8.04	13.80	91.30	6.63	29.40 >	320.00 >	10.88	4.39	24.35	+
11223	80	10/08/91	AEH	0.862	13.20	187.00	14.22	22.30	291.00	13.03	0.00 >	320.00	0.00	8.38	32.54	+
11224	80	07/10/91	72S	0.934	2.21	19.60	8.91	4.33	29.30	6.76	9.65	270.00	27.98	4.24 >	24.56	+
11224	80	10/08/91	AEI	1.076	4.17	47.00	11.26	8.09	81.00	10.01	0.00 >	320.00	0.00	5.80	23.68	+
11447	81	07/31/91	7U3	0.860	0.00	389.00	0.00	0.00	604.00	0.00	0.00	991.00	0.00	0.00	2.61	-
11447	82	08/14/91	8H9	0.796	141.00	541.00	3.83	212.00	762.00	3.58	0.00 >	3200.00	0.00	2.55	12.84	+

Table 12 (Cont'd)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25		TC 25		AI 25	IC 50		TC 50		AI 50	IC 95		TC 95		AI 95	SI		TAI	ACT
11678	81	07/16/91	760	1.089	203.00	>	320.00	>	1.58	0.00	>	320.00		0.00	0.00	>	320.00		0.00	0.00	>	1.08	+
11678	81	10/08/91	AEK	0.914	129.00		635.00		4.90	205.00	>	1000.00	>	4.87	0.00	>	1000.00		0.00	3.09		13.43	+
11679	81	07/10/91	730	0.772	< 1.00		12.60	>	12.59	6.31		19.10		3.02	0.00		30.70		0.00	2.00	>	14.84	+
11679	81	10/08/91	AEK	0.914	4.25		11.10		2.61	0.00		18.10		0.00	0.00		30.60		0.00	0.00		4.14	+

DIFRNTL = The differential is the difference in the cell control and the virus control optical densities.

$IC_{25,50,95}$ = (Viral) inhibitory concentration 25%, 50% and 95% = The drug concentration (µg/ml) that inhibited viral CPE by 25%, 50% or 95% calculated by using a regression analysis for semilog curve fitting.

$TC_{25,50,95}$ = (Cell) toxicity concentration 25%, 50% and 95% = The drug concentration (µg/ml) that reduced cell viability by 25%, 50% or 95%.

$AI_{25,50,95}$ = Antiviral Index = A single point ratio of the antiviral and anticellular effect of the compound, calculated with 25%, 50% or 95% reduction values (calculated by dividing the $TC_{25,50,95}$ by the $IC_{25,50,95}$).

SI = Selectivity Index = A ratio calculated by dividing the TC_{25} by the IC_{50} (based upon 6 one-half-log_{10} dilutions, µg/ml, the maximum scale is 0-320).

TAI = Total Antiviral Index = The area between the cytotoxicity and the antiviral curves (based upon a scale of 0-100%).

ACT = Activity = A "+" denotes a test that produced ≥25% reduction in CPE. A "-" denotes an inactive test (i.e. ≤25% reduction in CPE).

4.3.3 Japanese Encephalitis Virus (JE):

The total output of JE-testing during this reporting period is summarized in monthly increments in **Figure 40**. During this period 3938 tests were performed against JE-virus with MTT-assay format. Out of these, 192 were control compound assays - Selenazofurin (AVS-0253) and 122 were control compounds - 2-Thio-6-Azauridine (AVS-6724). Two hundred fifty-one tests were internal (+ + + +) virus load, cell load, and other quality control tests. The 31 tests were considered unsatisfactory based on the criteria of the quality controls set during this reporting period. The rest, totaling 3342, were actual single drug assays. The total number of assays represents approximately 67% above our yearly contractual obligations (i.e. 3342/2000).

Out of the 3342 test compounds, 82 demonstrated antiviral activity at greater than 50% reduction levels. This represents around 2.5% of the tested compounds having *in vitro* antiviral activity against JE-virus. The remainder, 3260 compounds (97.5%), are to be considered inactive with this assay protocol.

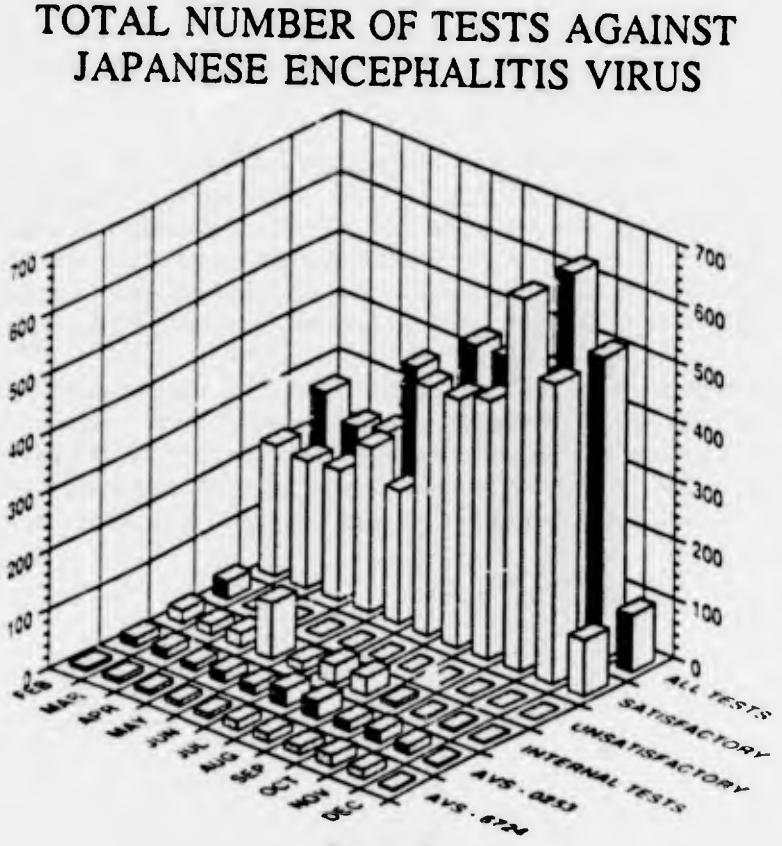


Figure 40

Quality Control Tests =	KPC (Positive Control - 2-Thio-6-Azauridine =	122
	KPC (Positive Control - Selenazofurin) =	192
	(+ + + +) (Internal Virus and Cell Load Controls) =	251
	(UT) Unsatisfactory Test (QC rejects) =	31
Accepted Single Drug Tests =		3342
Total Number of JE Tests =		3938

4.3.3.1 JE-Quality Controls:

4.3.3.1.1 Antiviral Activity of Selenazofurin vs JE Virus:

Control Compound-Antiviral Performance: Selenazofurin (AVS-0253) has been the sole control compound against JE in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of the positive control drug (Selenazofurin) are illustrated in **Figure 41-A**.

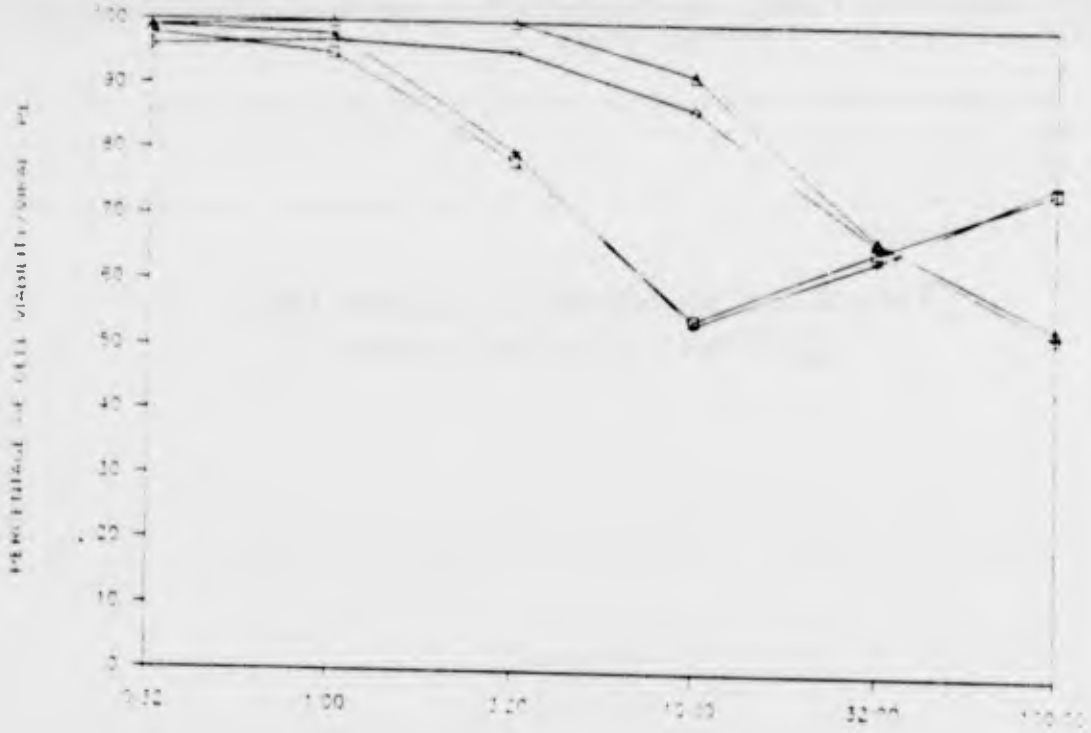
The 192 control tests performed with Selenazofurin gave a mean **Total Antiviral Index** (TAI) of 11% (SD ± 9.8) and the median value was 8.44%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from 0 - >51.64% during this period. The mean **Selectivity Index (SI)** was only 1.99 (SD ± 5.03) and the median SI value was 0, indicating poor antiviral selectivity for Selenazofurin and it ranged from 0 - >41.38 during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25% (AI_{25})** value was 7.76 (SD ± 15.95). The median AI_{25} value was 4.06 (range .049 - >114.4). The mean **Antiviral Index 50% (AI_{50})** was 5.53 (SD ± 8.59) with a median of 0 (range 0 - >42.6). This indicates that Selenazofurin does not consistently reach 50% antiviral reduction levels. The mean **Antiviral Index 95% (AI_{95})** was not attainable with Selenazofurin versus Japanese Encephalitis Virus.

The mean **Antiviral Inhibitory Concentration 25% (IC_{25})** was 4.54 μg/ml (SD ± 5.62). The median IC_{25} value was 3.8 μg/ml (range = 0.45 - 72.2 μg/ml). The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 3.06 μg/ml (SD ± 6.12). The median IC_{50} value was 0 μg/ml (range = 0 - 63.4 μg/ml). This discrepancy indicates that the control compound Selenazofurin does not consistently reach 50% reduction levels. The mean **Antiviral Inhibitory Concentration 95% (IC_{95})** could not be attained with Selenazofurin versus Japanese Encephalitis Virus.

The average maximum antiviral inhibitory level of 192 Selenazofurin tests (**Figure 41-A**) was reached at 10 μg/ml of the compound with 55% antiviral effect. Further increase of the drug concentration does not improve its antiviral activity. Maximum antiviral effect (~55%) was found with a simultaneous ~15% cytotoxic suppression. Above 10 μg/ml concentration the antiviral protection levels drop to ~25% at 100 μg/ml, while simultaneously the Selenazofurin becomes maximally toxic (~45%).

SELENAZOFURIN - VS - JE VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability Δ Median % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	0.32	1	3.2	10	32	100	0.32	1	3.2	10	32	100
Mean	98	95	79	55	∞	75	96	97	94	80	58	49
Median	99	98	80	54	64	76	98	100	97	82	57	49
Std. Dev.	0.04	0.07	0.11	0.15	0.15	0.14	0.05	0.05	0.07	0.14	0.11	0.10

Figure 41-A
Average Antiviral and Cytotoxicity Values for 192 Positive Control Compound Tests

4.3.3.1.2 Maximum Antiviral Effect of Selenazofurin vs JE Virus:

A bar graph scatter plot (**Figure 42-A**) depicts the distribution of the maximum antiviral reduction values of all 192 control compound assays for Selenazofurin. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 47% (SD ± 13.3) reduction levels. The maximum reduction levels vary from 25 - 82% but remain quite consistently around the median of 46%. The assay control values give a relatively broad, bell-shaped distribution curve. This indicates quite a consistent day-to-day performance of the control compound in the JE-MTT assay.

The positive control compound performance criteria for Selenazofurin versus the JE virus was set at 25% reduction level. Assays in which Selenazofurin did not meet this accepted quality control level (≥ 25%) were rejected (i.e., 31 unsatisfactory tests).

Since Selenazofurin is only marginally active against JE virus, better quality control compounds are needed. However, regardless of the poor performance of the JE quality control drug Selenazofurin, around 82 different compounds have equal or better antiviral activity against JE virus than AVS-0253. Some of these could certainly be used as a better *in vitro* antiviral control compound in this large-scale antiviral screening program.

Variation of the Maximum Antiviral Effect
JE Virus - VS - Selenazofurin

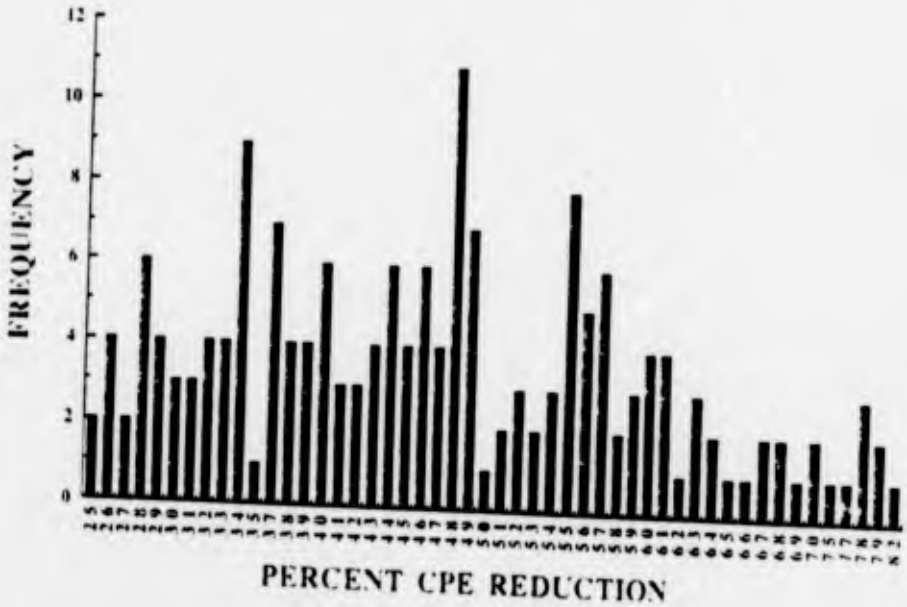


Figure 42-A
Maximum Antiviral CPE Reduction (%).
Summary of 192 Control Tests.

4.3.3.1.3 Cellular Cytotoxicity of Selenazofurin vs JE Virus:

JE-Control Compound-Cytotoxicity Performance: The 192 cytotoxicity values of the positive control compound Selenazofurin are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 19.31 μg/ml (SD ± 18.1) and the median was 16.7 μg/ml (range of 0.743 - > 100 μg/ml). The mean cell **Toxic Concentration 50% (TC_{50})** value was 73.3 μg/ml (SD ± 31.68) and the median was 90.3 μg/ml (range of 8.51 - > 100 μg/ml). The mean cell **Toxic Concentration 95% (TC_{95})** value was 99.32 μg/ml (SD ± 7.2) and the median was 100 μg/ml (range of > 32 - > 100 μg/ml).

As can be seen from **Figure 41-A**, the toxicity starts to become measurable above the concentration of 3.2 μg/ml and the maximum toxicity has not been reached at 100 μg/ml.

When the cytotoxicity reaches around 15% (10 μg/ml), the control compound (Selenazofurin) loses its maximum antiviral effect (45%). Above 10 μg/ml the antiviral protection of Selenazofurin starts to decrease down to (~25%), Selenazofurin becomes maximally toxic (~45%) at 100 μg/ml concentration. The highest Selenazofurin concentration tested in these assays was 100 μg/ml.

Selenazofurin has a definite cytotoxic suppression on cellular metabolism and growth. The TC_{25} and TC_{50} toxicity can be achieved with relative consistency at 100 μg/ml.

4.3.3.1.4 JE-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (Selenazofurin):

JE-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 192 control assays is plotted in **Figure 43-A.** The results indicate that the cell O.D. readings reached a mean of 1.178 (SD ± 0.12) with a median of 1.174 (range of 0.87 - 1.441). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

JE-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 192 control assays is presented in **Figure 44-A.** The results indicate that the average load O.D. reading is 0.32 (SD ± 0.107) with a median of 0.335 (range of 0.082 - 0.610). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

JE-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 192 control assays is provided in **Figure 45-A.** The results indicate that the average differential O.D. reading is 0.848 (SD ± 0.143) with a median of 0.829 (range 0.465 - 1.296). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 85% measurement accuracy.

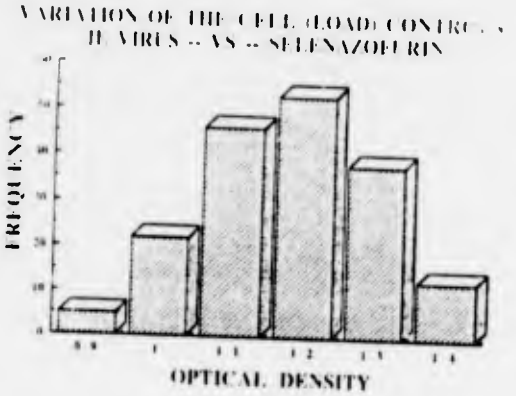


Figure 43-A

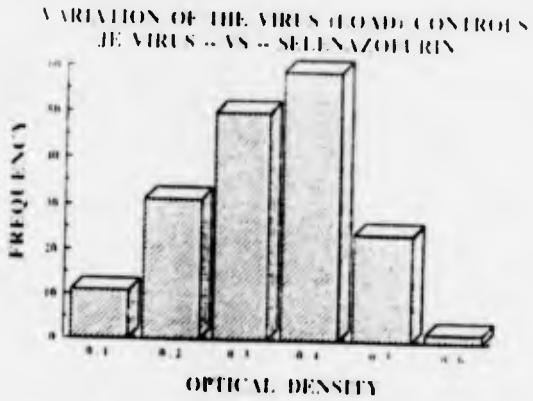


Figure 44-A

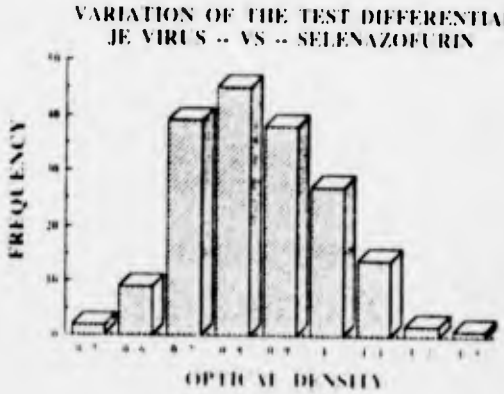


Figure 45-A

4.3.3.1 JE-Quality Controls:

4.3.3.1.1 Antiviral Activity of 2-Thio-6-Azauridine vs JE Virus:

Second Control Compound-Antiviral Performance: 2-Thio-6-Azauridine (AVS-6724) has been tested as a second control compound against JE in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of this second positive control drug are illustrated in **Figure 41-B**.

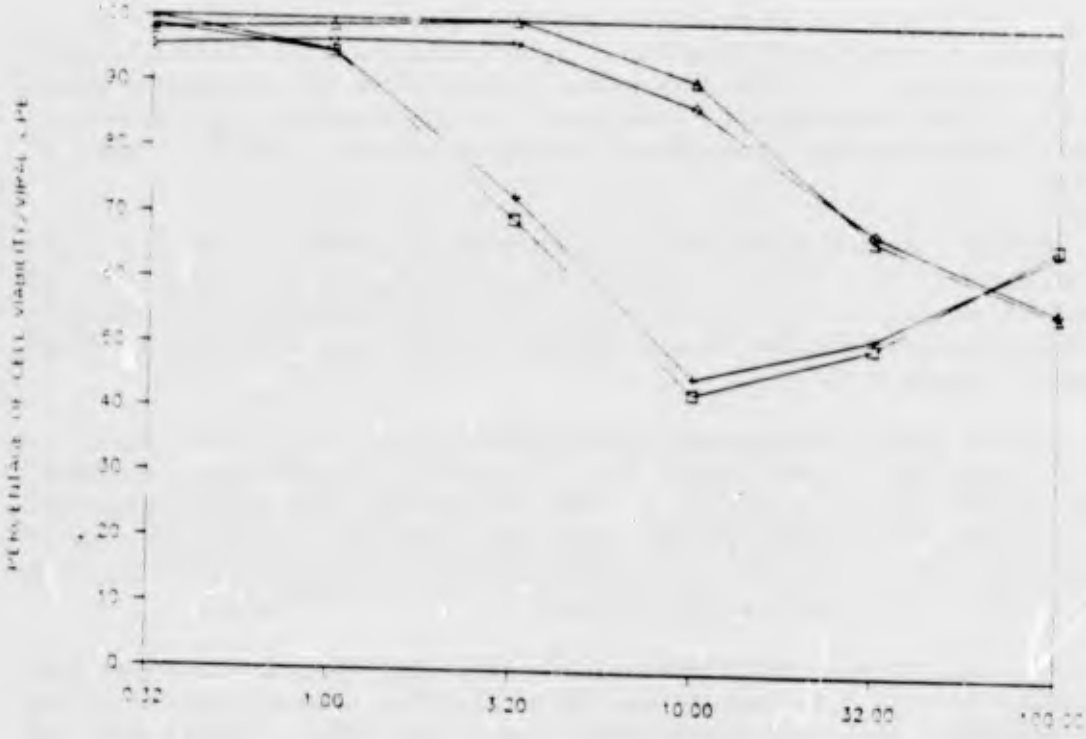
The 122 control tests performed with 2-Thio-6-Azauridine gave a mean **Total Antiviral Index** (TAI) of 20% (SD ± 13) and the median value was 18%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from 0 - >59.8% during this period. The mean **Selectivity Index (SI)** was only 6.1 (SD ± 8.98) and the median SI value was 2.98, indicating moderate antiviral selectivity for 2-Thio-6-Azauridine and it ranged from 0 - >43.25 during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25% (AI_{25})** value was 14.64 (SD ± 16.58). The median AI_{25} value was 7.68 (range 0.85 - 66.52). The mean **Antiviral Index 50% (AI_{50})** was 12.86 (SD ± 11.52) with a median of 11.01 (range 0 - >45.12). This indicates that 2-Thio-6-Azauridine does not consistently reach 50% antiviral reduction levels. The mean **Antiviral Index 95% (AI_{95})** was 0.32 (SD ± 1.9) with a median of 0 (range 0 - >12.02).

The mean **Antiviral Inhibitory Concentration 25% (IC_{25})** was 3.03 μg/ml (SD ± 1.4). The median IC_{25} value was 2.9 μg/ml (range = 1.03 - 8.05 μg/ml). The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 4 μg/ml (SD ± 10.07). The median IC_{50} value was 4.75 μg/ml (range = 0 - 94.2 μg/ml). This discrepancy indicates that the control compound 2-Thio-6-Azauridine does not consistently reach 50% reduction levels. The mean **Antiviral Inhibitory Concentration 95% (IC_{95})** was 0.24 μg/ml (SD ± 1.44). The median IC_{95} value was 0 μg/ml (range 0 - 8.98 μg/ml).

The average maximum antiviral inhibitory level of 122 2-Thio-6-Azauridine tests (**Figure 41-B**) was reached at 10 μg/ml of the compound with 55% antiviral effect. Further increase of the drug concentration does not improve its antiviral activity. Maximum antiviral effect (~57%) was found with a simultaneous ~10% cytotoxic suppression. Above 10 μg/ml concentration the antiviral protection levels off to ~30% reduction level at 100 μg/ml, while simultaneously the 2-Thio-6-Azauridine becomes maximally toxic (~45%).

2-THIO-6-AZAURIDINE - VS - JE VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability Δ Median % Cell Viability

% Viral CPE

% Cell Viability

Conc.(μg/ml)	0.32	1	3.2	10	32	100	0.32	1	3.2	10	32	100
Mean	99	95	70	43	51	66	96	97	97	87	68	57
Median	100	95	73	46	52	66	98	99	100	91	67	56
Std. Dev.	0.03	0.05	0.13	0.18	0.20	0.16	0.05	0.05	0.06	0.13	0.15	0.12

Figure 41-B
Average Antiviral and Cytotoxicity Values for 122 Positive Control Compound Tests

4.3.3.1.2 Maximum Antiviral Effect of 2-Thio-6-Azauridine vs JE Virus:

A bar graph scatter plot (Figur 42-B) depicts the distribution of the maximum antiviral reduction values of all 122 control compound assays for 2-Thio-6-Azauridine. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 61% (SD ± 15.95) reduction levels. The maximum reduction levels vary from 29 - 100% but remain quite consistently around the median of 59.5%. The assay control values give a relatively broad, bell-shaped distribution curve. This indicates quite a consistent day-to-day performance of the control compound in the JE-MTT assay.

Recommendations:

Based upon the data obtained in parallel studies with Selenazofurin, we recommend that 2-Thio-6-Azauridine (AVS #6724) be used as a second control compound against JE virus. It's overall performance is much better than the present control, Selenazofurin. It is readily available from Sigma Chemical Company, it is inexpensive and works as effectively at low drug concentrations as Selenazofurin.

Variation of the Maximum Antiviral Effect JE Virus - VS - 2-Thio-6-Azauridine

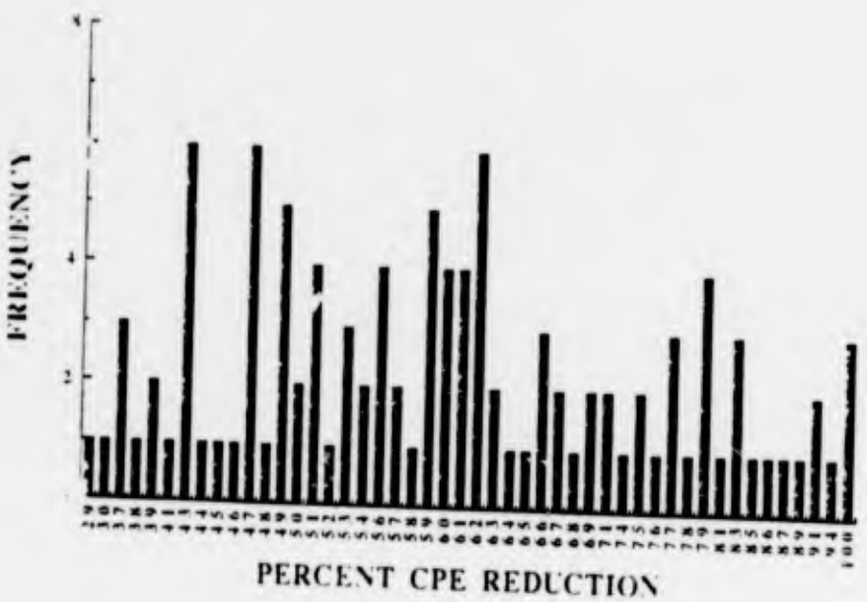


Figure 42-B
Maximum Antiviral CPE Reduction (%).
Summary of 122 Control Tests.

4.3.3.1.3 Cellular Cytotoxicity of 2-Thio-6-Azauridine vs JE Virus:

JE-Control Compound-Cytotoxicity Performance: The 122 cytotoxicity values of the positive control compound 2-Thio-6-Azauridine are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 32.85 μg/ml (SD ± 27.08) and the median was 24.7 μg/ml (range of 2.9 - > 100 μg/ml). The mean cell **Toxic Concentration 50%** (TC_{50}) value was 87.31 μg/ml (SD ± 25.6) and the median was 100 μg/ml (range of 8.4 - > 100 μg/ml). The mean cell **Toxic Concentration 95%** (TC_{95}) value was 99.37 μg/ml (SD ± 6.5) and the median was 100 μg/ml (range of > 32 - > 100 μg/ml).

As can be seen from **Figure 41-B**, the toxicity starts to become measurable above the concentration of 3.2 μg/ml and the maximum toxicity has not been reached at 100 μg/ml.

When the cytotoxicity reaches around 10% (10 μg/ml), the control compound (2-Thio-6-Azauridine) loses its maximum antiviral effect (55%). Above 10 μg/ml the antiviral protection of 2-Thio-6-Azauridine starts to decrease down to (~30%). 2-Thio-6-Azauridine becomes maximally toxic at 100 μg/ml concentration (40%). The highest 2-Thio-6-Azauridine concentration tested in these assays was 100 μg/ml.

2-Thio-6-Azauridine has a definite cytotoxic suppression on cellular metabolism and growth. The TC_{25} and TC_{50} toxicity can be achieved with relative consistency at 100 μg/ml.

4.3.3.1.4 **JE-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (2-Thio-6-Azauridine):**

JE-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 122 control assays is plotted in **Figure 43-B**. The results indicate that the cell O.D. readings reached a mean of 1.200 (SD ± 0.098) with a median of 1.194 (range of 0.866 - 1.429). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

JE-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 122 control assays is presented in **Figure 44-B**. The results indicate that the average load O.D. reading is 0.324 (SD ± 0.102) with a median of 0.330 (range of 0.080 - 0.558). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

JE-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 122 control assays is provided in **Figure 45-B**. The results indicate that the average differential O.D. reading is 0.876 (SD ± 0.121) with a median of 0.879 (range 0.533 - 1.175). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 88% measurement accuracy.

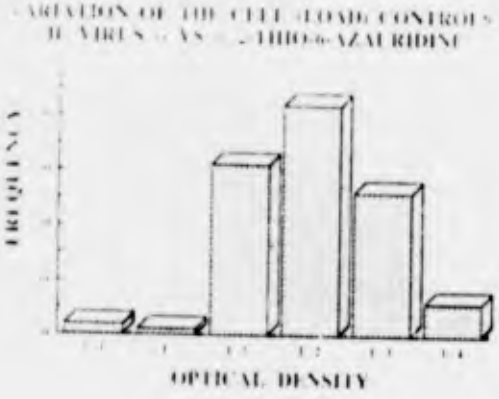


Figure 43-B

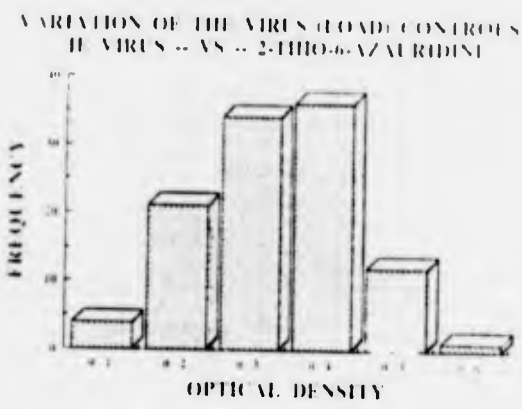


Figure 44-B

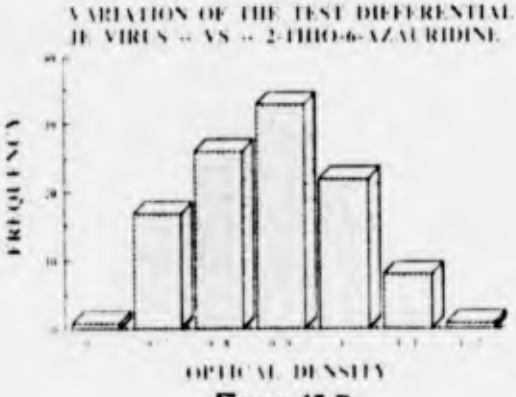


Figure 45-B

4.3.3.1.5 Overall JE-Assay Plate Quality Controls:

JE-Overall-Cell Load Performance: A bar graph scatter plot of the overall mean cell control (O.D. reading) of 3342 accepted assays is plotted in **Figure 43-C**. The results indicate that the overall cell O.D. readings reached a mean 1.180 (SD ± 0.121) with a median of 1.183 (range of 0.804 - 1.604). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

JE-Overall-Virus Load Performance: A bar graph scatter plot of the overall mean virus load O.D. readings of the 3342 accepted assays is presented in **Figure 44-C**. The results indicate that the overall average virus load O.D. reading is 0.302 (SD ± 0.108) with a median of 0.307 (range of 0.002 - 0.648). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

JE-Overall-Assay Differential Performance: A bar graph scatter plot of the overall mean O.D. differential values of the 3342 accepted assays is provided in **Figure 45-C**. The results indicate that the overall average differential O.D. reading is 0.878 (SD ± 0.129) with a median of 0.873 (range 0.444 - 1.351). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 88% measurement accuracy.

GRAPHIC ILLUSTRATION OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ALL ACCEPTED JE PLATE DATA

Figure 43-C

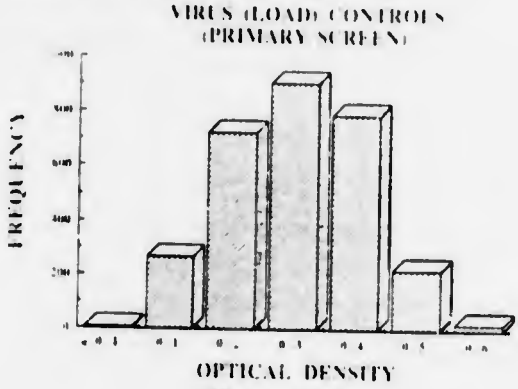


Figure 44-C

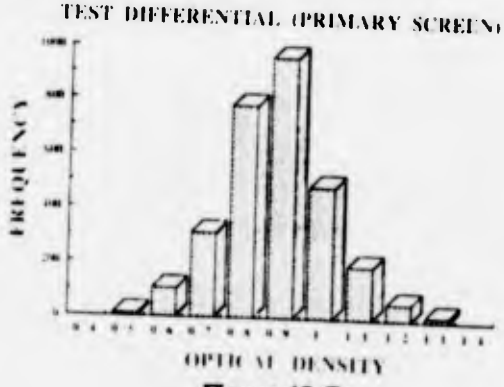


Figure 45-C

4.3.3.2 JE-Antiviral Activity Results:

New Drugs with 95% Antiviral Reduction Levels: Out of the 3342 actual single drug tests, 12 new compounds demonstrated excellent antiviral activity having antiviral reduction values of equal to or better than 95%. This represents around 0.4% of the compounds having antiviral activity at this excellent reduction level. These data is summarized in **Table 13**. Compounds AVS-11679, 9589, and 11506 demonstrated the greatest *in vitro* promise having TAI's that ranged from 42 - 57 and SI values that ranged from 9 - 39. Six compounds demonstrated moderate antiviral activity with TAI's that ranged from 14 - 29 and SI values that ranged from 0.5 - 9.7.

Table 13
AVS Compounds Active Against Japanese Encephalitis Virus (JE) at AI_{95} Level

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 95	TC 95	AI 95	SI	TAI
JE 11679	81	10/09/91	0.691	6.52	30.90	4.74	38.91 >	56.90
JE 9589	79	05/02/91	0.657	90.30 >	320.00 >	3.54 >	8.85 >	46.62
JE 11506	81	07/31/91	0.720	911.00	9660.00	10.60	12.34 >	42.38
JE 9916	79	06/12/91	1.135	909.00 >	3200.00 >	3.52	5.82	29.14
JE 9416	82	09/04/91	0.765	30.80 >	320.00 >	10.40	9.74 >	28.75
JE 11224	80	10/09/91	0.785	29.80	320.00	10.75	3.58 >	24.89
JE 11155	78	10/10/91	0.913	293.00 >	320.00 >	1.09 >	2.38 >	22.52
JE 9414	81	10/08/91	0.647	931.00 >	1000.00 >	1.07 >	2.04 >	15.72
JE 11376	81	07/18/91	1.075	945.00 >	3200.00 >	3.39	0.46 >	13.97
JE 11238	80	09/25/91	0.856	302.00 >	320.00 >	1.06 >	1.79 >	8.60
JE 10429	83	10/17/91	0.904	302.00 >	320.00 >	1.06	0.49 >	8.20
JE 9590	79	10/02/91	0.648	945.00	308.00	0.33	0.23 >	5.86

New Drugs with 50% Antiviral Reduction Levels: Out of the 3342 actual single drug tests, 70 new compounds demonstrated good antiviral activity, having antiviral reduction values equal to or better than 50%. This represents around 2.0% of the test compounds being active at this good antiviral reduction level. These compounds are summarized in **Table 14** according to the highest **Total Antiviral Index (TAI)**. AVS-11796 and 11907 demonstrated the best TAI's of 28 and 24% and SI's of 6.8 and 2.1, respectively. Other compounds demonstrated moderate antiviral activity, having TAI's that ranged from 0 - 22% and SI's from 0.4 - 4.2.

Table 14
Compounds Active Against Japanese Encephalitis (JE) at AI_{50} Level

AVS No.	Ship-ment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
** VIRUS JE								
JE 11796	82	08/21/91	0.573	56.60	590.00	10.44	6.82	> 28.24
JE 11907	82	09/26/91	1.040	14.70 >	320.00 >	21.72	2.10	> 24.07
JE 9909	81	07/10/91	0.784	60.40 >	320.00 >	5.29	4.90	> 22.13
JE 9202	77	02/27/91	0.849	508.00	2030.00	3.99	2.82	> 21.97
JE 11717	82	09/26/91	0.946	26.00 >	320.00 >	12.30	3.65	> 21.60
JE 10031	79	07/03/91	1.022	277.00	1840.00	6.64	4.19	20.27
JE 11897	82	08/29/91	0.697	10.40	59.90	5.77	3.80	20.10
JE 9248	77	02/14/91	0.767	1270.00 >	3200.00 >	2.52 >	2.52	> 19.70
JE 11495	81	07/31/91	0.779	50.50	304.00	6.02	4.00	19.14
JE 11583	81	08/07/91	0.725	684.00	1770.00	2.59	1.55	> 18.74
JE 11223	80	07/10/91	0.847	18.30	94.80	5.17	3.38	> 18.03
JE 11597	81	08/07/91	0.864	534.00	2040.00	3.82	2.74	> 17.89
JE 11403	81	07/23/91	0.766	15.80	80.90	5.12	3.23	> 17.59
JE 11577	81	08/07/91	0.774	33.60	245.00	7.29	3.57	17.15
JE 11451	81	07/31/91	0.661	543.00	2200.00	4.05	2.94	> 16.79
JE 11044	78	10/10/91	0.932	19.10	193.00	10.09	3.29	16.51
JE 11467	81	07/30/91	0.853	510.00	2030.00	3.98	2.83	16.48
JE 9439	79	04/24/91	0.682	158.00 >	320.00 >	2.02 >	2.02	> 16.10
JE 9970	79	06/18/91	1.024	402.00	1710.00	4.26	2.42	> 15.88
JE 9990	79	06/19/91	1.014	169.00	839.00	4.97	3.14	> 15.64
JE 10030	79	07/03/91	1.022	59.00	311.00	5.27	3.28	15.52
JE 11622	81	08/08/91	0.886	4.91	19.30	3.93	2.45	> 15.15
JE 11463	81	07/30/91	0.851	217.00	1270.00	5.85	3.29	14.78
JE 11844	82	08/22/91	0.952	17.60	63.40	3.61	2.57	13.93
JE 11830	82	08/21/91	0.879	706.00	2220.00	3.15	2.07	> 13.61
JE 9433	80	10/08/91	0.752	590.00	2100.00	3.56	2.63	> 13.43
JE 11045	78	04/04/91	0.963	171.00	660.00	3.85	1.79	> 13.06
JE 11639	81	08/13/91	0.965	2.60	17.30	6.66	3.84	12.69
JE 9430	80	07/10/91	0.799	17.80	59.00	3.32	2.17	12.50
JE 11043	78	04/03/91	0.779	1830.00	6330.00	3.47	2.42	12.42
JE 11895	82	08/29/91	0.902	47.70	148.00	3.11	1.54	12.39
JE 9429	80	07/10/91	0.799	64.70	210.00	3.25	2.40	> 12.26
JE 11847	82	08/22/91	0.955	94.60	650.00	6.87	2.50	11.83
JE 11584	81	08/07/91	0.898	9.74	64.20	6.59	2.78	> 11.59
JE 11827	82	08/21/91	0.919	63.10	262.00	4.15	2.51	11.45
JE 9255	77	10/10/91	0.950	2810.00	6000.00	2.13	0.34	> 11.00
JE 11122	78	04/17/91	0.465	902.00	2100.00	2.33	1.59	10.84
JE 11580	81	08/07/91	0.831	20.90	92.10	4.41	2.51	10.47
JE 11206	80	09/24/91	0.771	70.80	203.00	2.87	2.04	> 10.34
JE 11101	78	04/11/91	0.614	232.00	660.00	2.85	1.91	10.33
JE 11910	82	09/26/91	1.076	205.00 >	320.00 >	1.56 >	1.56	> 9.90
JE 11832	82	08/22/91	0.923	731.00	2360.00	3.23	2.04	9.44
JE 9450	79	10/02/91	0.848	67.10	186.00	2.77	1.77	> 9.34
JE 9235	77	02/14/91	0.764	896.00	1840.00	2.06	1.30	8.93
JE 9446	79	04/24/91	0.897	66.40	199.00	2.99	2.06	8.88
JE 11500	81	07/31/91	0.688	684.00	1950.00	2.85	1.91	7.75
JE 11621	81	08/08/91	0.886	230.00	861.00	3.75	2.24	7.65
JE 11941	82	09/24/91	0.891	264.00 >	320.00 >	1.21 >	1.21	> 7.55

Table 14 (Cont'd)

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
JE 9199	77	02/27/91	0.998	765.00	1610.00	2.10	0.90 >	6.96
JE 9449	79	04/24/91	0.924	63.10	169.00	2.68	1.42	6.89
JE 11076	78	10/10/91	0.838	2640.00	6090.00	2.31	1.57	6.45
JE 11604	81	08/07/91	0.952	647.00	1790.00	2.77	1.68	6.43
JE 11489	81	07/31/91	0.791	21.40	56.40	2.64	1.62	6.26
JE 9184	77	02/07/91	0.785	89.00	194.00	2.18	0.96 >	6.09
JE 11722	82	08/14/91	0.847	238.00	590.00	2.48	1.62	5.99
JE 11349	81	07/17/91	0.816	749.00	1770.00	2.37	1.41	5.70
JE 11805	82	08/21/91	0.901	300.00	636.00	2.12	1.45 >	5.62
JE 11575	81	08/07/91	0.910	790.00	1830.00	2.31	1.44 >	4.97
JE 11911	82	09/26/91	1.076	294.00 >	320.00 >	1.09 >	1.09 >	4.86
JE 10478	83	10/23/91	0.907	227.00	52.30	0.23	0.12 >	4.5[illegible]
JE 9826	79	06/05/91	1.212	71.40	165.00	2.31	1.15	4.36
JE 9669	79	05/15/91	0.925	176.00 >	320.00 >	1.82	0.08 >	4.23
JE 11207	80	09/24/91	0.884	97.60	175.00	1.80	1.05	3.50
JE 11535	81	08/06/91	0.781	855.00	1610.00	1.88	0.31 >	3.04
JE 11527	81	08/01/91	0.952	814.00	1560.00	1.91	0.9[illegible]	2.89
JE 9225	77	10/09/91	0.675	320.00	528.00	1.65	0.93	2.61
JE 9448	79	04/24/91	0.924	219.00 >	320.00 >	1.46	0.40 >	2.39
JE 9227	77	02/14/91	0.853	283.00	485.00	1.72	0.80	1.79
JE 11536	81	08/06/91	0.862	277.00	415.00	1.50	0.44	0.91
JE 9221	77	10/09/91	0.842	2450.00	2090.00	0.85	0.62	0.00

New Drugs with 25% Antiviral Reduction Levels: Of the 3342 actual single drug tests, 143 new compounds demonstrated moderate antiviral activity, having antiviral reduction values equal to or better than 25%. This represents around 4.3% of the test compounds being active at this minimal antiviral reduction level.

In general, when compared to the 95% and 50% antiviral activity categories, the compounds in this (25%) category do not appear to have any significant antiviral promise and probably do not need to be presently confirmed any further.

4.3.3.3 Confirmatory Assays:

Some of the compounds were sent to us in more than one separate drug shipment. These compounds were tested more than once. Data from the confirmatory assays are summarized in **Table 15.** If a compound showed $\geq$50% reduction in CPE during this contract period, then it was considered a candidate for confirmatory testing. The confirmatory tests are from active compounds taken from the prescreen and primary MTT assays. Out of 58 confirmatory tests, 41 compounds were confirmed active during this reporting period and the remaining 17 compounds gave conflicting results. The criteria for activity is that the confirmatory test has to show $\geq$25% reduction in CPE. Failure to confirm the activity in these compounds was probably due to differences during the assay conditions:

1) In confirmatory assays the concentration range is adjusted to a more accurate semilog scale to maximize the TAI window.

2) Differences in the "differential" of the two runs can cause the compound to read positive or negative, falsely. The variability in the differential can cause false positive or negative bias to be introduced into the calculations, thus reflecting the variability in the maximum activity of the compound.

3) The metabolic rate, cell and virus load/well, age, and passage number of the cells may cause the above observed variability in the confirmatory results.

4) Problems associated with stability and storage of the compound (i.e., different lot numbers, solubility, light sensitivity, hygroscopic, etc.).

5) Problems associated with technical execution of large numbers of plates by different technicians.

During this reporting period the overall confirmatory rate against JE was 71%. The conflicting results should be retested at a later date based on the availability of the compound.

Some of the compounds have not been confirmed due to the discontinuation of this project by the sponsor.

4.3.3.4 Recommendations of JE-Actives Based Upon the *In Vitro* Results with MTT Assay (Vero Cells).

Based upon the *in vitro* results with the MTT assay (Vero cells) we recommend the following:

1) Compounds with the highest TAI in the 95% activity category that have confirmed results with the exception of "colored" compounds should receive the highest priority for further profile studies and *in vivo* animal testing.

2) Compounds with the highest TAI in the 50% activity category that have confirmed results with the exception of "colored" compounds should receive the next highest priority for further profile studies and *in vivo* animal testing.

Table 15

Confirmatory Assays for Compounds Active Against Japanese Encephalitis Virus (JE)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
0206	5	05/10/89	00X	0.932	115.00	253.00	2.20	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	6.26	+
0206	4	12/05/89	SRV	0.770	159.00	566.00	3.56	0.00	813.00	0.00	0.00 >	1000.00	0.00	0.00	9.73	+
0206	67	06/21/90	WYX	0.579	34.40	635.00	18.47	49.10	950.00	19.36	93.10 >	1000.00 >	10.74	12.94	36.80	+
0206	5/67	11/20/91	BZS	0.966	146.00	335.00	2.29	275.00	704.00	2.56	0.00 >	1000.00	0.00	1.22	4.58	+
0360	48	02/06/89	OKC	0.722	0.01	0.28	25.80	0.04	0.67	15.90	0.00	2.83	0.00	6.61 >	30.84	+
0360	2	08/23/89	RAT	0.464	0.03	0.25	9.59	0.04	0.54	11.97	0.09 >	1.00 >	10.84	5.64	27.74	+
0360	2	11/01/90	ZWU	1.014	0.00	0.54	0.00	0.00	0.94	0.00	0.00 >	1.00	0.00	0.00	0.00	-
0360	2/48	03/14/91	3VU	1.039	0.46 >	1.00 >	2.17	0.88 >	1.00 >	1.14	0.00 >	1.00	0.00 >	1.14 >	4.31	+
0361	48	02/06/89	OKD	0.660 <	0.00	0.04 >	14.00	0.01	0.09	8.29	0.03	0.30	10.40	4.30 >	29.61	+
0361	2	08/23/89	RAU	0.427 <	0.03	0.06 >	1.92 <	0.03	0.09 >	2.83	0.00	0.31	0.00 >	1.92 >	3.71	+
0361	48	02/13/90	TZX	0.725	0.01	0.06	4.29	0.02	0.08	3.54	0.00	0.30	0.00	2.45 >	14.49	+
0361	2	11/01/90	ZWV	1.057	0.03	0.04	1.57	0.00	0.07	0.00	0.00 >	0.32	0.00	0.00	0.74	+
0361	2/48	03/14/91	3VV	0.941	0.02	0.06	3.52	0.03	0.11	3.31	0.00	0.77	0.00	1.82	6.11	+
2318	13	08/30/89	RE4	0.686	0.17	6.60	39.98	0.27 >	10.00 >	36.69	0.87 >	10.00 >	11.45	24.21 >	43.71	+
2318	67	05/31/90	WGP	0.972	0.54	0.93	1.73	0.00	2.95	0.00	0.00 >	10.00	0.00	0.00	2.68	+
2318	67	07/12/90	XG6	0.871	0.34	7.38	21.60	0.52 >	10.00 >	19.36	0.00 >	10.00	0.00	14.30 >	34.12	+
2318	82	10/01/91	A3R	0.824 <	1.00	7.73 >	7.73	1.56	90.30	57.97	0.00 >	320.00	0.00	4.97 >	18.71	+
2563	48	02/06/89	OKE	0.554	0.14 >	3.20 >	23.50	0.32 >	3.20 >	10.00	0.89 >	3.20 >	3.59 >	10.00 >	41.49	+
2563	15	05/23/89	QST	0.850	1.61	12.40	7.68	2.59	20.80	8.03	0.00 >	32.00	0.00	4.77	20.32	+
2563	21	09/06/89	RFV	1.087	0.08	1.39	16.86	0.16	2.21	13.50	0.00	8.57	0.0	8.49 >	18.23	+
2563	48	12/05/89	SRT	0.822	0.00 >	3.20	0.00	0.00 >	3.20	0.00	0.00 >	3.20	0.00	0.00 >	0.65	-
2563	15/21	11/01/90	ZWX	1.046	0.29	0.61	2.12	0.00	1.24	0.00	0.00 >	10.00	0.00	0.00	2.32	+
2563	21/48	03/14/91	3VV	0.941	0.33	1.57	4.75	0.51	2.67	5.28	0.00 >	10.00	0.00	3.10	16.56	+
2811	48	02/06/89	OKH	0.629	0.01	0.24	32.70	0.02	0.47	22.20	0.09	0.91	10.40	11.00 >	37.18	+
2811	RS-1	03/14/91	3VW	0.984	0.04	0.14	3.59	0.06	0.27	4.86	0.00 >	1.00	0.00	2.53	12.28	+
2812	48	02/06/89	OKI	0.576	0.00 >	0.01 >	4.89	0.00 >	0.01 >	2.57	0.01 >	0.01 >	1.06 >	2.57	18.14	+
2812	61	11/29/89	SQL	0.963	0.01	0.02	4.11	0.00	0.04	0.00	0.00	0.26	0.00	0.00 >	8.53	+
2812	61	12/06/90	1PP	0.918	0.00 <	0.32	0.00	0.00 <	0.32	0.00	0.00 <	0.32	0.00	0.00	0.00	-
2812	RS-1	03/14/91	3VX	0.895	0.00	0.02	4.12	0.01	0.03	4.08	0.00	0.24	0.00	2.43	9.08	+
2978	25	02/28/89	OU3	0.820	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	2.00	-
2978	27	04/19/89	PMD	0.683	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	4.69	-
2978	27	02/13/90	TZZ	0.827	233.00	674.00	2.89	0.00 >	1000.00	0.00	0.00 >	1000.00	0.00	0.00 >	10.35	+
2978	25/27	03/14/91	3VX	0.895	423.00	683.00	1.61	724.00	2020.00	2.78	0.00 >	3200.00	0.00	0.94 >	4.39	+
2980	48	02/07/89	OLM	1.142	0.42	1.72	4.13	0.69 >	3.20 >	4.62	0.00 >	3.20	0.00	2.48 >	15.31	+
2980	48	03/10/89	OZY	0.737	0.11	2.10	19.10	0.18 >	3.20 >	18.20	0.73 >	3.20 >	4.39	12.00	40.06	+
2980	25	09/07/89	RHS	0.875	0.15	0.90	5.90	0.27	1.94	7.26	0.00 >	10.00	0.00	3.37 >	17.45	+
2980	61	11/29/89	SQU	1.012	0.24	1.76	7.45	0.50	2.58	5.15	0.00 >	3.20	0.00	3.51 >	21.71	+
2980	48/61	03/14/91	3VY	0.960	0.47	1.84	3.88	0.78	3.15	4.06	0.00 >	10.00	0.00	2.37	8.37	+

Table 15 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
4074	48	02/07/89	OLP	1.229	0.00 >	0.03	0.00	0.00 >	0.03	0.00	0.00 >	0.03	0.00	0.00	0.00	-
4074	48	03/10/89	OZZ	0.762	0.01 >	0.03 >	4.20	0.01 >	0.03 >	2.30	0.03 >	0.03 >	1.09 >	2.30 >	19.27	+
4074	48	12/07/89	STO	0.617 <	1.00	1.98 >	1.98 <	1.00	2.96 >	2.96 <	1.00	26.80 >	26.82 >	1.98 >	7.52	+
4074	48	02/13/90	U00	0.746	0.20	0.81	3.93	0.68	1.80	2.64	0.00 >	10.00	0.00	1.18 >	11.27	+
4074	RS-1	03/14/91	3V2	0.923	0.37	1.81	4.94	0.51	2.72	5.32	0.94	29.50	31.50	3.53 >	19.13	+
4113	48	02/07/89	OLQ	1.210	0.26 >	0.32 >	1.21	0.00 >	0.32	0.00	0.00 >	0.32	0.00	0.00 >	2.35	+
4113	48	03/10/89	P00	0.827	0.12 >	0.32 >	2.75	0.20 >	0.32 >	1.61	0.00 >	0.32	0.00 >	1.61 >	12.65	+
4113	39	05/24/89	Q5T	0.809	0.05	0.36	7.02	0.09	0.69	7.67	0.28	2.85	10.10	4.01 >	27.24	+
4113	39	09/12/89	RJK	0.700	0.12 >	1.00 >	8.60	0.17 >	1.00 >	5.89	0.00 >	1.00	0.00 >	5.89 >	22.13	+
4113	39	11/01/90	ZWY	1.028	0.19 >	0.32 >	1.71	0.00 >	0.32	0.00	0.00 >	0.32	0.00	0.00 >	2.46	+
4113	39/48	03/14/91	3W0	1.023	0.35	1.46	4.23	0.57	2.43	4.29	0.00	8.87	0.00	2.59	12.11	+
4223	62	01/18/90	TFT	1.098	1.51	2.28	1.[illegible]	0.00	4.28	0.00	0.00	23.50	0.00	0.00 >	1.62	+
4223	62	02/06/90	TR9	0.956	0.69 >	1.00 >	1.44	0.00 >	1.00	0.00	0.00 >	1.00	0.00	0.00 >	0.55	+
4223	63	02/13/90	TZU	0.874	0.00	0.60	0.00	0.00	0.91	0.00	0.00 >	1.00	0.00	0.00	2.75	-
4223	RS-1	03/14/91	3W2	0.952	0.08	0.30	4.01	0.00	1.17	0.00	0.00	7.91	0.00	0.00	5.62	+
4223	RS-1	03/14/91	3W2	0.952	0.36	0.59	1.66	0.72	1.53	2.14	0.00	9.22	0.00	0.83	3.65	+
4591	63	02/13/90	TZW	0.734	0.01	0.02	3.68	0.00	0.03	0.00	0.00	0.09	0.00	0.00 >	8.11	+
4591	RS-1	03/14/91	3W4	0.988	0.00 <	1.00	0.00	0.00 <	1.00	0.00	0.00 <	1.00	0.00	0.00	0.00	-
4591	RS-1	04/18/91	4RR	0.750	0.00	0.02	8.45	0.00	0.02	5.57	0.00	0.09	0.00	3.50 >	18.42	+
4591	RS-1	10/15/91	ALN	0.916 <	0.00 <	0.00 -	1.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00 >	0.04	+
4591	RS-1	10/15/91	ALG	0.840	0.00 <	0.32	0.00	0.00 <	0.32	0.00	0.00 <	0.32	0.00	0.00	0.00	-
4592	48	02/28/89	OU1	0.764	4.34	50.10	11.60	6.97	92.50	13.30	26.50 >	100.00 >	3.77	7.19	32.32	+
4592	42	09/13/89	RLN	0.615	4.12	23.10	5.61	5.96	50.40	8.46	0.00 >	100.00	0.00	3.87 >	19.07	+
4592	61	11/29/89	SQN	1.006	6.34	32.00	5.05	17.90	71.50	4.00	0.00 >	100.00	0.00	1.79	15.38	+
4592	48	02/06/90	TRB	0.965	14.20	45.60	3.22	25.40	76.50	3.02	0.00 >	100.00	0.00	1.80 >	10.40	+
4592	63	02/13/90	TZW	0.734	2.81	7.25	2.58	0.00	14.30	0.00	0.00	80.00	0.00	0.00 >	7.17	+
4592	RS-1	03/14/91	3W3	0.975	4.21	8.57	2.04	7.44	21.80	2.94	0.00	91.20	0.00	1.15	4.94	+
4855	48	02/21/89	OQT	0.598	2.77	112.00	40.50	4.79	200.00	41.70	15.50 >	320.00 >	20.70	23.40 >	59.97	+
4855	48	09/13/89	RLT	0.615	4.91 >	320.00 >	65.23	11.10 >	320.00 >	28.79	28.80 >	320.00 >	11.12 >	28.79 >	62.18	+
4855	61	11/29/89	SQR	0.975	6.27	190.00	30.31	14.10	280.00	19.86	62.20 >	320.00 >	5.14	13.47 >	44.23	+
4855	48	12/06/90	1PQ	1.032	7.17 >	100.00 >	13.94	13.60 >	100.00 >	7.35	29.40 >	100.00 >	3.40 >	7.35 >	35.99	+
4855	48	03/13/91	3TF	0.787	13.00	160.00	12.35	26.90	221.00	8.22	0.00 >	320.00	0.00	5.97 >	28.55	+
8221	75	11/21/90	183	0.859	13.60	28.30	2.09	29.20	60.60	2.08	0.00	255.00	0.00	0.97	2.95	+
8221	75	03/20/91	40G	0.895	7.27	33.80	4.66	18.50	79.80	4.31	0.00	292.00	0.0	1.83 >	8.86	+
8263	76	12/12/90	1UI	0.741	41.60	153.00	3.69	66.30	232.00	3.50	0.00 >	1000.00	0.00	2.31	11.47	+
8263	76	03/20/91	40P	1.011	53.50	125.00	2.34	93.50	250.00	2.67	0.00 >	1000.00	0.00	1.34	3.71	+
8352	75	11/29/90	1KT	0.727	1.11	5.70	5.15	1.59	8.20	5.15	3.06	32.00	10.45	3.58 >	17.41	+
8352	75	03/28/91	48V	0.725	1.84	13.80	7.51	3.85	24.40	6.34	0.00 >	32.00	0.00	3.59 >	18.16	+

Table 15 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
8353	75	11/29/90	1KT	0.727	19.40 >	320.00 >	16.52	36.50 >	320.00 >	8.77	97.80 >	320.00 >	3.27 >	8.77 >	35.30	+
8353	75	03/27/91	46Q	0.921	26.40	51.80	1.97	0.00	378.00	0.00	0.00 >	1000.00	0.00	0.00	1.91	+
8353	74	11/20/91	C03	0.979	5.25	64.70	12.33	9.76	336.00	34.42	0.00 >	1000.00	0.00	6.64 >	23.78	+
8372	76	12/13/90	1XS	0.889	1600.0	2760.0	1.73	2550.00 >	3200.00 >	1.25	0.00 >	3200.00	0.00	1.08 >	2.29	+
8372	76	03/21/91	43U	0.932	1560.0	2470.0	1.58	2470.00	8170.00	3.31	0.00 >	10000.0	0.00	1.00 >	4.95	+
9121	77	01/31/91	2XY	0.591	0.00 >	3200.0	0.00	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	0.70	-
9121	77	10/09/91	AGY	0.840	5210.0	6600.0	1.27	9330.00 >	10000.0 >	1.07	0.00 >	10000.0	0.00	0.71 >	1.06	+
9184	77	02/07/91	34S	0.785	42.90	85.10	1.99	89.00	194.00	2.18	0.00	915.00	0.00	0.96 >	6.09	+
9184	77	10/09/91	AN8	0.778	151.00	300.00	1.98	230.00	581.00	2.53	0.00 >	1000.00	0.00	1.31	5.65	+
9199	77	02/27/91	3FZ	0.998	438.00	686.00	1.57	765.00	1610.00	2.10	0.00	3040.00	0.00	0.90 >	6.96	+
9199	77	10/09/91	AH9	0.841	740.00	456.00	0.62	0.00	1340.00	0.00	0.00	3010.00	0.00	0.00	0.00	+
9202	77	02/27/91	3G0	0.849	336.00	1430.0	4.25	508.00	2030.00	3.99	0.00	3100.00	0.00	2.82 >	21.97	+
9202	77	10/09/91	AH9	0.841	579.00	709.00	1.22	0.00	1670.00	0.00	0.00	3050.00	0.00	0.00	1.30	+
9221	77	02/27/91	3G9	1.000	0.00	2410.0	0.00	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	7.80	-
9221	77	10/09/91	AHB	0.842	1560.0	1510.0	0.97	2450.00	2090.00	0.85	0.00	3130.00	0.00	0.62	0.00	+
9225	77	02/14/91	39A	0.805	171.00	256.00	1.49	294.00	500.00	1.70	0.00	950.00	0.00	0.87	2.02	+
9225	77	10/09/91	AHC	0.675	179.00	296.00	1.66	320.00	528.00	1.65	0.00	953.00	0.00	0.93	2.61	+
9227	77	02/14/91	398	0.853	168.00	226.00	1.34	283.00	485.00	1.72	0.00	948.00	0.00	0.80	1.79	+
9227	77	10/09/91	AHC	0.675	186.00	290.00	1.56	0.00	521.00	0.00	0.00	952.00	0.00	0.00	2.09	+
9235	77	02/14/91	39F	0.764	518.00	1160.0	2.24	896.00	1840.00	2.06	0.00	3060.00	0.00	1.30	8.93	+
9235	77	10/10/91	AJE	0.942	264.00	1210.0	4.60	0.00	1870.00	0.00	0.00	3070.00	0.00	0.00 >	9.85	+
9248	77	02/14/91	39L	0.767	135.00 >	3200.0 >	23.78	1270.00 >	3200.00 >	2.52	0.00 >	3200.00	0.00 >	2.52 >	19.70	+
9248	77	10/10/91	AJG	0.974	155.00	2100.0	13.58	566.00	4930.00	8.71	0.00	9490.00	0.00	3.71	17.09	+
9255	77	02/20/91	38F	0.776	1840.0	3200.0	1.74	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00	3.51	+
9255	77	10/10/91	AJI	0.950	320.00	955.00	2.98	2810.00	6000.00	2.13	0.00	9600.00	0.00	0.34 >	11.00	+
9414	81	07/10/91	72E	0.983	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00	0.00	-
9414	81	10/08/91	ACQ	0.647	344.00 >	1000.0 >	2.91	491.00 >	1000.00 >	2.04	931.00 >	1000.00 >	1.07 >	2.04 >	15.72	+
9416	82	09/04/91	94U	0.765	12.50	168.00	13.46	17.20 >	320.00 >	18.60	30.80 >	320.00 >	10.40	9.74 >	28.75	+
9416	82	11/20/91	C04	1.008	13.10	54.00	4.11	19.10	121.00	6.32	0.00 >	320.00	0.00	2.82	12.15	+
9430	80	07/10/91	724	0.799	12.60	38.60	3.06	17.80	59.00	3.32	0.00	95.90	0.00	2.17	12.50	+
9430	80	10/08/91	ACV	0.867	0.00	49.50	0.00	0.00	67.10	0.00	0.00	98.60	0.00	0.00	2.85	-
9433	80	07/10/91	728	0.908	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00	0.00	-
9433	80	10/08/91	ACW	0.752	435.00	1550.0	3.57	590.00	2100.00	3.56	0.00	3090.00	0.00	2.63 >	13.43	+
9439	79	04/24/91	4VT	0.682	66.90 >	320.00 >	4.78	158.00 >	320.00 >	2.02	0.00 >	320.00	0.00 >	2.02 >	16.10	+
9439	79	10/01/91	A43	0.746	0.00 >	3200.0	0.00	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	5.69	-

Table 15 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9446	79	04/24/91	4VX	0.897	46.10	137.00	2.97	66.40	199.00	2.99	0.00	310.00	0.00	2.06	8.88	+
9446	79	10/01/91	A44	0.858	0.00	159.00	0.00	0.00	218.00	0.00	0.00	514.00	0.00	0.00	5.27	-
9448	79	04/24/91	4VY	0.924	148.00	88.70	0.60	219.00 >	320.00 >	1.46	0.00 >	320.00	0.00	0.40 >	2.39	+
9448	79	10/01/91	A45	0.839	0.00	492.00	0.00	0.00	663.00	0.00	0.00	973.00	0.00	0.00 >	3.29	-
9449	79	04/24/91	4VY	0.924	44.90	89.80	2.00	63.10	169.00	2.68	0.00	311.00	0.00	1.42	6.89	+
9449	79	10/01/91	A46	0.932	0.00	161.00	0.00	0.00	222.00	0.00	0.00	660.00	0.00	0.00	0.00	-
9450	79	04/24/9	4V.	0.897	0.00	51.30	[illegible]	0.00	76.70	0.00	0.00	[illegible]79.00	0.00	0.00	0.00	-
9450	79	10/02/91	A7A	0.848	46.30	119.00	[illegible]56	67.10	186.00	2.77	0.00	307.00	0.00	1.77 >	9.34	+
9589	79	05/02/91	568	0.657	13.70 >	320.00 >	23.40	36.20 >	320.00 >	8.85	90.30 >	320.00 >	3.54 >	8.85 >	46.62	+
9589	79	10/02/91	A7J	0.741	42.50	13.40	0.32	56.60	20.00	0.35	94.50	31.70	0.34	0.24 >	20.81	+
9590	79	05/02/91	569	0.814	81.00	153.00	1.89	0.00	216.00	0.00	0.00 >	320.00	0.00	0.00	3.61	+
9590	79	10/02/91	A7K	0.648	425.00	133.00	0.31	566.00	195.00	0.34	945.00	308.00	0.33	0.23 >	5.86	+
9669	79	05/15/91	5HV	0.925	80.20	14.60	0.18	176.00 >	320.00 >	1.82	0.00 >	320.00	0.00	0.08 >	4.23	+
9669	79	10/02/91	A7T	0.836	0.00	9.69	0.00	0.00	28.90	0.00	0.00	858.00	0.00	0.00	0.00	-
9826	79	06/05/91	63V	1.212	47.80	81.90	1.71	71.40	165.00	2.31	0.00	305.00	0.00	1.15	4.36	+
9826	79	10/03/91	A9P	1.030	0.00	54.40	0.00	0.00	76.70	0.00	0.00	274.00	0.00	0.00	0.00	-
9916	79	06/12/91	6CT	1.135	189.00	2250.0	11.93	387.00 >	3200.00 >	8.27	909.00 >	3200.00 >	3.52	5.82	29.14	+
9916	79/C2	08/14/91	8CF	0.746	738.00	1730.0	2.35	0.00	2650.00	0.00	0.00 >	3200.00	0.00	0.00 >	3.76	+
9970	79	06/18/91	6JY	1.024	179.00	974.00	5.44	402.00	1710.00	4.26	0.00	3050.00	0.00	2.42 >	15.88	+
9970	79/C2	08/14/91	8CL	0.900	462.00	1110.0	2.41	768.00	1810.00	2.35	0.00	3060.00	0.0	1.45	5.40	+
9990	79	06/19/91	6NB	1.014	112.00	530.00	4.73	169.00	839.00	4.97	0.00 >	1000.00	0.00	3.14 >	15.64	+
9990	79/C2	08/14/91	8CM	0.657	0.00	474.00	0.00	0.00	649.00	0.00	0.00	965.00	0.00	0.00	0.00	-
10030	79	07/03/91	6UH	1.022	39.00	194.00	4.96	59.00	311.00	5.27	0.00	929.00	0.00	3.28	15.52	+
10030	79/C2	08/14/91	8CQ	0.924	43.80	177.00	4.06	76.50	255.00	3.33	0.00	883.00	0.00	2.32 >	14.16	+
10031	79	07/03/91	6UH	1.022	167.00	1160.0	6.98	277.00	1840.00	6.64	0.00	3060.00	0.00	4.19	20.27	+
10031	79/C2	08/14/91	8CR	0.876	0.00	478.00	0.00	0.00	711.00	0.00	0.00	2620.00	0.00	0.00	0.12	-
11043	78	04/03/91	4D8	0.779	1110.0	4420.0	4.00	1830.00	6330.00	3.47	0.00	9770.00	0.00	2.42	12.42	+
11043	78	10/10/91	AJK	0.932	1890.0	3630 0	1.92	0.00	5750.00	0.00	0.00	9580.00	0.00	0.00	3.14	+
11044	78	04/04/91	4EZ	1.004	10.00	30.20	3.02	24.10	67.50	2.80	0.00 >	320.00	0.00	1.25	6.79	+
11044	78	10/10/91	AJK	0.932	12.70	62.70	4.92	19.10	193.00	10.09	0.00 >	320.00	0.00	3.29	16.51	+
11045	78	04/04/91	4F0	0.963	69.80	306.00	4.39	171.00	660.00	3.85	0.00 >	3200.00	0.00	1.79 >	13.06	+
11045	78	10/10/91	AJL	0.850	129.00	408.00	3.17	190.00	723.00	3.80	0.00	2810.00	0.00	2.14 >	10.52	+
11076	78	04/10/91	4J7	0.785	0.00 >	3200.0	0.00	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00	0.35	-
11076	78	10/10/91	AJP	0.838	1440.0	4140.0	2.88	2640.00	6090.00	2.31	0.00	9610.00	0.00	1.57	6.45	+

Table 15 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
11101	78	04/11/91	4N7	0.614	125.00	442.00	3.54	232.00	660.00	2.85	0.00	2200.00	0.00	1.91	10.33	+
11101	78	10/15/91	AL6	0.862	0.00	9.26	0.00	0.00	590.00	0.00	0.00	959.00	0.00	0.00	0.00	-
11122	78	04/17/91	40U	0.465	262.00	1430.0	5.46	902.00	2100.00	2.33	0.00	6220.00	0.00	1.59	10.84	+
11122	78	10/15/91	ALC	0.782	566.00	1500.0	2.65	0.00	2070.00	0.00	0.00	3090.00	0.00	0.00 >	7.50	+
11155	78	04/18/91	4RI	0.753	15.80	48.30	3.05	0.00	82.30	0.00	0.00	290.00	0.00	0.00	4.04	+
11155	78	10/10/91	AJQ	0.913	25.60 >	320.00 >	12.51	134.00 >	320.00 >	2.38	293.00 >	320.00 >	1.09 >	2.38 >	22.52	+
11223	80	07/10/91	71X	0.847	12.00	62.10	5.16	18.30	96.80	5.17	0.00 >	320.00	0.00	3.38 >	18.03	+
11223	80	10/09/91	AEX	0.860	39.30	169.00	4.31	56.60	248.00	4.38	0.00 >	320.00	0.00	2.99	14.76	+
11224	80	07/10/91	71Y	0.808	3.66	19.80	5.42	5.12	29.60	5.79	9.35	272.00	29.10	3.87 >	20.48	+
11224	80	10/09/91	AEY	0.785	10.80	55.40	5.16	15.50	84.80	5.48	29.80	320.00	10.75	3.58 >	24.89	+
11679	81	07/10/91	72H	1.000 <	1.00	0.78 >	0.78	1.81	18.30	10.08	0.00	30.60	0.00	0.43	17.47	+
11679	81	10/09/91	AF0	0.691	0.15	15.50	102.59	0.40	21.00	52.71	6.52	30.90	4.74	38.91 >	56.90	+
11679	81	11/20/91	BZW	1.167	1.14	5.60	4.92	3.77	14.80	3.93	0.00	30.30	0.00	1.49	8.24	+

DIFRNTL = The differential is the difference in the cell control and the virus control optical densities.

$IC_{25,50,95}$ = (Viral) inhibitory concentration 25%, 50% and 95% = The drug concentration (µg/ml) that inhibited viral CPE by 25%, 50% or 95% calculated by using a regression analysis for semilog curve fitting.

$TC_{25,50,95}$ = (Cell) toxicity concentration 25%, 50% and 95% = The drug concentration (µg/ml) that reduced cell viability by 25%, 50% or 95%.

$AI_{25,50,95}$ = Antiviral Index = A single point ratio of the antiviral and anticellular effect of the compound, calculated with 25%, 50% or 95% reduction values (calculated by dividing the $TC_{25,50,95}$ by the $IC_{25,50,95}$).

SI = Selectivity Index = A ratio calculated by dividing the TC_{25} by the IC_{50} (based upon 6 one-half-log_{10} dilutions, µg/ml, the maximum scale is 0-320).

TAI = Total Antiviral Index = The area between the cytotoxicity and the antiviral curves (based upon a scale of 0-100%).

ACT = Activity = A "+" denotes a test that produced ≥25% reduction in CPE. A "-" denotes an inactive test (i.e. ≤25% reduction in CPE).

4.3.4 Venezuelan Equine Encephalomyelitis Virus (VE):

The total output of VE-testing during this reporting period is summarized in monthly increments in **Figure 46**. During this period 3892 tests were performed against VE-virus with MTT-assay format. Out of these, 202 were control compound assays-Selenazofurin (AVS-0253) and 111 were control compound assays - 2-Thio-6-Azauridine (AVS-6724). One hundred eighty-six tests were internal (+++) virus load, cell load, and other quality control tests. Seven tests were considered unsatisfactory based on the criteria of the quality controls set during this reporting period. The rest, totaling 3386 were actual single drug assays. The total number of assays represents approximately 69% above our yearly contractual obligation (i.e. 3386/2000). The 7 unsatisfactory tests represent a 0.2% rejection rate based on present quality control parameters for the VE virus.

Out of the 3386 test compounds, 174 demonstrated antiviral activity at greater than 50% reduction levels. This represents around 5% of the tested compounds having *in vitro* antiviral activity against VE-virus. The remainder, 3212 compounds (95%), are to be considered inactive with this assay protocol.

TOTAL NUMBER OF TESTS AGAINST VENEZUELAN EQUINE ENCEPHALOMYELITIS VIRUS (VE)

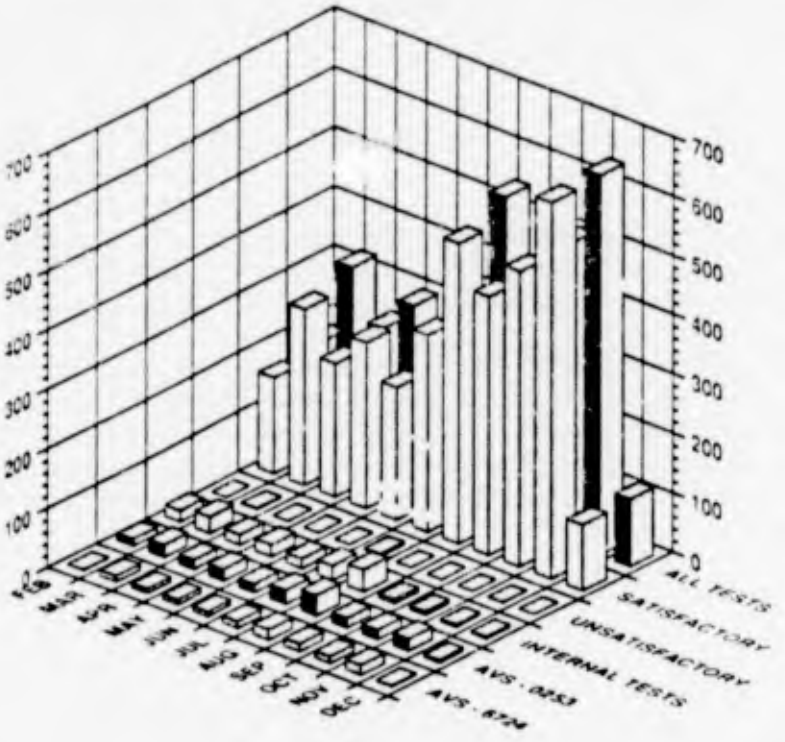


Figure 46

Quality Control Tests =	KPC (Positive Cor. ol - 2-Thio-6-Azauridine) =	111
	KPC (Positive Control - Selenazofurin) =	202
	(+++++) (Internal virus and Cell Load Controls) =	186
	(UT) Unsatisfactory Test (QC rejects) =	7
Accepted Single Drug Tests =		3386
Total Number of VE Tests =		3892

4.3.4.1 VE-Quality Controls:

4.3.4.1.1 Antiviral Activity of Selenazofurin vs VE Virus:

Control Compound-Antiviral Performance: Selenazofurin (AVS-0253) has been the primary control compound against VE in these MIT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of the positive control drug (Selenazofurin) are illustrated in **Figure 47-A.**

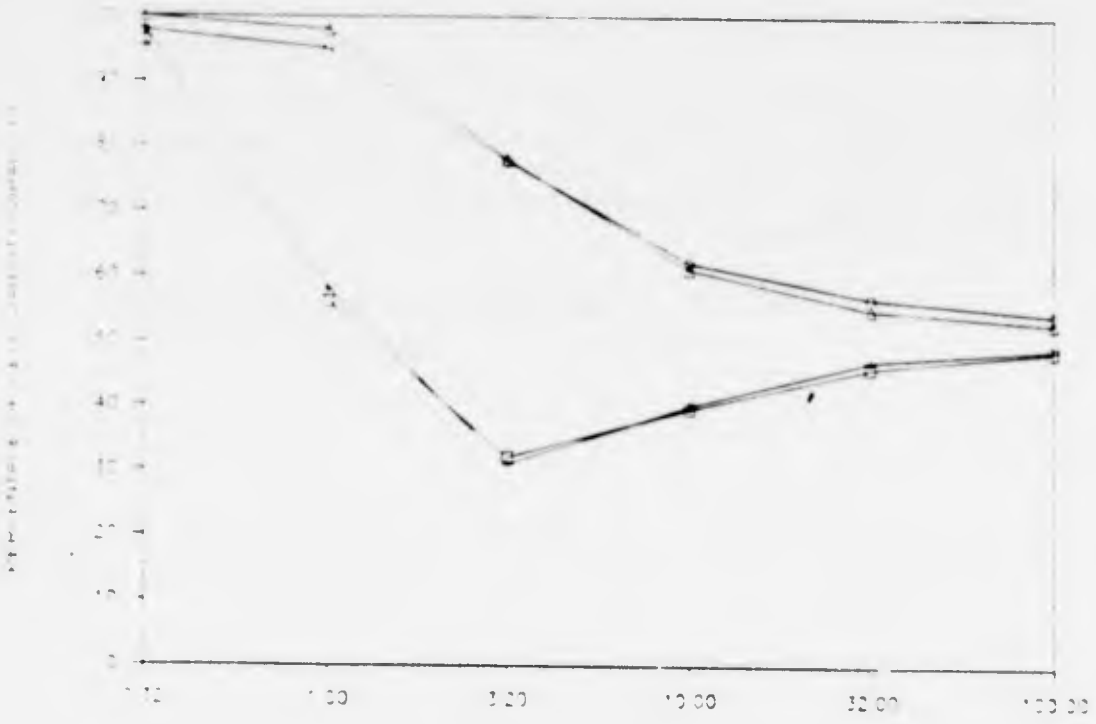
The 202 control tests performed with Selenazofurin gave a mean **Total Antiviral Index** (TAI) of 26% (SD ± 16) and the median value was 22.5%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from ~1.3 - >71% during this period. The mean **Selectivity Index (SI)** was 10.57 (SD ± 25.16) and the median SI value was 3.11, indicating poor antiviral selectivity for Selenazofurin and it ranged from 0 - >182% during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25%** (AI_{25}) value was 18.39 (SD ± 39.5). The median AI_{25} value was 6.43 (range 1.0 - >259.68). The mean **Antiviral Index 50%** (AI_{50}) was 63.59 (SD ± 51.15) with a median of 57.94 (range 0 - >219.23). The mean **Antiviral Index 95%** (AI_{95}) was 1.33 (SD ± 7.1) with a median of 0 (range of 0 - >55.9).

The mean **Antiviral Inhibitory Concentration 25%** (IC_{25}) was 0.72 μg/ml (SD ± 0.618). The median IC_{25} value was 0.611 μg/ml (range <0.32 - 6.34 μg/ml). The mean **Antiviral Inhibitory Concentration 50%** (IC_{50}) was 2.84 μg/ml (SD ± 9.68). The median IC_{50} value was 1.31 μg/ml (range = 0 - 100 μg/ml). The mean **Antiviral Inhibitory Concentration 95%** (IC_{95}) was 0.117 μg/ml (SD ± 0.641). The median IC_{95} value was 0 μg/ml (range 0 - 5.96 μg/ml). This discrepancy indicates that the control compound Selenazofurin does not consistently reach 50 or 95% reduction levels.

The average maximum antiviral inhibitory level of 202 Selenazofurin tests (**Figure 47-A**) was reached at 3.2 μg/ml of the compound with 70% antiviral effect. Further increase of the drug concentration does not improve its antiviral activity. Maximum antiviral effect (~70%) was found with a simultaneous ~20% cytotoxic suppression. Above the 3.2 μg/ml concentration the antiviral protection levels off to ~50% reduction level at 100 μg/ml while simultaneously the Selenazofurin becomes maximally toxic (~50%).

SELENAZOFURIN -VS- VENEZUELAN EQUINE ENCEPHALITIS (VE)



CONCENTRATION (μg/ml)

□ Mean % Viral CPE

\+ Median % Viral CPE

◊ Mean % Cell Viability

Δ Median % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	0.32	1	3.2	10	32	100	0.32	1	3.2	10	32	100
Mean	96	56	32	40	46	49	98	95	78	62	57	54
Median	98	58	31	40	47	49	100	98	78	61	55	53
Std. Dev.	0.06	0.17	0.15	0.12	0.10	0.10	0.04	0.07	0.13	0.12	0.10	0.11

Figure 47-A
Average Antiviral and Cytotoxicity Values for 20? Positive Control Compound Tests

4.3.4.1.2 Maximum Antiviral Effect of Selenazofurin vs VE Virus (VE):

A bar graph scatter plot (**Figure 48-A**) depicts the distribution of the maximum antiviral reduction values of all 202 control compound assays for Selenazofurin. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 69% (SD ± 13.4) reduction levels. The maximum reduction levels vary from 42 - 100% but remain quite consistently around the median of 69%. The assay control values give a relatively broad, bell-shaped distribution curve. This indicates quite a consistent day-to-day performance of the control compound in the VE-MTT assay.

During this period the positive control compound performance criteria for Selenazofurin versus the VE virus was set at 25% reduction level. All assays in which Selenazofurin did not meet this accepted quality control level (≥25%) were rejected (i.e., 7 unsatisfactory tests).

Since Selenazofurin is only marginally active against VE virus,better quality control compounds are needed. However, regardless of the poor performance of the VE quality control drug Selenazofurin, around 174 different compounds have equal or better antiviral activity against VE virus than AVS-0253. Some of these could certainly be used as a better *in vitro* antiviral control compound in this large-scale antiviral screening program.

Variation of the Maximum Antiviral Effect
VE Virus - VS- Selenazofurin

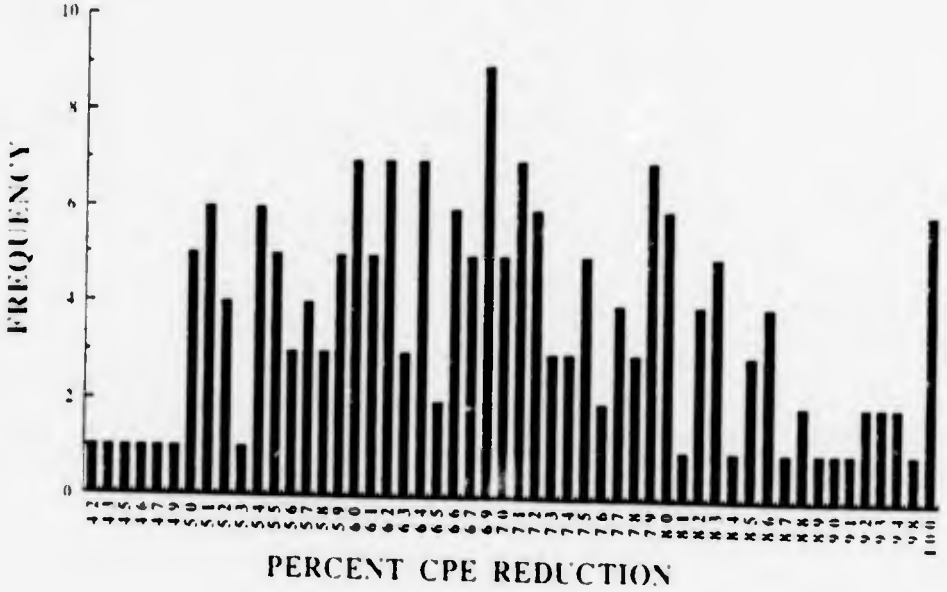


Figure 48-A
Maximum Antiviral CPE Reduction (%).
Summary of 202 Control Tests.

4.3.4.1.3 Cellular Cytotoxicity of Selenazofurin vs VE Virus:

VE-Control Compound-Cytotoxicity Performance: The 202 cytotoxicity values of the positive control compound Selenazofurin are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 10.3 μg/ml (SD ± 20.11) and the median was 4.63 μg/ml (range of 0.66 - > 100 μg/ml). The mean cell **Toxic Concentration 50%** (TC_{50}) value was 71.41 μg/ml (SD ± 37.98) and the median was 100 μg/ml (range of 2.72 - 100 μg/ml). The mean cell **Toxic Concentration 95%** (TC_{95}) value cannot be attained in most assays with Selenazofurin versus Venezuelan Equine Encephalomyelitis Virus.

As can be seen from **Figure 47-A**, the toxicity starts to become measurable above the concentration of 1.0 μg/ml and the maximum toxicity has not been reached at 100 μg/ml.

When the cytotoxicity reaches around 40% (10 μg/ml), the control compound (Selenazofurin) has reached close to its maximum toxicity. After 3.2 μg/ml the antiviral protection of Selenazofurin starts to decrease down to ~50%. Selenazofurin becomes maximally toxic between 10 - 100 μg/ml (50%). The highest Selenazofurin concentration tested in these assays was 100 μg/ml.

Selenazofurin has a definite cytotoxic suppression on cellular metabolism and growth. The TC_{25} and TC_{50} toxicity can be achieved with relative consistency at 100 μg/ml. A peculiar feature of the control compound is that the cytotoxicity levels off after reaching its maximum cytotoxic value (±50%) at 10 μg/ml and continues at that stationary level at increasing drug concentrations.

4.3.4.1.4 VE-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (Selenazofurin):

VE-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 202 control assays is plotted in **Figure 49-A**. The results indicate that the cell O.D. readings reached a mean of 1.124 (SD ± 0.148) with a median of 1.134 (range of 0.731 - 1.498). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

VE-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 202 control assays is presented in **Figure 50-A**. The results indicate that the average virus load O.D. reading is 0.028 (SD ± 0.035) with a median of 0.022 (range of -0.038 - 0.283). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

VE-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 187 control assays is provided in **Figure 51-A**. The results indicate that the average differential O.D. reading is 1.096 (SD ± 0.151) with a median of 1.105 (range 0.669 - 1.475). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 90% measurement accuracy.

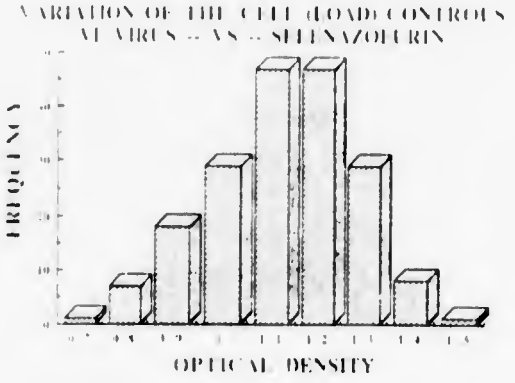


Figure 49-A

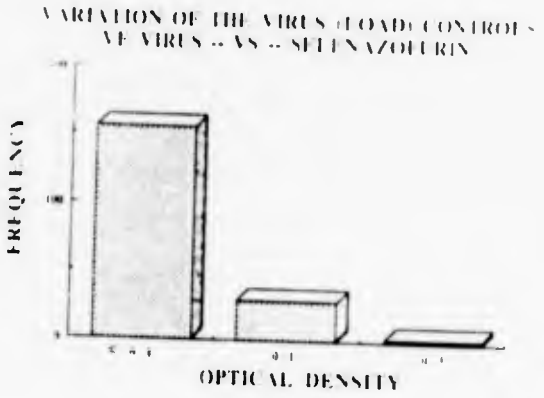


Figure 50-A

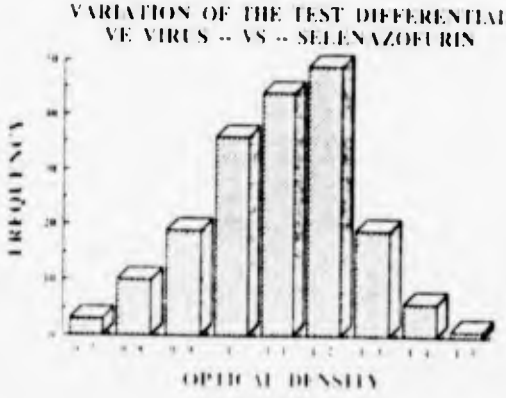


Figure 51-A

4.3.4.1.1 Antiviral Activity of AVS-6724 (2-Thio-6-Azauridine) vs VE Virus:

Second Control Compound-Antiviral Performance: 2-Thio-6-Azauridine (AVS-6724) has been tested as a second control compound against VE in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of this second positive control drug are illustrated in **Figure 47-B**.

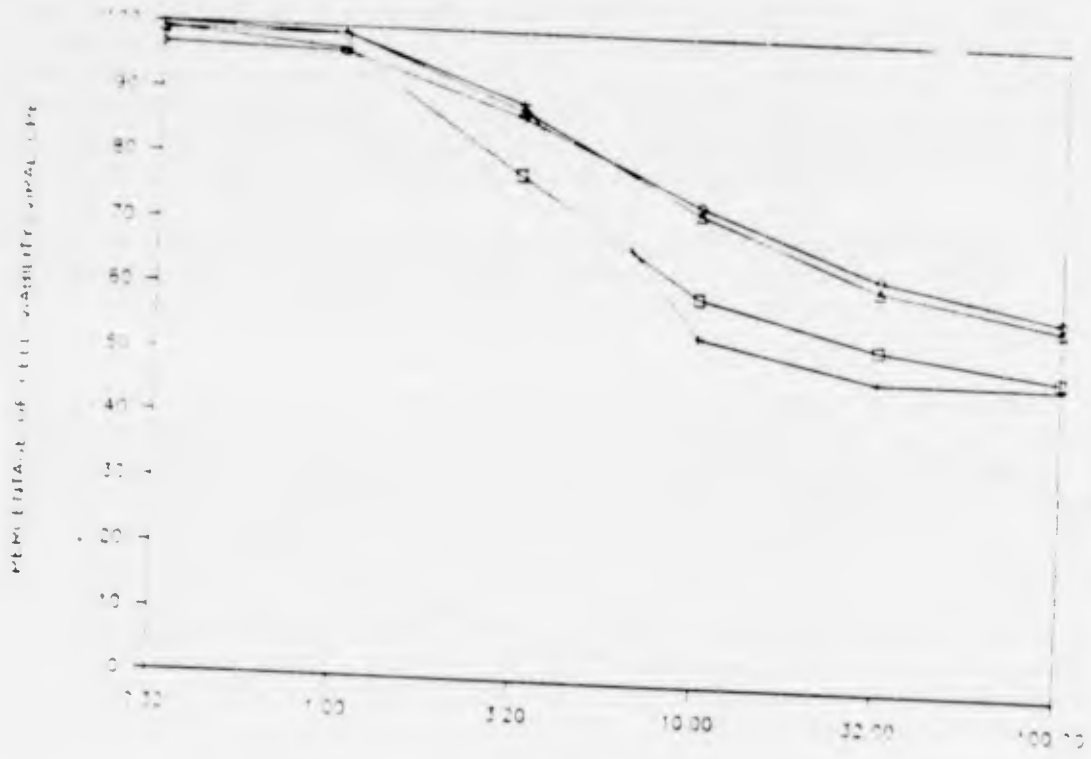
The 111 control tests performed with 2-Thio-6-Azauridine gave a mean **Total Antiviral Index** (TAI) of 12.2% (SD ± 14) and the median value was 6.5%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from 0 - >56% during this period. The mean **Selectivity Index (SI)** was 3.6 (SD ± 8.8) and the median SI value was 0.55, indicating poor antiviral selectivity for 2-Thio-6-Azauridine and it ranged from 0 - >57.5 during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25% (AI_{25})** value was 7.14 (SD ± 14.21). The median AI_{25} value was 1.7 (range 0.085 - >81.25). The mean **Antiviral Index 50% (AI_{50})** was 11.01 (SD ± 15.45) with a median of 4.01 (range 0 - >68.64). The mean **Antiviral Index 95% (AI_{95})** was 0.84 (SD ± 4.9) with a median of 0 (range 0 - >33.24).

The mean **Antiviral Inhibitory Concentration 25% (IC_{25})** was 15.72 μg/ml (SD ± 21.94). The median IC_{25} value was 5.02 μg/ml (range 0.88 - 92.7 μg/ml). The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 17.39 μg/ml (SD ± 33.06). The median IC_{50} value was 4.97 μg/ml (range = 0 - 205 μg/ml). The mean **Antiviral Inhibitory Concentration 95% (IC_{95})** was 0.105 (SD ± 0.624). The median IC_{95} value was 0 (range = 0 - >4.68 μg/ml). This discrepancy indicates that the control compound 2-Thio-6-Azauridine does not consistently reach 50 or 95% reduction levels.

The average maximum antiviral inhibitory level of 111 2-Thio-6-Azauridine tests **(Figure 47-B)** was reached at 10 μg/ml of the compound with 50% antiviral effect. Further increase of the drug concentration does not improve its antiviral activity. Maximum antiviral effect (~50%) was found with a simultaneous ~25% cytotoxic suppression. Above 10 μg/ml concentration, the antiviral protection levels off to approximately 50% reduction level at 100 μg/ml while simultaneously 2-Thio-6-Azauridine becomes maximally toxic around 40%.

2-THIO-6-AZAURIDINE - VE VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability Δ Median % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	0.32	1	3.2	10	32	100	0.32	1	3.2	10	32	100
Mean	99	97	78	60	53	49	97	96	87	74	64	58
Median	100	99	89	54	48	48	99	99	88	73	62	57
Std. Dev.	0.01	0.05	0.25	0.28	0.19	0.11	0.04	0.05	0.10	0.11	0.11	0.10

Figure 47-B
Average Antiviral and Cytotoxicity Values for 111 Positive Control Compound Tests

4.3.4.1.2 Maximum Antiviral Effect of 2-Thio-6-Azauridine vs VE Virus (VE):

A bar graph scatter plot (**Figure 48-B**) depicts the distribution of the maximum antiviral reduction values of all 111 control compound assays for 2-Thio-6-Azauridine. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 57% (SD ± 15.9) reduction levels. The maximum reduction levels vary from 26 - 100% but remain quite consistently around the median of 55%. The assay control values give a relatively broad, bell-shaped distribution curve. This indicates quite a consistent day-to-day performance of the control compound in the VE-MTT assay.

Recommendations:

Based upon the data obtained in parallel studies with Selenazofurin, we do not recommend that 2-Thio-6-Azauridine (AVS-6724) be used as a second control compound against the VE virus. Although its overall performance is not as good as that obtained with the present control compound, Selenazofurin, it is readily available from Sigma Chemical Company, it is inexpensive and works as effectively at low drug concentrations as Selenazofurin.

Variation of the Maximum Antiviral Effect
VE Virus - VS - 2-Thio-6-Azauridine

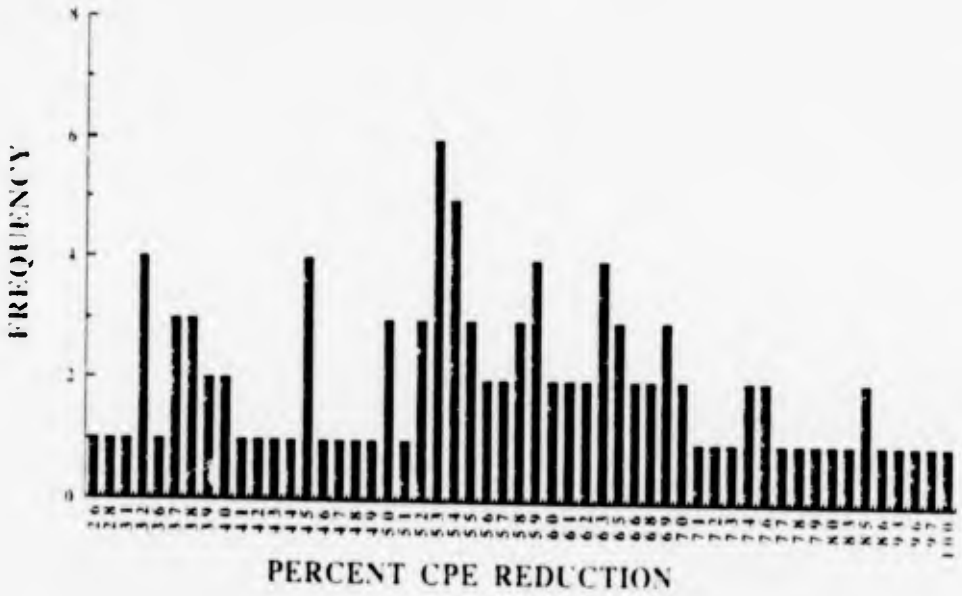


Figure 48-B
Maximum Antiviral CPE Reduction (%).
Summary of 111 Control Tests.

4.3.4.1.3 Cellular Cytotoxicity of 2-Thio-6-Azauridine vs VE Virus:

VE-Control Compound-Cytotoxicity Performance: The 111 cytotoxicity values of the positive control compound 2-Thio-6-Azauridine are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 23.04 μg/ml (SD ± 39.55) and the median was 9.03 μg/ml (range of 1.69 - >320 μg/ml). The mean cell **Toxic Concentration 50%** (TC_{50}) value was 91.08 μg/ml (SD ± 33.2) and the median was 100 μg/ml (range of 10 - >320 μg/ml). The mean cell **Toxic Concentration 95%** (TC_{95}) value was 102.14 μg/ml (SD ± 21.68) and the median was 100 μg/ml (range of >100 - >320 μg/ml).

As can be seen from **Figure 47-B**, the toxicity starts to become measurable above the concentration of 1.0 μg/ml and the maximum toxicity has not been reached at 100 μg/ml.

When the cytotoxicity reaches around 40% (100 μg/ml), the control compound (2-Thio-6-Azauridine) has not reached its maximum toxicity. Also, at 100 μg/ml the antiviral protection of 2-Thio-6-Azauridine has reached its maximum antiviral effect (~50%). The highest 2-Thio-6-Azauridine concentration tested in these assays was 100 μg/ml.

2-Thio-6-Azauridine has a definite cytotoxic suppression on cellular metabolism and growth. The TC_{25} and TC_{50} toxicity can be achieved with relative consistency at 100 μg/ml.

4.3.4.1.4 VE-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (2-Thio-6-Azauridine):

VE-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 111 control assays is plotted in **Figure 49-B**. The results indicate that the cell O.D. readings reached a mean 1.155 (SD ± 0.125) with a median of 1.167 (range of 0.81 - 1.374). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

VE-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 111 control assays is presented in **Figure 50-B**. The results indicate that the average virus load O.D. reading is 0.024 (SD ± 0.033) with a median of 0.021 (range of -0.017 - 0.283). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

VE-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 111 control assays is provided in **Figure 51-B**. The results indicate that the average differential O.D. reading is 1.131 (SD ± 0.125) with a median of 1.142 (range 0.744 - 1.377). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 86% measurement accuracy.

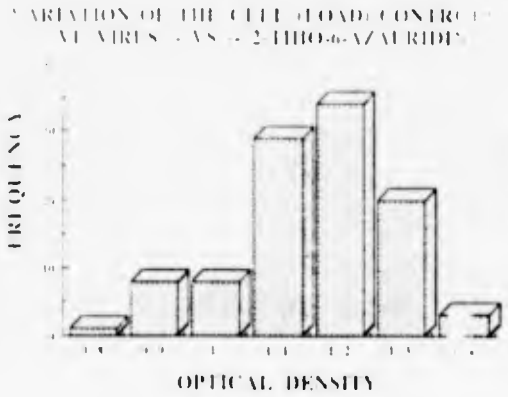


Figure 49-B

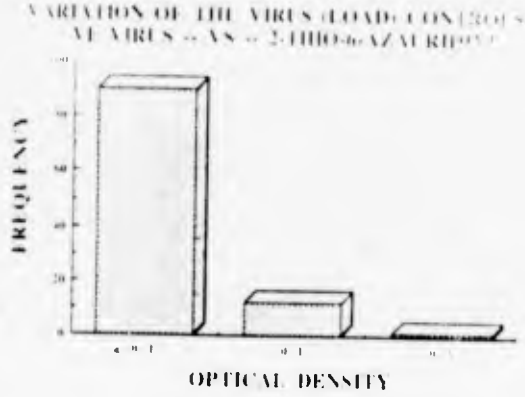


Figure 50-B

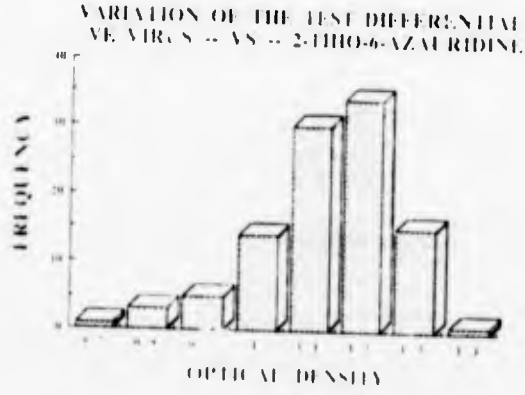


Figure 51-B

4.3.4.1.5 Overall VE-Assay Plate Quality Controls:

VE-Overall-Cell Load Performance: A bar graph scatter plot of the overall mean cell control (O.D. reading) of 3386 accepted assays is plotted in **Figure 49-C**. The results indicate that the overall cell O.D. readings reached a mean of 1.136 (SD ± 0.126) with a median of 1.145 (range of 0.545 - 1.498). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

VE-Overall-Virus Load Performance: A bar graph scatter plot of the overall mean virus load O.D. readings of the 3386 accepted assays is presented in **Figure 50-C**. The results indicate that the overall average virus load O.D. reading is 0.028 (SD ± 0.032) with a median of 0.022 (range of 0.00 - 0.438). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

VE-Overall-Assay Differential Performance: A bar graph scatter plot of the overall mean O.D. differential values of the 3386 accepted assays is provided in **Figure 51-C**. The results indicate that the overall average differential O.D. reading is 1.109 (SD ± 0.129) with a median of 1.121 (range 0.562 - 1.475). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 89% measurement accuracy.

GRAPHIC ILLUSTRATION OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ALL ACCEPTED VE PLATE DATA

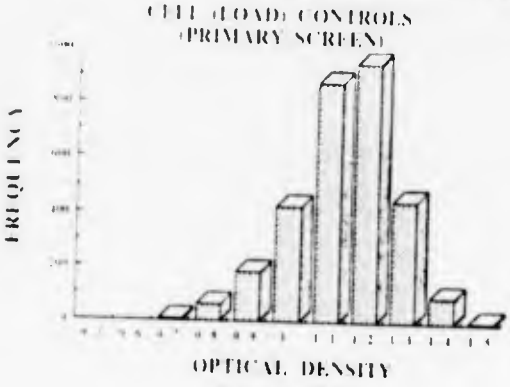


Figure 49-C

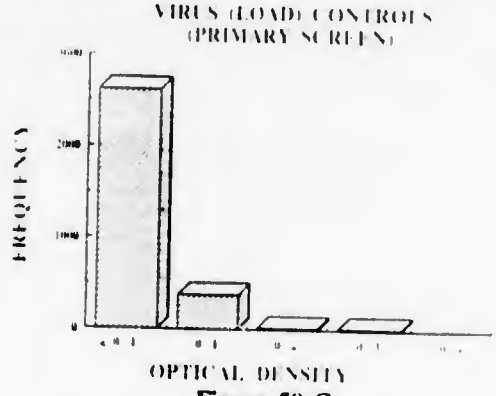


Figure 50-C

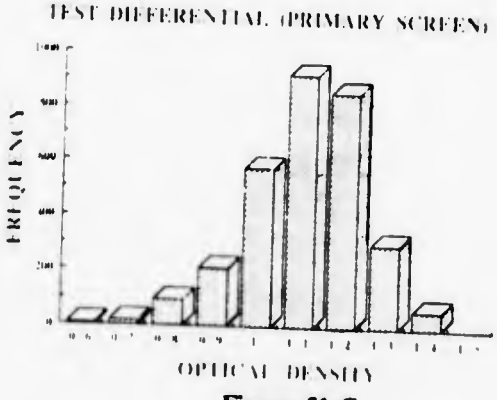


Figure 51-C

4.3.4.2 VE-Antiviral Activity Results:

New Drugs with 95% Antiviral Reduction Levels: Out of the 3386 actual single drug tests, 15 new compounds demonstrated excellent antiviral activity, having antiviral reduction values of equal to or better than 95%. This represents around 0.4% of the test compounds being active at this excellent reduction level. These compounds are summarized in **Table 16** according to the highest **Total Antiviral Index (TAI)**. AVS-11907 demonstrated the greatest *in vitro* promise with a TAI of 70% and a SI value of 142. AVS-11717, 9589 and 11095, demonstrated moderate antiviral activity with TAI's that ranged from 41 - 47, and SI values that ranged from 12 - 28.

Table 16

AVS Compounds Active Against Venezuelan Equine Encephalomyelitis (VE) at AI_{95} Level

	AVS No.	Shipment#	Test Date	Differntl.	IC 95	TC 95	AI 95	SI	TAI
VE	11907	82	09/27/91	1.022	3.01 >	320.00 >	106.31	142.18 >	69.91
VE	11717	82	09/27/91	1.125	28.30 >	320.00 >	11.30	28.04 >	47.43
VE	9589	79	05/03/91	0.937	203.00 >	320.00 >	1.57 >	14.13 >	44.09
VE	11095	78	10/11/91	0.965	12400.0 >	10000.0 >	0.81 >	11.57 >	40.98
VE	9896	79	06/14/91	0.999	2.97 >	320.00 >	107.74	5.84 >	27.91
VE	11279	81	07/12/91	1.134	1000.00	3200.00	3.20	6.69	27.45
VE	11086	78	04/12/91	0.826	943.00 >	3200.00 >	3.39	2.95	16.37
VE	11506	81	08/09/91	1.089	3040.00	9710.00	3.19	2.70 >	15.76
VE	11100	78	04/12/91	0.806	301.00	965.00	3.21	2.76	15.48
VE	9394	77	03/08/91	1.080	3000.00 >	3200.00 >	1.07 >	1.94 >	12.57
VE	9414	81	10/08/91	1.041	937.00 >	1000.00 >	1.07 >	1.92 >	11.88
VE	9217	77	10/11/91	0.998	3160.00 >	3200.00 >	1.01 >	1.82 >	10.26
VE	10348	82	09/27/91	1.199	299.00 >	320.00 >	1.07	0.29 >	7.70
VE	9969	79/C2	08/16/91	1.067	3020.00	906.00	0.30	0.10 >	5.14
VE	9590	79	10/04/91	0.879	945.00	95.80	0.10	0.07 >	4.96

New Drugs with 50% Antiviral Reduction Levels: Out of the 3386 actual single drug tests, 159 new compounds demonstrated good antiviral activity, having antiviral reduction values equal to or better than 50%. This represents around 4.7% of the test compounds being active at this good antiviral reduction level. These compounds are summarized in **Table 17** according to the highest **Total Antiviral Index (TAI)**. AVS-11121 demonstrated the best TAI of 33% and a SI of 5.2. Six other compounds demonstrated moderate antiviral activity, having TAI's from 20 - 29% and SI's from 1.5 - 7.2. The rest (152 compounds) showed marginal antiviral activity with TAI's from 0 to 18% and SI's from 0.02 to 4.1.

Table 17

AVS Compounds Active Against Venezuelan Equine Encephalomyelitis (VE) at AI_{50} Level

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
VE 11121	78	04/12/91	0.714	1930.00 >	10000.0 >	5.18 >	5.18 >	33.38
VE 11838	82	08/23/91	1.135	431.00 >	3200.00 >	7.43	7.20 >	28.63
VE 11924	82	10/04/91	1.198	217.00 >	320.00 >	1.47 >	1.47 >	27.49
VE 11191	80	10/11/91	1.164	800.00 >	1000.00 >	1.25 >	1.25 >	23.09
VE 11078	78	10/11/91	0.974	827.00	6000.00	7.25	4.84	22.94
VE 11827	82	08/23/91	1.113	63.80	616.00	9.66	5.36	20.67
VE 11313	81	07/19/91	1.120	1350.00	5750.00	4.27	2.69 >	20.24
VE 11060	78	04/05/91	1.068	977.00	6050.00	6.19	4.05	17.84
VE 11111	78	04/12/91	0.755	556.00	1810.00	3.25	2.00 >	17.68
VE 9298	77	03/01/91	1.177	1370.00 >	3200.00 >	2.33	1.70 >	17.00
VE 11839	82	08/23/91	1.135	1080.00 >	3200.00 >	2.97 >	2.97 >	16.74
VE 9849	79	06/07/91	1.288	9.50	51.40	5.42	2.86 >	16.38
VE 11188	80	10/11/91	1.124	161.00	634.00	3.94	2.81	16.19
VE 9449	79	04/26/91	0.855	57.40	220.00	3.83	2.78 >	16.13
VE 11186	80	07/12/91	1.235	181.00	660.00	3.64	2.70	15.77
VE 11286	81	07/12/91	1.134	550.00	2480.00	4.51	2.78	15.61
VE 9264	77	02/26/91	0.897	531.00	1940.00	3.65	2.45	15.50
VE 9389	77	03/08/91	0.968	1790.00 >	3200.00 >	1.79 >	1.79 >	15.14
VE 11400	81	07/26/91	0.924	452.00	1560.00	3.45	1.85 >	14.84
VE 9450	79	10/04/91	1.092	53.10	190.00	3.57	2.29	14.75
VE 9253	77	02/26/91	1.033	1490.00 >	3200.00 >	2.15 >	2.15 >	14.52
VE 11306	81	07/19/91	1.064	689.00	2100.00	3.05	2.25 >	13.42
VE 11848	82	08/23/91	1.042	609.00	2040.00	3.35	2.40	13.24
VE 11108	78	10/18/91	1.002	11300.0 >	10000.0 >	0.88	0.58 >	13.10
VE 11420	81	07/26/91	1.011	1900.00	5410.00	2.84	1.39 >	12.64
VE 11329	81	07/19/91	1.159	1590.00 >	3200.00 >	2.01 >	2.01 >	12.63
VE 11911	82	09/27/91	0.974	214.00 >	320.00 >	1.50 >	1.50 >	12.53
VE 11290	81	07/12/91	1.168	733.00 >	3200.00 >	4.37	2.50 >	12.39
VE 11048	78	10/11/91	1.019	505.00	1670.00	3.31	1.82	12.34
VE 9268	77	02/26/91	0.988	566.00	1870.00	3.31	2.14	11.98
VE 11064	78	04/05/91	0.914	1770.00	5690.00	3.21	1.86 >	11.81
VE 11737	82	08/16/91	1.129	479.00	1130.00	2.36	1.41	11.42
VE 9366	77	03/08/91	1.084	1520.00 >	3200.00 >	2.10	1.40 >	11.32
VE 11097	78	04/12/91	0.800	660.00	2080.00	3.15	2.30	11.25
VE 11804	82	08/23/91	0.851	626.00	2050.00	3.28	2.37	11.22
VE 11507	81	08/09/91	1.089	616.00	1860.00	3.02	1.86	11.05
VE 11795	82	08/23/91	0.863	865.00	2150.00	2.48	1.82 >	10.77
VE 9173	77	02/08/91	0.990	3040.00 >	3200.00 >	1.05 >	1.05 >	10.75
VE 11109	78	04/12/91	0.682	1000.00	1710.00	1.71	0.97 >	10.73
VE 11611	81	08/09/91	1.171	589.00	1960.00	3.33	2.26	10.66
VE 11201	80	09/27/91	1.074	241.00 >	320.00 >	1.33 >	1.33 >	10.63
VE 11102	78	04/12/91	0.803	6260.00 >	10000.0 >	1.60 >	1.60 >	10.57
VE 11613	81	08/09/91	1.163	202.00	626.00	3.10	2.15	10.45
VE 9917	79	06/14/91	1.091	607.00 >	1000.00 >	1.65 >	1.65 >	10.23
VE 11854	82	08/23/91	1.155	229.00	618.00	2.70	1.87	10.15
VE 9162	77	02/08/91	1.175	773.00	1740.00	2.26	1.05 >	10.05
VE 11144	78	04/19/91	1.067	2350.00	6460.00	2.75	2.00	9.83
VE 9172	77	02/08/91	0.990	922.00	1940.00	2.10	0.96 >	9.68
VE 11287	81	07/12/91	1.161	649.00	1870.00	2.89	1.87	9.67

Table 17 (Cont'd)

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
VE 9489	80	10/11/91	1.050	201.00	544.00	2.70	1.55	9.57
VE 9452	79	10/04/91	1.092	65.70	192.00	2.92	1.52	9.56
VE 11610	81	08/09/91	1.162	590.00	1870.00	3.18	2.06	9.44
VE 11117	78	04/12/91	0.806	2520.00	5470.00	2.17	1.23 >	9.27
VE 11131	78	04/19/91	1.123	1930.00	5140.00	2.67	1.47 >	9.05
VE 11125	78	10/18/91	1.021	5440.00 >	10000.0 >	1.84	1.72 >	8.97
VE 11093	78	10/11/91	0.965	2060.00	5280.00	2.56	1.44	8.75
VE 11719	82	08/16/91	1.028	872.00	2630.00	3.01	1.85	8.62
VE 11211	80	09/27/91	0.978	90.70	204.00	2.25	1.61	8.51
VE 11566	81	08/09/91	1.033	684.00 >	1000.00 >	1.46	1.21 >	8.49
VE 9121	77	10/11/91	1.242	5820.00 >	10000.0 >	1.72 >	1.72 >	8.36
VE 11103	78	04/12/91	0.810	1000.00	2100.00	2.10	1.55	8.32
VE 11411	81	07/26/91	1.121	668.00	1860.00	2.78	1.78	8.31
VE 11550	81	08/09/91	1.065	320.00 >	1000.00 >	3.13	0.51 >	7.97
VE 9451	79	10/04/91	1.092	65.60	142.00	2.16	1.15 >	7.92
VE 9842	79	06/07/91	1.312	93.40	266.00	2.85	1.74	7.68
VE 9487	80	10/11/91	1.042	301.00 >	320.00 >	1.06 >	1.06 >	7.65
VE 11489	81	08/09/91	0.998	23.90	60.50	2.53	1.70 >	7.49
VE 11202	80	09/27/91	1.074	93.00	211.00	2.27	1.67	7.30
VE 11119	78	04/12/91	0.792	2900.00 >	3200.00 >	1.10	0.66 >	7.19
VE 11302	81	07/19/91	1.199	808.00	1690.00	2.09	0.93 >	7.17
VE 11881	82	08/30/91	0.562	1000.00	1980.00	1.98	1.37	7.12
VE 11043	78	04/05/91	0.928	6800.00 >	10000.0 >	1.47	1.18 >	6.97
VE 11574	81	08/09/91	0.913	657.00	1630.00	2.44	1.04 >	6.90
VE 11331	81	07/19/91	1.114	2220.00 >	3200.00 >	1.44 >	1.44 >	6.74
VE 9163	77	02/08/91	1.096	669.00	1480.00	2.22	0.94 >	6.70
VE 11842	82	08/23/91	1.126	776.00	1980.00	2.54	1.74	6.65
VE 11206	80	09/27/91	1.118	97.70	211.00	2.16	1.59	6.60
VE 9448	79	04/26/91	0.855	265.00 >	320.00 >	1.21 >	1.21 >	6.50
VE 11806	82	08/23/91	1.022	688.00	1870.00	2.72	1.76	6.34
VE 11780	82	08/23/91	0.793	951.00	1670.00	1.76	0.98 >	6.24
VE 11612	81	08/09/91	1.171	523.00	1370.00	2.62	0.76	6.17
VE 11904	82	08/30/91	0.912	951.00	1670.00	1.76	0.98 >	6.14
VE 11582	81	08/09/91	0.969	787.00	1760.00	2.24	1.31 >	6.11
VE 11650	81	08/16/91	1.112	894.00	1890.00	2.11	1.38	5.79
VE 11150	78	04/19/91	1.051	701.00	1370.00	1.95	1.06	5.52
VE 11727	82	08/16/91	1.136	236.00	607.00	2.57	1.56	5.47
VE 9511	80	10/11/91	1.050	69.50	180.00	2.59	1.24	5.38
VE 9970	79	06/28/91	1.172	865.00	1870.00	2.17	1.40	5.34
VE 11635	81	08/16/91	1.127	693.00	1650.00	2.38	1.18 >	5.24
VE 11658	81	08/16/91	1.215	739.00	1550.00	2.10	1.07	5.06
VE 9886	79	06/14/91	1.387	7.38	15.20	2.05	1.03 >	5.01
VE 11447	82	08/16/91	1.103	2020.00	370.00	0.18	0.10 >	4.90
VE 11568	81	08/09/91	1.025	915.00	1810.00	1.98	1.22	4.88
VE 11688	82	09/27/91	1.181	84.50	193.00	2.28	1.37	4.38
VE 9381	77	03/08/91	1.142	2260.00 >	3200.00 >	1.42	0.53 >	4.20
VE 9871	79	06/07/91	1.349	100.00	192.00	1.92	1.28	4.01
VE 9221	77	02/15/91	1.072	2030.00 >	3200.00 >	1.58	0.46 >	3.75
VE 9772	79	05/31/91	0.997	8.11	15.80	1.95	1.05	3.72
VE 11368	81	07/19/91	1.162	2190.00	5000.00	2.28	0.38	3.51
VE 10663	83	11/01/91	1.117	290.00 >	320.00 >	1.10	0.69 >	.56
VE 9360	77	03/08/91	1.163	7070.00	5750.00	0.81	0.51	3.56
VE 11216	80	09/27/91	1.041	82.70	146.00	1.76	0.88	3.53
VE 11873	82	08/30/91	1.000	2630.00 >	3200.00 >	1.22	0.81 >	3.51
VE 11418	81	07/26/91	1.065	2240.00 >	3200.00 >	1.43	0.98 >	3.42
VE 11560	81	08/09/91	0.995	793.00	1510.00	1.90	0.81 >	3.38
VE 9476	79	04/26/91	0.862	87.70	176.00	2.01	1.17	3.36
VE 11423	81	07/26/91	0.861	768.00 >	1000.00 >	1.30	0.29 >	3.33
VE 11409	81	07/26/91	0.967	2600.00	5070.00	1.95	1.02	3.31
VE 11490	81	08/09/91	1.049	2590.00	3930.00	1.52	0.79	3.19
VE 9166	77	02/08/91	1.121	856.00	1820.00	2.12	0.35	3.19

Table 17 (Cont'd)

	AVS No.	Shipment#	Test Date	Differntl.	IC 50	TC 50	AI 50	SI	TAI
VE	11155	78	04/19/91	1.160	81.00	143.00	1.76	0.93	3.17
VE	11372	81	07/19/91	1.311	2480.00 >	3200.00 >	1.29	0.79 >	3.11
VE	11570	81	08/09/91	1.097	839.00	1040.00	1.25	0.80	3.11
VE	9276	77	03/01/91	1.129	2860.00 >	3200.00 >	1.12	0.71 >	3.10
VE	9350	77	03/01/91	1.132	904.00	1610.00	1.78	0.73 >	2.80
VE	11623	81	08/09/91	1.086	2330.00 >	3200.00 >	1.37	0.63 >	2.79
VE	11185	80	07/12/91	1.235	948.00	779.00	0.82	0.58 >	2.77
VE	11720	82	08/16/91	1.028	963.00	1040.00	1.08	0.63	2.66
VE	9134	77	02/01/91	1.182	2520.00 >	3200.00 >	1.27	0.87 >	2.54
VE	11278	81	07/12/91	1.179	744.00	1450.00	1.95	0.50	2.33
VE	11828	82	08/23/91	1.113	855.00	1590.00	1.86	0.89	2.30
VE	10637	83	11/01/91	1.125	227.00 >	320.00 >	1.41	1.41 >	2.25
VE	11424	81	07/26/91	0.861	885.00	213.00	0.24	0.18 >	1.94
VE	11656	81	08/16/91	1.119	969.00	1150.00	1.19	0.65	1.92
VE	11866	82	08/30/91	1.087	1890.00	239.00	0.13	0.04 >	1.90
VE	11738	82	08/16/91	1.101	2860.00	4770.00	1.66	0.71	1.89
VE	9267	77	02/26/91	1.002	2820.00 >	3200.00 >	1.14	0.79 >	1.59
VE	11360	81	07/19/91	1.285	661.00	7.81	0.01 <	0.02	1.44
VE	9513	80	07/12/91	1.099	90.[illegible]	66.70	0.74	0.34 >	1.34
VE	11308	81	07/19/91	1.213	2740.00	5920.00	2.16	0.69	1.30
VE	10031	79/C2	08/16/91	1.063	2270.00	558.00	0.25	0.15 >	1.20
VE	11075	78	10/11/91	1.040	2780.00	2960.00	1.07	0.71	1.20
VE	11168	78	04/19/91	0.998	1000.00 >	1000.00 >	1.00	0.67 >	1.20
VE	11296	81	07/19/91	1.283	2660.00 >	3200.00 >	1.20	0.73 >	1.17
VE	11383	81	07/19/91	1.191	3040.00	2100.00	0.69	0.51	1.11
VE	9171	77	02/08/91	1.020	957.00	1000.00	1.04	0.28 >	1.10
VE	9889	79	06/14/91	1.386	2.66	3.94	1.48	0.71	1.10
VE	10568	83	11/01/91	1.113	259.00 >	320.00 >	1.24	0.31 >	1.01
VE	11864	82	08/30/91	1.024	1000.00	1430.00	1.43	0.68	1.01
VE	11781	82	08/23/91	0.756	264.00	291.00	1.10	0.24	1.00
VE	11101	78	04/12/91	0.803	930.00	1200.00	1.29	0.71	0.96
VE	11768	82	08/23/91	0.977	3200.00	2210.00	0.69	0.50	0.91
VE	11536	81	08/09/91	1.050	268.00	316.00	1.18	0.78	0.87
VE	9236	77	02/15/91	1.176	1000.00	320.00	0.32	0.18	0.80
VE	10062	82	09/06/91	1.199	238.00	61.80	0.26	0.18 >	0.74
VE	11618	81	08/09/91	1.209	1000.00	1490.00	1.49	0.38	0.68
VE	11395	81	07/26/91	1.037	8160.00 >	10000.0 >	1.23	0.46 >	0.59
VE	11615	81	08/09/91	1.165	3120.00 >	3200.00 >	1.02	0.66 >	0.52
VE	11734	82	08/16/91	1.095	819.00	799.00	0.98	0.68 >	0.47
VE	11366	81	07/19/91	1.181	2390.00	24.70	0.01 <	0.00 >	0.41
VE	11233	80	09/27/91	1.178	30.60	38.20	1.25	0.55	0.26
VE	11224	80	10/11/91	0.931	320.00	89.10	0.28	0.09 >	0.13
VE	11630	81	08/09/91	1.229	3130.00	3820.00	1.22	0.17	0.13
VE	9768	79	05/31/91	1.008	10.00	1.00	0.10 <	0.10	0.12
VE	9816	79	10/04/91	1.038	28.80	6.00	0.21	0.14 >	0.11
VE	9796	79	05/31/91	1.039	29.40	27.20	0.93	0.63 >	0.10
VE	11731	82	08/16/91	1.217	955.00	975.00	1.02	0.36	0.01
VE	11769	82	08/23/91	0.991	684.00	248.00	0.36	0.18	0.00
VE	11843	82	08/23/91	1.126	918.00	603.00	0.66	0.40	0.00

New Drugs with 25% Antiviral Reduction Levels: Of the 3386 actual single drug tests, 492 new compounds demonstrated moderate antiviral activity, having antiviral reduction values equal to or better than 25%. This represents around 15% of the test compounds being active at this minimal antiviral reduction level.

In general, when compared to the 95% and 50% antiviral activity categories, the compounds in this (25%) category do not appear to have any significant antiviral promise and probably do not need to be presently confirmed any further.

4.3.4.3 Confirmatory Assays:

Some of the compounds were sent to us in more than one separate drug shipment. These compounds were tested more than once. Data from the confirmatory assays are summarized in **Table 18**. If a compound showed $\geq$50% reduction in CPE during this contract period, then it was considered a candidate for confirmatory testing. The confirmatory tests are from active compounds taken from the prescreen and primary MTT assays. Out of 81 confirmatory tests, 59 compounds were confirmed active during this reporting period and the remaining 22 compounds gave conflicting results. The criteria for activity is that the confirmatory test has to show $\geq$25% reduction in CPE. Failure to confirm the activity in these compounds was probably due to differences during the assay conditions:

1) In confirmatory assays the concentration range is adjusted to a more accurate semilog scale to maximize the TAI window.

2) Differences in the "differential" of the two runs can cause the compound to read positive or negative, falsely. The variability in the differential can cause false positive or negative bias to be introduced into the calculations, thus reflecting the variability in the maximum activity of the compound.

3) The metabolic rate, cell and virus load/well, age, and passage number of the cells may cause the above observed variability in the confirmatory results.

4) Problems associated with stability and storage of the compound (i.e., different lot numbers, solubility, light sensitivity, hygroscopic, etc.).

5) Problems associated with technical execution of large numbers of plates by different technicians.

During this reporting period the overall confirmatory rate against VE was 73%. The conflicting results should be retested at a later date based on the availability of the compound.

Some of the compounds have not been confirmed due to the discontinuation of this project by the sponsor.

4.3.4.4 Recommendations of VE-Actives Based Upon the *In Vitro* Results with MTT Assay (Vero Cells).

Based upon the *in vitro* results with the MTT assay (Vero cells) we recommend the following:

1) Compounds with the highest TAI in the 95% activity category that have confirmed results with the exception of "colored" compounds should receive the highest priority for further profile studies and *in vivo* animal testing.

2) Compounds with the highest TAI in the 50% activity category that have confirmed results with the exception of "colored" compounds should receive the next highest priority for further profile studies and *in vivo* animal testing.

Table 18

Confirmatory Assays for Compounds Active Against Venezuelan Equine Encephalomyelitis Virus (VE)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
10031	79	07/03/91	6TN	1.036	0.00	1360.0	0.00	0.00	2000.00	0.00	0.00	3150.00	0.00	0.00 >	2.21	-
10031	79/C2	08/16/91	806	1.063	1510.0	338.00	0.22	2270.00	558.00	0.25	0.00	956.00	0.00	0.15 >	1.20	+
11043	78	04/05/91	4DN	0.928	4190.0	8020.0	1.91	6800.00 >	10000.0 >	1.47	0.00 >	10000.0	0.00	1.18 >	6.97	+
11043	78	10/11/91	AHL	1.104	7300.0	5050.0	0.69	0.00	8150.00	0.00	0.00 >	10000.0	0.00	0.00	0.40	+
11048	78	04/05/91	4EM	0.967	224.00	688.00	3.07	606.00	1480.00	2.45	0.00	3030.00	0.00	1.14	7.31	+
11048	78	10/11/91	AHN	1.018	224.00	918.00	4.09	505.00	1670.00	3.31	0.00	3050.00	0.00	1.82	12.34	+
11060	78	04/05/91	4ES	1.068	553.00	3960.0	7.15	977.00	6050.00	6.19	0.00	9830.00	0.00	4.05	17.84	+
11060	78	10/11/91	AHN	1.018	543.00	1210.0	2.23	0.00	1870.00	0.00	0.00	3070.00	0.00	0.00	5.08	+
11064	78	04/05/91	4EU	0.914	1040.0	3300.0	3.16	1770.00	5690.00	3.21	0.00	10000.0	0.00	1.86 >	11.81	+
11064	78	10/11/91	AHO	1.038	1460.0	3630.0	2.48	2310.00	5780.00	2.51	0.00	9660.00	0.00	1.57 >	6.55	+
11075	78	04/12/91	4KX	0.850	1350.0	2450.0	1.81	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	6.20	+
11075	78	10/11/91	AHQ	1.040	1560.0	1980.0	1.27	2780.00	2960.00	1.07	0.00	9230.00	0.00	0.71	1.20	+
11078	78	04/12/91	4KZ	0.835	468.00	2630.0	5.61	971.00 >	3200.00 >	3.29	0.00 >	3200.00	0.00	2.70 >	15.48	+
11078	78	10/11/91	AFJ	0.974	420.00	4000.0	9.53	827.00	6000.00	7.25	0.00	9600.00	0.00	4.84	22.94	+
11086	78	04/12/91	4L3	0.826	414.00	1640.0	3.96	556.00	2280.00	4.10	943.00 >	3200.00 >	3.39	2.95	16.37	+
11086	78	10/11/91	AFJ	0.974	443.00	1300.0	2.94	666.00	1990.00	2.99	0.00	4560.00	0.00	1.96	9.80	+
11093	78	04/12/91	4L6	0.821	1220.0	2620.0	2.15	1990.00 >	3200.00 >	1.61	0.00 >	3200.00	0.00	1.31 >	6.85	+
11093	78	10/11/91	AFK	0.965	1280.0	2960.0	2.31	2060.00	5280.00	2.56	0.00	9530.00	0.00	1.44	8.75	+
11095	78	04/12/91	4LP	0.758	3200.0 >	10000 >	3.13	5370.00 >	10000.0 >	1.86	0.00 >	10000.0	0.00 >	1.86 >	20.07	+
11095	78	10/11/91	AFK	0.965	197.00 >	10000 >	50.73	864.00 >	10000.0 >	11.57	12400 >	10000.0 >	0.81 >	11.57 >	40.98	+
11097	78	04/12/91	4LQ	0.800	449.00	1520.0	3.38	660.00	2080.00	3.15	0.00	3090.00	0.00	2.30	11.23	+
11097	78	10/11/91	AFL	1.069	583.00	1190.0	2.03	0.00	1960.00	0.00	0.00 >	3200.00	0.00	0.00 >	4.40	+
11100	78	04/12/91	4LR	0.806	128.00	480.00	3.75	173.00	653.00	3.76	301.00	965.00	3.21	2.76	15.48	+
11100	78	10/18/91	ALY	1.003	0.00	320.00	0.00	0.00	641.00	0.00	0.00	2780.00	0.00	0.00	0.66	-
11101	78	04/12/91	4LS	0.803	507.00	660.00	1.30	930.00	1200.00	1.29	0.00	3000.00	0.00	0.71	0.96	+
11101	78	10/18/91	ALZ	1.011	833.00	618.00	0.74	0.00	1530.00	0.00	0.00	3030.00	0.00	0.00	0.06	+
11102	78	04/12/91	4LS	0.803	3770.0 >	10000 >	2.66	6260.00 >	10000.0 >	1.60	0.00 >	10000.0	0.00 >	1.60 >	10.57	+
11102	78	10/18/91	ALZ	1.011	7520.0	5260.0	0.70	0.00	9210.00	0.00	0.00 >	10000.0	0.00	0.00	0.01	+
11103	78	04/12/91	4LT	0.810	499.00	1550.0	3.11	1000.00	2100.00	2.10	0.00	3090.00	0.00	1.55	8.32	+
11103	78	10/18/91	AMO	0.989	952.00	1120.0	1.18	0.00	1860.00	0.00	0.00	3200.00	0.00	0.00 >	1.62	+

Table 18 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
11108	78	04/12/91	4LV	0.861	0.00	2320.0	0.00	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	2.91	-
11108	78	10/18/91	AM1	1.002	780.00	6600.0	8.46	11300.0 >	10000.0 >	0.88	0.00 >	10000.0	0.00	0.58 >	13.10	+
11109	78	04/12/91	4LW	0.682	411.00	974.00	2.37	1000.00	1710.00	1.71	0.00	3050.00	0.00	0.97 >	10.73	+
11109	78	10/18/91	AM1	1.002	0.00	519.00	0.00	0.00	1080.00	0.00	0.00	2990.00	0.00	0.00	1.30	-
11111	78	04/12/91	4LX	0.755	134.00	1110.0	8.31	556.00	1810.00	3.25	0.00	3060.00	0.00	2.00 >	17.68	+
11111	78	10/18/91	AM2	0.884	239.00	526.00	2.20	0.00	1100.00	0.00	0.00 >	3200.00	0.00	0.00	4.43	+
11117	78	04/12/91	4LY	0.806	1300.0	3100.0	2.38	2520.00	5470.00	2.17	0.00	9720.00	0.00	1.23 >	9.27	+
11117	78	10/18/91	AM3	0.991	1190.0	2050.0	1.73	0.00	3200.00	0.00	0.00	9320.00	0.00	0.00	4.63	+
11119	78	04/12/91	4LZ	0.792	598.00	1930.0	3.22	2900.00 >	3200.00 >	1.10	0.00 >	3200.00	0.00	0.66 >	7.19	+
11119	78	10/18/91	AM4	1.040	810.00	575.00	0.71	0.00	1440.00	0.00	0.00	9110.00	0.00	0.00	0.00	+
11121	78	04/12/91	4M0	0.714	1030.0 >	10000 >	9.75	1930.00 >	10000.0 >	5.18	0.00 >	10000.0	0.00 >	5.18 >	33.38	+
11121	78	10/18/91	AM5	0.974	2830.0 >	10000 >	3.53	7260.00 >	10000.0 >	1.38	0.00 >	10000.0	0.00 >	1.38 >	13.16	+
11125	78	04/19/91	4PB	1.130	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00	0.57	-
11125	78	10/18/91	AM6	1.021	942.00	9380.0	9.96	5440.00 >	10000.0 >	1.84	0.00 >	10000.0	0.00	1.72 >	8.97	+
11131	78	04/19/91	4PE	1.123	1330.0	2830.0	2.13	1930.00	5140.00	2.67	0.00	9510.00	0.00	1.47 >	9.05	+
11131	78	10/18/91	AM7	0.988	4470.0 >	10000 >	2.24	7820.00 >	10000.0 >	1.28	0.00 >	10000.0	0.00 >	1.28 >	7.11	+
11144	78	04/19/91	4PK	1.067	1450.0	4690.0	3.24	2350.00	6460.00	2.75	0.00	9650.00	0.00	2.00	9.83	+
11144	78	10/18/91	AMK	0.760	1470.0	3790.0	2.57	2340.00	5890.00	2.52	0.00	9660.00	0.00	1.62 >	9.57	+
11150	78	04/19/91	4QJ	1.051	440.00	745.00	1.69	701.00	1370.00	1.95	0.00	3020.00	0.0	1.06	5.52	+
11150	78	10/11/91	AHR	1.078	752.00	1220.0	1.62	0.00	1900.00	0.00	0.00	3120.00	0.00	0.00 >	2.53	+
11155	78	04/19/91	4QM	1.160	47.80	75.40	1.58	81.00	143.00	1.76	0.00	302.00	0.00	0.93	3.17	+
11155	78	10/11/91	AHR	1.078	174.00	215.00	1.23	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00	1.05	+
11168	78	04/19/91	4QS	0.998	552.00	674.00	1.22	1000.00 >	1000.00 >	1.00	0.00 >	1000.00	0.00	0.67 >	1.20	+
11168	78	10/11/91	AHT	1.064	0.00 >	3200.0	0.00	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	0.81	-
11185	80	07/12/91	6YX	1.235	489.00	550.00	1.12	948.00	779.00	0.82	0.00 >	1000.00	0.00	0.58 >	2.77	+
11185	80	10/11/91	AFB	1.128	556.00	674.00	1.19	0.00 >	1000.00	0.00	0.00 >	1000.00	0.00	0.00	1.56	+
11186	80	07/12/91	6YX	1.235	131.00	490.00	3.74	181.00	660.00	3.64	0.00	966.00	0.00	2.70	15.77	+
11186	80	10/11/91	AFB	1.128	0.00	492.00	0.00	0.00	663.00	0.00	0.00	973.00	0.00	0.00 >	3.06	-
11188	80	07/12/91	6YY	1.238	57.30	190.00	3.32	0.00	280.00	0.00	0.00	913.00	0.00	0.00	8.42	+
11188	80	10/11/91	AFC	1.124	76.70	452.00	5.89	161.00	634.00	3.94	0.00	963.00	0.00	2.81	16.19	+
11191	80	07/12/91	620	1.304	94.20	1400.0	14.89	0.00	2070.00	0.00	0.00 >	3200.00	0.00	0.00 >	12.72	+
11191	80	10/11/91	AFD	1.164	44.00 >	1000.0 >	22.73	800.00 >	1000.00 >	1.25	0.00 >	1000.00	0.00 >	1.25 >	23.09	+

Table 18 (Cont'd)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
11224	80	07/12/91	70P	1.209	52.40	21.20	0.40	0.00	43.30	0.00	0.00 >	320.00	0.00	0.00 >	0.62	+
11224	80	10/11/91	AFE	0.931	109.00	29.00	0.27	320.00	89.10	0.28	0.00 >	320.00	0.00	0.09 >	0.13	+
11447	81	08/02/91	7PY	1.180	0.00	470.00	0.00	0.00	649.00	0.00	0.00	971.00	0.00	0.00	1.46	-
11447	82	08/16/91	8GU	1.103	1420.0	205.00	0.14	2020.00	370.00	0.18	0.00	937.00	0.00	0.10 >	4.90	+
2318	13	03/17/89	P4V	1.040	0.36	0.25	0.69	1.00	1.44	1.44	0.00 >	10.00	0.00	0.25 >	0.42	+
2318	13	09/11/8[illegible]	RI	0.848 <	0.03 >	10.00 >	312.[illegible]	0.52 >	10.00 >	19.40	0.94 >	0.00 >	10.68 >	19.40 >	49.94	+
2318	67	06/01/9[illegible]	WH4	0.987	0.56	0.31	0.56	0.00	2.22	0.00	0.00 >	10.00	0.00	0.00 >	0.10	+
2318	67	07/13/90	XGK	1.079	1.21	4.17	[illegible].45	2.05 >	10.00 >	4.87	0.00 >	10.00	0.00	2.03 >	14.40	+
2318	82	10/04/91	A47	1.103	4.23	4.90	1.16	7.96	51.40	6.46	0.00 >	320.00	0.00	0.62 >	5.37	+
8212	75	11/30/90	11G	1.127	950.00 >	3200.0 >	3.37	2390.00 >	3200.00 >	1.34	0.00 >	3200.00	0 00 >	1.34 >	9.72	+
8212	75	03/22/91	40T	1.191	407.00	1300.0	3.20	861.00	1940.00	2.25	0.00	3070.00	6.00	1.51	9.55	+
8221	75	11/20/90	1AI	1.108 <	10.00 <	10.00 -	1.00	19.90	62.20	3.13	0.00	283.00	0.00 <	0.50	2.79	+
8221	75	03/22/91	40V	1.237	13.00	155.00	11.89	17.90	210.00	11.74	31.60	309.00	9.78	8.66	33.28	+
8240	75	11/20/90	1AR	1.088	578.00	660.00	1.14	0.00	2180.00	0.00	0.00 >	3200.00	0.00	0.00	0.26	+
8240	75	03/22/91	40Z	1.301	1100.0	2860.0	2.59	2050.00 >	3200.00 >	1.56	0.00 >	3200.00	0.00	1.40 >	9.23	+
8262	76	12/11/90	1TN	1.033	0.00 >	1000.0	0.00	0.00 >	1000.00	0.00	0.00 >	1000.00	0.00	0.00 >	4.10	-
8262	76	03/22/91	413	1.257	1040.0	3780.0	3.62	1770.00	5850.00	3.31	0.00	9590.00	0.00	2.14 >	12.41	+
8270	76	12/14/90	1WC	1.190	1070.0	2070.0	1.94	2450.00 >	3200.00 >	1.30	0.00 >	3200.00	0.00	0.84 >	5.90	+
8270	76	03/22/91	415	1.240	931.00	4000.0	4.30	1610.00	6000.00	3.74	0.00	9600.00	0.00	2.49	14.04	+
8315	76	12/14/90	1WH	1.175	330.00	1350.0	4.08	729.00	1960.00	2.70	0.00	3080.00	0.00	1.85 >	9.33	+
8315	76	03/22/91	418	1.214	198.00	592.00	2.99	666.00	1040.00	1.57	0.00	2980.00	0.00	0.89	8.13	+
8318	76	12/14/90	1WJ	0.465	335.00	1620.0	4.83	592.00	2240.00	3.78	0.00 >	3200.00	0.00	2.73	15.27	+
8318	76	03/22/91	41B	1.214	298.00	943.00	3.17	733.00	1690.00	2.31	0.00	3050.00	0.00	1.29 >	9.87	+
8374	76	12/14/90	1WZ	1.174	553.00	974.00	1.76	957.00	1780.00	1.86	0.00 >	3200.00	0.00	1.02	3.14	+
8374	76	03/22/91	41G	1.132	1020.0	3200.0	3.12	1880.00 >	3200.00 >	1.70	0.00 >	3200.00	0.00	1.70 >	8.87	+
8499	75	11/30/90	1KH	1.183	187.00	162.00	0.87	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	1.26	+
8499	75	03/29/91	475	1.236	163.00	175.00	1.07	312.00 >	1000.00 >	3.21	0.00 >	1000.00	0.00	0.56	0.51	+
8513	76	12/21/90	219	1.069	403.00	1550.0	3.85	545.00	2100.00	3.85	941.00	3090.00	3.28	2.84 >	14.49	+
8513	76	03/22/91	411	1.200	273.00	1430.0	5.22	458.00	2020.00	4.41	952.00	3080.00	3.24	3.11	17.35	+
9121	77	02/01/91	2YE	0.920	0.00 >	3200.0	0.00	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	2.02	-
9121	77	10/11/91	AGI	1.242	4110.0 >	10000 >	2.43	5820.00 >	10000.0 >	1.72	0.00 >	10000.0	0.00 >	1.72 >	8.36	+
9134	77	02/01/91	2YL	1.182	1390.0	2200.0	1.58	2520.00 >	3200.00 >	1.27	0.00 >	3200.00	0.00	0.87 >	2.54	+
9134	77	10/11/91	AGK	1.176	198.00	474.00	2.40	0.00	649.00	0.00	0.00	965.00	0.00	0.00	4.75	+

Table 18 (Cont'd)

AVS No.	Ship-ment	Test Date	Pit #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9162	77	02/08/91	32K	1.175	293.00	815.00	2.78	773.00	1740.00	2.26	0.00 >	3200.00	0.00	1.05 >	10.05	+
9162	77	10/11/91	AGN	1.137	486.00	1110.0	2.29	0.00	1810.00	0.00	0.00	3060.00	0.00	0.00	2.96	+
9163	77	02/08/91	32L	1.096	278.00	629.00	2.26	669.00	1460.00	2.22	0.00	3160.00	0.00	0.94 >	6.70	+
9163	77	10/11/91	AGN	1.137	2880.0	932.00	0.32	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	0.26	+
9166	77	02/08/91	32M	1.121	448.00	304.00	0.68	856.00	1820.00	2.12	0.00 >	3200.00	0.00	0.35	3.19	+
9166	77	10/11/91	AGO	1.167	897.00	1420.0	1.58	0.00	2070.00	0.00	0.00 >	3200.00	0.00	0.00 >	2.29	+
9171	77	02/08/91	32R	1.020	320.00	269.00	0.84	957.00	1000.00	1.04	0.00	2980.00	0.00	0.28 >	1.10	+
9171	77	10/11/91	AGQ	1.174	551.00	950.00	1.72	0.00	1690.00	0.00	0.00	3050.00	0.00	0.00	3.82	+
9172	77	02/08/91	32S	0.990	333.00	887.00	2.66	922.00	1940.00	2.10	0.00 >	3200.00	0.00	0.96 >	9.68	+
9172	77	10/11/91	AGQ	1.174	0.00	927.00	0.00	0.00	1670.00	0.00	0.00	3050.00	0.00	0.00 >	0.30	-
9173	77	02/08/91	32S	0.990	704.00 >	3200.0 >	4.54	3040.00 >	3200.00 >	1.05	0.00 >	3200.00	0.00 >	1.05 >	10.75	+
9173	77	10/11/91	AGR	1.114	4200.0 >	10000 >	2.38	8270.00 >	10000.0 >	1.21	0.00 >	10000.0	0.00 >	1.21 >	7.18	+
9217	77	02/15/91	37V	1.136	616.00	898.00	1.46	0.00	2310.00	0.00	0.00 >	3200.00	0.0	0.00	1.50	+
9217	77	10/11/91	AGV	0.998	1270.0 >	3200.0 >	2.53	1750.00 >	3200.00 >	1.82	3160.0 >	3200.00 >	1.01 >	1.82 >	10.26	+
9221	77	02/15/91	37X	1.072	730.00	924.00	1.27	2030.00 >	3200.00 >	1.58	0.00 >	3200.00	0.00	0.46 >	3.75	+
9221	77	10/11/91	AGV	0.998	2120.0	3540.0	1.67	0.00	5700.00	0.00	0.00	9570.00	0.00	0.00 >	3.01	+
9236	77	02/15/91	391	1.176	443.00	183.00	0.41	1000.00	320.00	0.32	0.00	3040.00	0.00	0.18	0.80	+
9236	12	10/11/91	AHF	1.088	955.00	269.00	0.28	0.00	578.00	0.00	0.00	2620.00	0.00	0.00	0.15	+
9253	77	02/26/91	3CN	1.033	566.00 >	3200.0 >	5.66	1490.00 >	3200.00 >	2.15	0.00 >	3200.00	0.00 >	2.15 >	14.52	+
9253	77	10/11/91	AHI	1.076	521.00	361.00	0.69	0.00	619.00	0.00	0.00	2600.00	0.00	0.00 >	0.23	+
9414	80	07/12/91	715	1.186	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00	0.05	-
9414	81	10/08/91	AD6	1.041	374.00 >	1000.0 >	2.67	520.00 >	1000.00 >	1.92	937.00 >	1000.00 >	1.07 >	1.92 >	11.88	+
9448	79	04/26/91	4WD	0.855	156.00 >	320.00 >	2.05	265.00 >	320.00 >	1.21	0.00 >	320.00	0.00 >	1.21 >	6.50	+
9448	79	10/04/91	A4L	1.080	462.00	618.00	1.34	0.00	916.00	0.00	0.00	2940.00	0.00	0.00	4.28	+
9449	79	04/26/91	4WD	0.855	39.80	160.00	4.01	57.40	220.00	3.83	0.00 >	320.00	0.00	2.78 >	16.13	+
9449	79	10/04/91	A4M	1.221	64.50	181.00	2.80	0.00	262.00	0.00	0.00	894.00	0.00	0.00	7.32	+
9450	79	04/26/91	4WE	0.861	39.10	70.60	1.81	79.60	124.00	1.55	0.00	300.00	0.00	0.89 >	6.15	+
9450	79	10/04/91	A5Z	1.092	33.80	122.00	3.60	53.10	190.00	3.57	0.00	312.00	0.00	2.29	14.75	+
9451	79	04/26/91	4WE	0.861	22.30	53.00	2.38	0.00	74.00	0.00	0.00	271.00	0.00	0.00 >	6.22	+
9451	79	10/04/91	A60	1.092	38.70	75.60	1.95	65.60	142.00	2.16	0.00	312.00	0.00	1.15 >	7.92	+
9452	79	04/26/91	4WF	0.918	27.70	66.70	2.41	90.90	105.00	1.15	0.00	315.00	0.00	0.73 >	6.99	+
9452	79	10/04/91	A60	1.092	39.90	100.00	2.51	65.70	192.00	2.92	0.00 >	320.00	0.00	1.52	9.56	+

Table 18 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9487	80	07/12/91	6Z3	1.145	59.30	134.00	2.26	0.00	212.00	0.00	0.00 >	320.00	0.00	0.00 >	2.84	+
9487	80	10/11/91	AF7	1.042	143.00 >	320.00 >	2.24	301.00 >	320.00 >	1.06	0.00 >	320.00	0.00 >	1.06 >	7.65	+
9489	80	07/12/91	6Z4	1.189	233.00	181.00	0.78	0.00	262.00	0.00	0.00 >	320.00	0.00	0.00	0.46	+
9489	80	10/11/91	AF8	1.050	133.00	312.00	2.35	201.00	544.00	2.70	0.00	963.00	0.00	1.55	9.57	+
9511	80	07/12/91	70J	1.146	71.00	154.00	2.16	0.00	215.00	0.00	0.00 >	320.00	0.00	0.00 >	4.31	+
9511	80	10/11/91	AF8	1.050	46.80	86.40	1.85	69.50	180.00	2.59	0.00 >	320.00	0.00	1.24	5.38	+
9513	80	07/12/91	70K	1.099	26.60	31.10	1.17	90.60	66.70	0.74	0.00 >	320.00	0.00	0.34 >	1.34	+
9513	80	10/11/91	AF9	1.111	20.10	19.50	0.97	0.00	44.40	0.00	0.00 >	320.00	0.00	0.00	0.00	+
9589	79	05/03/91	588	0.937	6.69 >	320.00 >	47.85	22.60 >	320.00 >	14.13	203.00 >	320.00 >	1.57 >	14.13 >	44.09	+
9589	79	10/04/91	A68	1.094	21.00	262.00	12.49	42.90	485.00	11.30	93.30	948.00	10.16	6.10	25.19	+
9590	79	05/03/91	58C	1.017	0.00	49.00	0.00	0.00	66.00	0.00	0.00	96.60	0.00	0.00	3.46	-
9590	79	10/04/91	A69	0.879	425.00	37.00	0.09	566.00	58.00	0.10	945.00	95.80	0.10	0.07 >	4.96	+
9768	79	05/31/91	5VR	1.008	2.30 <	1.00 <	0.44	10.00	1.00	0.10	0.00	43.30	0.00 <	0.10	0.12	+
9768	79	10/04/91	A6X	1.037	3.20	0.14	0.04	0.00	0.27	0.00	0.00 >	32.00	0.00	0.00	0.00	+
9772	79	05/31/91	5[illegible]	0.997	5.05	8.51	1.69	8.11	15.80	1.95	0.00	30.40	0.00	1.05	3.72	+
9772	79	10/04/91	A6[illegible]	1.037	0.00	10.00	[illegible]	0.00	17.30	0.00	0.00	30.50	0.00	0.00	0.00	-
9796	79	05/31/91	5X2	1.039	17.10	18.60	1.09	29.40	27.20	0.93	0.00	90.60	0.00	0.63 >	0.10	+
9796	79	10/04/91	A6Z	1.009	0.00	15.50	0.00	0.00	21.00	0.00	0.00	30.90	0.00	0.00	0.00	-
9816	79	06/07/91	62E	1.252	0.00	5.63	0.00	0.00	9.68	0.00	0.00	29.70	[illegible].00	0.00	0.00	-
9816	79	10/04/91	A73	1.038	17.00	4.00	0.24	28.80	6.00	0.21	0.00	9.60	0.00	0.14 >	0.11	+
9842	79	06/07/91	641	1.312	52.80	162.00	3.07	93.40	266.00	2.85	0.00 >	320.00	0.00	1.74	7.68	+
9842	79	10/04/91	A76	1.053	0.00	161.00	0.00	0.00	225.00	0.00	0.00	738.00	0.00	0.00	1.54	-
9849	79	06/07/91	651	1.288	2.61	27.20	10.40	9.50	51.40	5.42	0.00	100.00	0.00	2.86 >	16.38	+
9849	79	10/04/91	A78	1.024	0.00	8.35	0.00	0.00	23.90	0.00	0.00	93.00	0.00	0.00	2.93	-
9871	79	06/07/91	65T	1.349	56.60	128.00	2.27	100.00	192.00	1.92	0.00	307.00	0.00	1.28	4.01	+
9871	79	10/08/91	AD1	0.986	66.40	61.10	0.92	0.00	134.00	0.00	0.00	301.00	0.00	0.00	0.12	+
9886	79	06/14/91	684	1.387	4.59	8.00	1.74	7.38	15.20	2.05	0.00	30.60	0.00	1.08 >	5.01	+
9886	79	10/08/91	AD3	1.007	12.10	12.90	1.06	0.00	23.00	0.00	0.00	89.90	0.00	0.00 >	1.37	+
9889	79	06/14/91	685	1.386	1.60	1.89	1.18	2.66	3.94	1.48	0.00	9.51	0.00	0.71	1.10	+
9889	79	10/08/91	AD4	1.024	0.00	2.25	0.00	0.00	4.26	0.00	0.00	9.53	0.00	0.00	0.33	-
9896	79	06/14/91	689	0.999	1.05	8.87	8.48	1.52	24.20	15.94	2.97 >	320.00 >	107.74	5.84 >	27.91	+
9896	79	10/08/91	AD4	1.024	0.00	15.90	0.00	0.00	40.50	0.00	0.00 >	100.00	0.00	0.00	0.31	-

Table 18 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25		TC 25		AI 25	IC 50		TC 50		AI 50	IC 95		TC 95		AI 95	SI		TAI	ACT
9917	79	06/14/91	6D8	1.091	425.00	>	1000.0	>	2.35	607.00	>	1000.00	>	1.65	0.00	>	1000.00		0.00	>	1.65	>	10.23	+
9917	79/C2	08/16/91	8CV	1.050	0.00		906.00		0.00	0.00		1650.00		0.00	0.00		3050.00		0.00	>	1.85		-
9969	79	06/28/91	6KC	1.250	0.00		436.00		0.00	0.00		660.00		0.00	0.00		2280.00		0.00		0.00		-
9969	79/C2	08/16/91	8D0	1.067	1340.0		177.00		0.13	1790.00		269.00		0.15	3020.0		906.00		0.30	>	5.14		+
9970	79	06/28/91	6KD	1.172	515.00		1210.0		2.35	865.00		1870.00		2.17	0.00		3070.00		0.00		1.40		5.34	+
9970	79/C2	08/16/91	8D0	1.067	631.00		647.00		1.03	0.00		974.00		0.00	0.00		2970.00		0.00		0.00		0.80	+

DIFRNTL = The differential is the difference in the cell control and the virus control optical densities.

$IC_{25,50,95}$ = (Viral) inhibitory concentration 25%, 50% and 95% = The drug concentration (µg/ml) that inhibited viral CPE by 25%, 50% or 95% calculated by using a regression analysis for semilog curve fitting.

$TC_{25,50,95}$ = (Cell) toxicity concentration 25%, 50% and 95% = The drug concentration (µg/ml) that reduced cell viability by 25%, 50% or 95%.

$AI_{25,50,95}$ = Antiviral Index = A single point ratio of the antiviral and anticellular effect of the compound, calculated with 25%, 50% or 95% reduction values (calculated by dividing the $TC_{25,50,95}$ by the $IC_{25,50,95}$).

SI = Selectivity Index = A ratio calculated by dividing the TC_{25} by the IC_{50} (based upon 6 one-half-log_{10} dilutions, µg/ml, the maximum scale is 0-320).

TAI = Total Antiviral Index = The area between the cytotoxicity and the antiviral curves (based upon a scale of 0-100%).

ACT = Activity = A "+" denotes a rest that produced ≥25% reduction in CPE. A "-" denotes an inactive test (i.e. ≤25% reduction in CPE).

4.3.5 Punta Toro Virus (PT):

The total output of PT-testing during this reporting period is summarized in monthly increments in **Figure 52**. During this period 4148 tests were performed against PT-virus with MTT-assay format. Out of these, 176 were control compound assays with Ribavirin (AVS-0001) and 86 were control compound assays with 2-Thio-6-Azauridine (AVS-6724). Two hundred eleven tests were internal (+++) virus load, cell load, and other quality control tests. Four hundred four tests were considered unsatisfactory based on the criteria of the quality controls set during this reporting period. The rest, totaling 3271 were actual single drug assays. The 404 unsatisfactory tests represent a 9.7% rejection rate based on present quality control parameters for the PT virus. The total number of assays represents approximately 64% above our yearly contractual obligation (i.e., 3271/2000).

Out of the 3271 test compounds, 161 demonstrated antiviral activity at greater than 50% reduction levels. This represents around 4.9% of the tested compounds having *in vitro* antiviral activity against PT-virus. The remainder, 3110 compounds (90%), are to be considered inactive with this assay protocol.

TOTAL NUMBER OF TESTS AGAINST PUNTA TORO VIRUS

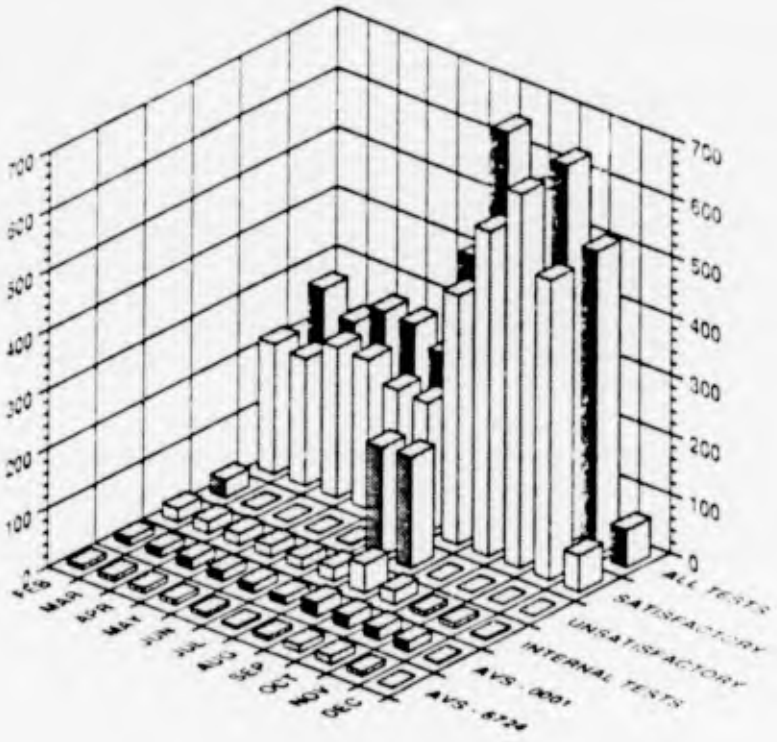


Figure 52

Quality Control Tests =	KPC (Positive Control - 2-Thio-6-Azauridine) =	86
	KPC (Positive Control - Ribavirin) =	176
	(+++) (Internal Virus and Cell Load Controls) =	211
	(UT) Unsatisfactory Test (QC rejects) =	404
Accepted Single Drug Tests =		3271
Total number of PT Tests =		4148

4.3.5.1 **PT-Quality Controls:**

4.3.5.1.1 **Antiviral Activity of Ribavirin vs PT Virus:**

Control Compound-Antiviral Performance: Ribavirin (AVS-0001) has been the primary control compound against PT in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of the positive control drug (Ribavirin) are illustrated in **Figure 53-A.**

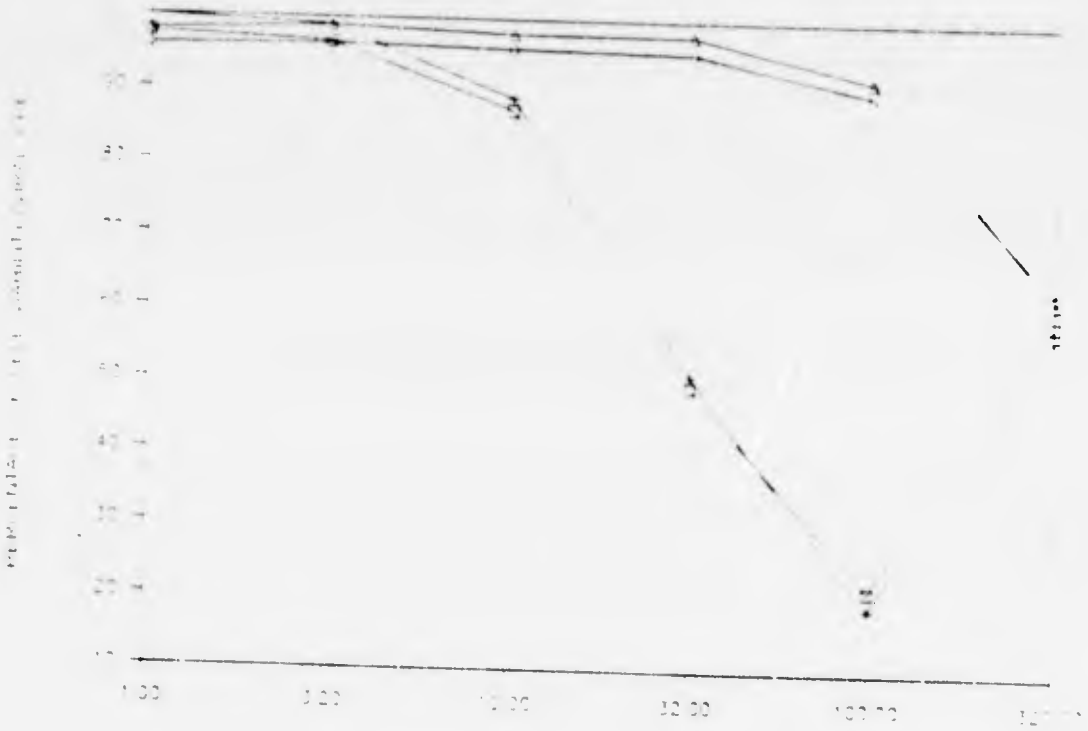
The 176 control tests performed with Ribavirin gave a mean **Total Antiviral Index** (TAI) of 26.3% (SD ± 12.4) and the median value was 25%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from >3.03 - >64.25% during this period. The mean **Selectivity Index (SI)** was only 7.99 (SD ± 6.78) and the median SI value was 5.73, indicating moderate antiviral selectivity for Ribavirin against PT virus. The SI ranged from 0 - >49.06) during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25% (AI_{25})** value was 19.44 (SD ± 34.19). The median AI_{25} value was 11.41 (range .029 - >320). The mean **Antiviral Index 50% (AI_{50})** was 11.12 (SD ± 7.2) with a median of 9.42 (range 0 - 49.06). The mean **Antiviral Index 95% (AI_{95})** was 1.58 (SD ± 3.15), with a median of 0 (range 0 - >11.65). This indicates that the control compound, Ribavirin, does not consistently reach 95% antiviral reduction levels.

The mean **Antiviral Inhibitory Concentration 25% (IC_{25})** was 21.91 μg/ml (SD ± 19.32). The median IC_{25} value was 13.9 μg/ml (range = <1 - 169 μg/ml). The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 41.15 μg/ml (SD ± 34.65). The median IC_{50} value was 32 μg/ml (range = 0 - 293 μg/ml). The mean **Antiviral Inhibitory Concentration 95% (IC_{95})** was 18.14 μg/ml (SD ± 33.8). The median IC_{95} value was 0 μg/ml (range from 0 - 100 μg/ml). This discrepancy indicates that the control compound Ribavirin does not consistently reach 95% reduction levels. The highest starting concentration of Ribavirin (100 μg/ml) was set at 320 μg/ml to properly evaluate the maximum antiviral effects of Ribavirin.

The average maximum antiviral inhibitory level of 176 Ribavirin tests (**Figure 53-A**) was reached at 100 μg/ml of the compound with 80% antiviral effect. Maximum antiviral effect (~80%) was found with a simultaneous ~10% cytotoxic suppression. Above (100 μg/ml) concentration Ribavirin starts to lose its antiviral potency (~40%) at 320 μg/ml while simultaneously the Ribavirin becomes maximally toxic (~40%).

RIBAVIRIN -VS- PT VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊Mean % Cell Viability ΔMedian % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	**1**	**3.2**	**10**	**32**	**100**	**320**	**1**	**3.2**	**10**	**32**	**100**	**320**
Mean	98	97	87	50	21	59	96	96	96	96	90	63
Median	100	99	89	51	19	57	98	99	98	98	92	62
Std. Dev.	0 05	0.05	0.13	0.30	0.18	0.22	0.06	0.06	0.05	0.06	0.11	0.13

Figure 53-A
Average Antiviral and Cytotoxicity Values for 176 Positive Control Compound Tests

4.3.5.1.2 Maximum Antiviral Effect of Ribavirin vs PT Virus:

A bar graph scatter plot (**Figure 54-A**) depicts the distribution of the maximum antiviral reduction values of all 176 control compound assays for Ribavirin. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 81% (SD ± 15.15) reduction levels. The maximum reduction levels vary from 47 - 100% but remain quite consistently around the median of 83%. The assay control values give a shifted half-bell-shaped distribution curve toward the maximum 100% reduction level. This indicates quite a consistent day-to-day performance of the control compound in the PT-MTT assay.

The positive control compound performance criteria for Ribavirin versus the PT virus is set at 50% reduction level. Assays in which Ribavirin did not meet this accepted quality control level ($\geq$50%) were rejected (i.e., 404 unsatisfactory tests).

Ribavirin is active *in vitro* against PT virus and functions as a reasonable quality control compound. On the other hand, regardless of the performance of the PT-quality control drug Ribavirin, around 161 other compounds have equal or better antiviral activity against PT virus than AVS-0001. (See 95% and 50% reduction summaries).

Variation of the Maximum Antiviral Effect
PT Virus - VS - Ribavirin

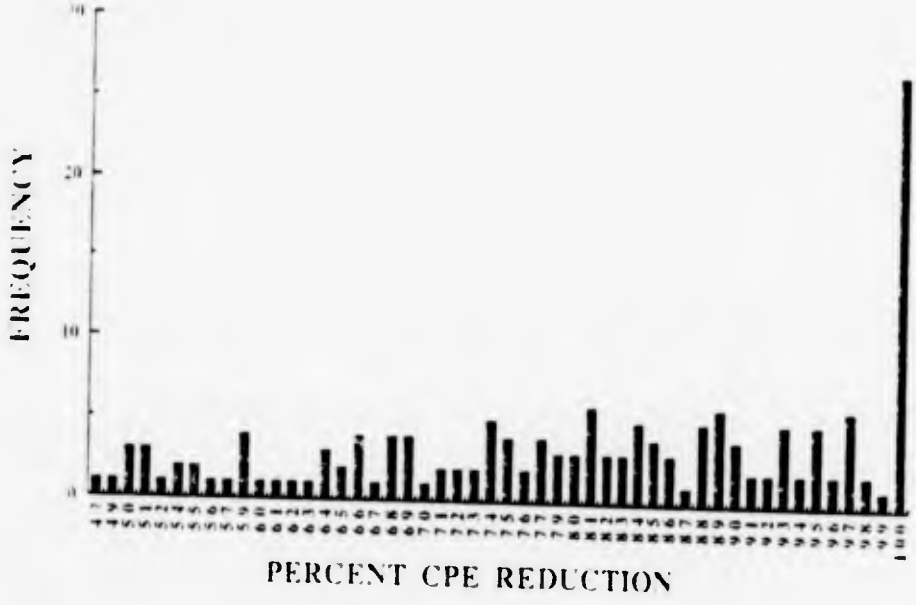


Figure 54-A
Maximum Antiviral CPE Reduction (%).
Summary of 176 Control Tests.

4.3.5.1.3 Cellular Cytotoxicity of Ribavirin vs PT Virus:

PT-Control Compound-Cytotoxicity Performance: The 176 cytotoxicity values of the positive control compound Ribavirin are also very consistent. The mean cell **Toxic Concentration 25% (TC_{25})** was 221.2 μg/ml (SD ± 80.88) and the median was 224 μg/ml (range of 0.893 - >320 μg/ml). The mean cell **Toxic Concentration 50% (TC_{50})** value was 311 (SD ± 29.1) and the median was 320 μg/ml (range of >100 - >320 μg/ml). The mean cell **Toxic Concentration 95% (TC_{95})** value was 318.7 (SD ± 17.23) and the median was 320 μg/ml (range of >100 - >320 μg/ml). This discrepancy indicates that the control compound Ribavirin does not consistently reach 95% cytotoxicity levels at the highest concentration tested (i.e. 320 μg/ml).

As can be seen from **Figure 53-A**, the toxicity starts to become measurable above the concentration of 32 μg/ml and the maximum toxicity has not been reached at 320 μg/ml. Further increase of the concentration of Ribavirin would be needed to properly evaluate the maximum cytotoxicity of Ribavirin.

Also **Figure 53-A**, indicates that when the cytotoxicity reaches ~10% at 100 μg/ml, the control compound (Ribavirin) has reached simultaneously its maximum antiviral effect (80%). The cytotoxic effect of Ribavirin is insignificant between 1 and 100 μg/ml. The maximum cytotoxicity reached ~40% at 320 μg/ml, which is the highest Ribavirin concentration tested.

4.3.5.1.4 **PT-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (Ribavirin):**

PT-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 176 control assays is plotted in **Figure 55-A**. The results indicate that the cell O.D. readings reached a mean of 1.206 (SD ± 0.137) with a median of 1.204 (range of 0.913 - 1.570). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

PT-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 176 control assays is presented in **Figure 56-A**. The results indicate that the average load O.D. reading is 0.339 (SD ± 0.230) with a median of 0.331 (range of 0.035 - 0.873). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

PT-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 176 control assays is provided in **Figure 57-A**. The results indicate that the average differential O.D. reading is 0.867 (SD ± 0.278) with a median of 0.806 (range 0.376 - 1.502). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 87% measurement accuracy.

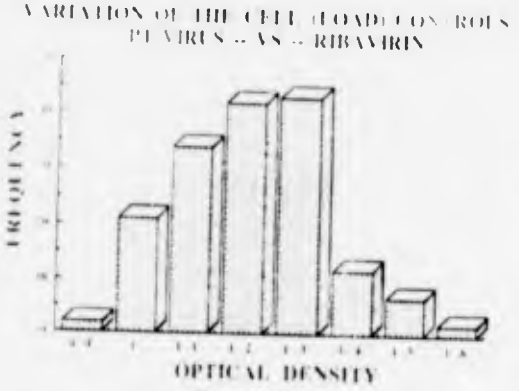


Figure 55-A

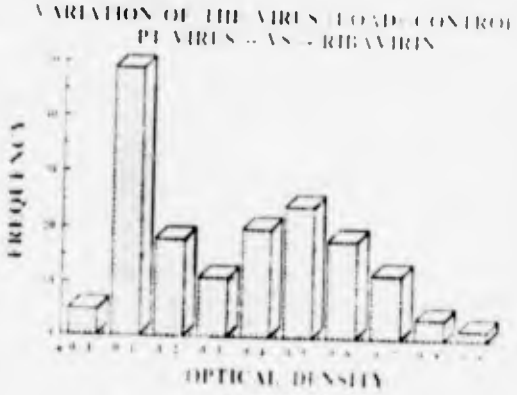


Figure 56-A

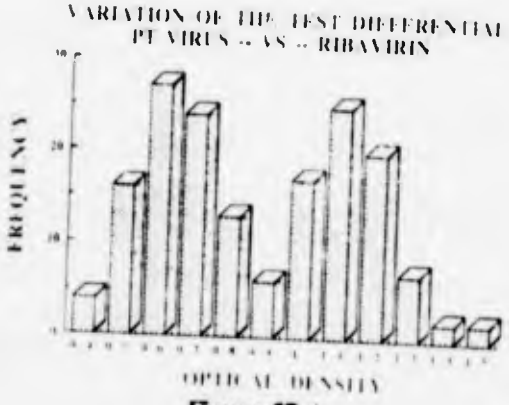


Figure 57-A

4.3.5.1.1 Antiviral Activity of AVS-6724 (2-Thio-6-Azauridine) vs PT Virus:

Second Control Compound-Antiviral Performance: 2-Thio-6-Azauridine (AVS-6724) has been tested as a second control compound against PT in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of this second positive control drug are illustrated in **Figure 53-B**.

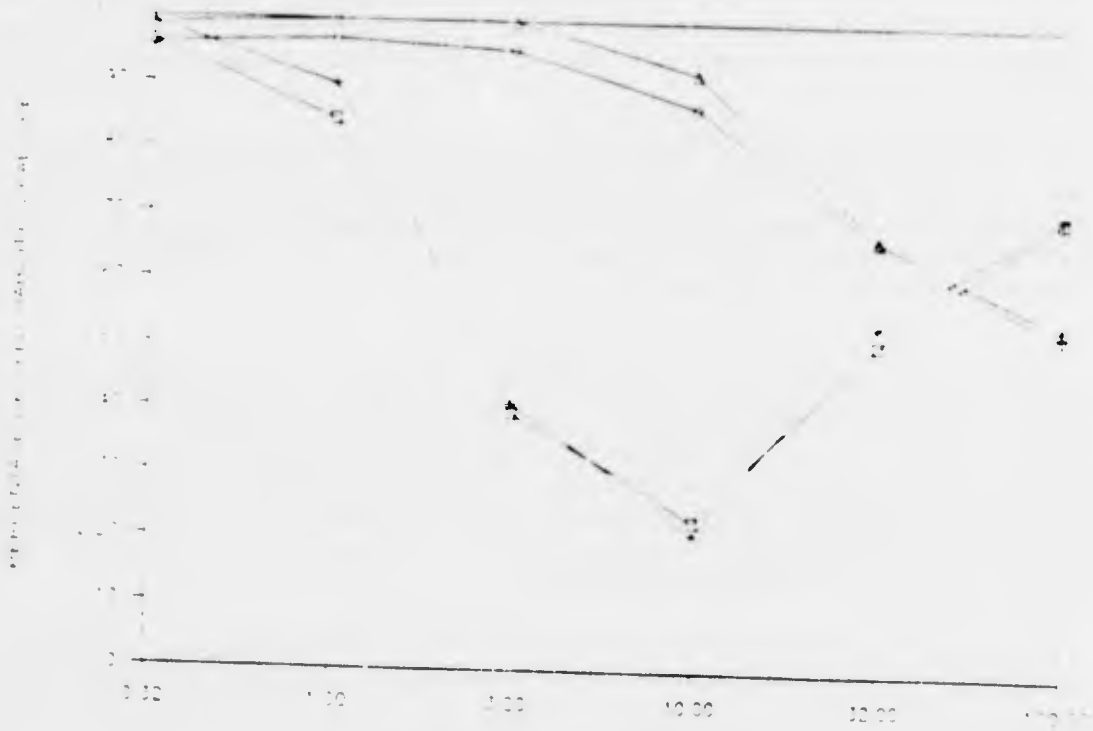
The 86 control tests performed with 2-Thio-6-Azauridine gave a mean **Total Antiviral Index** (TAI) of 32% (SD ± 17) and the median value was 29%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from 3.0 - > 77% during this period. The mean **Selectivity Index (SI)** was only 15.17 (SD ± 21.66) and the median SI value was 6.87, indicating moderate antiviral selectivity for 2-Thio-6-Azauridine against PT virus. The SI ranged from 0 - 149 during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25% (AI_{25})** value was 26.47 (SD ± 37.66). The median AI_{25} value was 15.11 (range 0.064 - > 212.5). The mean **Antiviral Index 50% (AI_{50})** was 39.34 (SD ± 36.71) with a median of 29.10 (range 0 - > 214). The mean **Antiviral Index 95% (AI_{95})** was 10.57 (SD ± 17.17), with a median of 0 (range 0 - > 104). This indicates that the, 2-Thio-6-Azauridine, does not consistently reach 95% antiviral reduction levels.

The mean **Antiviral Inhibitory Concentration 25% (IC_{25})** was 1.84 μg/ml (SD ± 1.65) The median IC_{25} value was 1.41 μg/ml (range = < 0.32 - 12.3 μg/ml). The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 3.25 μg/ml (SD ± 2.75). The median IC_{50} value was 2.41 μg/ml (range = 0 - 19.4 μg/ml). The mean **Antiviral Inhibitory Concentration 95% (IC_{95})** was 2.46 μg/ml (SD ± 4.57). The median IC_{95} value was 0 μg/ml (range from 0 - 32 μg/ml). This discrepancy indicates that the 2-Thio-6-Azauridine does not consistently reach 95% reduction levels. The highest starting concentration of 2-Thio-6-Azauridine was set to 100 μg/ml to properly evaluate the maximum antiviral and cytotoxicity effects of 2-Thio-6-Azauridine.

The average maximum antiviral inhibitory level of 86, 2-Thio-6-Azauridine, tests **(Figure 53-B)** was reached at 10 μg/ml of the compound with 80% antiviral effect. Maximum antiviral effect (~80%) was found with a simultaneous ~10% cytotoxic suppression. Above 10 μg/ml concentration 2-Thio-6-Azauridine starts to lose its antiviral potency (~30%) at 100 μg/ml, while simultaneously the 2-Thio-6-Azauridine becomes maximally toxic (~45%) with increasing cytotoxicity.

2-THIO-6-AZAURIDINE -VS- PT VIRUS



CONCENTRATION (μg/ml)

□Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability ΔMedian % Cell Viability

% Viral CPE

% Cell Viability

Conc.(μg/ml)	0.32	1	3.2	10	32	100	0.32	1	3.2	10	32	100
Mean	96	85	40	23	51	70	96	97	95	87	66	53
Median	99	90	41	21	54	71	99	100	100	92	67	54
Std. Dev.	0.06	0.19	0.29	0.23	0.23	0.21	0.06	0.05	0.08	0.16	0.14	0.2

Figure 53-B
Average Antiviral and Cytotoxicity Values for 86 Positive Control Compound Tests

4.3.5.1.2 Maximum Antiviral Effect of 2-Thio-6-Azauridine vs PT Virus:

A bar graph scatter plot (**Figure 54-B**) depicts the distribution of the maximum antiviral reduction values of all 86 control compound assays for 2-Thio-6-Azauridine. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 82% (SD ± 17.52) reduction levels. The maximum reduction levels vary from 30 - 100% but remain quite consistently around the median of 86%. The assay control values give a shifted half-bell-shaped distribution curve toward the maximum 100% reduction level. This indicates quite a consistent day-to-day performance of the control compound in the PT-MTT assay.

Recommendations:

Based upon the data obtained in parallel studies with Ribavirin, we recommend that 2-Thio-6-Azauridine (AVS #6724) will be used as a second control compound against PT virus. It's overall performance is slightly better than the present control, Ribavirin. It is readily available from Sigma Chemical Company, it is inexpensive and works at ~ 10-fold lower concentrations than Ribavirin.

Variation of the Maximum Antiviral Effect PT Virus - VS - 2-Thio-6-Azauridine

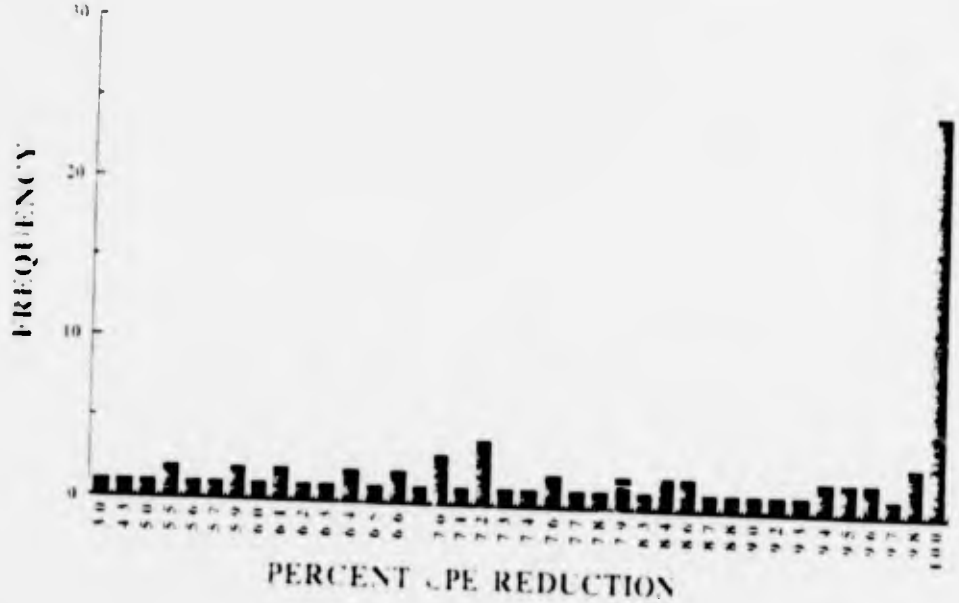


Fig. 54-B
Maximum Antiviral CPE Reduction (%).
Summary of 86 Control Tests.

4.3.5.1.3 Cellular Cytotoxicity of 2-Thio-6-Azauridine vs PT Virus:

PT-Control Compound-Cytotoxicity Performance: The 86 cytotoxicity values of the positive control compound 2-Thio-6-Azauridine are also very consistent. The mean cell **Toxic Concentration 25% (TC_{25})** was 29.4 μg/ml (SD ± 22.72) and the median was 24.7 μg/ml (range of 0.296 - 87.3 μg/ml). The mean cell **Toxic Concentration 50% (TC_{50})** value was 83.65 (SD ± 29.22) and the median was 100 μg/ml (range of 7.28 - > 100 μg/ml). The mean cell **Toxic Concentration 95% (TC_{95})** value was 99.95 (SD ± 0.48) and the median was 100 (range of 95.7 - > 100 μg/ml). This discrepancy indicates that the control compound 2-Thio-6-Azauridine does not consistently reach 95% cytotoxicity levels.

As can be seen from **Figure 53-B**, the toxicity starts to become measurable above the concentration of 3.2 μg/ml and the maximum toxicity has not been reached at 100 μg/ml. Further increase of the concentration of 2-Thio-6-Azauridine would be needed to properly evaluate the maximum cytotoxicity of 2-Thio-6-Azauridine.

Also **Figure 53-B** indicates that when the cytotoxicity reaches ~10% at 10 μg/ml, the control compound (2-Thio-6-Azauridine) has reached simultaneously its maximum antiviral effect (80%). The cytotoxic effect of 2-Thio-6-Azauridine is insignificant between 1 and 10 μg/ml. The maximum cytotoxicity reached ~45% at 100 μg/ml, which is the highest 2-Thio-6-Azauridine concentration tested.

4.3.5.1.4 PT-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (2-Thio-6-Azauridine):

Second PT-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 86 control assays is plotted in **Figure 55-B**. The results indicate that the cell O.D. readings reached a mean of 1.199 (SD ± 0.118) with a median of 1.198 (range of 0.907 - 1.559). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

Second PT-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 86 control assays is presented in **Figure 56-B**. The results indicate that the average load O.D. reading is 0.323 (SD ± 0.226) with a median of 0.273 (range of 0.035 - 0.758). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

Second PT-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 86 control assays is provided in **Figure 57-B**. The results indicate that the average differential O.D. reading is 0.876 (SD ± 0.268) with a median of 0.838 (range 0.437 - 1.488) The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 88% measurement accuracy.

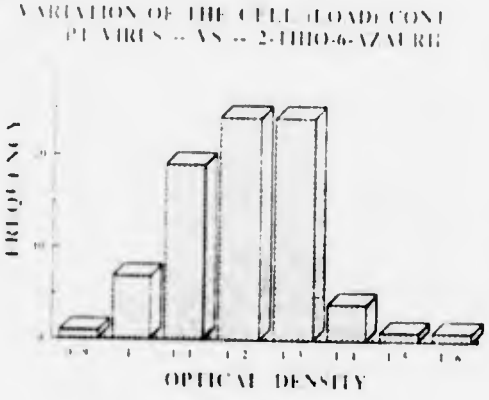


Figure 55-B

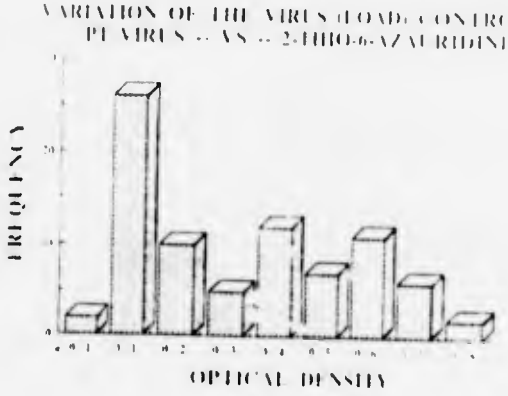


Figure 56-B

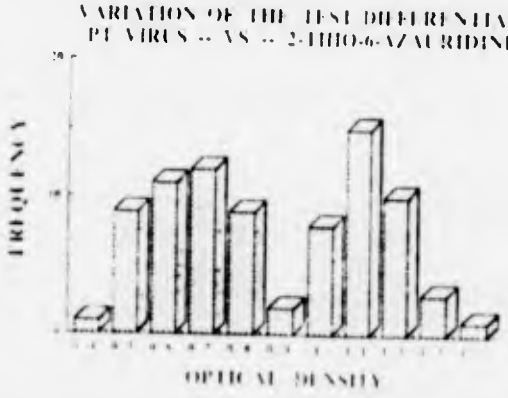


Figure 57-B

4.3.5.1.5 Overall PT-Assay Plate Quality Controls:

PT-Overall-Cell Load Performance: A bar graph scatter plot of the overall mean cell control (O.D. reading) of 3271 accepted assays is plotted in **Figure 55-C**. The results indicate that the overall cell O.D. readings reached a mean of 1.185 (SD ± 0.138) with a median of 1.180 (range of 0.827 - 1.651). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

PT-Overall-Virus Load Performance: A bar graph scatter plot of the overall mean virus load O.D. readings of the 3271 accepted assays is presented in **Figure 56-C**. The results indicate that the overall average virus load O.D. reading is 0.386 (SD ± 0.216) with a median of 0.422 (range of -0.009 - 0.892). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

PT-Overall-Assay Differential Performance: A bar graph scatter plot of the overall mean O.D. differential values of the 3271 accepted assays is provided in **Figure 57-C**. The results indicate that the overall average differential O.D. reading is 0.799 (SD ± 0.273) with a median of 0.709 (range 0.182 - 1.579). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 80% measurement accuracy.

GRAPHIC ILLUSTRATION OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ACCEPTED PT PLATE DATA

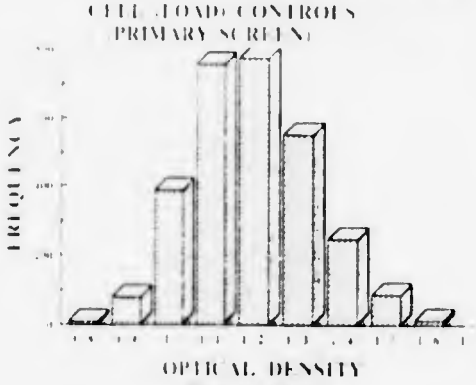


Figure 55-C

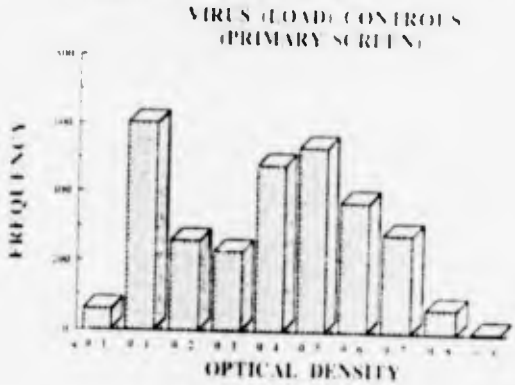


Figure 56-C

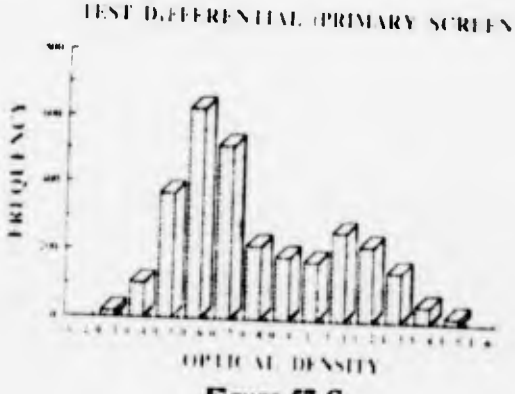


Figure 57-C

4.3.5.2 **PT-Antiviral Activity Results:**

New Drugs with 95% Antiviral Reduction Levels: Out of the 3271 actual single drug tests, 20 new compounds demonstrated excellent antiviral activity, having antiviral reduction values of equal to or better than 95%. This represents around 0.6% of the test compounds being active at this excellent reduction level. These compounds are summarized in **Table 19** according to the highest **Total Antiviral Index (TAI)**. Compound AVS-11506 demonstrated the greatest *in vitro* promise, having a TAI of 56% and **Selectivity Index (SI)** of 28. The other compounds demonstrated moderate antiviral activity with TAI's that ranged from 15 - 48% and SI values that ranged from 2.8 - 20.

Table 19

AVS Compounds Active Against Punta Toro (PT) at AI_{95} Level

AVS No.	Shipment#	Test Date	Diffrntl.	IC 95	TC 95	AI 95	SI	TAI
PT 11506	81	08/01/91	0.595	684.00	9660.00	14.12	28.11 >	55.96
PT 11123	78	04/16/91	1.100	914.00 >	10000.0 >	10.95	16.75 >	48.26
PT 11498	81	08/01/91	0.579	292.00	9550.00	32.67	15.45 >	46.24
PT 11501	81	08/01/91	0.595	906.00 >	3200.00 >	3.53	15.92 >	43.26
PT 11191	80	07/09/91	1.181	271.00 >	3200.00 >	11.81	20.10	43.03
PT 9539	79	10/03/91	0.558	92.60	966.00	10.43	10.58 >	40.63
PT 11121	78	04/11/91	1.015	3200.00 >	10000.0 >	3.13 >	8.24 >	38.02
PT 9423	80	10/08/91	0.681	93.80	966.00	10.30	9.29	36.11
PT 9970	79	06/18/91	0.738	236.00	2700.00	11.46	8.32	35.57
PT 9422	80	10/08/91	0.681	94.50	966.00	10.23	8.66	35.12
PT 11129	78	10/15/91	0.532	2910.00 >	10000.0 >	3 44	5.59 >	27.13
PT 11376	81	08/22/91	0.788	945.00 >	3200.00 >	3.39	4.47 >	26.56
PT 9842	79	06/05/91	1.192	90.30 >	320.00 >	3.54	4.49	25.63
PT 10030	79	07/03/91	1.155	290.00	966.00	3.33	4.04	22.66
PT 11134	78	10/15/91	0.569	920.00	3110.00	3.38	3.60 >	21.91
PT 11375	81	08/22/91	0.687	927.00	8490.00	9.16	3.59 >	21.76
PT 10031	79	07/03/91	1.155	914.00	3090.00	3.38	3.83	21.18
PT 11133	78	04/16/91	1.091	943.00	9230.00	9.79	3.57	19.88
PT 9414	81	10/08/91	0.430	903.00 >	1000.00 >	1.11 >	2.77 >	18.74
PT 9451	79	04/23/91	1.038	30.20	198.00	6.55	2.83	15.36

New Drugs with 50% Antiviral Reduction Levels: Out of the 3271 actual single drug tests, 141 new compounds demonstrated good antiviral activity, having antiviral reduction values equal to or better than 50%. This represents around 4.3% of the test compounds being active at this good antiviral reduction level. These compounds are summarized in **Table 20** according to the highest **Total Antiviral Index (TAI)**. AVS-9610 demonstrated the best activity with a TAI of 31% and a SI of 10. Fifteen (15) other compounds demonstrated moderate antiviral activity, having TAI's that ranged from 21 - 29% and SI's from 1.1 - 11. The rest (125 compounds) showed marginal antiviral activity with TAI's from < 1 to 18% and SI's from < 0.1 to > 250.

Table 20

AVS Compounds Active Against Punta Toro (PT) at AI_{50} Level

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
PT 9610	79	05/07/91	1.210	1.88	28.30	15.11	10.22 >	30.75
PT 11118	78	04/11/91	1.013	1710.00 >	10000.0 >	5.85 >	5.85 >	28.50
PT 9416	82	09/04/91	0.470	15.20 >	320.00 >	21.05	5.30 >	28.28
PT 11931	82	10/01/91	0.828	55.20 >	320.00 >	5.80 >	5.80 >	27.88
PT 9529	79	04/25/91	1.107	2.07	52.10	25.14	11.19 >	27.65
PT 10543	83	10/24/91	0.492	25.60 >	320.00 >	12.51	9.88	27.59
PT 9990	79	06/19/91	1.155	61.40	820.00	13.37	8.14	26.79
PT 11924	82	10/01/91	0.690	85.00 >	320.00 >	3.77 >	3.77 >	24.78
PT 9908	82	09/04/91	0.521	86.70 >	320.00 >	3.69 >	3.69 >	24.61
PT 11490	81	08/01/91	0.628	230.00	1860.00	8.08	5.16	24.59
PT 11832	82	08/22/91	0.574	1000.00	2260.00	2.26	1.36 >	24.46
PT 9258	77	02/20/91	1.069	72.90	1530.00	21.02	3.81	23.56
PT 9388	77	03/07/91	1.123	64.50	575.00	8.91	5.62 >	23.27
PT 11115	78	04/11/91	1.057	768.00 >	3200.00 >	4.16	1.11 >	21.78
PT 11110	78	04/11/91	1.020	1230.00 >	3200.00 >	2.61 >	2.61 >	21.19
PT 9909	81	08/06/91	0.701	54.10 >	320.00 >	5.91	5.48 >	21.15
PT 9916	79	06/12/91	1.381	511.00	2910.00	5.70	3.71	18.49
PT 9324	77	02/26/91	0.978	806.00 >	3200.00 >	3.97	2.61 >	18.21
PT 9238	77	02/20/91	0.955	239.00	611.00	2.56	1.54 >	17.89
PT 9899	79	06/12/91	1.217	8.02	88.70	11.05	5.17	17.33
PT 11047	78	04/04/91	0.738	561.00	3200.00	5.71	3.48	16.97
PT 9962	79	06/18/91	0.913	320.00	1940.00	6.05	4.07	16.68
PT 10394	83	10/16/91	0.559	95.90	200.00	2.09	1.45 >	16.40
PT 9935	79	06/13/91	1.112	781.00	3330.00	4.27	2.63 >	16.11
PT 9394	77	03/07/91	0.910	959.00	2120.00	2.21	1.63 >	15.69
PT 9937	79	06/13/91	1.250	498.00	2010.00	4.03	2.70	15.45
PT 11107	78	04/11/91	1.019	1760.00	6350.00	3.61	1.82 >	15.40
PT 9149	77	02/26/91	0.986	2260.00 >	3200.00 >	1.42 >	1.42 >	15.20
PT 11190	80	07/09/91	1.234	512.00 >	3200.00 >	6.26	2.35 >	15.01
PT 9981	79	06/19/91	1.213	2940.00 >	10000.0 >	3.41 >	3.41 >	14.99
PT 9530	79	04/25/91	1.107	17.50	63.90	3.66	2.46	14.90
PT 9225	77	02/20/91	0.895	197.00	748.00	3.80	2.20	14.89
PT 9689	79	05/16/91	1.316	77.60 >	320.00 >	4.12	2.80 >	14.77
PT 9389	77	03/07/91	1.123	468.00	3200.00	6.84	3.28 >	14.60
PT 9809	79	06/04/91	1.090	2.30	20.40	8.89	1.25 >	14.54
PT 9448	79	04/23/91	1.104	136.00 >	320.00 >	2.36 >	2.36 >	14.43
PT 9229	77	02/20/91	0.944	582.00	2600.00	4.46	2.75	14.31
PT 9971	79	06/18/91	0.738	195.00	730.00	3.74	2.69	14.29
PT 9445	79	04/23/91	1.251	6.38	25.10	3.93	2.75	14.12
PT 9874	79	06/11/91	0.944	8.73	37.00	4.24	2.51 >	14.08
PT 11795	82	08/22/91	0.608	590.00	2120.00	3.59	2.64	13.81
PT 9847	79	06/05/91	1.142	7.06	43.30	6.14	1.94 >	13.49
PT 9225	77	02/26/91	0.978	209.00	1000.00	4.78	0.83 >	13.40
PT 10066	82	09/04/91	0.682 <	1.00 >	320.00 >	320.00 >	250.00 >	12.77
PT 10583	83	10/29/91	0.458	214.00 >	320.00 >	1.49 >	1.49 >	12.60
PT 9883	79	06/11/91	0.905	1.72	6.42	3.74	2.67 >	12.45
PT 11486	81	08/01/91	0.654	210.00	660.00	3.14	2.33	11.98
PT 9446	79	04/23/91	1.389	58.20	190.00	3.27	2.13	11.73
PT 9452	79	04/23/91	1.121	23.00	82.70	3.60	2.49	11.71
PT 10062	82	09/04/91	0.694 <	1.00	58.00 >	58.02 >	37.04 >	11.66
PT 9745	79	05/23/91	1.034	7.26	21.10	2.91	2.14 >	11.58

Table 20 (Cont'd)

AVS No.	Shipment#	Test Date	Differntl.	IC 50	TC 50	AI 50	SI	TAI
PT 9322	77	02/26/91	0.976	538.00	2430.00	4.52	0.39	11.40
PT 9424	80	07/11/91	0.648	93.40 >	320.00 >	3.43 >	3.43 >	11.28
PT 11738	82	08/15/91	0.682	703.00	2770.00	3.95	2.69	11.26
PT 9806	79	06/04/91	1.305	6.84	19.60	2.87	1.97 >	11.02
PT 9607	79	05/07/91	1.024	9.43	25.00	2.65	1.84 >	10.69
PT 9475	79	04/25/91	1.342	7.39	39.70	5.37	2.51	10.51
PT 10064	82	09/04/91	0.664 <	1.00	43.30 >	43.33 >	13.67 >	10.32
PT 9426	80	07/11/91	0.670	249.00 >	320.00 >	1.29 >	1.29 >	10.14
PT 9227	77	02/20/91	0.949	217.00	460.00	2.12	1.15	9.99
PT 9969	79	06/18/91	0.945	217.00	1060.00	4.90	1.40	9.48
PT 11907	82	09/26/91	0.544	9.16	194.00	21.21	1.95	9.45
PT 11487	81	08/01/91	0.654	158.00	784.00	4.97	0.54	9.43
PT 10586	83	10/29/91	0.474	76.90	210.00	2.73	2.02 >	9.20
PT 9802	79	06/04/91	1.116	8.27	21.50	2.59	1.90 >	9.19
PT 9974	79	06/18/91	0.823	68.40	214.00	3.13	2.06	9.11
PT 9269	77	02/20/91	1.017	762.00	2050.00	2.69	1.94 >	9.06
PT 11122	78	04/16/91	0.989	888.00	2100.00	2.36	1.75	8.83
PT 9248	77	02/20/91	0.970	1950.00 >	3200.00 >	1.64 >	1.64 >	8.64
PT 10047	79	07/03/91	1.361	203.00	618.00	3.04	2.10	8.56
PT 11108	78	04/11/91	1.019	757.00 >	3200.00 >	4.23	1.25 >	8.55
PT 9992	79	06/19/91	1.244	603.00	1630.00	2.71	1.35	8.40
PT 9678	79	05/16/91	1.181	2.66	6.67	2.50	1.85 >	8.34
PT 9317	77	02/26/91	1.090	1150.00 >	3200.00 >	2.78	0.24 >	8.05
PT 9791	79	05/30/91	1.089	23.50	60.90	2.59	1.76	7.72
PT 11224	80	10/10/91	0.492	25.90	89.10	3.43	2.12	7.65
PT 9603	79	05/07/91	1.306	9.40	19.20	2.04	1.36 >	7.61
PT 11941	82	09/24/91	0.633	260.00 >	320.00 >	1.23 >	1.23 >	7.52
PT 11496	81	08/01/91	0.696	277.00	656.00	2.37	1.73	7.42
PT 9887	79	06/11/91	0.946	2.77	6.34	2.29	1.63 >	7.34
PT 9696	79	05/16/91	1.257	28.80	64.60	2.24	1.61 >	7.30
PT 11816	82	08/21/91	0.489	885.00	2100.00	2.37	1.75	7.22
PT 10001	79	06/19/91	1.134	320.00	972.00	3.04	0.95	7.04
PT 9856	79	10/03/91	0.387 <	0.10	6.60 >	66.00 >	49.00 >	6.86
PT 9781	79	10/03/91	0.536 <	1.00	14.80 >	14.81 >	7.92 >	6.69
PT 9931	79	06/13/91	1.257	307.00	709.00	2.30	1.18	6.61
PT 9659	79	05/14/91	1.153	7.76	18.90	2.44	1.59	6.51
PT 9455	79	04/23/91	1.232	282.00 >	320.00 >	1.13	1.08 >	6.42
PT 9173	77	02/07/91	0.883	1910.00 >	3200.00 >	1.68	0.27 >	6.23
PT 11166	78	04/18/91	1.276	938.00	2870.00	3.06	1.59	6.08
PT 11467	81	07/30/91	0.742	814.00	1890.00	2.32	1.52	5.85
PT 10892	84	11/13/91	0.595	288.00 >	320.00 >	1.11 >	1.11 >	5.55
PT 9470	79	04/23/91	1.097	288.00 >	320.00 >	1.11 >	1.11 >	5.55
PT 11131	78	04/16/91	1.092	2480.00	4770.00	1.93	1.04 >	5.34
PT 9814	79	06/04/91	1.076	26.50	54.70	2.06	1.21 >	5.28
PT 9339	77	02/28/91	1.052	881.00	2030.00	2.31	0.03	5.17
PT 11155	78	04/18/91	1.216	75.90	171.00	2.26	1.28	5.10
PT 11135	78	04/16/91	1.130	938.00	2800.00	2.99	1.21	5.09
PT 9439	79	10/01/91	0.658	2480.00 >	3200.00 >	1.29 >	1.29 >	4.88
PT 11139	78	04/16/91	1.287	2670.00	5620.00	2.11	1.24	4.75
PT 10260	82	09/19/91	0.775	277.00 >	320.00 >	1.16 >	1.16 >	4.57
PT 9443	79	04/23/91	1.204	84.50	163.00	1.93	1.05	4.38
PT 9844	79	06/05/91	1.116	95.50	161.00	1.68	0.76	3.80
PT 9235	77	02/20/91	1.027	799.00	1430.00	1.78	0.96	3.70
PT 9393	77	03/07/91	0.910	835.00	1520.00	1.81	0.34	3.70
PT 9797	79	05/30/91	0.964	82.70	171.00	2.07	1.16	3.59
PT 11371	81	08/22/91	0.719	978.00	1450.00	1.49	0.80	3.55
PT 9825	79	06/05/91	1.133	28.30	54.70	1.94	1.13	3.49
PT 11144	78	04/16/91	1.114	2970.00	4350.00	1.46	0.80 >	3.47
PT 10640	83	10/30/91	0.869	271.00 >	320.00 >	1.18	0.34 >	3.43
PT 11140	78	04/16/91	1.287	260.00	601.00	2.31	0.95	3.30
PT 9487	80	07/09/91	1.210	28.30	48.70	1.72	0.46	3.19
PT 9253	77	02/20/91	1.179	2390.00 >	3200.00 >	1.34	0.75 >	3.15
PT 9327	77	02/28/91	0.924	710.00	2100.00	2.96	0.04	2.97
PT 9669	79	05/14/91	1.137	80.60	200.00	2.48	0.39 >	2.87

Table 20 (Cont'd)

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
PT 9276	77	02/21/91	0.879	2680.00 >	3200.00 >	1.19 >	1.19 >	2.71
PT 9271	77	02/20/91	0.971	924.00	2470.00	2.67	0.75	2.60
PT 9246	77	02/20/91	0.931	3130.00 >	3200.00 >	1.02	0.72 >	2.51
PT 9900	79	06/12/91	1.217 <	1.00	1.89 >	1.89 >	1.24 >	2.39
PT 9319	77	02/26/91	1.151	1710.00 >	3200.00 >	1.87	0.19 >	2.19
PT 9391	77	03/07/91	0.864	3200.00 >	3200.00 >	1.00 >	1.00 >	2.17
PT 9255	77	02/20/91	1.261	1970.00 >	3200.00 >	1.62	0.36 >	1.95
PT 9590	79	10/03/91	0.532	722.00	64.60	0.09	0.06 >	1.94
PT 11495	81	08/01/91	0.787	294.00	469.00	1.60	0.80	1.53
PT 9153	77	02/26/91	1.131	3010.00 >	3200.00 >	1.06	0.65 >	1.48
PT 11074	78	04/09/91	1.496	809.00	1340.00	1.65	0.40	1.31
PT 9270	77	03/07/91	1.030	892.00 >	1000.00 >	1.12	0.56 >	1.29
PT 11086	78	04/09/91	1.526	957.00	1530.00	1.60	0.67	1.19
PT 11364	81	08/22/91	0.576	3040.00	2930.00	0.96	0.65	1.10
PT 9649	79	05/14/91	1.135	97.80	127.00	1.30	0.73	0.85
PT 10679	83	10/31/91	0.554	28.80	179.00	6.21	0.27	0.77
PT 9342	77	02/28/91	1.072	2770.00	4240.00	1.53	0.07	0.76
PT 1103C	78	04/02/91	1.320	3130.00 >	3200.00 >	1.02	0.70 >	0.74
PT 9396	77	03/12/91	1.369	937.00	1860.00	1.98 <	0.01	0.50
PT 10733	84	11/07/91	0.550	2.68	19.60	7.32 <	0.37	0.49
PT 11076	78	04/09/91	1.570	2760.00 >	3200.00 >	1.16	0.18 >	0.44
PT 11150	78	04/18/91	1.015	827.00	927.00	1.12	0.75	0.41
PT 9680	79	05/16/91	1.171 <	1.00	1.40 >	1.40 ~	1.00 >	0.28
PT 9259	77	02/20/91	1.069	3010.00	2810.00	0.93	0.53	0.02
PT 9167	77	02/26/91	1.302	2300.00	2440.00	1.06	0.04	0.00
PT 9263	77	02/20/91	1.346	2520.00	76.00	0.03 <	0.00	0.00

New Drugs with 25% Antiviral Reduction Levels: Of the 3271 actual singl. drug tests, 290 new compounds demonstrated marginal antiviral activity, having antiviral reduction values equal to or better than 25%. This represents around 9% of the test compounds being active at this marginal antiviral reduction level.

In general, when compared to the 95% and 50% antiviral activity categories, the compounds in this (25%) category do not appear to have any significant antiviral promise and probably do not need to be presently confirmed any further.

4.3.5.3 Confirmatory Assays:

Some of the compounds were sent to us in more than one separate drug shipment. These compounds were tested more than once. Data from the confirmatory assays are summarized in **Table 21**. If a compound showed $\geq$50% reduction in CPE during this contract period, then it was considered a candidate for confirmatory testing. The confirmatory tests are from active compounds taken from the prescreen and primary MTT assays. Out of 142 confirmatory tests, 68 compounds were confirmed active during this reporting period and the remaining 74 compounds gave conflicting results. The criteria for activity is that the confirmatory test has to show $\geq$25% reduction in CPE. Failure to confirm the activity in these compounds was probably due to differences during the assay conditions:

1) In confirmatory assays the concentration range is adjusted to a more accurate semilog scale to maximize the TAI window.

2) Differences in the "differential" of the two runs can cause the compound to read positive or negative, falsely. The variability in the differential can cause false positive or negative bias to be introduced into the calculations, thus reflecting the variability in the maximum activity of the compound.

3) The metabolic rate, cell and virus load/well, age, and passage number of the cells may cause the above observed variability in the confirmatory results.

4) Problems associated with stability and storage of the compound (i.e., different lot numbers, solubility, light sensitivity, hygroscopic, etc.).

5) Problems associated with technical execution of large numbers of plates by different technicians.

During this reporting period the overall confirmatory rate against PT was 48%. The conflicting results should be retested at a later date based on the availability of the compound.

Some of the compounds have not been confirmed due to the discontinuation of this project by the sponsor.

4.3.5.4 Recommendations of PT-Actives Based Upon the *In Vitro* Results with MTT Assay (Vero Cells).

Based upon the *in vitro* results with the MTT assay (Vero cells) we recommend the following:

1) Compounds with the highest TAI in the 95% activity category that have confirmed results with the exception of "colored" compounds should receive the highest priority for further profile studies and *in vivo* animal testing.

2) Compounds with the highest TAI in the 50% activity category that have confirmed results with the exception of "colored" compounds should receive the next highest priority for further profile studies and *in vivo* animal testing.

Table 21

Confirmatory Assays for Compounds Active Against Punta Toro Virus (PT)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
0360	48	02/07/89	OKL	0.992	0.02	0.18	9.21	0.04	0.44	10.80	0.09	2.93	32.00	4.50 >	28.15	+
0360	2	08/23/89	RBJ	0.736	0.06	0.20	3.44	0.00	0.29	0.00	0.00 >	1.00	0.00	0.00	6.96	+
0360	2	11/01/90	ZXR	1.0[illegible]	0.75	0.52	0.70	0.00	0.95	0.00	0.00 >	1.00	0.00	0.00	0.00	+
0360	2/48	05/14/91	3WM	1.222	0.33	0.03	0.10	0.52 >	1.00 >	1.92	0.00 >	1.00	0.00	0.06 >	10.37	+
10001	79	06/19/91	6N9	1.134	129.00	303.00	2.35	320.00	972.00	3.04	0.00	2980.00	0.00	0.95	7.04	+
10001	79/C2	08/15/91	8DI	0.553	0.00	1660.0	0.00	0.00	3040.00	0.00	0.00 >	3200.00	0.00	0.00	0.00	-
10030	79	07/03/91	6AW	1.155	65.20	490.00	7.51	121.00	660.00	5.44	290.00	966.00	3.33	4.04	22.66	+
10030	79/C2	08/15/91	8DK	0.683	0.00	217.00	0.00	0.00	358.00	0.00	0.00	936.00	0.00	0.00	0.00	-
10031	79	07/03/91	6AW	1.155	219.00	1550.0	7.06	405.00	2100.00	5.19	914.00	3090.00	3.38	3.83	21.18	+
10031	79/C2	08/15/91	8DL	0.671	1000.0	619.00	0.62	0.00	1400.00	0.00	0.00	3020.00	0.00	0.00	0.00	+
10047	79	07/03/91	6V4	1.361	133.00	427.00	3.21	203.00	618.00	3.04	0.00	962.00	0.00	2.10	8.56	+
10047	79/C2	08/15/91	8DL	0.671	0.00	151.00	0.00	0.00	221.00	0.00	0.00	773.00	0.00	0.00	0.00	-
11047	78	04/04/91	4GT	0.738	357.00	1950.0	5.47	561.00	3200.00	5.71	0.00	9320.00	0.00	3.48	16.97	+
11047	78	10/10/91	AKH	0.512	1410.0	4850.0	3.43	2000.00	6570.00	3.29	0.00	9660.00	0.00	2.43	12.88	+
11074	78	04/09/91	4LC	1.496	417.00	320.00	0.77	809.00	1340.00	1.65	0.00	3010.00	0.00	0.40	1.31	+
11074	78	10/10/91	AKK	0.568	0.00	453.00	0.00	0.00	638.00	0.00	0.00	970.00	0.00	0.00	0.00	-
11076	78	04/09/91	4LD	1.570	1310.0	505.00	0.39	2760.00 >	3200.00 >	1.16	0.00 >	3200.00	0.00	0.18 >	0.44	+
11076	78	10/10/91	AKL	0.578	2000.0	4570.0	2.29	0.00	6380.00	0.00	0.00	9640.00	0.00	0.00	3.80	+
11086	78	04/09/91	4LI	1.526	553.00	640.00	1.16	957.00	1530.00	1.60	0.00	3030.00	0.00	0.67	1.19	+
11086	78	10/10/91	AFZ	0.517	0.00	717.00	0.00	0.00	1520.00	0.00	0.00	3040.00	0.00	0.00	0.00	-
11107	78	04/11/91	4NS	1.019	684.00	3200.0	4.68	1760.00	6350.00	3.61	0.00 >	10000.0	0.00	1.82 >	15.40	+
11107	78	10/15/91	AMD	0.651	0.00	1940.0	0.00	0.00	4930.00	0.00	0.00 >	10000.0	0.00	0.00	0.00	-
11108	78	04/11/91	4NS	1.019	378.00	948.00	2.51	757.00 >	3200.00 >	4.23	0.00 >	3200.00	0.00	1.25 >	8.55	+
11108	78	10/15/91	AME	0.619	0.00	1370.0	0.00	0.00	3200.00	0.00	0.00 >	10000.0	0.00	0.00 >	0.45	-
11110	78	04/11/91	4NT	1.020	357.00 >	3200.0 >	8.97	1230.00 >	3200.00 >	2.61	0.00 >	3200.00	0.00 >	2.61 >	21.19	+
11110	78	10/15/91	AMF	0.600	0.00	3570.0	0.00	0.00	8160.00	0.00	0.00 >	10000.0	0.00	0.00	0.64	-
11115	78	04/11/91	4NV	1.057	73.20	854.00	11.67	768.00 >	3200.00 >	4.16	0.00 >	3200.00	0.00	1.11 >	21.78	+
11115	78	10/15/91	AMG	0.576	255.00	417.00	1.63	0.00	2400.00	0.00	0.00 >	10000.0	0.00	0.00	0.71	+
11118	78	04/11/91	4NW	1.013	939.00 >	10000 >	10.65	1710.00 >	10000.0 >	5.85	0.00 >	10000.0	0.00 >	5.85 >	28.50	+
11118	78	10/15/91	AMH	0.577	0.00	7730.0	0.00	0.00 >	10000.0	0.00	0.00 >	10000.0	0.00	0.00 >	1.25	-
11121	78	04/11/91	4NX	1.015	468.00 >	10000 >	21.37	1210.00 >	10000.0 >	8.24	3200.0 >	10000.0 >	3.13 >	8.24 >	38.02	+
11121	78	10/15/91	AMI	0.531	0.00	4560.0	0.00	0.00 >	10000.0	0.00	0.00 >	10000.0	0.00	0.00	0.00	-
11122	78	04/16/91	4PO	0.989	491.00	1550.0	3.16	888.00	2100.00	2.36	0.00	3090.00	0.00	1.75	8.83	+
11122	78	10/15/91	AMI	0.531	0.00	1370.0	0.00	0.00	1980.00	0.00	0.00	3080.00	0.00	0.00	0.00	-

Table 21 (Cont'd)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
11123	78	04/16/91	4PP	1.100	201.00	6780.0	33.73	405.00 >	10000.0 >	24.70	914.00 >	10000.0 >	10.95	16.75 >	48.26	+
11123	78	10/15/91	AMJ	0.515	957.00	6680.0	6.97	1910.00 >	10000.0 >	5.22	0.00 >	10000.0	0.00	3.49	15.90	+
11129	78	04/16/91	4PS	1.216	0.00 >	1000.0	0.00	0.00 >	1000.00	0.00	0.00 >	1000.00	0.00	0.00	0.00	-
11129	78	10/15/91	AMH	0.532	607.00	6900.0	11.35	1230.00 >	10000.0 >	8.11	2910.0 >	10000.0 >	3.44	5.59 >	27.13	+
11131	78	04/16/91	4PT	1.092	1510.0	2570.0	1.70	2480.00	4770.00	1.93	0.00	9480.00	0.00	1.04 >	5.34	+
11131	78	10/15/91	AMH	0.544	0.00	1650.0	0.00	0.00	2310.00	0.00	0.00	7880.00	0.00	0.00 >	2.94	-
11133	78	04/16/91	4PU	1.091	414.00	1980.0	4.78	556.00	2960.00	5.33	945.00	9230.00	9.79	3.57	19.88	+
11133	78	10/15/91	AMH	0.544	0.00	1650.0	0.00	0.00	2900.00	0.00	0.00	9230.00	0.00	0.00 >	0.60	-
11134	78	04/16/91	4PU	1.091	0.00	674.00	0.00	0.00	1080.00	0.00	0.00	2990.00	0.00	0.00 >	3.47	-
11134	78	10/15/91	AMO	0.569	248.00	1560.0	6.27	433.00	2110.00	4.88	920.00	3110.00	3.38	3.60 >	21.91	+
11139	78	04/16/91	4PX	1.287	1510.0	3290.0	2.18	2670.00	5620.00	2.11	0.00	9810.00	0.00	1.24	4.75	+
11139	78	10/15/91	AMO	0.569	0.00	2020.0	0.00	0.00	3040.00	0.00	0.00	9260.00	0.00	0.00	0.00	-
11140	78	04/16/91	4PX	1.287	155.00	247.00	1.59	260.00	601.00	2.31	0.00	2720.00	0.00	0.95	3.30	+
11140	78	10/15/91	AMP	0.629	0.00	80.60	0.00	0.00	722.00	0.00	0.00	2930.00	0.0	0.00 >	1.30	-
11144	78	04/16/91	4PZ	1.114	1600.0	2380.0	1.48	2970.00	4350.00	1.46	0.00	9540.00	0.00	0.80 >	3.47	+
11144	78	10/17/91	APC	0.775	0.00	477.00	0.00	0.00	738.00	0.00	0.00	2760.00	0.00	0.00	0.00	-
11150	78	04/18/91	4RV	1.015	514.00	624.00	1.21	827.00	927.00	1.12	0.00	2950.00	0.00	0.75	0.41	+
11150	78	10/10/91	AKM	0.548	0.00	461.00	0.00	0.00	656.00	0.00	0.00	1370.00	0.00	0.00	0.00	-
11155	78	04/18/91	4RY	1.216	69.30	97.40	1.98	75.90	171.00	2.26	0.00	305.00	0.00	1.28	5.10	+
11155	78	10/10/91	AKM	0.548	158.00 >	320.00 >	2.03	248.00 >	320.00 >	1.29	0.00 >	320.00	0.00 >	1.29 >	4.23	+
11166	78	04/18/91	4S3	1.276	548.00	1500.0	2.73	938.00	2870.00	3.06	0.00 >	3200.00	0.00	1.59	6.08	+
11166	78	10/10/91	AKN	0.528	2480.0	2830.0	1.14	0.00	5140.00	0.00	0.00	9510.00	0.00	0.00	0.21	+
11190	80	07/09/91	62E	1.234	268.00	1200.0	4.48	512.00 >	3200.00 >	6.26	0.00 >	3200.00	0.00	2.35 >	15.01	+
11190	80	10/10/91	AFS	0.499	542.00 >	1000.0 >	1.84	919.00 >	1000.00 >	1.09	0.00 >	1000.00	0.00 >	1.09 >	3.33	+
11191	80	07/09/91	62F	1.181	44.30	1480.0	33.43	73.70	3200.00	43.42	271.00 >	3200.00 >	11.81	20.10	43.03	+
11191	80	10/10/91	AFY	0.662	140.00 >	1000.0 >	7.13	197.00 >	1000.00 >	5.09	0.00 >	1000.00	0.00 >	5.09 >	16.22	+
11224	80	07/11/91	737	0.695	0.00	17.60	0.00	0.00	26.60	0.00	0.00	265.00	0.00	0.00	2.27	-
11224	80	10/10/91	AFU	0.492	16.10	55.10	3.42	25.90	89.10	3.43	0.00 >	320.00	0.00	2.12	7.65	+
2318	53	06/19/89	PFL	0.701 <	1.00	1.50 >	1.50 <	1.00	3.65 >	3.65	0.00 >	320.00	0.00 >	1.50 >	2.76	+
2318	13	08/30/89	RF9	0.608	0.58	2.18	3.75	0.00	6.00	0.00	0.00 >	10.00	0.00	0.00	4.86	+
2318	67	05/31/90	WMJ	0.653	0.26	2.13	8.09	0.67	6.60	9.89	0.00 >	10.00	0.00	3.19 >	13.75	+
2318	67	07/12/90	XGV	0.619	0.42	2.90	6.88	0.69 >	10.00 >	14.52	0.00 >	10.00	0.00	4.21	16.14	+
2318	82	10/01/91	A4N	0.814 <	1.00	3.20 >	3.20 <	1.00	49.00 >	49.00	0.00 >	320.00	0.00 >	3.20 >	6.66	+

Table 21 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
2563	48	02/07/89	OKN	1.002	0.21	1.77	8.50	0.39 >	3.20 >	8.14	0.91 >	3.20 >	3.51	4.51 >	28.54	+
2563	15	05/23/89	052	0.724	0.00	5.33	0.00	0.00	7.45	0.00	0.00 >	32.00	0.00	0.00	0.12	-
2563	21	11/28/89	SLM	0.491	1.57	4.41	2.81	2.51	8.19	3.26	0.00 >	10.00	0.00	1.76	9.32	+
2563	48	12/05/89	S..	0.733	0.00 >	3.20	0.00	0.00 >	3.20	0.00	0.00 >	3.20	0.00	0.00 >	0.31	-
2563	15/21	11/01/90	ZXU	1.116	0.21	0.73	3.54	0.77	1.56	2.03	0.00	10.00	0.00	0.95	8.65	+
2563	21/48	03/14/91	3UH	1.232	0.15	0.86	5.81	0.34	2.19	6.33	0.00 >	10.00	0.00	2.48	12.15	+
2811	48	02/07/89	OKQ	0.862	0.02	0.16	9.50	0.03	0.26	8.65	0.09 >	1.00 >	11.30	5.49	25.35	+
2811	RS-1	03/14/91	3U0	1.116	0.02	0.13	5.94	0.05	0.27	5.03	0.00	0.96	0.00	2.37	11.43	+
2812	48	02/07/89	OKR	0.837	0.00 >	0.01 >	5.31	0.00 >	0.01 >	2.87	0.01 >	0.01 >	1.11 >	2.87 >	19.34	+
2812	61	12/06/89	SQ8	0.814	0.00	0.02	4.83	0.01	0.03	4.58	0.00	0.10	0.00	3.05 >	13.37	+
2812	61	12/05/90	101	0.912	0.00 <	0.32	0.00	0.00 <	0.32	0.00	0.00 <	0.32	0.00	0.00	0.00	-
2812	RS-1	03/14/91	3UP	1.106	0.00	0.01	3.56	0.01	0.03	3.14	0.00	0.09	1.00	1.68	7.41	+
2980	48	02/08/89	OLV	0.686	0.42 >	3.20 >	7.61	0.78 >	3.20 >	4.10	2.77 >	3.20 >	1.16 >	4.10 >	23.21	+
2980	25	09/07/89	RIV	0.532	0.39	1.25	3.23	0.59	2.02	3.43	0.00	10.00	0.00	2.11	11.88	+
2980	61	12/06/89	SQH	0.832	0.19	2.28	12.22	0.43 >	3.20 >	7.43	0.00 >	3.20	0.00	5.29	24.37	+
2980	48/61	03/14/91	3U0	1.268	0.42	1.58	3.74	0.85	3.03	3.58	0.00 >	10.00	0.00	1.87	9.45	+
4070	48	02/08/89	OLU	0.678	1.05 >	3.20 >	3.06	1.52 >	3.20 >	2.11	2.97 >	3.20 >	1.08 >	2.11 >	14.01	+
4070	48	12/07/89	SUD	0.723	0.96	6.42	6.71	2.46	12.40	5.06	0.00	80.60	0.00	2.61	13.05	+
4070	48	03/14/91	3UR	1.152	1.50	5.55	3.71	2.62	8.82	3.37	0.00	45.60	0.00	2.12 >	10.78	+
4074	48	02/08/89	OLV	0.718	0.01 >	0.03 >	4.37	0.00 >	0.03	0.00	0.00 >	0.03	0.00	0.00 >	6.49	+
4074	48	12/07/89	SUE	0.894 <	1.00	1.69 >	1.69 <	1.00	2.71 >	2.71	0.00	26.50	0.00 >	1.69 >	3.55	+
4074	48	02/13/90	U00	0.699	0.00	0.67	0.00	0.00	1.16	0.00	0.00	9.66	0.00	0.00	1.98	-
4074	RS-1	03/14/91	3UR	1.152	0.30	1.09	3.63	0.61	2.26	3.69	0.00	22.00	0.00	1.78	9.68	+
4105	48	02/08/89	OLX	0.679	3.68	11.10	3.02	7.37	22.00	2.99	0.00 >	32.00	0.00	1.51	10.07	+
4105	48	03/14/91	3US	1.101	150.00 >	320.00 >	2.13	244.00 >	320.00 >	1.31	0.00 >	320.00	0.00 >	1.31 >	5.58	+
4113	48	02/08/89	OLZ	0.674	0.00 >	0.32	0.00	0.00 >	0.32	0.00	0.00 >	0.32	0.00	0.00	0.11	-
4113	39	05/24/89	062	1.102	0.00	0.23	0.00	0.00	0.59	0.00	0.00	2.70	0.00	0.00	0.00	-
4113	39	11/28/89	SLX	0.611	0.00	0.77	0.00	0.00 >	1.00	0.00	0.00 >	1.00	0.00	0.00 >	0.00	-
4113	39	11/01/90	ZXV	0.771	0.00 >	0.32	0.00	0.00 >	0.32	0.00	0.00 >	0.32	0.00	0.00 >	1.51	-
4113	39/48	03/14/91	3US	1.101	0.33	1.29	3.94	0.60	2.33	3.87	0.00	8.83	0.00	2.15	10.94	+
4223	62	01/18/90	TF3	0.783 <	1.00 <	1.00 -	1.00	0.00 <	1.00	0.00	0.00	2.85	0.00	0.00	0.00	+
4223	62	02/06/90	TRW	0.628	0.00 >	1.00	0.00	0.00 >	1.00	0.00	0.00 >	1.00	0.0	0.00 >	3.36	-
4223	63	02/13/90	U07	0.598	0.13	0.28	2.16	0.26	0.61	2.37	0.00 >	1.00	0.00	1.08	6.39	+
4223	RS-1	03/14/91	3UU	1.139	0.14	0.68	4.79	0.28	1.42	5.09	0.00	6.60	0.00	2.44	11.75	+
4280	42	11/28/89	SM4	0.505	0.00	0.10	0.00	0.00 >	10.00	0.00	0.00 >	10.00	0.00	0.00 >	1.68	-
4280	42	11/01/90	ZXU	0.885	5.72	13.40	2.34	0.00	21.80	0.00	0.00	88.70	0.00	0.00	4.51	+
4280	77	03/12/91	3UA	1.263	15.80	17.70	1.11	29.00	52.60	1.81	0.00	279.00	0.00	0.61	1.09	+

Table 21 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
4590	48	05/01/89	QUO	0.998	1.14	2.72	2.39	2.55	5.98	2.34	0.00	26.80	0.00	1.07 >	8.63	+
4590	42	11/28/89	SM7	0.353	0.00	2.57	0.00	0.00	5.82	0.00	0.00 >	10.00	0.00	0.00 >	2.20	-
4590	61	12/06/89	SQA	0.855	0.25	1.31	5.30	0.53	2.62	4.91	0.00	9.13	0.00	2.46	14.26	+
4590	48	02/06/90	TRN	0.635	1.22	5.14	4.20	2.30	8.18	3.55	0.00 >	10.00	0.00	2.23 >	12.80	+
4590	63	02/13/90	U08	0.588	1.64	5.82	3.54	2.81	8.43	3.00	0.00 >	10.00	0.00	2.07	8.44	+
4590	RS-1	03/14/91	3UV	1.210	1.23	7.01	5.71	2.89	14.80	5.13	0.00 >	32.00	0.00	2.43	15.91	+
4592	48	03/01/89	QUP	0.968	10.50	48.10	4.60	15.20	88.40	5.82	29.70 >	100.00 >	3.37	3.17	24.18	+
4592	42	11/28/89	SM7	0.353	0.00	22.60	0.00	0.00	59.60	0.00	0.00 >	100.00	0.00	0.00 >	0.89	-
4592	61	12/06/89	SQA	0.855	4.97	43.30	8.72	9.50	83.60	8.83	0.00 >	100.00	0.00	4.56	19.51	+
4592	48	02/06/90	TRO	0.701	11.30	45.10	3.98	24.90	77.80	3.13	0.00 >	100.00	0.00	1.81	9.83	+
4592	63	02/13/90	U09	0.615	0.00	7.06	0.00	0.00	12.80	0.00	0.00	57.50	0.00	0.00 >	4.95	-
4592	RS-1	03/14/91	3UV	1.210	2.81	8.93	3.17	6.63	21.50	3.25	0.00	90.60	0.00	1.35	9.34	+
4855	48	02/22/89	QPX	0.470 <	1.00	104.00 >	104.00	5.08	184.00	36.20	16.80	328.00	19.50	20.50 >	60.25	+
4855	48	11/28/89	SME	0.444	8.55	159.00	18.62	14.10	218.00	15.47	29.50 >	320.00 >	10.85	11.28 >	41.15	+
4855	61	12/06/89	SQE	0.853	8.97 >	320.00 >	35.67	15.30 >	320.00 >	20.93	56.60 >	320.00 >	5.66 >	20.93 >	53.71	+
4855	48	12/05/90	1Q2	0.856	3.00 >	100.00 >	33.30	11.90 >	100.00 >	8.41	142.00 >	100.00 >	0.71 >	8.41 >	33.70	+
4855	48	03/12/91	3UD	1.343	11.60	277.00	23.88	29.70 >	320.00 >	10.78	95.10 >	320.00 >	3.37	9.34 >	38.16	+
7940	74	10/16/90	ZJT	1.042	0.00	251.00	0.00	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00	0.00	-
7940	75	11/08/90	1ST	0.777	49.40	427.00	8.64	77.60	618.00	7.96	320.00	962.00	3.01	5.50 >	25.33	+
7940	75	03/28/91	49W	0.908	390.00	845.00	2.17	725.00	1630.00	2.25	0.00	3040.00	0.00	1.17	5.88	+
7947	75	12/04/90	1N5	1.039	919.00	547.00	0.59	0.00	1800.00	0.00	0.00 >	3200.00	0.00	0.00	0.00	+
7947	75	03/28/91	49K	0.894	510.00	830.00	1.63	814.00	1640.00	2.01	0.00	3070.00	0.00	1.02	3.06	+
8212	75	12/21/90	277	0.866	130.00	535.00	4.11	0.00	1430.00	0.00	0.00	3020.00	0.00	0.00 >	8.49	+
8212	75	03/19/91	438	1.287	92.70	456.00	4.92	305.00	1040.00	3.42	0.00	2980.00	0.00	1.49 >	8.57	+
8228	75	11/20/90	1C0	1.052	32.00	234.00	7.32	56.60	426.00	7.52	0.00	3200.00	0.00	4.14 >	22.15	+
8228	75	03/19/91	43C	1.315	168.00	461.00	2.75	287.00	682.00	2.37	0.00 >	1000.00	0.00	1.61 >	7.47	+
8233	75	11/20/90	1C3	1.169	31.70	276.00	8.71	367.00 >	3200.00 >	8.72	0.00 >	3200.00	0.00	0.75 >	19.73	+
8233	75	03/19/91	43D	1.102	449.00	1400.0	3.12	970.00	3070.00	3.16	0.00 >	3200.00	0.00	1.44	4.12	+
8234	75	11/20/90	1C3	1.169 <	1.00	18.50 >	18.46	1.14	100.00	87.88	0.00 >	320.00	0.00	16.22 >	25.79	+
8234	75	03/19/91	43D	1.102	15.90	73.60	4.63	147.00 >	320.00 >	2.18	0.00 >	320.00	0.00	0.50 >	11.3	+
8237	75	11/20/90	1C5	0.982	85.50	849.00	9.93	196.00 >	3200.00 >	16.54	0.00 >	3200.00	0.00	4.39 >	20.03	+
8237	75	03/19/91	43E	1.079	241.00	1230.0	5.10	548.00 >	3200.00 >	5.84	0.00 >	3200.00	0.00	2.25 >	12.38	+
8240	75	11/20/90	1C6	1.077	68.40	1160.0	17.02	182.00	1990.00	10.91	0.00 >	3200.00	0.00	6.40 >	23.34	+
8240	75	03/19/91	43E	1.079	368.00	1120.0	3.03	607.00	1840.00	3.03	0.00	3140.00	0.00	1.84	6.75	+

Table 21 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
8251	76	12/11/90	1SM	0.701	53.60	198.00	3.70	91.80	296.00	3.23	0.00	923.00	0.00	2.16	8.21	+
8251	75	03/19/91	43G	1.038	67.70	490.00	7.24	157.00	660.00	4.21	0.00	966.00	0.00	3.12 >	16.46	+
8259	76	12/11/90	1SQ	0.772	61.10	600.00	9.81	128.00 >	3200.00 >	24.94	0.00 >	3200.00	0.00	4.68	19.80	+
8259	75	03/19/9[illegible]	43[illegible]	1.178	56.60	200.00	[illegible]	179.00	2570.00	14.37	0.00 >	[illegible]00.00	0.00	1.12	9.71	+
8260	76	12/11/90	1S[illegible]	0.772	137.00	728.00	[illegible].32	239.00 >	1000.00 >	4.18	0.00 >	1000.00	0.00	3.04 >	11.53	+
8260	75	03/19/91	43H	1.178	81.00	276.00	3.41	264.00	1930.00	7.32	0.00 >	3200.00	0.00	1.05	10.03	+
8261	76	12/11/90	1SR	0.779	145.00	490.00	3.38	0.00	2670.00	0.00	0.00 >	3200.00	[illegible].00	0.00	6.03	+
8261	75	03/19/91	43I	1.225	109.00	135.00	1.24	320.00	728.00	2.27	0.00 >	3200.00	[illegible].00	0.42	1.36	+
8263	76	12/11/90	1SS	0.660	29.40	77.90	2.65	47.90	164.00	3.42	0.00	951.00	0.00	1.63	13.00	+
8263	75	03/[illegible]9/91	43J	1.098	78.90	223.00	2.82	207.00	440.00	2.13	0.00 >	1000.00	0.00	1.08 >	9.64	+
8271	76	12/12/90	1UO	0.910	129.00	507.00	3.92	198.00	694.00	3.50	0.00 >	1000.00	0.00	2.56 >	15.67	+
8271	76	03/21/91	45H	0.964	42.30	490.00	11.59	79.60	660.00	8.29	0.00	966.00	0.00	6.15 >	22.36	+
8309	76	12/12/90	1UQ	1.075	1340.0 >	3200.0 >	2.39	1920.00 >	3200.00 >	1.66	0.00 >	3200.00	0.00 >	1.66 >	5.48	+
8309	76	03/21/91	45I	0.782	885.00	5300.0	5.99	1430.00	7400.00	5.18	2950.0 >	10600.0 >	3.39	3.71	21.44	+
8311	76	12/12/90	1UR	0.953	402.00	9.62	0.02	819.00	1830.00	2.23	0.00	3060.00	0.00	0.01	4.39	+
8311	76	03/21/91	45J	0.892	435.00	1280.0	2.95	665.00	1920.00	2.89	0.00	3070.00	0.00	1.93 >	9.61	+
8312	76	12/12/90	1US	0.885	239.00	1210.0	5.07	718.00	1870.00	2.61	0.00	3070.00	0.0	1.69	13.14	+
8312	76	03/21/91	45J	0.892	91.30	1550.0	16.98	672.00	2100.00	3.12	0.00	3090.00	0.00	2.31 >	19.89	+
8339	76	12/20/90	25U	0.954	0.00	32.00	0.00	0.00	312.00	0.00	0.00	931.00	0.00	0.00	0.00	-
8339	77	03/07/91	3RN	0.982	143.00	329.00	2.29	286.00	553.00	1.93	0.00	955.00	0.00	1.15 >	4.46	+
8353	75	11/29/90	1K1	0.934	11.80 >	320.00 >	27.05	16.80 >	320.00 >	19.05	31.60 >	320.00 >	10.[illegible] >	19.05 >	53.70	+
8353	75	[illegible]/26/91	47G	1.028	13.40	333.00	24.87	17.90	647.00	36.19	30.20 >	1000.00 >	33.12	18.59 >	43.49	+
8355	76	12/20/90	25W	0.776	6.26	61.40	9.82	16.90	149.00	8.82	0.00 >	320.00	0.00	3.64 >	21.43	+
8355	76	03/26/91	47N	1.165	11.10	84.90	7.64	16.20 >	320.00 >	19.73	32.00 >	320.00 >	10.00	5.24	25.98	+
8358	76	12/20/90	25Y	0.937	37.30	139.00	3.72	54.90	204.00	3.71	0.00	320.00	0.00	2.53	12.83	+
8358	76	03/26/91	47N	1.165	37.80	146.00	3.85	63.40	209.00	3.29	0.00 >	320.00	0.00	2.30	11.46	+
8372	76	12/13/90	1Y7	1.049	566.00	444.00	0.78	1000.00 >	3200.00 >	3.20	0.00 >	3200.00	0.00	0.44	1.68	+
8372	76	03/21/91	45O	0.925	1390.0	2320.0	1.66	2520.00	6690.00	2.65	0.00 >	10000.0	0.00	0.92 >	6.24	+
8376	76	12/13/90	1Y9	0.827 <	10.00	66.70 >	6.67	17.30	110.00	6.37	0.00	2710.00	0.00	3.84 >	16.33	+
8376	76	03/21/91	45Q	1.156	13.10	46.70	3.56	24.40	92.60	3.80	0.00 >	320.00	0.00	1.92 >	9.88	+
8377	76	12/13/90	1Y9	0.827	14.90	165.00	11.04	37.30	255.00	6.85	0.00 >	1000.00	0.00	4.43 >	26.15	+
8377	76	03/21/91	45Q	1.156	19.20	136.00	7.08	49.80	264.00	5.29	0.00 >	1000.00	0.00	2.72	12.70	+
8378	76	12/13/90	1YA	0.992	88.50	453.00	5.12	143.00	638.00	4.47	295.00	970.00	3.29	3.18	17.44	+
8378	76	03/21/91	45R	0.847	153.00	407.00	2.65	235.00	605.00	2.57	0.00	960.00	0.00	1.73	6.04	+

Table 21 (Cont'd)

AVS No.	Ship-ment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
8499	75	11/29/90	1K3	0.915	36.00	228.00	6.35	74.70 >	320.00 >	4.29	0.00 >	320.00	0.00	3.06 >	19.70	+
8499	75	03/26/91	47I	1.106	19.70	265.00	13.45	54.60 >	1000.00 >	18.32	0.00 >	1000.00	0.00	4.85 >	19.84	+
8511	76	12/19/90	25K	0.669	459.00 >	3200.0 >	6.97	658.00 >	3200.00 >	4.86	0.00 >	3200.00	0.00 >	4.86 >	20.21	+
8511	76	03/21/91	45S	0.808	573.00	3290.0	5.74	1200.00	5530.00	4.62	0.00	9550.00	0.00	2.75	12.95	+
8601	76	01/23/91	2UG	0.710	0.00	3.20	0.00	0.00	8.68	0.00	0.00	30.40	0.00	0.00	0.00	-
8601	76	03/26/91	47L	0.927	2.51	18.10	7.21	8.62	27.80	3.22	0.00	91.30	0.00	2.10	10.26	+
8701	76	01/22/91	2TL	0.755	8.02 >	320.00 >	39.90	39.40 >	320.00 >	8.13	0.00 >	320.00	0.00 >	8.13 >	20.17	+
8701	76	04/02/91	4DS	1.243	5.21 >	1000.0 >	191.77	20.80 >	1000.00 >	48.08	0.00 >	1000.00	0.00 >	48.08 >	42.93	+
8702	76	01/22/91	2TN	0.768	4.99 >	320.00 >	64.10	10.00 >	320.00 >	32.00	0.00 >	320.00	0.00 >	32.00 >	24.65	+
8702	76	04/02/91	4DS	1.243	4.07 >	320.00 >	78.53	9.66 >	320.00 >	33.12	0.00 >	320.00	0.00 >	33.12 >	41.17	+
9149	77	02/26/91	3JO	0.986	493.00 >	3200.0 >	6.49	2260.00 >	3200.00 >	1.42	0.00 >	3200.00	0.00 >	1.42 >	15.20	+
9149	77	10/09/91	AIE	0.549	0.00	1940.0	0.00	0.00	6360.00	0.00	0.00 >	10000.0	0.00	0.00	0.05	-
9153	77	02/26/91	3JQ	1.131	1400.0	1960.0	1.40	3010.00 >	3200.00 >	1.06	0.00 >	3200.00	0.00	0.65 >	1.48	+
9153	77	10/09/91	AIE	0.549	0.00	660.00	0.00	0.00	5580.00	0.00	0.00	9560.00	0.00	0.00	0.00	-
9167	77	02/26/91	3JX	1.302	789.00	94.80	0.12	2300.00	2440.00	1.06	0.00 >	3200.00	0.00	0.04	0.00	+
9167	77	10/09/91	AIH	0.561	0.00	183.00	0.00	0.00	1370.00	0.00	0.00	3020.00	0.00	0.00	0.00	-
9173	77	02/07/91	351	0.883	1380.0	524.00	0.38	1910.00 >	3200.00 >	1.68	0.00 >	3200.00	0.00	0.27 >	6.23	+
9173	77	10/09/91	AIJ	0.574	2410.0	3920.0	1.63	0.00	7540.00	0.00	0.00 >	10000.0	0.00	0.00	0.70	+
9225	77	02/20/91	3AJ	0.895	58.30	433.00	7.43	197.00	748.00	3.80	0.00	2830.00	0.00	2.20	14.89	+
9225	77	10/09/91	AIO	0.572	0.00	143.00	0.00	0.00	210.00	0.00	0.00	622.00	0.00	0.00	0.00	-
9227	77	02/20/91	3AK	0.949	68.40	249.60	3.64	217.00	460.00	2.12	0.00	946.00	0.00	1.15	9.99	+
9227	77	10/09/91	AIO	0.572	0.00	152.00	0.00	0.00	252.00	0.00	0.00	897.00	0.00	0.00	0.00	-
9229	77	02/20/91	3AL	0.944	359.00	1600.0	4.65	582.00	2600.00	4.46	0.00 >	3200.00	0.00	2.75	14.31	+
9229	77	10/09/91	AIP	0.598	0.00	477.00	0.00	0.00	1510.00	0.00	0.00	3030.00	0.00	0.00 >	0.03	-
9235	77	02/20/91	3AO	1.027	481.00	767.00	1.60	799.00	1430.00	1.78	0.00	3020.00	0.00	0.96	3.70	+
9235	77	10/10/91	AKA	0.647	759.00	765.00	1.01	0.00	1530.00	0.00	0.00	3030.00	0.00	0.00	0.00	+
9238	77	02/20/91	3AP	0.955	43.90	369.00	8.39	239.00	611.00	2.56	0.00 >	1000.00	0.00	1.54 >	17.62	+
9238	77	10/10/91	AKB	0.567	0.00	329.00	0.00	0.00	553.00	0.00	0.00	955.00	0.00	0.00	0.00	-
9246	77	02/20/91	3AT	0.931	1770.0	2250.0	1.27	3130.00 >	3200.00 >	1.02	0.00 >	3200.00	0.00	0.72 >	2.51	+
9246	77	10/10/91	AKB	0.567	0.00	1500.0	0.00	0.00	2070.00	0.00	0.00	3090.00	0.00	0.00	0.00	-
9248	77	02/20/91	3AU	0.970	1360.0 >	3200.0 >	2.36	1950.00 >	3200.00 >	1.64	0.00 >	3200.00	0.00 >	1.64 >	8.64	+
9248	77	10/10/91	AKC	0.583	0.00	4570.0	0.00	0.00	6380.00	0.00	0.00	9640.00	0.00	0.00	0.00	-
9253	77	02/20/91	3BT	1.179	957.00	1790.0	1.87	2390.00 >	3200.00 >	1.34	0.00 >	3200.00	0.00	0.75 >	3.15	+
9253	77	10/10/91	AKD	0.599	0.00	1110.0	0.00	0.00	1810.00	0.00	0.00	3060.00	0.00	0.00	0.00	-

Table 21 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	AC
9255	77	02/20/91	38U	1.261	827.00	703.00	0.85	1970.00 >	3200.00 >	1.62	0.00 >	3200.00	0.00	0.36 >	1.95	+
9255	77	10/10/91	AKE	0.558	2190.0	4120.0	1.88	0.00	6220.00	0.00	0.00	10000.0	0.00	0.00	1.89	+
9258	77	02/20/91	38U	1.069	47.10	278.00	5.90	72.90	1530.00	21.02	0.00	3030.00	0.00	3.81	23.56	+
9258	77	10/10/91	AKF	0.588	75.90	274.00	3.62	0.00	1120.00	0.00	0.00 >	3200.00	0.00	0.00	4.62	+
9259	77	02/20/91	38U	1.069	1400.0	1590.0	1.13	3010.00	2810.00	0.93	0.00 >	3200.00	0.00	0.53	0.02	+
9259	77	10/10/91	AKF	0.588	0.00	1310.0	0.00	0.00	1950.00	0.00	0.00	3100.00	0.00	0.00	0.00	-
9414	81	08/06/91	82Z	0.794	81.80 >	320.00 >	3.91	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	3.59	+
9414	81	10/08/91	ADM	0.430	194.00 >	1000.0 >	5.16	362.00 >	1000.00 >	2.77	903.00 >	1000.00 >	1.1 >	2.77 >	18.74	+
9423	80	07/11/91	73A	0.648	40.20 >	320.00 >	7.96	62.30 >	320.00 >	5.14	0.00 >	320.00	0.00 >	5.14	17.66	+
9423	80	10/08/91	ADP	0.681	38.30	490.00	12.80	52.70	660.00	12.52	93.80	966.00	10.30	9.29	36.11	+
9439	79	04/23/91	4YE	1.095	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00	0.00	-
9439	79	10/01/91	A4Z	0.658	1590.0 >	3200.0 >	2.03	2480.00 >	3200.00 >	1.29	0.00 >	3200.00	0.00 >	1.29 >	4.88	+
9443	79	04/23/91	4YF	1.204	49.80	88.70	1.78	84.50	163.00	1.93	0.00	304.00	0.00	1.05	4.38	+
9443	79	10/01/91	A4A	0.658	0.00	119.00	[illegible]	0.00	186.00	0.00	0.00	307.00	0.00	0.00	0.00	-
9445	79	04/23/91	4YH	1.251	4.15	17.50	4.23	6.38	25.10	3.93	0.00	89.50	0.00	2.75	14.12	+
9445	79	10/01/91	A50	0.658	0.00	2.17	0.00	0.00	25.60	0.00	0.00	94.00	0.00	0.00	0.00	-
9446	79	04/23/91	4YI	1.389	41.10	124.00	3.01	58.20	190.00	3.27	0.00	309.00	[illegible].00	2.13	11.73	+
9446	79	10/01/91	A50	0.658	0.00	156.00	0.00	0.00	212.00	0.00	0.00	313.00	0.00	0.00	0.74	-
9448	79	04/23/91	4YJ	1.104	63.00 >	320.00 >	5.08	136.00 >	320.00 >	2.36	0.00 >	320.00	0.00 >	2.36 >	14.43	+
9448	79	10/01/91	A51	0.728	273.00	493.00	1.81	0.00	667.00	0.00	0.00	979.00	0.00	0.00	3.15	+
9451	79	04/23/91	4YK	1.038	13.40	50.70	3.79	17.90	69.40	3.88	30.20	198.00	6.55	2.83	15.36	+
9451	79	10/03/91	ABN	0.563	0.00	33.50	0.00	0.00	70.40	0.00	0.00	283.00	0.00	0.00	0.00	-
9452	79	04/23/91	4YL	1.121	14.90	57.40	3.85	23.00	82.70	3.60	0.00	293.00	0.00	2.49	11.71	+
9452	79	10/03/91	ABN	0.563	0.00	56.10	0.00	0.00	83.50	0.00	0.00	313.00	0.00	0.00	0.00	-
9455	79	04/23/91	4YM	1.232	150.00	304.00	2.03	282.00 >	320.00 >	1.13	0.00 >	320.00	0.00	1.08 >	6.42	+
9455	79	10/03/91	ABO	0.568	0.00	496.00	0.00	0.00	696.00	0.00	0.00 >	1000.00	0.00	0.00	0.00	-
9470	79	04/23/91	4YP	1.097	170.00 >	320.00 >	1.89	288.00 >	320.00 >	1.11	0.00 >	320.00	0.00 >	1.11 >	5.55	+
9470	79	10/03/91	ABQ	0.542	0.00	550.00	0.00	0.00	779.00	0.00	0.00 >	1000.00	0.00	0.00	0.00	-
9475	79	04/25/91	50P	1.342	4.73	18.50	3.91	7.39	39.70	5.37	0.00	97.40	0.00	2.51	10.51	+
9475	79	10/03/91	ABQ	0.542	0.00	14.30	0.00	0.00	25.10	0.00	0.00	91.80	0.00	0.00	0.00	-
9487	80	07/09/91	6ZI	1.210	7.59	13.10	1.73	28.30	48.70	1.72	0.00	320.00	0.00	0.46	3.19	+
9487	80	10/10/91	AFN	0.536	0.00	43.30	0.00	0.00	137.00	0.00	0.00	302.00	0.00	0.00	0.00	-
9529	79	04/25/91	50T	1.107	1.34	23.20	17.27	2.07	52.10	25.14	0.00	95.20	0.00	11.19 >	27.65	+
9529	79	10/03/91	ABR	0.597	0.00	2.74	0.00	0.00	6.37	0.00	0.00	84.50	0.00	0.00	0.00	-

Table 21 (Cont'd)

AVS No.	Shipment	Test Date	Flt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9530	79	04/25/91	5DT	1.107	11.60	42.90	3.70	17.50	63.90	3.66	0.00 >	320.00	0.00	2.46	14.90	+
9530	79	10/03/91	ABS	0.603	0.00	49.90	0.00	0.00	67.80	0.00	0.00	100.00	0.00	0.00	0.00	-
9589	79	05/02/91	5BQ	1.396	0.00	12.80	0.00	0.00	19.20	0.00	0.00	30.70	0.00	0.00	0.00	-
9589	79	10/03/91	ABV	0.558	30.60	490.00	16.01	46.30	660.00	14.25	92.60	966.00	10.43	10.58 >	40.63	+
9590	79	05/02/91	5BR	1.229	0.00	55.60	0.00	0.00	90.30	0.00	0.00	294.00	0.00	0.00	0.11	-
9590	79	10/03/91	ABW	0.532	481.30	46.90	0.10	722.00	64.60	0.09	0.00	96.50	0.00	0.06 >	1.94	+
9603	79	05/07/91	5C0	1.306	4.35	12.80	2.94	9.40	19.20	2.04	0.00	30.70	0.00	1.36 >	7.61	+
9603	79	10/03/91	ABY	0.525	0.00	15.60	0.00	0.00	21.10	0.00	0.00	31.10	0.00	0.00	0.00	-
9607	79	05/07/91	5C2	1.024	4.54	17.30	3.81	9.43	25.00	2.65	0.00	87.40	0.00	1.84 >	10.69	+
9607	79	10/03/91	AB2	0.561	0.00	12.60	0.00	0.00	21.70	0.00	0.00	87.00	0.00	0.00	0.00	-
9610	79	05/07/91	5C3	1.210 <	1.00	19.20 >	19.17	1.88	28.30	15.11	0.00	91.50	0.00	10.22 >	30.75	+
9610	79	10/03/91	AC0	0.610	0.00	13.50	0.00	0.00	23.10	0.00	0.00	87.40	0.00	0.00	0.01	-
9649	79	05/14/91	5IF	1.135	55.90	71.50	1.28	97.80	127.00	1.30	0.00	301.00	0.00	0.73	0.85	+
9649	79	10/02/91	ABL	0.624	0.00	66.70	0.00	0.00	104.00	0.00	0.00	302.00	0.00	0.00	0.00	-
9659	79	05/14/91	5IK	1.153	4.94	12.40	2.50	7.76	18.90	2.44	0.00	30.70	0.00	1.59	6.51	+
9659	79	10/02/91	ABN	0.778	0.00	8.35	0.00	0.00	15.60	0.00	0.00	30.40	0.00	0.00 >	3.79	-
9669	79	05/14/91	5IP	1.137	49.30	31.20	0.63	80.60	200.00	2.48	0.00 >	320.00	0.00	0.39 >	2.87	+
9669	79	10/02/91	ABN	0.594	0.00	14.20	0.00	0.00	29.00	0.00	0.00	894.00	0.00	0.00	0.00	-
9678	79	05/16/91	5KK	1.181	1.60	4.93	3.09	2.66	6.67	2.50	0.00	9.79	0.00	1.85 >	8.34	+
9678	79	10/02/91	ABO	0.682	2.77	4.95	1.79	0.00	6.71	0.00	0.00	9.86	0.00	0.00 >	5.74	+
9680	79	05/16/91	5KL	1.171 <	1.00 <	1.00 -	1.00 <	1.00	1.40 >	1.40	0.00	3.02	0.00 -	1.00 >	0.28	+
9680	79	10/02/91	ABP	0.674	0.00	1.55	0.00	0.00	2.10	0.00	0.00	3.09	0.00	0.00	1.35	-
9696	79	05/16/91	5KT	1.257	17.00	46.50	2.74	28.00	64.60	2.24	0.00	97.10	0.00	1.61 >	7.30	+
9696	79	10/02/91	ABQ	0.615	16.80	49.20	2.93	0.00	66.30	0.00	0.00	97.30	0.00	0.00 >	12.01	+
9745	79	05/23/91	5TC	1.034	4.61	15.60	3.53	7.26	21.10	2.91	0.00	31.10	0.00	2.14 >	11.58	+
9745	79	10/02/91	ABV	0.561	0.00	8.20	0.00	0.00	16.90	0.00	0.00	80.60	0.00	0.00 >	0.21	-
9781	79	05/30/91	5Y3	1.046	0.00	5.40	0.00	0.00	8.01	0.00	0.00	28.50	0.00	0.00	0.27	-
9781	79	10/03/91	AAE	0.536 <	1.00	7.92 >	7.92 <	1.00	14.80 >	14.81	0.00	30.30	0.00 >	7.92 >	6.69	+
9791	79	05/30/91	5Y8	1.089	15.30	41.40	2.70	23.50	60.90	2.59	0.00	96.10	0.00	1.76	7.72	+
9791	79	10/03/91	AAE	0.536 <	1.00	44.80 >	44.84 <	1.00	63.70 >	63.73	0.00	97.70	0.00 >	44.84 >	7.37	+
9797	79	05/30/91	5Y8	0.964	51.40	96.20	1.87	82.70	121.00	2.07	0.00	305.00	0.00	1.16	3.59	+
9797	79	10/03/91	AAG	0.451	0.00	29.50	0.00	0.00	52.80	0.00	0.00	95.30	0.00	0.00	0.00	-
9802	79	06/04/91	62H	1.116	4.88	15.70	3.22	8.27	21.50	2.59	0.00	31.80	0.0	1.90 >	9.19	+
9802	79	10/03/91	AAG	0.451	0.00	15.70	0.00	0.00	21.50	0.00	0.00	31.80	0.00	0.00 >	3.90	-

Table 21 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9806	79	06/04/91	620	1.305	3.29	13.50	4.09	6.84	19.60	2.87	0.00	30.80	0.00	1.97 >	11.02	+
9806	79	10/03/91	AAH	0.529	0.00	15.20	0.00	0.00	20.80	0.00	0.00	30.90	0.00	0.00	0.00	-
9809	79	06/04/91	629	1.090 <	1.00	2.86 >	2.86	2.30	20.40	8.89	0.00	31.10	0.00	1.25 >	14.54	+
9809	79	10/03/91	AAI	0.355	0.00	3.44	0.00	0.00	6.42	0.00	0.00	26.50	0.00	0.00	0.00	-
9814	79	06/04/91	62S	1.076	15.80	32.00	2.03	26.50	54.70	2.06	0.00	95.50	0.00	1.21 >	5.28	+
9814	79	10/03/91	AAI	0.355	0.00	14.80	0.00	0.00	20.50	0.00	0.00	30.90	0.00	0.00	0.00	-
9825	79	06/05/91	640	1.133	16.80	32.00	1.90	28.30	54.70	1.94	0.00	95.50	0.00	1.13	3.49	+
9825	79	10/03/91	AAK	0.293	0.00	15.50	0.00	0.00	21.00	0.00	0.00	30.90	0.00	0.00	0.00	-
9842	79	06/05/91	64X	1.192	17.90	163.00	9.08	36.20	225.00	6.22	90.30 >	320.00 >	3.54	4.49	25.63	+
9842	79	[illegible]/03/91	AAN	0.316	77.90	156.00	2.00	0.00	212.00	0.00	0.00	313.00	0.00	0.00	3.97	+
9844	79	06/05/91	64Y	1.116	30.60	72.80	2.38	95.50	161.00	1.68	0.00	304.00	0.00	0.76	3.80	+
9844	79	10/03/91	AAN	0.331	0.00	67.50	0.00	0.00	112.00	0.00	0.00	299.00	0.00	0.00	0.00	-
9847	79	06/05/91	642	1.142	3.20	13.70	4.27	7.06	43.30	6.14	0.00	94.30	0.00	1.94 >	13.49	+
9847	79	10/03/91	AAN	0.331	0.00	30.00	0.00	0.00	54.10	0.00	0.00	95.40	0.00	0.00	0.00	-
9856	79	06/06/91	66U	1.267	0.00	4.90	0.00	0.00	6.60	0.00	0.00	9.66	0.00	0.00 >	0.20	-
9856	79	10/03/91	AAO	0.387 <	0.10	4.90 >	49.00 <	0.10	6.60 >	66.00	0.00	9.66	0.00 >	49.00 >	6.86	+
9874	79	06/11/91	68D	0.944	4.43	22.00	4.95	8.73	37.00	4.24	0.00	93.70	0.00	2.51 >	14.08	+
9874	79	10/08/91	ADI	0.612	0.00	12.10	0.00	0.00	18.70	0.00	0.00	30.70	0.00	0.00	0.00	-
9883	79	06/11/91	68N	0.905 <	1.00	4.59 >	4.59	1.72	6.42	3.74	0.00	9.71	0.00	2.67 >	12.45	+
9883	79	10/08/91	ADI	0.612	0.00	5.47	0.00	0.00	12.80	0.00	0.00	30.40	0.00	0.00	0.00	-
9887	79	06/11/91	[illegible]	0.946	1.51	4.52	2.99	2.77	6.34	2.29	0.00	9.63	0.00	1.63 >	7.34	+
9887	79	10/08/91	ADJ	0.709	0.00	1.52	0.00	0.00	2.14	0.00	0.00	5.47	0.00	0.00	0.00	-
9899	79	06/12/91	6F[illegible]	1.217	5.07	41.40	[illegible]	8.02	88.70	11.05	0.00	[illegible]5.00	0.00	5.17	17.33	+
9899	79	10/08/91	ADL	0.558	6.42	29.10	[illegible].53	0.00	53.40	0.00	0.00	95.30	0.00	0.00	4.16	+
9900	79	06/12/91	6FB	1.217 <	1.00	1.24 >	1.24 <	1.00	1.89 >	1.89	0.00	3.07	0.00 >	1.24 >	2.39	+
9900	79	10/08/91	ADL	0.558	0.00	0.26	0.00	0.00	0.53	0.00	0.00	0.95	0.00	0.00	0.00	-
9916	79	06/12/91	6FF	1.381	346.00	1900.0	5.48	511.00	2910.00	5.70	0.00 >	3200.00	0.00	3.71	18.49	+
9916	79/C2	08/15/91	BD9	0.669	470.00	2340.0	4.98	691.00 >	3200.00 >	4.63	0.00 >	3200.00	0.00	3.39 >	15.78	+
9931	79	06/13/91	6II	1.257	113.00	362.00	3.21	307.00	709.00	2.30	0.00	2820.00	0.00	1.18	6.61	+
9931	79/C2	08/15/91	BD8	0.738	0.00	559.00	0.00	0.00	1070.00	0.00	0.00 >	3200.00	0.00	0.00	0.74	-
9937	79	06/13/91	6IL	1.250	224.00	1340.0	5.99	498.00	2010.00	4.03	0.00	3200.00	0.00	2.70	15.45	+
9937	79/C2	08/15/91	BD8	0.738	0.00	1290.0	0.00	0.00	2010.00	0.00	0.00 >	3200.00	0.00	0.00	0.00	-
9962	79	06/18/91	6KO	0.913	179.00	1300.0	7.29	320.00	1940.00	6.05	0.00	3070.00	0.00	4.07	16.68	+
9962	79/C2	08/15/91	BDO	0.750	499.00	969.00	1.94	779.00	1710.00	2.20	0.00	3050.00	0.00	1.24	4.45	+

Table 21 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9969	79	[illegible]	[illegible]	0.945	109.00	303.00	2.79	217.00	1060.00	4.90	0.00	> 3200.00	0.00	1.40	9.48	+
9969	79/C2	08/15/91	8DF	0.683	0.00	456.00	0.00	0.00	834.00	0.00	0.00	2920.00	0.00	0.00	0.71	-
9970	79	06/18/91	6KS	0.738	45.50	538.00	11.83	64.70	756.00	11.69	236.00	2700.00	11.46	8.32	35.57	+
9970	79/C2	08/15/91	8DF	0.683	425.00	835.00	1.96	566.00	1560.00	2.75	945.00	3040.00	3.21	1.48	2.33	+
9971	79	06/18/91	6KS	0.738	140.00	525.00	3.76	195.00	730.00	3.74	0.00	> 1000.00	0.00	2.69	14.29	+
9971	79/C2	08/15/91	8DG	0.815	0.00	477.00	0.00	0.00	664.00	0.00	0.00	1000.00	0.00	0.00	> 0.10	-
9974	79	06/18/91	6KU	0.823	46.80	141.00	3.02	68.40	214.00	3.13	0.00	757.00	0.00	2.06	9.11	+
9974	79/C2	08/15/91	8DG	0.815	0.00	210.00	0.00	0.00	405.00	0.00	0.00	951.00	0.00	0.00	0.00	-
9990	79	06/19/91	6N4	1.155	39.00	500.00	12.80	61.40	820.00	13.37	0.00	> 1000.00	0.00	8.14	26.79	+
9990	79/C2	08/15/91	8DH	0.463	177.00	653.00	3.69	313.00	987.00	3.15	0.00	> 1000.00	0.00	2.09	8.09	+
9992	79	06/19/91	6N5	1.244	262.00	815.00	3.11	603.00	1630.00	2.71	0.00	3130.00	0.00	1.35	8.40	+
9992	79/C2	08/15/91	8DH	0.463	237.00	1650.0	6.98	0.00	2400.00	0.00	0.00	> 3200.00	0.00	0.00	6.12	+

DIFFNTL = The differential is the difference in the cell control and the virus control optical densities.

$IC_{25,50,95}$ = (Viral) inhibitory concentration 25%, 50% and 95% = The drug concentration (µg/ml) that inhibited viral CPE by 25%, 50% or 95% calculated by using a regression analysis for semilog curve fitting.

$TC_{25,50,95}$ = Cell) toxicity concentration 25%, 50% and 95% = The drug concentration (µg/ml) that reduced cell viability by 25%, 50% or 95%.

$AI_{25,50,95}$ = Antiviral Index = A single point ratio of the antiviral and anticellular effect of the compound, calculated with 25%, 50% or 95% reduction values (calculated by dividing the $TC_{25,50,95}$ by the $IC_{25,50,95}$).

SI = Selectivity Index = A ratio calculated by dividing the TC_{25} by the IC_{50} (based upon 6 one-half-log_{10} dilutions, µg/ml, the maximum scale is 0-320).

TAI = Total Antiviral Index = The area between the cytotoxicity and the antiviral curves (based upon a scale of 0-100%).

ACT = Activity = A "+" denotes a test that produced ≥25% reduction in CPE. A "-" denotes an inactive test (i.e. ≤25% reduction in CPE).

4.3.6 Sandfly Fever Virus (SF):

The total output of SF testing during this reporting period is summarized in monthly increments in **Figure 58**. During this period, 3970 tests were performed against the SF virus with the MTT assay format. Of these, 194 were control compound assays with Ribavirin (AVS-0001) and 95 were control compound assays with 2-Thio-6-Azauridine (AVS-6724). Two hundred thirteen tests were internal (+++) virus load and cell load quality control tests. The 155 tests were considered unsatisfactory based on the criteria of the quality control set during this reporting period. The rest, totaling 3313, were actual single drug assays. The total number of assays represents approximately 66% above our yearly contractual obligation (i.e., 3313/2000).

Out of the 3313 test compounds, 230 demonstrated antiviral activity at greater than 50% reduction levels. This represents around 7% of the tested compounds having *in vitro* antiviral activity against the SF virus. The remainder, 3083 compounds (93%), are to be considered inactive with this assay protocol.

TOTAL NUMBER OF TESTS AGAINST SANDFLY FEVER VIRUS

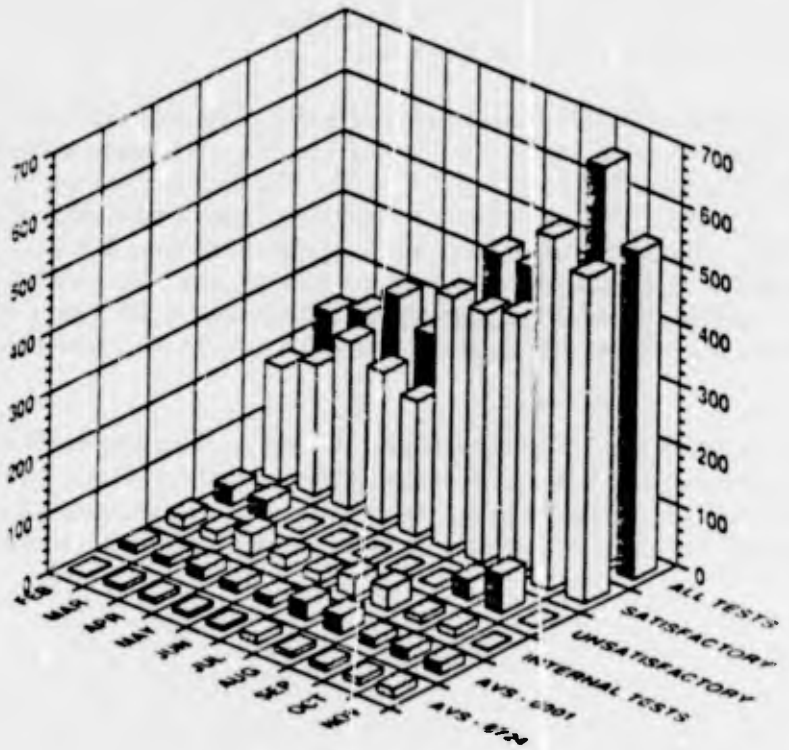


Figure 58

Quality Control Tests =	KPC (Positive Control - 2-Thio-6-Azauridine) =	95
	KPC (Positive Control - Ribavirin) =	194
	(++++) (Internal Virus and Cell Load Controls) =	213
Accepted Single Drug Tests =	(UT) Unsatisfactory Test (QC rejects) =	155
		3313
Total Number of SF Tests =		3970

4.3.6.1 SF-Quality Controls:

4.3.6.1.1 Antiviral Activity of Ribavirin vs SF Virus:

Control Compound-Antiviral Performance: Ribavirin (AVS-0001) has been the primary control compound against SF in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of the positive control drug (Ribavirin) are illustrated in Figure 59-A.

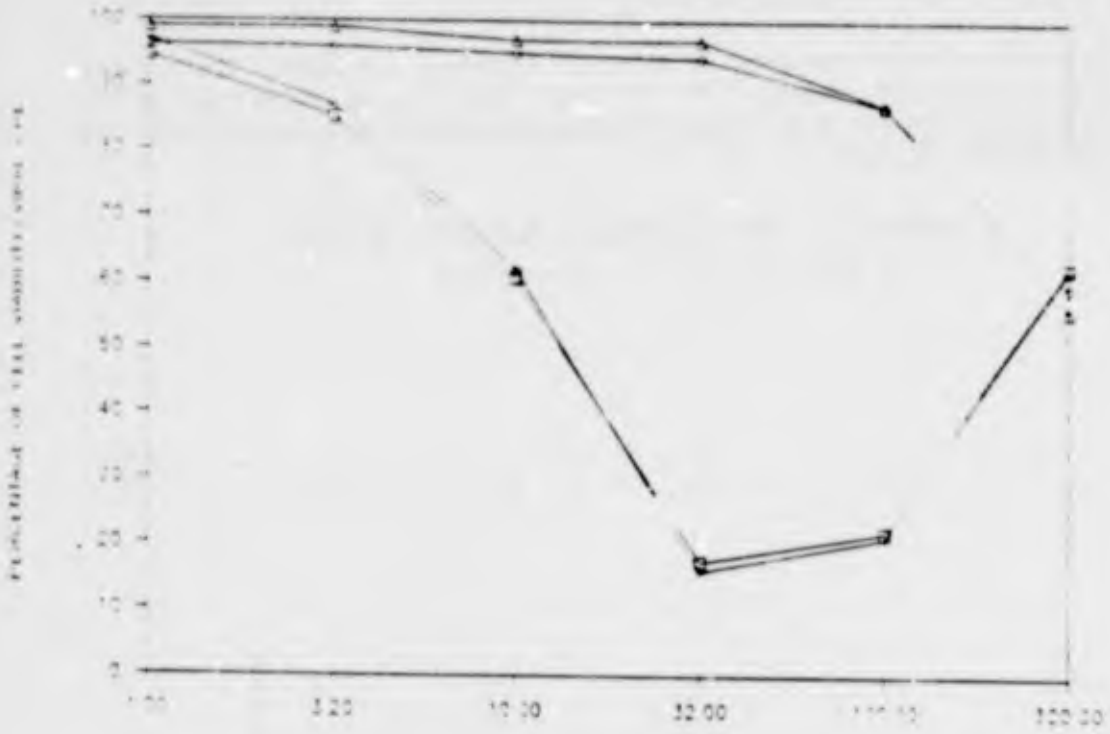
The 194 control tests performed with Ribavirin gave a mean **Total Antiviral Index** (TAI) of 38% (SD ± 13.7) and the median value was 37%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from 7.13 - >82.5% during this period. The mean **Selectivity Index (SI)** was 20.1 (SD ± 20.76) and the median SI value was 14.23, indicating moderate antiviral selectivity for Ribavirin against SF virus. The SI ranged from 0.07 - >160.9 during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25%** (AI_{25}) value was 51.45 (SD ± 62.36). The median AI_{25} value was 33.68 (range 0.192 - >474.53). The mean **Antiviral Index 50%** (AI_{50}) was 28.55 (SD ± 19.45) with a median of 22.79 (range 4.14 - >160.94). The mean **Antiviral Index 95%** (AI_{95}) was 2.98 (SD ± 4.96), with a median of 0 (range 0 - >22.95). This indicates that the control compound, Ribavirin, does not consistently reach 95% antiviral reduction levels.

The mean **Antiviral Inhibitory Concentration 25%** (IC_{25}) was 6.61 μg/ml (SD ± 3.7). The median IC_{25} value was 5.66 μg/ml (range = 0.674 - 18.7 μg/ml). The mean **Antiviral Inhibitory Concentration 50%** (IC_{50}) was 14.26 μg/ml (SD ± 7.96). The median IC_{50} value was 13.7 μg/ml (range = 1.99 - 66.10 μg/ml). The mean **Antiviral Inhibitory Concentration 95%** (IC_{95}) was 12.2 μg/ml (SD ± 24.69). The median IC_{95} value was 0 μg/ml (range from 0 - 199 μg/ml). This discrepancy indicates that the control compound Ribavirin does not consistently reach 95% reduction levels. During this reporting period, the highest starting concentration of Ribavirin (100 μg/ml) was increased from high dose of 100 to 320 μg/ml to properly evaluate the maximum antiviral effect of Ribavirin.

The average maximum antiviral inhibitory level of 194 Ribavirin tests (**Figure 59-A**) was reached at 32 μg/ml of the compound with 85% antiviral effect. Maximum antiviral effect (~85%) was found with a simultaneous ~5% cytotoxic suppression. Above (100 μg/ml) concentration Ribavirin starts to lose its antiviral potency (~40%) at 320 μg/ml while simultaneously the Ribavirin becomes maximally toxic (~40%).

RIBAVIRIN -VS- SF VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability ΔMedian % Cell Viability

	% Viral CPE						% Cell Viability					
Conc.(μg/ml)	1	3.2	10	32	100	320	1	3.2	10	32	100	320
Mean	95	85	61	17	22	62	96	96	94	94	87	59
Median	97	87	62	16	21	62	99	99	97	97	87	56
Std. Dev.	0.07	0.11	0.18	0.14	0.15	0.17	0.05	0.05	0.06	0.07	0.10	0.14

Figure 59-A
Average Antiviral and Cytotoxicity Values for 194 Positive Control Compound Tests

4.3.6.1.2 Maximum Antiviral Effect of Ribavirin vs SF Virus:

A bar graph scatter plot (**Figure 60-A**) depicts the distribution of the maximum antiviral reduction values of all 194 control compound assays for Ribavirin. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 85% (SD ± 12.5) reduction levels. The maximum reduction levels vary from 54 - 100% but remain quite consistently around the median of 87%. The assay control values give a shifted half-bell-shaped distribution curve toward the maximum 100% reduction level. This indicates quite a consistent day-to-day performance of the control compound in the SF-MTT assay.

During this period the positive control compound performance criteria for Ribavirin versus the SF virus was set at 50% reduction level. All assays in which Ribavirin did not meet this accepted quality control level (≥50%) were rejected (i.e., 155 unsatisfactory tests).

Ribavirin is active *in vitro* against SF virus and functions as a reasonable quality control compound. On the other hand, regardless of the performance of the SF-quality control drug Ribavirin, around 230 other compounds have equal or better antiviral activity against SF virus than AVS-0001. (See 95% and 50% reduction summaries).

Variation of the Maximum Antiviral Effect SF Virus - VS - Ribavirin

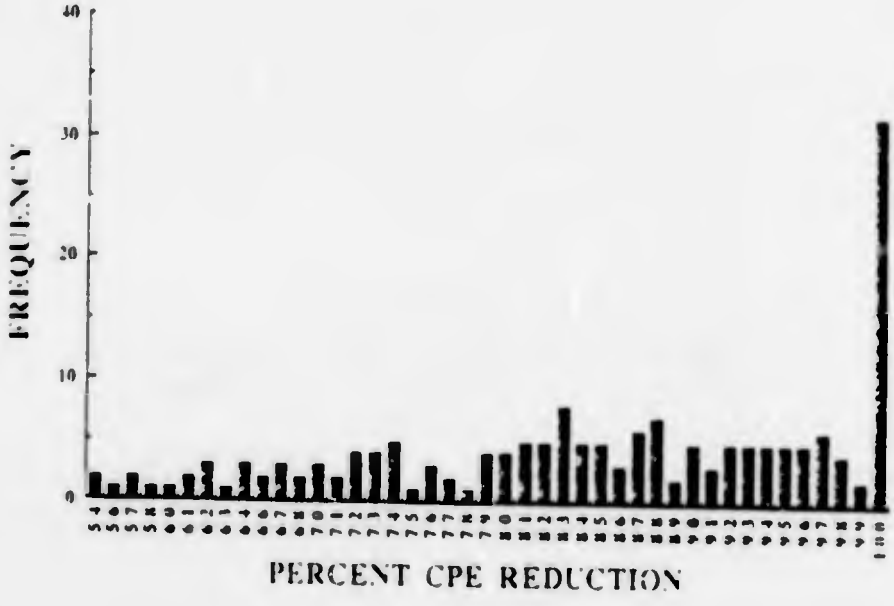


Figure 60-A
Maximum Antiviral CPE Reduction (%).
Summary of 194 Control Tests.

4.3.6.1.3 Cellular Cytotoxicity of Ribavirin vs SF Virus:

SF-Control Compound-Cytotoxicity Performance: The 194 cytotoxicity values of the positive control compound Ribavirin are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 193.64 μg/ml (SD ± 84.57) and the median was 193 μg/ml (range of 0.96 - > 320 μg/ml). The mean cell **Toxic Concentration 50%** (TC_{50}) value was 309.61 μg/ml (SD ± 26.97) and the median was 320 μg/ml (range of 96 - > 320 μg/ml). The mean cell **Toxic Concentration 95%** (TC_{95}) value was 318.77 (SD ± 16.44) and the median was 320 μg/ml (range of > 100 - > 320 μg/ml). This discrepancy indicates that the control compound Ribavirin does not consistently reach 95% cytotoxicity levels at the highest concentration tested (i.e. 320 μg/ml).

As can be seen from **Figure 59-A**, the toxicity starts to become measurable above the concentration of 32 μg/ml and the maximum toxicity has not been reached at 320 μg/ml. Further increase of the concentration of Ribavirin would be needed to properly evaluate the maximum cytotoxicity of Ribavirin.

Also, **Figure 59-A** indicates that when the cytotoxicity reaches ~ 10% at 100 μg/ml, the control compound (Ribavirin) has reached simultaneously its maximum antiviral effect (80%). The cytotoxic effect of Ribavirin is insignificant between 1 and 100 μg/ml. The average cytotoxicity reached ~ 40% at 320 μg/ml, which is the highest Ribavirin concentration tested.

4.3.6.1.4 SF-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (Ribavirin):

SF-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 194 control assays is plotted in **Figure 61-A**. The results indicate that the cell O.D. readings reached a mean of 1.146 (SD ± 0.136) with a median of 1.144 (range of 0.76 - 1.566). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

SF-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 194 control assays is presented in **Figure 62-A**. The results indicate that the average virus load O.D. reading reached a mean of 0.305 (SD ± 0.182 with a median of 0.234 (range of 0.018 -0.748). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

SF-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 194 control assays is provided in **Figure 63-A**. The results indicate that the average differential O.D. reading is 0.841 (SD ± 0.201) with a median of 0.846 (range 0.412 - 1.368). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 85% measurement accuracy.

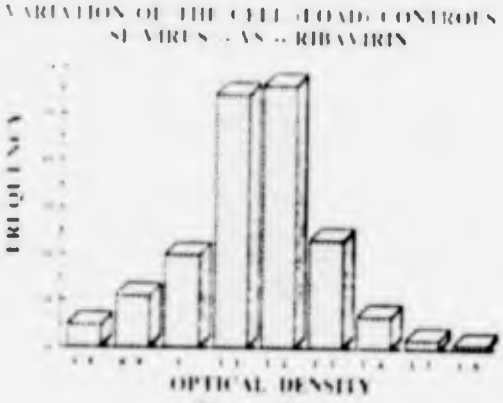


Figure 61-A

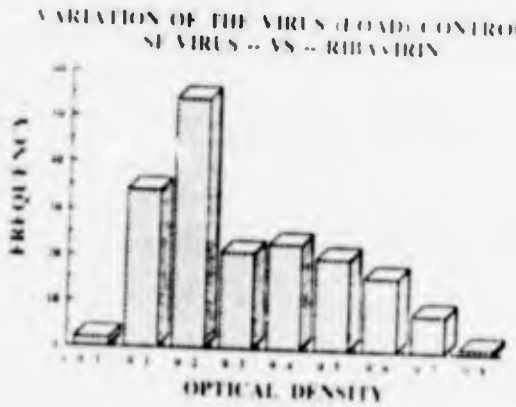


Figure 62-A

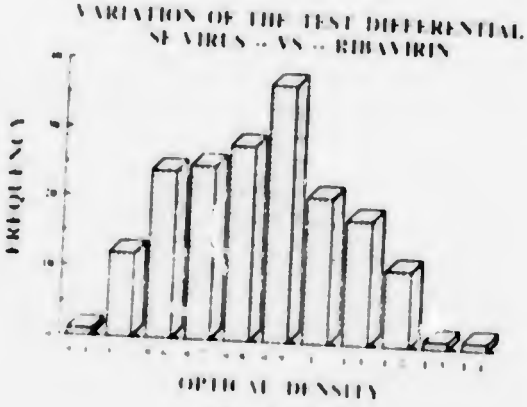


Figure 63-A

4.3.6.1.1 Antiviral Activity of AVS-6724 (2-Thio-6-Azauridine) vs SF Virus:

Control Compound-Antiviral Performance: 2-Thio-6-Azauridine (AVS-6724) has been tested as a second control compound against SF in these MTT-assay screens. The mean and median antiviral inhibition and cytotoxicity patterns of this second positive control drug are illustrated in **Figure 59-B**.

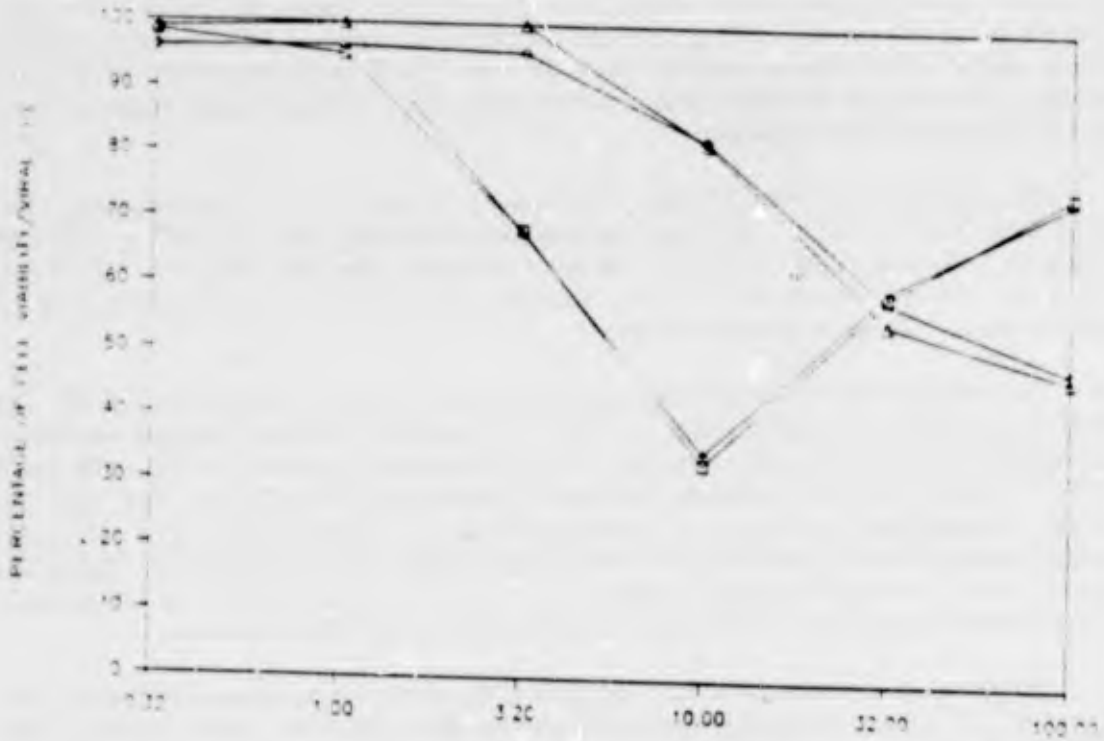
The 95 control tests performed with 2-Thio-6-Azauridine gave a mean **Total Antiviral Index** (TAI) of 17% (SD ± 11.48) and the median value was 14%. The TAI measures the overall antiviral effectiveness of the compound and it ranged from 2.4 - >51.8% during this period. The mean **Selectivity Index (SI)** was 5.76 (SD ± 9.59) and the median SI value was 2.57, indicating moderate antiviral selectivity for 2-Thio-6-Azauridine against SF virus. The SI ranged from 0.03 - 75.63 during this period. However, the closeness of the mean and median values indicate that the present execution of the SOP is consistent and repeatable.

The mean **Antiviral Index 25% (AI_{25})** value was 11.53 (SD ± 21.15). The median AI_{25} value was 5.4 (range 0.057 - 174.21). The mean **Antiviral Index 50% (AI_{50})** was 17.83 (SD ± 16.2) with a median of 12.21 (range 2.04 - >77.94). The mean **Antiviral Index 95% (AI_{95})** was 1.21 (SD ± 4.88), with a median of 0 (range 0 - >33.46). This indicates that the, 2-Thio-6-Azauridine, does not consistently reach 95% antiviral reduction levels.

The mean **Antiviral Inhibitory Concentration 25% (IC_{25})** was 3.01 μg/ml (SD ± 1.58). The median IC_{25} value was 2.41 μg/ml (range = <0.32 - 5.66 μg/ml). The mean **Antiviral Inhibitory Concentration 50% (IC_{50})** was 5.57 μg/ml (SD ± 2.65). The median IC_{50} value was 5.33 μg/ml (range = 1.28 - 10 μg/ml). The mean **Antiviral Inhibitory Concentration 95% (IC_{95})** was 1.21 μg/ml (SD ± 4.88). The median IC_{95} value was 0 μg/ml (range from 0 - >33.46 μg/ml). This discrepancy indicates that the 2-Thio-6-Azauridine does not consistently reach 95% reduction levels. During this reporting period, the highest starting concentration of 2-Thio-6-Azauridine was set to 100 μg/ml to properly evaluate the maximum antiviral and cytotoxicity effects of 2-Thio-6-Azauridine.

The average maximum antiviral inhibitory level of 95, 2-Thio-6-Azauridine, tests (**Figure 59-B**) was reached at 10 μg/ml of the compound with 70% antiviral effect. Maximum antiviral effect (~70%) was found with a simultaneous ~20% cytotoxic suppression. Above 10 μg/ml concentration, 2-Thio-6-Azauridine starts to lose its antiviral potency (~30%) at 100 μg/ml, while simultaneously the 2-Thio-6-Azauridine becomes maximally toxic (~50%) with increasing cytotoxicity.

2-THIO-6-AZAURIDINE - VS- SF VIRUS



CONCENTRATION (μg/ml)

□ Mean % Viral CPE + Median % Viral CPE ◊ Mean % Cell Viability ΔMedian % Cell Viability

% Viral CPE % Cell Viability

Conc.(μg/ml)	0.32	1	3.2	10	32	100	0.32	1	3.2	10	32	100
Mean	99	95	68	33	59	75	96	97	96	82	58	48
Median	100	100	68	35	60	74	99	100	100	82	55	47
Std. Dev.	0.04	0.09	0.26	0.16	0.14	0.12	0.06	0.05	0.07	0.14	0.12	0.09

Figure 59-B
Average Antiviral and Cytotoxicity Values for 95 Positive Control Compound Tests

4.2.6.1.2 Maximum Antiviral Effect of 2-Thio-6-Azauridine vs SF Virus:

A bar graph scatter plot (**Figure 60-B**) depicts the distribution of the maximum antiviral reduction values of all 95 control compound assays for 2-Thio-6-Azau..Jine. The results indicate that the average maximum antiviral reduction obtained with the present SOP is around 69% (SD ± 14.34) reduction levels. The maximum reduction levels vary from 50 - 100% but remain quite consistently around the median of 65%. The assay control values give a double bell-shaped distribution curve toward the maximum reduction level. This indicates quite a consistent day-to-day performance of the control compound in the SF-MTT assay.

Recommendations:

Based upon the data obtained in parallel studies with Ribavirin, we recommend that 2-Thio-6-Azauridine (AVS #6724) will be used as a second control compound against SF virus. It's overall performance is slightly inferior to the present control, Ribavirin. However, it is readily available from Sigma Chemical Company, it is inexpensive and works at ~5 - 10-fold lower concentrations than Ribavirin.

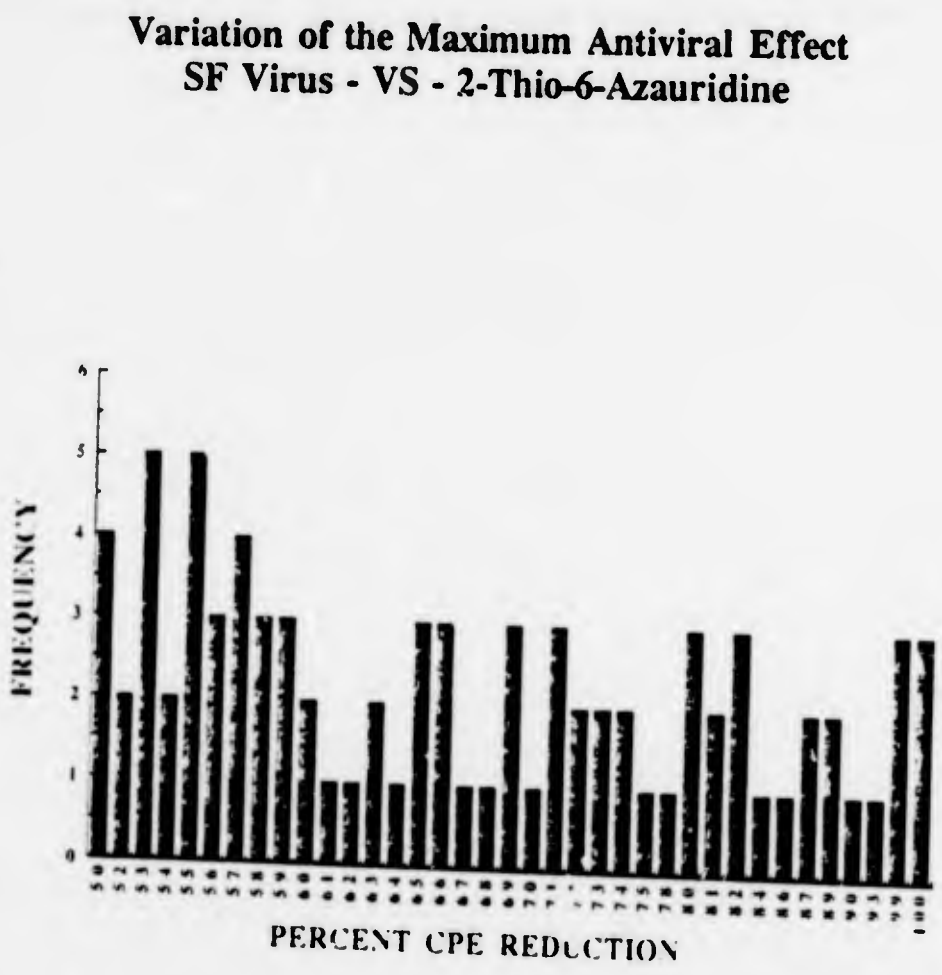


Figure 60-B
Maximum Antiviral CPE Reduction (%).
Summary of 95 Control Tests.

4.3.6.1.3 Cellular Cytotoxicity of 2-Thio-6-Azauridine vs SF Virus:

SF-Control Compound-Cytotoxicity Performance: The 95 cytotoxicity values of the positive control compound 2-Thio-6-Azauridine are also very consistent. The mean cell **Toxic Concentration 25%** (TC_{25}) was 19.4 μg/ml (SD ± 15.8) and the median was 16.1 μg/ml (range of 0.267 - 97 μg/ml). The mean cell **Toxic Concentration 50%** (TC_{50}) value was 67.13 μg/ml (SD ± 32.81) and the median was 70.9 μg/ml (range of 7.5 - > 100 μg/ml). The mean cell **Toxic Concentration 95%** (TC_{95}) value was 100 (SD ± 0) and the median was 100 μg/ml. This indicates that the control compound 2-Thio-6-Azauridine does not consistently reach 95% cytotoxicity levels at the highest concentration tested (100 μg/ml).

As can be seen from **Figure 59-B**, the toxicity starts to become measurable above the concentration of 3.2 μg/ml and the maximum toxicity has not been reached at 100 μg/ml. Further increase of the concentration of 2-Thio-6-Azauridine would be needed to properly evaluate the maximum cytotoxicity of 2-Thio-6-Azauridine.

Also, **Figure 59-B** indicates that when the cytotoxicity reaches ~20% at 10 μg/ml, the control compound (2-Thio-6-Azauridine) has reached simultaneously its maximum antiviral effect (70%). The cytotoxic effect of 2-Thio-6-Azauridine is insignificant between 1 and 3.2 μg/ml. The average maximum cytotoxicity reached ~50% at 100 μg/ml, which is the hignest 2-Thio-6-Azauridine concentration tested.

4.3.6.1.4 SF-Assay Plate Quality Controls: Cell Load and Virus Load Parameters 2-Thio-6-Azauridine:

SF-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 95 control assays is plotted in **Figure 61-B**. The results indicate that the cell O.D. readings reached a mean of 1.102 (SD ± 0.122) with a median of 1.14 (range of 0.708 - 1.339). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

SF-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of the 95 control assays is presented in **Figure 62-B**. The results indicate that the average virus load O.D. reading reached a mean of 0.292 (SD ± 0.169 with a median of 0.237 (range of 0.032 - 0.672). This demonstrates that a good cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with very consistent viral CPE results.

SF-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of the 95 control assays is provided in **Figure 63-B**. The results indicate that the average differential O.D. reading is 0.810 (SD ± 0.183) with a median of 0.772 (range 0.461 - 1.171). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 81% measurement accuracy.

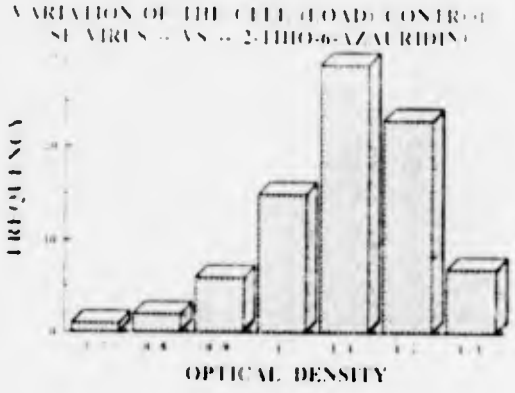


Figure 61-B

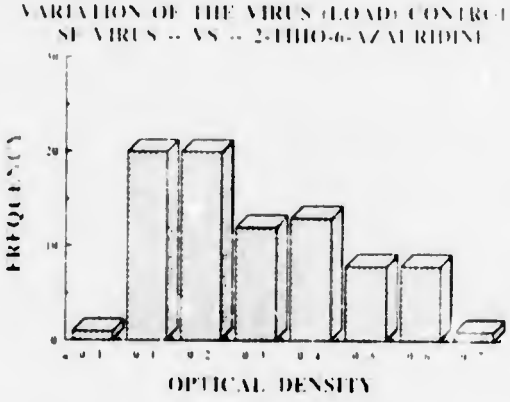


Figure 62-B

Figure 63-B

4.3.6.1.5 Overall SF-Assay Plate Quality Controls:

SF-Overall-Cell Load Performance: A bar graph scatter plot of the overall mean cell control (O.D. reading) of 3313 accepted assays is plotted in **Figure 61-C**. The results indicate that the overall cell O.D. readings reached a mean 1.116 (SD ± 0.135) with a median of 1.111 (range of 0.693 - 1.570). This indicates that a uniform and equal number (18,000 cells/well) of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

SF-Overall-Virus Load Performance: A bar graph scatter plot of the overall mean virus load O.D. readings of the 3313 accepted assays is presented in **Figure 62-C**. The results indicate that the overall average virus load O.D. reading is 0.311 (SD ± 0.169) with a median of 0.280 (range of .02 - 0.84). This demonstrates that a reasonable cell destruction is taking place and a uniform load of virus (32 $TCID_{50}$) is administered on the cell monolayer with consistent viral CPE results.

SF-Overall-Assay Differential Performance: A bar graph scatter plot of the overall mean O.D. differential values of the 3313 accepted assays is provided in **Figure 63-C**. The results indicate that the overall average differential O.D. reading is 0.805 (SD ± 0.208) with a median of 0.783 (range 0.301 - 1.368). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation with close to 80% measurement accuracy.

GRAPHIC ILLUSTRATION OF THE OVERALL PLATE VARIATIONS OBSERVED WITH ALL ACCEPTED SF PLATE DATA

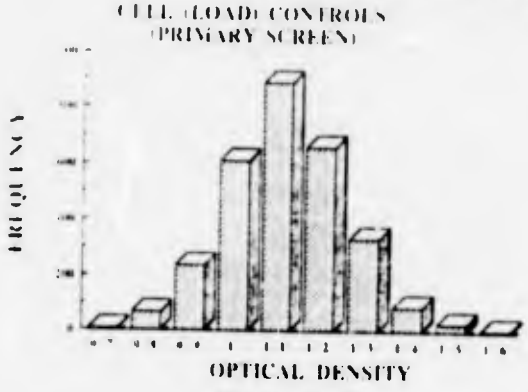


Figure 61-C

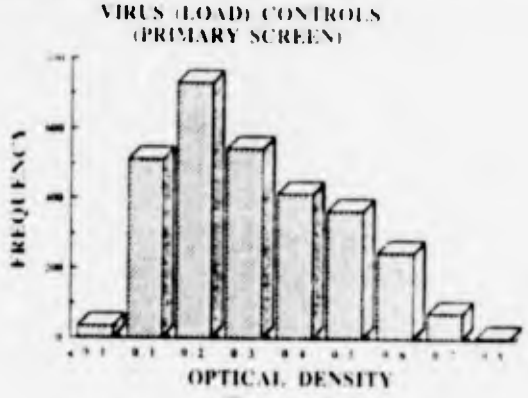


Figure 62-C

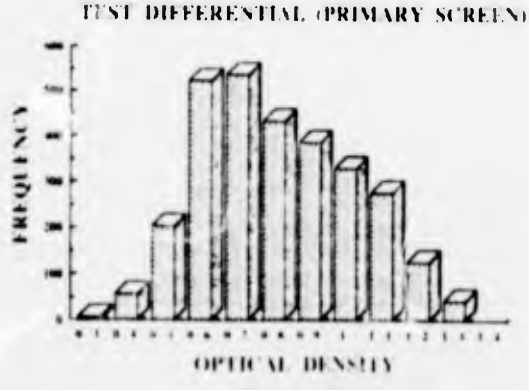


Figure 63-C

4.3.6.2 **SF-Antiviral Activity Results:**

New Drugs with 95% Antiviral Reduction Levels. Out of the 3313 actual single drug tests, 38 new compounds demonstrated excellent antiviral activity, having antiviral reduction values of equal to or better than 95%. This represents around 1.1% of the test compounds being active at this excellent reduction level. These compounds are summarized in Table 22 according to the highest Total Antiviral Index (TAI). Compound AVS-11830, 9248, 10260, 9859 and 10543 demonstrated the greatest *in vitro* promise, having TAI's that ranged from 52 - 59% and Selectivity Index (SI) values that ranged from 23 - > 320, respectively. The other 33 compounds demonstrated moderate antiviral activity with TAI's that ranged from 5% to 44% and SI values that ranged from 0.09 - 12.

Table 22
AVS Compounds Active Against Sandfly (SF) at AI_{95} Level

AVS No.	Shipment#	Test Date	Differntl.	IC 95	TC 95	AI 95	SI	TAI
SF 11830	82	08/27/91	0.729	270.00 >	3200.00 >	11.87	26.52 >	59.31
SF 9248	77	10/10/91	0.627	246.00	9520.00	38.76	49.20 >	57.32
SF 10260	82	09/19/91	0.543	286.00 >	320.00 >	1.12 >	320.00 >	52.95
SF 9859	79	10/08/91	0.691	15.70 >	32.00 >	2.04 >	22.77 >	52.51
SF 10543	83	10/24/91	0.863	26.80 >	320.00 >	11.93	27.89	51.84
SF 11829	82	08/27/91	0.729	781.00 >	3200.00 >	4.10	10.48 >	43.67
SF 9857	79	10/03/91	0.531	0.95	9.66	10.18	11.37 >	42.88
SF 9860	79	10/08/91	0.691	21.10 >	32.00 >	1.51 >	7.15 >	42.63
SF 9863	79	10/08/91	0.699	22.70 >	32.00 >	1.41 >	6.79 >	42.03
SF 11506	81	07/31/91	1.097	864.00	9520.00	11.02	11.58	37.79
SF 11827	82	08/27/91	0.764	82.70 >	1000.00 >	12.09	6.94 >	33.84
SF 9856	79	10/03/91	0.586	2.96	9.66	3.26	8.74	33.81
SF 9858	79	10/08/91	0.709	9.12	30.90	3.39	3.89 >	33.20
SF 10031	79	07/03/91	1.033	1000.00	3090.00	3.09	8.57 >	33.14
SF 9233	77	03/07/91	0.370	270.00 >	1000.00 >	3.71	10.12	31.97
SF 9842	79	06/05/91	0.681	83.70	311.00	3.72	7.33	31.91
SF 9414	81	10/08/91	0.731	896.00 >	1000.00 >	1.12 >	2.99 >	30.65
SF 9589	79	10/03/91	0.526	91.40	950.00	10.40	6.72 >	29.47
SF 10030	79	07/03/91	1.033	92.40	945.00	10.23	5.40 >	29.12
SF 9670	79	05/14/91	1.017	8.84	30.90	3.50	5.83 >	28.62
SF 9217	77	02/12/91	0.590	861.00	3150.00	3.66	6.13	27.17
SF 11104	80	07/09/91	1.172	863.00 >	1000.00 >	1.16 >	5.68 >	26.36
SF 11076	78	10/10/91	0.609	2830.00	9660.00	3.42	5.37 >	25.89
SF 10150	82	09/12/91	0.550	30.70	96.60	3.15	4.14	25.63
SF 9970	79	06/18/91	0.621	287.00	2280.00	7.96	4.80	25.21
SF 9916	79	06/12/91	0.712	846.00	3170.00	3.75	4.74	24.74
SF 11134	78	10/15/91	0.566	921.00	3090.00	3.36	3.54 >	24.10
SF 11463	81	07/30/91	1.033	299.00	2890.00	9.67	3.57 >	21.64
SF 9752	79	05/29/91	0.976	289.00 >	320.00 >	1.11 >	4.40 >	21.30
SF 9394	77	03/07/91	0.451	916.00	3180.00	3.47	3.78	20.81
SF 11451	81	07/30/91	0.907	940.00 >	3200.00 >	3.40	3.26	18.66
SF 9451	79	10/03/91	0.555	30.20	291.00	9.64	3.32	16.18
SF 10640	83	10/30/91	0.585	320.00 >	320.00 >	1.00 >	2.68 >	15.98
SF 9452	79	10/03/91	0.555	30.20	291.00	9.63	2.95	15.51
SF 10011	79/C2	08/13/91	0.918	965.00	3080.00	3.19	2.42 >	14.20
SF 9590	79	10/03/91	0.440	945.00	96.10	0.10	0.07 >	12.23
SF 10429	83	10/17/91	0.725	302.00 >	320.00 >	1.06	0.23 >	5.83
SF 9969	79/C2	08/13/91	1.002	3020.00	897.00	0.30	0.09 >	5.04

New Drugs with 50% Antiviral Reduction Levels: Out of the 3313 actual single drug tests, 192 new compounds demonstrated good antiviral activity, having antiviral reduction values equal to or better than 50%. This represents around 5.8% of the test compounds being active at this good antiviral reduction level. These compounds are summarized in **Table 23** according to the highest **Total Antiviral Index (TAI)**. AVS-11924, 11931, 9610 and 11679 demonstrated the best TAI's of that ranged from 46 - 57 and SI's that ranged from 25 - >126. Thirty-seven (37) other compounds demonstrated moderate antiviral activity, having TAI's that ranged from 20 - 38% and SI's from 1.3 - >320. The rest (151 compounds) showed marginal antiviral activity with TAI's that ranged from 0.3 to 19 and SI's from 0.03 - 7.7.

Table 23
AVS Compounds Active Against Sandfly (SF) at AI_{50} Level

AVS No.	Shipment#	Test Date	Differntl.	IC 50	TC 50	AI 50	SI	TAI
SF 11924	82	10/18/91	0.884	6.34 >	320.00 >	50.48 >	50.48 >	56.66
SF 11931	82	10/18/91	0.863	12.70 >	320.00 >	25.11 >	25.11 >	51.07
SF 9610	79	10/03/91	0.555	0.79	41.40	52.27	29.18 >	46.35
SF 11679	81	10/10/91	0.460 <	0.10	19.10 >	190.59 >	125.88 >	45.59
SF 11922	82	10/18/91	0.864	5.73	232.00	40.50	13.22 >	37.94
SF 11681	81	10/10/91	0.572	172.00	3160.00	18.39	11.98 >	36.17
SF 10264	82	09/19/91	0.570 <	1.00 >	320.00 >	320.00 >	320.00 >	34.42
SF 9276	77	02/21/91	0.464	505.00 >	3200.00 >	6.34	4.16 >	33.97
SF 9712	79	10/02/91	0.797	0.32	6.60	20.63	15.31 >	33.23
SF 9696	79	05/16/91	0.906	4.92	59.50	12.09	7.98 >	33.01
SF 9600	79	10/03/91	0.424	2.91	108.00	37.25	13.68 >	31.27
SF 11832	82	08/22/91	0.828	155.00	1560.00	10.03	5.38 >	30.64
SF 9604	79	05/07/91	1.242	10.00	236.00	23.60	13.60 >	30.64
SF 11921	82	10/18/91	0.872	8.90	204.00	22.88	8.78	30.49
SF 11131	78	10/15/91	0.575	266.00	2640.00	9.94	6.85 >	30.35
SF 9430	80	10/08/91	0.757	1.88	66.00	35.08	26.04	30.34
SF 9255	77	10/10/91	0.660	124.00	5170.00	41.85	17.81 >	30.24
SF 9632	79	05/09/91	1.232	7.69	66.30	8.63	6.40 >	29.82
SF 11638	81	08/13/91	0.989	846.00	7250.00	8.56	6.17 >	29.44
SF 9235	77	10/10/91	0.588	100.00	1160.00	11.63	6.60 >	27.56
SF 11824	82	08/27/91	0.609	8.35	81.90	9.81	6.63 >	27.35
SF 11095	78	10/10/91	0.524	3200.00 >	10000.0 >	3.13	2.33 >	26.97
SF 11876	82	08/27/91	0.914	1190.00	6600.00	5.56	4.13 >	26.77
SF 9264	77	02/20/91	0.658	59.60	657.00	11.02	8.14 >	26.09
SF 11928	82	10/18/91	0.797	134.00 >	320.00 >	2.39 >	2.39 >	25.90
SF 9753	79	05/29/91	1.043	29.90 >	320.00 >	10.69	7.13 >	25.43
SF 11247	80	09/26/91	0.601	75.90 >	320.00 >	4.22 >	4.22 >	25.42
SF 11376	81	07/18/91	1.091	512.00	2150.00	4.19	3.07 >	24.66
SF 9862	79	06/06/91	0.690	28.10	210.00	7.47	5.51 >	23.51
SF 11123	78	10/15/91	0.531	1000.00	8300.00	8.30	5.64	22.47
SF 9899	79	10/08/91	0.806	2.71	50.70	18.72	8.06	22.31
SF 9596	79	05/07/91	1.109	8.18	57.00	6.96	4.33 >	22.08
SF 11816	82	08/27/91	0.767	514.00	2100.00	4.08	3.01 >	20.79
SF 9433	80	10/08/91	0.732	147.00	626.00	4.25	2.98 >	20.69
SF 11897	82	08/29/91	0.696	8.85	58.00	6.56	4.18 >	20.40
SF 9699	79	10/02/91	0.869	59.60	210.00	3.52	2.60	20.30
SF 9247	77	10/10/91	0.627	68.40	642.00	9.39	5.03 >	20.08
SF 11926	82	10/18/91	0.845	243.00 >	320.00 >	1.32 >	1.32 >	20.02
SF 11828	82	08/27/91	0.764	250.00	1200.00	4.79	2.12 >	19.89
SF 11043	78	10/10/91	0.592	787.00	2150.00	2.74	1.63 >	19.75
SF 11242	80	09/26/91	0.603	164.00 >	320.00 >	1.95 >	1.95 >	19.75
SF 9954	79	06/18/91	0.602	135.00	665.00	4.94	3.26	19.43
SF 11930	82	10/18/91	0.863	254.00 >	320.00 >	1.26 >	1.26 >	19.22
SF 9847	79	06/05/91	0.721	1.65	22.40	13.58	7.72 >	18.94
SF 10148	82	09/12/91	0.578	5.17	21.00	4.06	3.00 >	18.85
SF 11890	82	08/29/91	0.553	354.00 >	1000.00 >	2.82 >	2.82 >	18.85

Table 23 (Cont'd)

AVS No.	Shipment#	Test Date	Differntl.	IC 50	TC 50	AI 50	SI	TAI
SF 11927	82	10/18/91	0.845	179.00 >	320.00 >	1.79 >	1.79 >	18.69
SF 11678	81	10/10/91	0.460	320.00	949.00	2.96	1.96	18.36
SF 10583	83	10/29/91	0.733	132.00 >	320.00 >	2.42 >	2.42 >	18.17
SF 9962	79	06/18/91	0.604	293.00	1830.00	6.22	3.88	17.93
SF 11344	81	07/17/91	1.057	844.00 >	3200.00 >	3.79	0.81 >	17.80
SF 9908	82	09/04/91	0.682	60.70 >	320.00 >	5.27	3.70	17.63
SF 9849	79	10/03/91	0.586	2.17	19.30	8.90	4.61 >	17.44
SF 9745	79	10/02/91	0.776	7.96	21.10	2.65	1.95 >	17.41
SF 11701	82	09/26/91	0.886 <	1.00	6.18 >	6.18 >	4.27 >	17.37
SF 11635	81	08/13/91	0.998	213.00	791.00	3.72	2.18 >	17.33
SF 11047	78	10/10/91	0.568	2840.00	6600.00	2.33	1.73 >	16.83
SF 11188	80	10/10/91	0.573	54.10	229.00	4.24	3.04 >	16.72
SF 1060	78	04/04/91	0.641	473.00	2020.00	4.26	2.98	16.67
SF 11625	81	08/08/91	1.105	71.40	210.00	2.94	2.17 >	16.60
SF 9603	79	05/07/91	1.242	5.14	19.20	3.73	2.49 >	16.34
SF 10881	84	11/13/91	0.755	121.00 >	320.00 >	2.65	0.08 >	16.20
SF 11885	82	08/29/91	0.702	551.00	1920.00	3.49	2.33	16.09
SF 11122	78	04/16/91	1.222	646.00	2100.00	3.25	2.40 >	15.97
SF 11062	78	04/04/91	0.649	581.00	1810.00	3.11	1.91	15.80
SF 9416	82	09/04/91	0.649	20.90 >	320.00 >	15.33	3.31 >	15.77
SF 11190	80	10/10/91	0.573	580.00 >	1000.00 >	1.72 >	1.72 >	15.60
SF 11907	82	09/26/91	0.607	8.61 >	320.00 >	37.18	2.89 >	15.55
SF 10164	82	09/11/91	0.758	216.00 >	320.00 >	1.48 >	1.48 >	15.00
SF 10047	79	07/03/91	1.036	43.40	175.00	4.04	2.37 >	14.84
SF 11185	80	10/10/91	0.556	211.00 >	1000.00 >	4.75	3.65	14.82
SF 9184	77	02/07/91	0.609 <	10.00	168.00 >	16.83 >	6.70 >	14.81
SF 10892	84	11/13/91	0.740	143.00 >	320.00 >	2.24 >	2.24 >	14.76
SF 11866	82	08/27/91	0.943	61.40	448.00	7.29	2.67 >	14.45
SF 11687	82	09/25/91	0.738	62.30	268.00	4.31	2.93	14.22
SF 11700	82	09/26/91	0.714 <	1.00	5.21 >	5.21 >	2.78 >	13.88
SF 11874	82	08/27/91	0.815	731.00	2320.00	3.17	1.50 >	13.68
SF 11865	82	08/27/91	0.943	23.20	156.00	6.73	1.65 >	13.50
SF 11420	81	07/25/91	1.151	819.00	3580.00	4.38	2.53	13.48
SF 10721	84	11/07/91	0.712	4.43	124.00	27.93	0.45 >	13.43
SF 11795	82	08/20/91	0.888	598.00	2100.00	3.51	2.59	13.41
SF 11467	81	07/30/91	0.940	498.00	1830.00	3.66	2.28	13.37
SF 10454	83	10/22/91	0.717	76.00	230.00	3.02	2.09 >	13.22
SF 11768	82	08/20/91	0.823	704.00	2100.00	2.98	2.20	13.16
SF 10719	84	11/07/91	0.816	4.61	134.00	28.98 <	0.22 >	13.09
SF 11812	82	08/27/91	0.850	7.15	25.30	3.53	1.96 >	13.02
SF 10336	82	09/25/91	0.594	48.60	269.00	5.54	0.19 >	12.84
SF 11407	81	07/23/91	1.009	27.90	85.10	3.05	2.10 >	12.75
SF 11303	81	07/16/91	1.000	684.00	2300.00	3.36	2.23	12.65
SF 11745	82	08/15/91	0.969	927.00	1700.00	1.83	0.97 >	12.57
SF 10525	83	10/23/91	0.804	18.00	68.20	3.78	2.77 >	12.54
SF 11699	82	09/26/91	0.714	32.00	71.10	2.22	1.61 >	12.54
SF 11680	81	07/10/91	0.981	173.00 >	320.00 >	1.85 >	1.85 >	12.36
SF 11498	81	07/31/91	1.114	547.00	1610.00	2.94	1.56	12.22
SF 11825	82	08/27/91	0.718	649.00	1940.00	2.98	2.01 >	12.06
SF 11133	78	04/16/91	1.196	815.00	2920.00	3.58	2.36 >	11.91
SF 11860	82	08/27/91	0.833	27.40	52.80	1.92	1.08 >	11.89
SF 9843	79	06/05/91	0.681	20.60	66.00	3.20	2.37	11.81
SF 11459	81	07/30/91	1.045	249.00	660.00	2.66	1.97 >	11.71
SF 9731	79	05/23/91	0.783	2.30	7.56	3.29	2.34 >	11.66
SF 9733	79	05/23/91	0.783	2.30	7.56	3.29	2.34 >	11.66
SF 9173	77	02/07/91	0.620	1160.00 >	3200.00 >	2.75	0.71 >	11.63
SF 11740	82	08/15/91	0.754	8.72	31.10	3.57	2.33 >	11.40
SF 11472	81	07/30/91	1.048	790.00	2610.00	3.31	1.76	11.27
SF 11496	81	07/31/91	1.139	137.00	575.00	4.19	0.68	11.27
SF 11565	81	08/06/91	1.025	302.00	622.00	2.06	1.37	11.12
SF 11822	82	08/27/91	0.676	78.90	159.00	2.02	1.08 >	11.04

Table 23 (Cont'd)

AVS No.	Shipment#	Test Date	Diff-rntl.	IC 50	TC 50	AI 50	SI	TAI
SF 9475	79	04/25/91	1.046	10.00	16.90	1.69	0.94 >	11.01
SF 11884	82	08/29/91	0.702	549.00	1860.00	3.39	2.16	10.99
SF 11941	82	09/24/91	0.601	235.00 >	320.00 >	1.36 >	1.36 >	10.97
SF 9935	79	06/13/91	0.487	924.00	3350.00	3.63	2.30 >	10.95
SF 11575	81	08/06/91	1.044	527.00	1690.00	3.21	1.68 >	10.93
SF 11637	81	08/13/91	1.071	21.50	52.10	2.43	1.33 >	10.85
SF 11878	82	08/27/91	0.992	693.00	1510.00	2.17	1.02 >	10.74
SF 11453	81	07/30/91	1.053	615.00	2420.00	3.93	1.94	10.64
SF 11814	82	08/27/91	0.642	827.00	2090.00	2.53	1.85 >	10.45
SF 9971	79	06/18/91	0.621	261.00	638.00	2.44	1.75 >	10.29
SF 11486	81	07/31/91	1.127	298.00	660.00	2.21	1.64	10.05
SF 11302	81	07/16/91	1.111	593.00	1670.00	2.82	1.46	9.87
SF 9445	79	10/18/91	0.904	20.10	68.40	3.40	0.33	9.80
SF 11512	81	08/01/91	1.115	710.00	1700.00	2.40	1.31	9.71
SF 10799	84	11/07/91	0.632	2.08	6.34	3.05	2.17 >	9.28
SF 11551	81	08/06/91	0.849	641.00	1890.00	2.95	1.93	9.20
SF 11495	81	07/31/91	0.991	97.80	247.00	2.52	1.67 >	9.13
SF 11848	82	08/22/91	0.842	243.00	599.00	2.46	1.62 >	9.08
SF 11923	82	10/18/91	0.864	95.20 >	320.00 >	3.36	3.36 >	8.89
SF 9598	79	05/07/91	1.032	2.98	10.00	3.35	1.89 >	8.71
SF 9480	80	07/09/91	1.155	67.70	218.00	3.22	0.35	8.68
SF 10073	82	09/05/91	0.701	290.00 >	320.00 >	1.10 >	1.10 >	8.63
SF 11186	80	10/10/91	0.556	194.00	609.00	3.13	2.13	8.47
SF 10647	83	10/30/91	0.537	92.20 >	320.00 >	3.47	2.46	8.41
SF 9817	79	06/04/91	0.529	7.06	19.90	2.82	1.96	8.39
SF 9749	79	05/29/91	1.001	8.31	19.90	2.39	1.65	8.35
SF 11528	81	08/01/91	0.991	919.00	1640.00	1.79	0.92	8.30
SF 9530	79	10/03/91	0.515	26.80	67.80	2.53	1.86	8.18
SF 9751	79	05/29/91	0.976	232.00 >	320.00 >	1.38 >	1.38 >	7.91
SF 11447	81	07/30/91	1.033	256.00	803.00	3.13	1.39	7.85
SF 9818	79	06/04/91	0.529	7.74	20.00	2.59	1.80	7.84
SF 9529	79	04/25/91	0.940	2.81	7.51	2.67	1.66 >	7.82
SF 9327	77	02/28/91	0.589	620.00	1910.00	3.09	1.34	7.75
SF 9937	79	06/13/91	0.558	827.00	2050.00	2.48	1.79	7.56
SF 11383	81	07/18/91	1.014	591.00	1690.00	2.86	1.40	7.43
SF 9748	79	05/29/91	0.972	25.70	59.00	2.30	1.50	7.39
SF 10432	83	10/17/91	0.595	76.80	244.00	3.18	2.01	7.24
SF 11782	82	08/20/91	0.678	843.00	1750.00	2.08	1.22	7.14
SF 10581	83	10/29/91	0.762	186.00 >	320.00 >	1.72	0.09 >	7.13
SF 11454	81	07/30/91	0.998	2110.00	5530.00	2.62	1.56	6.59
SF 11332	81	07/16/91	0.912	2960.00 >	3200.00 >	1.08 >	1.08 >	6.54
SF 9509	81	07/10/91	0.995	246.00 >	320.00 >	1.30	1.13 >	6.52
SF 11173	80	07/10/91	1.065	80.20	204.00	2.55	1.82	6.67
SF 10334	82	09/25/91	0.763	6.64	192.00	28.89	0.15	6.39
SF 11413	81	07/25/91	1.299	228.00	503.00	2.21	1.16	6.25
SF 10556	83	10/24/91	0.845	207.00 >	320.00 >	1.55 >	1.55 >	6.12
SF 11542	81	08/06/91	0.975	2520.00 >	3200.00 >	1.27 >	1.27 >	5.88
SF 11048	78	04/04/91	0.634	814.00	2720.00	3.35	1.52	5.71
SF 11895	82	08/29/91	0.532	90.20	163.00	1.81	0.98 >	5.57
SF 9354	77	03/05/91	0.760	791.00	2110.00	2.67	0.27	5.55
SF 10883	84	11/13/91	0.775	8.20	51.30	6.25	0.23 >	5.32
SF 10015	79	07/02/91	1.041	3050.00 >	3200.00 >	1.05 >	1.05 >	5.12
SF 9930	79	06/13/91	0.604	756.00	1840.00	2.44	1.54	4.85
SF 10342	82	09/25/91	0.579	264.00 >	320.00 >	1.21 >	1.21 >	4.73
SF 11739	82	08/15/91	1.091	93.80	198.00	2.11	1.46	4.69
SF 11650	81	08/13/91	0.993	300.00	709.00	2.36	1.21	4.54
SF 9675	79	05/16/91	0.838	93.00	165.00	1.78	0.97 >	4.54
SF 11509	81	08/01/91	1.118	806.00	1480.00	1.84	0.90	4.40
SF 11738	82	08/15/91	1.091	869.00	1990.00	2.29	1.43	4.18
SF 11327	81	07/16/91	0.930	1000.00	1870.00	1.87	1.21	4.10
SF 11735	82	08/15/91	1.000	957.00	1920.00	2.01	1.34	3.87

Table 23 (Cont'd)

	AVS No.	Shipment#	Test Date	Diff-rntl.	IC 5C		TC 50		AI 50		SI		TAI
SF	11306	81	07/16/91	1.064	814.00		1580.00		1.94		1.05	>	3.67
SF	10652	83	10/30/91	0.546	29.00		63.20		2.17		0.32	>	3.58
SF	11856	82	08/27/91	0.865	872.00		1900.00		2.18		0.11		3.39
SF	9328	77	02/28/91	0.589	858.00		1980.00		2.31		0.03		3.39
SF	11030	78	04/02/91	1.208	3050.00	>	3200.00	>	1.05		0.62	>	3.31
SF	9669	79	05/14/91	1.103	100.00		278.00		2.78		0.21	>	3.29
SF	9489	80	07/09/91	1.158	91.90		186 00		2.03		0.35		2.95
SF	9364	77	03/05/91	0.841	278.00		404.00		1.45		0.69	>	2.81
SF	9323	77	02/26/91	0.671	827.00		1630.00		1.97		0.06		2.23
SF	9338	77	02/28/91	0.513	802.00		1480.00		1.85		0.04		1.94
SF	11932	82	10/18/91	0.923	320.00	>	320.00	>	1.00		0.24	>	1.88
SF	10254	82	09/19/91	0.578	320.00	>	320.00	>	1.00	>	1.00	>	1.70
SF	9681	79	05/16/91	0.829	1.00		2.10		2.10		1.55	>	1.68
SF	11597	81	08/07/91	1.235	738.00		1270.00		1.72		0.41		1.65
SF	11500	81	07/31/91	1.185	313.00		626.00		2.00		0.71		1.57
SF	11719	82	08/14/91	0.852	1000.00		1660.00		1.66		0.81		1.55
SF	11807	82	08/27/91	0.883	2520.00	>	3200.00	>	1.27		0.11	>	1.25
SF	10705	84	12/05/91	0.830	26.60		7.09		0.27	<	0.04	>	1.19
SF	11489	81	07/31/91	1.078	31.30		42.60		1.36		0.75		1.10
SF	9447	79	04/23/91	0.993	100.00		152.00		1.52		0.66		1.09
SF	9446	79	10/18/91	0.904	93.60		151.00		1.61		0.33		1.03
SF	11535	81	09/06/91	0.949	938.00		1510.00		1.61		0.22	>	0.88
SF	11582	81	08/06/91	0.953	1000.00		1430.00		1.43		0.58		0.88
SF	11857	82	08/27/91	0.853	30.60		43.70		1.43		0.57		0.30

New Drugs with 25% Antiviral Reduction Levels: Of the 3313 actual single drug tests, 420 new compounds demonstrated minimal antiviral activity, having antiviral reduction values equal to or better than 25%. This represents around 13% of the test compounds being active at this marginal antiviral reduction level.

In general, when compared to the 95% and 50% antiviral activity categories, the compounds in this (25%) category do not appear to have any significant antiviral promise and probably do not need to be presently confirmed

4.3.6.3 Confirmatory Assays:

Some of the compounds were sent to us in more than one separate drug shipment. These compounds were tested more than once. Data from the confirmatory assays are summarized in **Table 24**. If a compound showed $\geq$50% reduction in CPE during this contract period, then it was considered a candidate for confirmatory testing. The confirmatory tests are from active compounds taken from the prescreen and primary MTT assays. Out of 110 confirmatory tests, 82 compounds were confirmed active during this reporting period and the remaining 28 compounds gave conflicting results. The criteria for activity is that the confirmatory test has to show $\geq$25% reduction in CPE. Failure to confirm the activity in these compounds was probably due to differences during the assay conditions:

1) In confirmatory assays the concentration range is adjusted to a more accurate semilog scale to maximize the TAI window.

2) Differences in the "differential" of the two runs can cause the compound to read positive or negative, falsely. The variability in the differential can cause false positive or negative bias to be introduced into the calculations, thus reflecting the variability in the maximum activity of the compound.

3) The metabolic rate, cell and virus load/well, age, and passage number of the cells may cause the above observed variability in the confirmatory results.

4) Problems associated with stability and storage of the compound (i.e., different lot numbers, solubility, light sensitivity, hygroscopic, etc.).

5) Problems associated with technical execution of large numbers of plates by different technicians.

During this reporting period the overall confirmatory rate against SF was 75%. The conflicting results should be retested at a later date based on the availability of the compound.

Some of the compounds have not been confirmed due to the discontinuation of this project by the sponsor.

4.3.6.4 Recommendations of SF-Actives Based Upon the *In Vitro* Results with MTT Assay (Vero Cells).

Based upon the *in vitro* results with the MTT assay (Vero cells) we recommend the following:

1) Compounds with the highest TAI in the 95% activity category that have confirmed results with the exception of "colored" compounds should receive the highest priority for further profile studies and *in vivo* animal testing.

2) Compounds with the highest TAI in the 50% activity category that have confirmed results with the exception of "colored" compounds should receive the next highest priority for further profile studies and *in vivo* animal testing.

Table 24

Confirmatory Assays for Compounds Active Against Sandfly Virus (SF)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	AI	ACT
2318	53	04/11/89	PGO	0.669	< 1.00	< 1.00	= 1.00	0.00	2.90	0.00	0.00	> 320.00	0.00	0.00	> 3.15	+
2318	13	08/30/89	REU	0.652	0.24	2.01	8.40	0.50	10.00	20.01	0.00	> 10.00	0.00	4.02	19.09	+
2318	67	05/31/90	WHY	0.856	0.00	0.64	0.00	0.00	2.71	0.00	0.00	> 10.00	0.00	0.00	0.00	-
2318	67	07/12/90	XHC	0.908	0.28	1.95	6.86	0.57	8.64	15.27	0.00	> 10.00	0.00	3.45	13.75	+
2318	82	10/18/91	AT2	0.869	< 1.00	2.41	> 2.41	< 1.00	10.00	> 10.00	0.00	> 320.00	0.00	> 2.41	> 7.40	+
2980	48	02/07/89	QM4	1.141	0.42	2.19	5.20	0.00	> 3.20	0.00	0.00	> 3.20	0.00	0.00	> 14.98	+
2980	25	09/07/89	RIE	0.725	0.03	1.75	54.73	0.20	3.09	15.60	0.00	> 10.00	0.00	8.83	> 32.54	+
2980	61	11/29/89	SG6	0.918	0.66	1.83	2.77	0.00	2.87	0.00	0.00	> 3.20	0.00	0.00	> 8.51	+
2980	28/61	04/26/91	S1N	0.936	1.49	2.45	1.64	3.20	5.92	1.85	0.00	> 10.00	0.00	0.76	5.16	+
4070	48	02/07/89	QM5	1.153	0.00	> 3.20	0.00	0.00	> 3.20	0.00	0.00	> 3.20	0.00	0.00	> 6.51	-
4070	48	12/07/89	SUG	0.583	1.00	8.41	8.41	0.00	16.60	0.00	0.00	61.10	0.00	0.00	> 15.64	+
4070	48	04/26/91	S1N	0.944	4.61	9.50	2.06	8.15	21.50	2.64	0.00	96.80	0.00	1.17	> 7.30	+
4074	48	02/07/89	QM7	1.222	0.00	> 0.03	0.00	0.00	> 0.03	0.00	0.00	> 0.03	0.00	0.00	> 6.49	-
4074	48	12/07/89	SUR	0.526	< 1.00	1.89	> 1.89	< 1.00	2.77	> 2.77	< 1.00	26.50	> 26.50	> 1.89	> 6.22	+
4074	48	02/13/90	UQQ	0.655	0.69	0.85	1.23	0.00	1.96	0.00	0.00	> 10.00	0.00	0.00	0.53	+
4074	RS-1	04/26/91	S1N	0.944	0.49	1.28	2.61	0 82	2.45	3.00	0.00	26.80	0.00	1.57	6.53	+
4855	48	02/22/89	QQ5	0.808	7.22	122.00	16.80	19.50	221.00	11.30	0.00	> 320.00	0.00	6.23	37.03	+
4855	61	11/29/89	SQ1	0.831	10.20	> 320.00	> 31.51	14.90	> 320.00	> 21.49	29.60	> 320.00	> 10.80	> 21.49	> 59.59	+
4855	48	12/05/90	1QE	1.070	1.18	> 100.00	> 84.69	12.50	> 100.00	> 7.98	0.00	> 100.00	0.00	> 7.98	> 42.18	+
4855	48	03/12/91	3US	0.744	9.31	156.00	16.76	18.10	212.00	11.72	81.00	313.00	3.87	8.62	> 36.43	+
8228	75	11/20/90	1CF	0.491	82.70	203.00	2.45	0.00	424.00	0.00	0.00	942.00	0.00	0.00	2.48	+
8228	75	03/22/91	44L	0.756	113.00	490.00	4.32	177.00	660.00	3.72	0.00	966.00	0.00	2.76	15.74	+
8240	75	11/20/90	1CL	0.580	296.00	1000.0	3.41	0.00	1960.00	0.00	0.00	> 3200.00	0.00	0.00	2.32	+
8240	75	03/22/91	44M	0.772	458.00	1140.0	2.49	800.00	1830.00	2.28	0.00	3060.00	0.00	1.42	5.10	+
8251	76	12/11/90	1T2	0.768	33.60	427.00	12.70	50.80	618.00	12.16	0.00	962.00	0.00	8.40	> 22.87	+
8251	76	03/22/91	44P	0.626	60.90	490.00	8.04	320.00	660.00	2.06	0.00	966.00	0.00	1.53	16.13	+
8269	76	12/12/90	1V2	0.855	0.00	500.00	0.00	0.00	1000.00	0.00	0.00	3020.00	0.00	0.00	> 1.92	-
8269	76	03/22/91	44T	0.661	161.00	568.00	3.53	259.00	895.00	3.45	0.00	> 1000.00	0.00	2.19	8.22	+
8271	76	12/12/90	1V3	0.803	32.00	493.00	15.42	77.90	667.00	8.56	280.00	979.00	3.50	6.33	> 35.25	+
8271	76	03/21/91	45W	0.807	118.00	433.00	3.66	201.00	622.00	3.10	0.00	962.00	0.00	2.16	8.25	+
8326	76	12/13/90	1YD	0.720	177.00	283.00	1.60	313.00	537.00	1.72	0.00	962.00	0.00	0.91	1.96	+
8326	76	03/21/91	460	0.767	165.00	66.00	0.40	292.00	516.00	1.76	0.00	951.00	0.00	0.23	1.58	+
8353	75	11/29/90	1JH	0.765	14.70	68.00	18.20	33.30	> 320.00	> 9.60	0.00	> 320.00	0.00	8.05	> 31.38	+
8353	75	03/26/91	47T	0.633	11.40	242.00	21.33	16.20	601.00	37.10	30.70	> 1000.00	> 3..61	14.96	33.07	+
8355	76	01/18/91	2RW	0.634	19.40	53.90	2.79	8.00	159.00	0.00	0.00	> 320.00	0.00	0.00	5.19	+
8355	76	03/26/91	47U	0.622	11.80	85.10	7.19	18.90	> 320 00	> 16.91	0.00	> 320.00	0.00	4.50	> 19.72	+

Table 24 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	S.	TAI	ACT
8358	76	01/18/91	28P	0.778	37.60	170.00	4.51	54.10	239.00	4.42	0.00 >	320.00	0.00	3.13	16.26	+
8358	76	03/26/91	47U	0.622	19.70	156.00	7.92	38.70	212.00	5.49	90.90	313.00	3.44	4.03	23.75	+
8370	76	12/13/90	1YL	0.778	57.40	466.00	8.11	115.00	668.00	5.83	320.00	2260.00	7.05	4.06	20.33	+
8370	76	03/26/91	47R	0.661	115.00	552.00	4.79	278.00	938.00	3.38	0.00	2960.00	0.00	1.99	10.83	+
8374	76	01/16/91	20W	0.822	327.00	1480.0	4.53	554.00	2050.00	3.71	0.00	3090.00	0.00	2.67 >	16.87	+
8374	76	03/21/91	464	0.608	224.00	887.00	3.95	477.00	1670.00	3.51	0.00	3050.00	0.00	1.86	11.50	+
8378	76	12/13/90	1YP	0.548	109.00	427.00	3.91	170.00	618.00	3.64	0.00	962.00	0.00	2.52	14.33	+
8378	76	03/21/91	466	0.599	50.50	420.00	8.33	84.70	614.00	7.24	0.00	961.00	0.00	4.96 >	15.81	+
8499	75	11/29/90	1JP	0.728	37.50 >	320.00 >	8.65	58.10 >	320.00 >	5.51	0.00 >	320.00	0.00 >	5.51 >	26.49	+
8499	75	03/26/91	47V	0.658	45.30	173.00	3.74	96.50 >	1000.00 >	10.59	0.00 >	1000.00	0.00	1.83 >	11.56	+
8541	76	01/03/91	297	0.912	15.10	0.83	0.06	23.60	2.69	0.11	0.00	95.80	0.00	0.04	3.78	+
8541	76	03/26/91	47W	0.600	12.30 >	320.00 >	26.00	41.20 >	320.00 >	7.76	0.00 >	320.00	0.00 >	7.76 >	24.69	+
8696	76	01/22/91	21Z	0.716	0.00	93.80	0.00	0.00	170.00	0.00	0.00	308.00	0.00	0.00	0.45	-
8696	76	04/02/91	4E5	1.182	406.00	320.00	0.79	734.00 >	1000.00 >	1.36	0.00 >	1000.00	0.00	0.44 >	1.32	+
9121	77	01/31/91	228	0.636	850.00 >	3200.0 >	3.77	1860.00 >	3200.0 >	1.72	0.00 >	3200.00	0.00 >	1.72 >	16.13	+
9121	77	10/09/91	A1Q	0.514	4510.0 >	10000 >	2.22	6360.00 >	10000.0 >	1.57	0.00 >	10000.0	0.00 >	1.57 >	8.23	+
9173	77	02/07/91	35G	0.620	597.00	830.00	1.39	1160.00 >	3200.00 >	2.75	0.00 >	3200.00	0.00	0.71 >	11.63	+
9173	77	10/09/91	A1Z	0.684	1080.0	4760.0	4.40	1770.00	6530.00	3.69	0.00	9720.00	0.00	2.69	10.94	+
9184	77	02/07/91	35M	0.609 <	10.00	67.00 >	6.70 <	10.00	168.00 >	16.83	0.00	950.00	0.0 >	6.70 >	14.81	+
9184	77	10/09/91	AJ0	0.660	0.00	54.20	0.00	0.00	86.90	0.00	0.00	303.00	0.00	0.00 >	0.40	-
9217	77	02/12/91	38P	0.590	1[illegible]0.00	1570.0	9.80	255.00	2130.00	8.35	861.00	3150.00	3.66	6.13	27.17	+
9217	77	10/09/91	AJ3	0.656	396.00	1530.0	3.87	618.00	2090.00	3.38	0.00	3090.00	0.00	2.48 >	15.91	+
9233	77	03/07/91	3SJ	0.370	49.30	767.00	15.57	75.90 >	1000.00 >	13.18	270.00 >	1000.00 >	3.71	10.12	31.97	+
9233	77	10/10/91	AKP	0.510	0.00	1230.0	0.00	0.00	2180.00	0.00	0.00 >	3200.00	0.00	0.00 >	0.81	-
9235	77	03/06/91	3P	0.881	175.00	417.00	2.39	0.00	687.00	0.00	0.00	2490.00	0.00	0.00	5.29	+
9235	77	10/10/91	AK0	0.588	36.20	660.00	18.23	100.00	1160.00	11.63	0.00	3200.00	0.00	6.60 >	27.56	+
9247	77	03/06/91	3PZ	0.897	0.00	1570.0	0.00	0.00	2770.00	0.00	0.00 >	3200.00	0.00	0.00	0.36	-
9247	77	10/10/91	AKS	0.627	23.90	344.00	14.37	68.40	642.00	9.39	0.00	2830.00	0.00	5.03 >	20.08	+
9248	77	03/06/91	3PZ	0.897	120.00 >	3200.0 >	26.57	178.00 >	3200.00 >	18.03	622.00 >	3200.00 >	5.14 >	18.03 >	44.95	+
9248	77	10/10/91	AKS	0.627 <	32.00	2220.0 >	69.24	45.00	4150.00	92.24	246.00	9520.00	38.76	49.20 >	57.32	+
9255	77	02/20/91	3C9	0.624	2350.0	518.00	0.22	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	6.00	+
9255	77	10/10/91	AKU	0.660	35.30	2200.0	62.26	124.00	5170.00	41.85	0.00	9610.00	0.00	17.81 >	30.24	+
9414	81	07/10/91	74H	1.005	68.40 >	100.00 >	1.46	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00 >	5.50	+
9414	81	10/08/91	AE2	0.731	79.60 >	1000.0 >	12.56	334.00 >	1000.00 >	2.99	896.00 >	1000.00 >	1.12 >	2.99 >	30.65	+

Table 24 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9430	80	07/09/91	70G	1.129	0.00	35.40	0.00	0.00	57.00	0.00	0.00	95.70	0.00	0.00	0.00	-
9430	80	10/08/91	AE7	0.757	1.37	49.00	35.72	1.88	66.00	35.08	0.00	96.60	0.00	26.04	30.34	+
9433	80	07/10/91	748	0.977	146.00	272.00	1.87	233.00 >	320.00 >	1.38	0.00 >	320.00	0.00	1.17 >	4.53	+
9433	80	10/08/91	AE8	0.732	59.90	440.00	7.34	147.00	626.00	4.25	0.00	963.00	0.00	2.98 >	20.69	+
9445	79	04/23/91	4YW	0.889 <	1.00	2.61 >	2.61	0.00	8.12	0.00	0.00	29.40	0.00	0.00 >	3.64	+
9445	79	10/18/91	AUC	0.904	3.90	6.60	1.69	20.10	68.40	3.40	0.00	210.00	0.00	0.33	9.80	+
9446	79	04/23/91	4YX	0.993	0.00	7.65	0.00	0.00	19.30	0.00	0.00	94.60	0.00	0.00	0.00	-
9446	79	10/18/91	AUC	0.904	54.10	30.70	0.57	93.60	151.00	1.61	0.00	303.00	0.00	0.33	1.03	+
9447	79	04/23/91	4YX	0.993	53.10	66.00	1.24	100.00	152.00	1.52	0.00	310.00	0.00	0.66	1.09	+
9447	79	10/18/91	AUD	0.874	78.90	84.30	1.07	0.00	186.00	0.00	0.00	963.00	0.00	0.00	0.04	+
9451	79	04/23/91	4YZ	0.875	0.00	4.90	0.00	0.00	6.60	0.00	0.00	9.66	0.00	0.00 >	1.36	-
9451	79	10/03/91	AB7	0.555	13.40	59.40	4.44	17.90	86.80	4.85	30.20	291.00	9.64	3.32	16.18	+
9452	79	04/23/91	420	0.832	0.00	3.63	0.00	0.00	5.75	0.00	0.00	9.58	0.00	0.00	0.04	-
9452	79	10/03/91	AB7	0.555	13.40	52.70	3.94	17.90	73.50	4.11	30.20	291.00	9.63	2.95	15.51	+
9475	79	04/25/91	516	1.046	1.43	9.63	6.60	10.00	16.90	1.69	0.00	100.00	0.00	0.94 >	11.01	+
9475	79	10/03/91	ABA	0.583	5.38	14.20	2.64	10.00	24.80	2.48	0.00	137.00	0.00	1.42	4.48	+
9480	80	07/09/91	62V	1.155	46.60	24.00	0.52	67.70	218.00	3.22	0.00 >	320.00	0.00	0.35	8.68	+
9480	80	10/10/91	AG3	0.656	0.00	43.30	0.00	0.00	165.00	0.00	0.00	869.00	0.00	0.00	0.00	-
9489	80	07/09/91	62Y	1.158	54.20	32.00	0.59	91.90	186.00	2.03	0.00	312.00	0.00	0.35	2.95	+
9489	80	10/10/91	AG4	0.588	0.00	35.40	0.00	0.00	124.00	0.00	0.00	300.00	0.00	0.00	0.00	-
9529	79	04/25/91	518	0.940 <	1.00	4.67 >	4.67	2.81	7.51	2.67	0.00	28.10	0.00	1.66 >	7.82	+
9529	79	10/03/91	ABB	0.614	0.00	2.31	0.00	0.00	5.79	0.00	0.00	90.30	0.00	0.00 >	0.02	-
9530	79	04/25/91	518	0.940	16.00	43.20	2.70	25.50	63.20	2.47	0.00	99.20	0.00	1.69	7.29	+
9530	79	10/03/91	ABC	0.515	16.40	49.90	3.05	26.80	67.80	2.53	0.00	100.00	0.00	1.86	8.18	+
9589	79	05/02/91	595	0.926	134.00	18.70	0.14	179.00	27.70	0.15	302.00	91.10	0.30	0.10 >	6.19	+
9589	79	10/03/91	ABF	0.526	21.90	272.00	12.39	40.50	500.00	12.35	91.40	950.00	10.40	6.72 >	29.47	+
9590	79	05/02/91	596	0.798	13.90	49.00	3.51	25.20	66.00	2.62	0.00	96.60	0.00	1.94	9.06	+
9590	79	10/03/91	ABG	0.440	13.80	42.00	3.04	566.00	61.40	0.11	945.00	96.10	0.10	0.07 >	12.23	+
9596	79	05/07/91	5CB	1.109	3.54	35.40	10.02	8.18	57.00	6.96	0.00	95.70	0.00	4.33 >	22.08	+
9596	79	10/03/91	ABG	0.440	3.20	22.80	7.12	6.77	40.40	5.96	0.00	94.00	0.00	3.37	14.99	+
9598	79	05/07/91	5CC	1.032	1.24	5.64	4.57	2.98	10.00	3.35	0.00	29.80	0.00	1.89 >	8.71	+
9598	79	10/03/91	ABH	0.481	7.06	15.50	2.20	0.00	21.00	0.00	0.00	30.90	0.00	0.00	4.54	+
9600	79	05/07/91	5CD	1.130	3.62	40.00	11.06	10.00	60.00	6.00	0.00	96.00	0.00	4.00 >	21.33	+
9600	79	10/03/91	ABI	0.424	1.33	39.80	30.03	2.91	108.00	37.25	0.00	299.00	0.00	13.68 >	31.27	+

Table 24 (Cont'd)

AVS No.	Shipment	Test Date	Pit #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9603	79	05/07/91	5CF	1.242 <	1.00	12.80 >	12.81	5.14	19.20	3.73	0.00	30.70	0.00	7.49 >	16.34	+
9603	79	10/03/91	ABI	0.424	1.27	9.24	7.27	0.00	16.70	0.00	0.00	30.50	0.00	0.00	11.49	+
9604	79	05/07/91	5CF	1.242	1.34	136.00	101.68	10.00	236.00	23.60	0.00 >	320.00	0.00	13.60 >	30.64	+
9604	79	10/03/91	ABJ	0.585	17.90	238.00	13.28	65.70	528.00	8.03	0.00	953.00	0.00	3.61 >	18.76	+
9610	79	05/07/91	5CI	1.183 <	1.00	15.70 >	15.70	1.00	25.90	25.89	0.00	90.30	0.00	15.70 >	27.65	+
9610	79	10/03/91	ABK	0.555	0.38	23.10	60.57	0.79	41.40	52.27	0.00	94.10	0.00	29.18 >	46.35	+
9632	79	05/09/91	5EK	1.232	3.70	49.20	13.28	7.69	66.30	8.63	0.00	97.30	0.00	6.40 >	29.82	+
9632	79	10/02/91	ABZ	0.822	3.87	49.20	12.71	16.20	66.30	4.09	0.00	97.30	0.00	3.03 >	23.96	+
9669	79	05/14/91	5J4	1.103	51.60	20.70	0.40	100.00	278.00	2.78	0.00 >	320.00	0.00	0.21 >	3.29	+
9669	79	10/02/91	A92	0.844	0.00	6.89	0.00	0.00	18.60	0.00	0.00	514.00	0.0	0.00	0.00	-
9675	79	05/16/91	5KY	0.838	50.70	90.60	1.79	93.00	165.00	1.78	0.00	305.00	0.00	0.97 >	4.54	+
9675	79	10/02/91	A92	0.844	100.00	28.70	0.29	0.00	130.00	0.00	0.00	305.00	0.00	0.00 >	0.80	+
9681	79	05/16/91	5L1	0.829 <	1.00	1.55 >	1.55	1.00	2.10	2.10	0.00	3.09	0.00	1.55 >	1.68	+
9681	79	10/02/91	A94	0.844	0.63	1.50	2.36	0.00	2.07	0.00	0.00	3.09	0.00	0.00	6.65	+
9696	79	05/16/91	5LB	0.906	1.74	39.30	22.58	4.92	59.50	12.09	0.00	96.00	0.00	7.98 >	33.01	+
9696	79	10/02/91	A95	0.859	13.50	39.30	2.91	20.00	59.50	2.98	0.00	96.00	0.00	1.97 >	8.73	+
9699	79	05/22/91	5P0	0.929	3.94	38.60	9.79	18.60	59.00	3.17	0.00	95.90	0.00	2.07	16.73	+
9699	79	10/02/91	A96	0.869	12.90	155.00	11.97	59.60	210.00	3.52	0.00	309.00	0.00	2.60	20.30	+
9712	79	05/22/91	5P7	0.884 <	1.00	1.11 >	1.11 <	1.60	1.81 >	1.81	0.00	3.06	0.00 >	1.11 >	1.41	+
9712	79	10/02/91	A98	0.797	0.18	4.90	27.72	0.32	6.60	20.63	0.00	9.66	0.00	15.31 >	33.23	+
9731	79	05/23/91	5TK	0.783 <	1.00	5.38 >	5.38	2.30	7.56	3.29	0.00	27.80	0.00	2.34 >	11.66	+
9731	79	10/02/91	A99	0.766	1.57	5.66	3.60	0.00	9.28	0.00	0.00	29.60	0.00	0.00	7.15	+
9733	79	05/23/91	5TL	0.783 <	1.00	5.38 >	5.38	2.30	7.56	3.29	0.00	27.80	0.00	2.34 >	11.66	+
9733	79	10/02/91	A99	0.766	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00	0.00	-
9745	79	05/23/91	5T8	0.728	6.19	15.60	2.51	0.00	21.10	0.00	0.00	31.10	0.00	0.00	6.18	+
9745	79	10/02/91	A9A	0.776	2.71	15.60	5.74	7.95	21.10	2.65	0.00	31.10	0.00	1.95 >	17.41	+
9748	79	05/29/91	5WB	0.972	11.70	38.60	3.29	25.70	59.00	2.30	0.00	95.90	0.00	1.50	7.39	+
9748	79	10/02/91	A9B	0.705	0.00	44.00	0.00	0.00	62.60	0.00	0.00	96.30	0.00	0.00	0.00	-
9749	79	05/29/91	5WC	1.001	4.28	13.70	3.20	8.31	19.90	2.39	0.00	31.00	0.00	1.65	8.35	+
9749	79	10/02/91	A9B	0.705	0.00	15.40	0.00	0.00	21.00	0.00	0.00	31.10	0.00	0.00	0.00	-
9753	79	05/29/91	5WE	1.043	13.00	214.00	16.37	29.90 >	320.00 >	10.69	0.00 >	320.00	0.00	7.13 >	25.43	+
9753	79	10/02/91	A9C	0.791	113.00	585.00	5.20	172.00	873.00	5.09	0.00 >	1000.00	0.00	3.41	19.54	+
9817	79	06/04/91	639	0.529	4.75	13.90	2.92	7.06	19.90	2.82	0.00	30.80	0.00	1.96	8.39	+
9817	79	10/03/91	AAZ	0.493	2.94	5.25	1.79	0.00	7.30	0.00	0.00	25.50	0.00	0.00	3.29	+

Table 24 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9818	79	06/04/91	639	0.529	4.33	13.90	3.21	7.74	20.00	2.59	0.00	31.00	0.00	1.80	7.84	+
9818	79	10/03/91	A80	0.590	0.00	71.70	0.00	0.00 >	100.00	0.00	0.00 >	100.00	0.00	0.00	0.00	-
9842	79	06/05/91	65C	0.681	12.00	156.00	12.96	21.20	211.00	9.95	82.70	311.00	3.72	7.33	31.91	+
9842	79	10/03/91	A82	0.433	13.50	153.00	11.39	25.00	209.00	8.36	0.00	309.00	0.00	6.14	23.88	+
9843	79	06/05/91	65C	0.681	14.20	49.00	3.46	20.60	66.00	3.20	0.00	96.60	0.00	2.37	11.81	+
9843	79	10/03/91	A82	0.433	0.00	23.20	0.00	0.00	42.40	0.00	0.00	94.20	0.00	0.00	0.00	-
9847	79	06/05/91	65E	0.721 <	1.00	12.70 >	12.70	1.65	22.40	13.58	0.00	86.40	0.00	7.72 >	18.94	+
9847	79	10/03/91	A83	0.521	1.99	15.70	7.93	0.00	41.40	0.00	0.00	94.10	0.00	0.00	9.17	+
9849	79	06/06/91	676	0.858 <	1.00	19.30 >	19.31	2.01	43.30	21.56	0.00	94.30	0.00	9.61 >	16.06	+
9849	79	10/03/91	A84	0.586	0.95	10.00	10.49	2.17	19.30	8.90	0.00	78.80	0.00	4.61 >	17.44	+
9856	79	06/06/91	679	0.645 <	1.00	4.90 >	4.90 <	1.00	6.60 >	6.60	2.46	9.66	3.93 >	4.90 >	22.88	+
9856	79	10/03/91	A84	0.586	0.35	4.90	13.95	0.56	6.60	11.78	2.96	9.66	3.26	8.74	33.81	+
9857	79	06/06/91	67A	0.711 <	1.00	4.90 >	4.90 <	1.00	6.60 >	6.60 <	1.00	9.66 >	9.66 >	4.90 >	25.10	+
9857	79	10/03/91	A85	0.531	0.18	4.90	27.39	0.43	6.60	15.31	0.95	9.66	10.18	11.37 >	42.88	+
9858	79	06/06/91	67A	0.711 <	1.00	4.90 >	4.90 <	1.00	6.60 >	6.60 <	1.00	9.66 >	9.66 >	4.90 >	25.10	+
9858	79	10/08/91	ADU	0.709	0.83	15.50	18.74	3.99	21.00	5.26	9.12	30.90	3.39	3.89 >	33.20	+
9859	79	06/06/91	67B	0.567 <	1.00	4.90 >	4.90 <	1.00	6.60 >	6.60 <	1.00	9.66 >	9.66 >	4.90 >	25.10	+
9859	79	10/08/91	ADV	0.691	0.37 >	32.00 >	86.88	1.41 >	32.00 >	22.77	15.70 >	32.00 >	2.04 >	22.77 >	52.51	+
9860	79	06/06/91	67B	0.567 <	1.00	15.50 >	15.50 <	1.00	21.00 >	21.00	2.71	30.90	11.40 >	15.50 >	40.56	+
9860	79	10/08/91	ADV	0.691	1.21 >	32.00 >	26.36	4.47 >	32.00 >	7.15	21.10 >	00 >	1.51 >	7.15 >	42.63	+
9862	79	06/06/91	67C	0.690	14.70	155.00	10.52	28.10	210.00	7.47	0.00	3 .00	0.00	5.51 >	23.51	+
9862	79	10/08/91	ADW	0.699	0.00 >	1000.0	0.00	0.00 >	1000.00	0.00	0.00 >	1000.00	0.00	0.00 >	10.30	-
9863	79	06/06/91	67D	0.701 <	1.00	4.90 >	4.90 <	1.00	6.60 >	6.60	2.58	9.66	3.74 >	4.90 >	22.38	+
9863	79	10/08/91	ADW	0.699	1.79 >	32.00 >	17.89	4.71 >	32.00 >	6.79	22.70 >	32.00 >	1.41 >	6.79 >	42.03	+
9899	79	06/12/91	6FH	0.855	4.73	7.09	1.50	9.00	15.30	0.00	0.00	30.30	0.00	0.00	1.56	+
9899	79	10/08/91	AE1	0.806	1.61	21.80	13.55	2.71	50.70	18.72	0.00	95.10	0.00	8.06	22.31	+
9916	79	06/12/91	6FU	0.712	155.00	1140.0	7.37	241.00	1870.00	7.74	846.00	3170.00	3.75	4.74	24.74	+
9916	79/C2	08/13/91	BDO	0.906	459.00	728.00	1.59	920.00	1450.00	1.58	0.00	3030.00	0.00	0.79	2.55	+
9930	79	06/13/91	6IW	0.606	476.00	1160.0	2.46	756.00	1840.00	2.44	0.00	3060.00	0.00	1.54	4.85	+
9930	79/C2	08/13/91	BDP	1.015	0.00	1500.0	0.00	0.00	2070.00	0.00	0.00	3090.00	0.00	0.00	1.08	-
9937	79	06/13/91	6J0	0.558	514.00	1480.0	2.88	827.00	2050.00	2.48	0.00	3090.00	0.00	1.79	7.56	+
9937	79/C2	08/13/91	BDQ	1.033	587.00	745.00	1.27	0.00	1370.00	0.00	0.00	3020.00	0.00	0.00	0.74	+
9954	79	06/18/91	6KZ	0.602	66.10	438.00	6.63	135.00	665.00	4.94	0.00 >	1000.00	0.00	3.26	19.43	+
9954	79/C2	08/13/91	BDS	0.913	0.00	245.00	0.00	0.00	487.00	0.00	0.00 >	1000.00	0.00	0.00	0.00	-

Table 24 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
9962	79	06/18/91	6L3	0.604	142.00	1140.0	8.02	293.00	1830.00	6.22	0.00	3060.00	0.0	3.88	17.93	+
9962	79/C2	08/13/91	8DS	0.913	0.00	407.00	0.00	0.00	843.00	0.00	0.00	2930.00	0.00	0.00	0.00	-
9969	79	06/18/91	6L5	0.540	110.00	536.00	4.87	247.00	923.00	3.73	0.00	2960.00	0.00	2.17 >	11.87	+
9969	79/C2	08/13/91	8DU	1.002	1340.0	166.00	0.12	1790.00	258.00	0.14	3020.0	897.00	0.30	0.09 >	5.04	+
9970	79	06/18/91	6L7	0.621	58.70	513.00	8.75	107.00	706.00	6.61	287.00	2280.00	7.96	4.80	25.21	+
9970	79/C2	08/13/91	8DU	1.002	134.00	490.00	3.66	179.00	660.00	3.69	302.00	966.00	3.20	2.74	15.02	+
9971	79	06/18/91	6L7	0.621	139.00	457.00	3.29	261.00	638.00	2.44	0.00	964.00	0.00	1.75 >	10.29	+
9971	79/C2	08/13/91	8DV	1.008	320.00	463.00	1.45	0.00	642.00	0.00	0.00	964.00	0.00	0.00	2.36	+
10011	79	07/02/91	6T8	1.054	389.00	1590.0	4.09	779.00	2180.00	2.80	0.00 >	3200.00	0.00	2.04 >	14.62	+
10011	79/C2	08/13/91	8DY	0.918	420.00	1370.0	3.25	566.00	1980.00	3.50	965.00	3080.00	3.19	2.42 >	14.20	+
10030	79	07/03/91	6VB	1.033	26.90	245.00	9.11	45.30	452.00	9.96	92.40	945.00	10.23	5.40 >	29.12	+
10030	79/C2	08/13/91	8DZ	0.818	49.40	100.00	2.02	97.30	183.00	1.88	0.00	622.00	0.00	1.03	4.97	+
10031	79	07/03/91	6VB	1.033	118.00	1530.0	12.99	179.00	2090.00	11.68	1000.0	3090.00	3.09	8.57 >	33.14	+
10031	79/C2	08/13/91	8E0	0.942	194.00	459.00	2.37	0.00	677.00	0.00	0.00 >	3200.00	0.00	0.00	4.52	+
10047	79	07/03/91	6VJ	1.036	23.50	103.00	4.37	43.40	175.00	4.04	0.00	306.00	0.00	2.37 >	14.84	+
10047	79/C2	08/13/91	8E0	0.942	48.60	124.00	2.54	78.10	189.00	2.42	0.00	307.00	0.00	1.58	6.78	+
11043	78	04/02/91	4EH	1.112	0.00	4700.0	0.00	0.00	6490.00	0.00	0.00	9710.00	0.00	0.00	2.31	-
11043	78	10/10/91	AKW	0.592	46.80	1280.0	27.35	787.00	2150.00	2.74	0.00	8300.00	0.00	1.63 >	19.75	+
11047	78	04/04/91	4H8	0.634	2110.0	2720.0	1.29	0.00	6600.00	0.00	0.00 >	10000.0	0.00	0.00	0.78	+
11047	78	10/10/91	AKX	0.568	1040.0	4900.0	4.71	2840.00	6600.00	2.33	0.00	9660.00	0.00	1.73 >	16.83	+
11048	78	04/04/91	4H8	0.634	510.00	1240.0	2.42	814.00	2720.00	3.35	0.00	9190.00	0.00	1.52	5.71	+
11048	78	10/10/91	AKY	0.572	0.00	405.00	0.00	0.00	936.00	0.00	0.00	2970.00	0.00	0.00	0.00	-
11060	78	04/04/91	4HE	0.641	215.00	1410.0	6.56	473.00	2020.00	4.26	0.00	3100.00	0.00	2.98	16.67	+
11060	78	10/10/91	AKY	0.572 <	32.00	217.00 >	6.79	0.00	703.00	0.00	0.00	2890.00	0.00	0.00 >	8.61	+
11062	78	04/04/91	4HF	0.649	162.00	1110.0	6.85	581.00	1810.00	3.11	0.00	3060.00	0.00	1.91	15.80	+
11062	78	10/10/91	AKZ	0.617	588.00	547.00	0.93	0.00	1400.00	0.00	0.00	3020.00	0.00	0.00	0.25	+
11076	78	04/24/91	4ZC	1.006	2000.0 >	3200.0 >	1.60	0.00 >	3200.00	0.00	0.00 >	3200.00	0.00	0.00 >	1.17	+
11076	78	10/10/91	AL1	0.609	422.00	4900.0	11.61	912.00	6600.00	7.24	2830.0	9660.00	3.42	5.37 >	25.89	+
11095	78	04/24/91	401	1.053	5480.0	5750.0	1.05	0.00	10000.0	0.00	0.00 >	10000.0	0.00	0.00 >	3.24	+
11095	78	10/10/91	AGG	0.524	398.00	7450.0	18.74	3200.00 >	10000.0 >	3.13	0.00 >	10000.0	0.00	2.33 >	26.97	+
11122	78	04/16/91	4Q3	1.222	402.00	1550.0	3.86	646.00	2100.00	3.25	0.00	3090.00	0.00	2.40 >	15.97	+
11122	78	10/15/91	AMY	0.572	273.00	1550.0	5.69	622.00	2100.00	3.38	0.00	3090.00	0.00	2.49	14.69	+
11123	78	04/16/91	4Q4	1.282	1240.0	2990.0	2.42	2390.00 >	10000.0 >	4.18	0.00 >	10000.0	0.00	1.25 >	9.47	+
11123	78	10/15/91	AMZ	0.531	566.00	5640.0	9.98	1000.00	8300.00	8.30	0.00 >	10000.0	0.00	5.64	22.47	+

Table 24 (Cont'd)

AVS No.	Shipment	Test Date	Plt #	Diff.	IC 25	TC 25	AI 25	IC 50	TC 50	AI 50	IC 95	TC 95	AI 95	SI	TAI	ACT
11131	78	04/16/91	408	1.210	468.00	1300.0	2.79	1790.00	1940.00	1.08	0.00	3070.00	0.00	0.73	4.73	+
11131	78	10/15/91	AN0	0.575	68.40	1820.0	26.62	266.00	2640.00	9.94	0.00	8970.00	0.00	6.85 >	35	+
11133	78	04/16/91	409	1.196	461.00	1920.0	4.17	815.00	2920.00	3.58	0.00	9210.00	0.00	2.36 >	11.91	+
11133	78	10/15/91	AN0	0.575	0.00	1730.0	0.00	0.00	2650.00	0.00	0.00	9030.00	0.00	0.00	0.10	-
11134	78	04/16/91	409	1.196	339.00	909.00	2.68	486.00	1690.00	3.48	930.00	3050.00	3.28	1.87	11.52	+
11134	78	10/15/91	AN1	0.566	217.00	1550.0	7.14	438.00	2100.00	4.80	921.00	3090.00	3.36	3.54 >	24.10	+
11185	80	07/09/91	6ZR	1.165	143.00	458.00	3.20	205.00	649.00	3.16	0.00	992.00	0.00	2.23	9.96	+
11185	80	10/10/91	AG7	0.556	118.00	767.00	6.52	211.00 >	1000.00 >	4.75	0.00 >	1000.00	0.00	3.65	14.82	+
11186	80	07/09/91	6ZR	1.165	0.00	146.00	0.00	0.00	206.00	0.00	0.00	315.00	0.00	0.00	0.39	-
11186	80	10/10/91	AG7	0.556	116.00	414.00	3.58	194.00	609.00	3.13	0.00	961.00	0.00	2.13	8.47	+
11188	80	07/09/91	6ZS	1.164	50.70	111.00	2.20	80.20	183.00	2.28	0.00	311.00	0.00	1.39	5.10	+
11188	80	10/10/91	AG8	0.573	22.10	165.00	7.45	54.10	229.00	4.24	0.00 >	1000.00	0.00	3.04 >	16.72	+
11190	80	07/09/91	6ZT	1.242	1400.0	2100.0	1.50	2670.00 >	3200.00 >	1.20	0.00 >	3200.00	0.00	0.79 >	2.21	+
11190	80	10/10/91	AG8	0.573	150.00 >	1000.0 >	6.66	580.00 >	1000.00 >	1.72	0.00 >	1000.00	0.00 >	1.72 >	15.60	+
11447	81	07/30/91	7S0	1.033	128.00	356.00	2.77	256.00	803.00	3.13	0.00 >	1000.00	0.00	1.39	7.85	+
11447	82	08/14/91	BGF	0.895	207.00	278.00	1.34	0.00	891.00	0.00	0.00	2960.00	0.00	0.00 >	1.17	+
11678	81	07/16/91	78C	1.103	211.00 >	320.00 >	1.52	0.00 >	320.00	0.00	0.00 >	320.00	0.00	0.00 >	2.22	+
11678	81	10/10/91	AGC	0.460	44.00	628.00	14.26	320.00	949.00	2.96	0.00 >	1000.00	0.00	1.96	18.36	+
11679	81	07/10/91	74K	0.971 <	1.00	10.00 >	10.00	1.07	17.40	16.25	0.00	30.80	0.00	9.32 >	22.83	+
11679	81	10/10/91	AGC	0.460 <	0.10	12.60 >	125.88 <	0.10	19.10 >	190.59	0.00	30.70	0.00 >	125.88 >	45.59	+
11680	81	07/10/91	74L	0.981	102.00 >	320.00 >	3.13	173.00 >	320.00 >	1.85	0.00 >	320.00	0.00 >	1.85 >	12.36	+
11680	81	10/10/91	AGD	0.572	779.00	1470.0	1.89	0.00	2780.00	0.00	0.00	9190.00	0.00	0.00	2.00	+
11681	81	07/10/91	74L	0.981	0.00 >	320.00	C.00	0.00 >	320.00	0.00	0.00 >	320.00	0.0	0.00 >	0.30	-
11681	81	10/10/91	AGD	0.572 <	32.00	2060.0 >	64.25	172.00	3160.00	18.39	0.00	9310.00	0.00	11.98 >	36.17	+

DIFFNTL = The differential is the difference in the cell control and the virus control optical densities.

$IC_{25,50,95}$ = (Viral) inhibitory concentration 25%, 50% and 95% = The drug concentration (µg/ml) that inhibited viral CPE by 25%, 50% or 95% calculated by using a regression analysis for semilog curve fitting.

$TC_{25,50,95}$ = (Cell) toxicity concentration 25%, 50% and 95% = The drug concentration (µg/ml) that reduced cell viability by 25%, 50% or 95%.

$AI_{25,50,95}$ = Antiviral Index = A single point ratio of the antiviral and anticellular effect of the compound, calculated with 25%, 50% or 95% reduction values (calculated by dividing the $TC_{25,50,95}$ by the $IC_{25,50,95}$).

SI = Selectivity Index = A ratio calculated by dividing the TC_{25} by the IC_{50} (based upon 6 one-half-log_{10} dilutions, µg/ml, the maximum scale is 0-320).

TAI = Total Antiviral Index = The area between the cytotoxicity and the antiviral curves (based upon a scale of 0-100%).

ACT = Activity = A "+" denotes a rest that produced ≥25% reduction in CPE. A "-" denotes an inactive test (i.e. ≤25% reduction in CPE).

4.3.7 Antiviral Screening *in Vitro*: HIV-1 Virus (Primary Protocol):

During this ten-month reporting period from February 1, 1991 through December 31, 1991, a total of 409 primary screen test compounds were received at SRI for evaluation in the HIV-1 *in vitro* antiviral screen. A cumulative summary of the compound shipments received, and the number of compounds in each shipment, are presented below (Table 25).

Table 25

HIV Antiviral Screen
Number of Compounds Received Per Shipment Through December 31, 1991

Shipment	Date Received	No. of Compounds
RS-1	03/07/91	12
79	04/11/91	381
81	06/26/91	8
82	08/23/91	6
82	09/05/91	2

Total = 409

Approximately 856 *in vitro* antiviral assays were performed in CEM and/or MT-2 cells during this contract period. Thirty-five compounds were found to be active (i.e. reduction in CPE of $\geq$50%) against HIV (Table 26).

Positive control drugs, AZT and ddC, were tested in each experiment. The degree of activity of these positive control compounds were used to assess the quality of the assays as to acceptable or not acceptable.

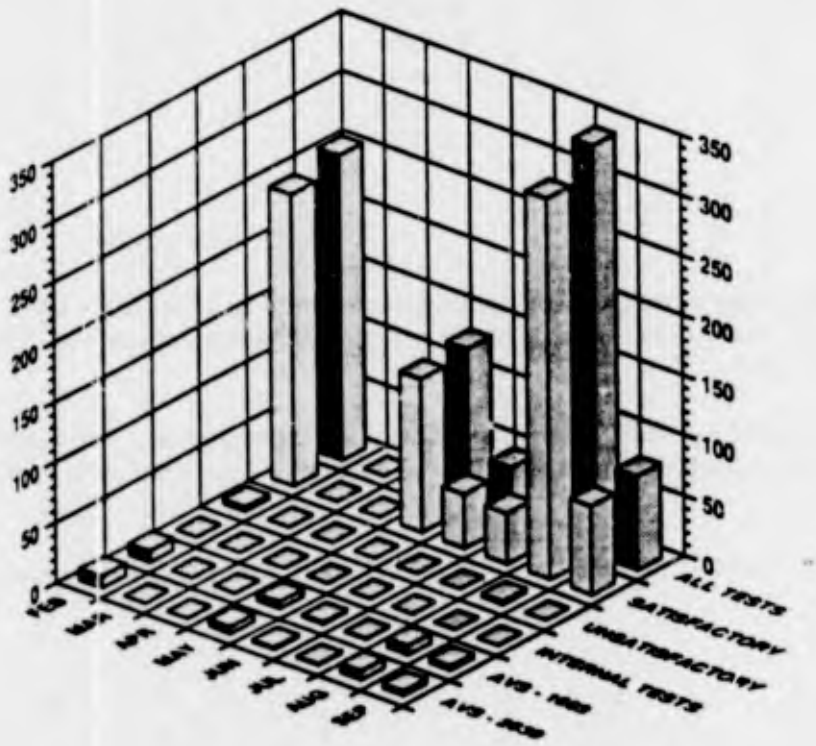
A cumulative summary presenting all of the satisfactory HIV testing during this reporting period is in Appendix C.

4.3.7.1 **Human Immunodeficiency Virus (HIV-1):**

During the time period covered by this report, compounds were screened for activity against HIV-1 in CEM and MT-2 cell cultures using an MTT-inhibition assay procedure.

A total of 856 assays were reported (787 excluding positive control drug testing); **Figure 64** shows the number of compounds evaluated each month for this ten-month period.

TOTAL NUMBER OF TESTS AGAINST
HUMAN IMMUNODEFICIENCY VIRUS (HIV-1)



MONTH

Figure 64

Quality Control Tests =	KPC1 = Positive Control, AZT =	31
	KPC2 = Positive Control, ddC =	31
	Unsatisfactory Tests =	7
Accepted Single Drug Tests =		787
Total Number of HIV-1 Tests =		856

4.3.7.2 Quality Control:

Two compounds were utilized as positive control drugs, AZT (AVS-1503) and ddC (AVS-2639). Both of these compounds were run in each experiment. If the positive control was not active (i.e. at least 50% CPE inhibition observed) in a given experiment, then the entire test was considered unsatisfactory and all compounds tested in parallel with this positive control drug were repeated. AZT and ddC continued to be reliable positive control drugs.

The following indices summarize the AZT data:

AZT:CEM Cells	IC_{50} µg/ml	TC_{25} µg/ml	SI	TAI
Mean	0.003	0.91	1416	56
S.D.	0.004	0.23	2014	18
Median	0.001	1.0	497	52

AZT:MT-2 Cells	IC_{50} µg/ml	TC_{25} µg/ml	SI	TAI
Mean	0.032	3.61	110	73
S.D.	0.007	2.26	73	11
Median	0.03	2.98	93	71

The following indices summarize the data for ddC:

ddC:CEM Cells	IC_{50} µg/ml	TC_{25} µg/ml	SI	TAI
Mean	0.03	1.39	180	30
S.D.	0.015	1.92	381	17
Median	0.06	0.39	35	28

ddC:MT-2 Cells	IC_{50} µg/ml	TC_{25} µg/ml	SI	TAI
Mean	0.19	2.65	20	42
S.D.	0.19	2.71	18	16
Median	0.13	1.69	18	44

The assays indicate that the positive control drugs inhibited CPE caused by the HIV virus. The magnitude of inhibition fell within acceptable distribution based on our historical experience with these compounds.

4.3.7.3 **HIV-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (CEM Cell Line):**

HIV-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 644 plates is plotted in **Figure 65-A**. The results indicate that the cell O.D. readings reached a mean 1.285 (SD ± 0.354) with a median of 1.219 (range of 0.627 - 2.231). This indicates that a uniform and equal number of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

HIV-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of 644 plates is presented in **Figure 66-A**. The results indicate that the average virus load O.D. reading is 0.131 (SD ± 0.097) with a median of 0.112 (range of -0.006 - 0.587). This demonstrates that reasonable cell destruction is taking place and a uniform load of virus is administered on the cell monolayer with consistent viral CPE results.

HIV-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of 644 plates is provided in **Figure 67-A**. The results indicate that the average differential O.D. reading is 1.154 (SD ± 0.343) with a median of 1.077 (range 0.491 - 2.035). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation.

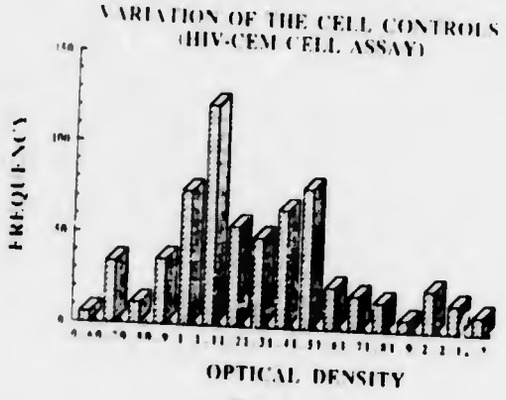


Figure 65-A

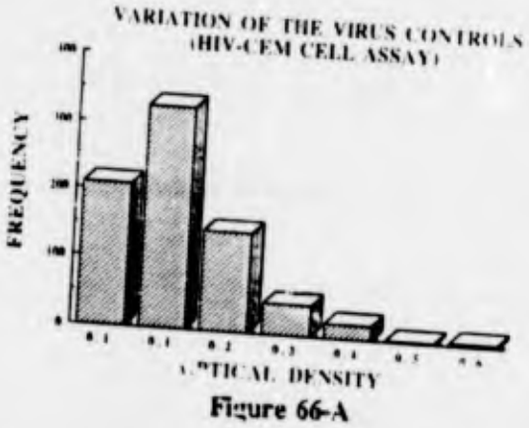


Figure 66-A

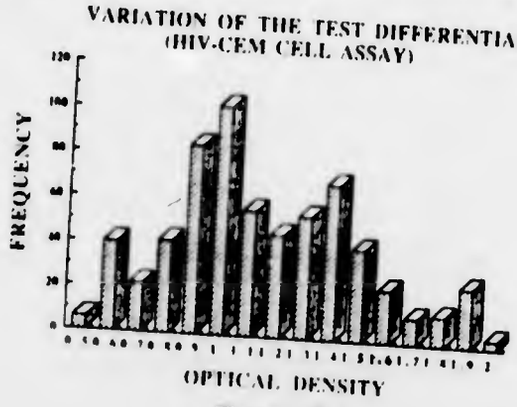


Figure 67-A

4.3.7.4 **HIV-Assay Plate Quality Controls: Cell Load and Virus Load Parameters (MT-2 Cell Line):**

HIV-Control Compound-Cell Load Performance: A bar graph scatter plot of the mean cell control (O.D. reading) of 205 plates is plotted in **Figure 65-B**. The results indicate that the cell O.D. readings reached a mean 1.393 (SD ± 0.177) with a median of 1.454 (range of 0.94 - 1.546). This indicates that a uniform and equal number of cells are being loaded into every well in the 96-well plate during the day-to-day operation. The cells reduced MTT to formazan giving maximum blue color uniformly and consistently.

HIV-Control Compound-Virus Load Performance: A bar graph scatter plot of the mean virus load O.D. readings of 205 plates is presented in **Figure 66-B**. The results indicate that the average virus load O.D. reading is 0.139 (SD ± 0.063) with a median of 0.151 (range of -0.016 - 0.258). This demonstrates that reasonable cell destruction is taking place and a uniform load of virus is administered on the cell monolayer with consistent viral CPE results.

HIV-Control Compound-Assay Differential Performance: A bar graph scatter plot of the mean O.D. differential values of 205 plates is provided in **Figure 67-B**. The results indicate that the average differential O.D. reading is 1.259 (SD ± 0.143) with a median of 1.301 (range 0.907 - 1.473). The single bell-shaped curve is reasonably sharp and uniform. This reflects that the assays are executed consistently and are repeatable during day-to-day operation.

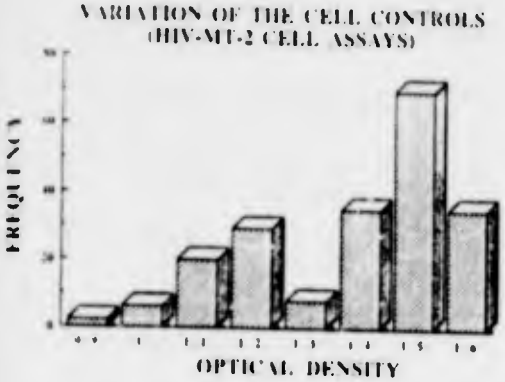


Figure 65-B

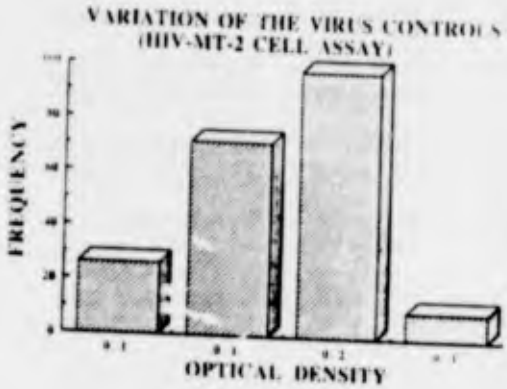


Figure 66-B

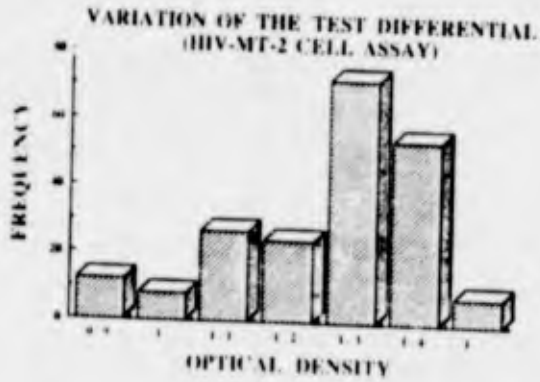


Figure 67-B

Results on compounds that suggested activity (i.e., a selectivity index (SI) of ≥ 1) are listed in Table 26 according to activity based on the SI. The IC_{50} is the minimum drug concentration (μg/ml) that inhibited CPE by 50% and was calculated by using a regression analysis program for semilog curve fitting. TC_{25} is the minimum drug concentration (μg/ml) that reduced cell viability by 25%. The SI is the selectivity index and is calculated by dividing the TC_{25} by the ID_{50}. TAI is the total antiviral index or the area between t he cytotoxicity and the antiviral curves. DIFRNTL is the abbreviation for the differential. The differential is the difference in the cell control and the virus control optical densities. A differential value of >0.5 is required for the assay to be considered satisfactory.

Table 26

AVS Compounds Most Active Against HIV-1 From the Primary Screen

AVS No.	Ship-ment	Plt #	Cell Line	Test Date	Diff-rntl.	IC 50	TC 25	SI	TAI
11912	82	2VK	CEM	09/27/91	0.884 <	0.32 >	100.00 >	312.50 >	58.00
4611	65	4CR	CEM	02/20/9_	3.724	0.61 >	100.00 >	163.56 >	83.70
4611	65	4CG	MT2	02/20/91	1.359	0.37	29.00	78.79 >	65.94
AM612	25A	49O	CEM	02/05/91	1.380 <	3.20	408.00 >	127.50 >	75.27
AM612	25A	496	MT2	02/05/91	1.110 <	3.20	64.60 >	20.20 >	48.26
AM612	25A	496	MT2	02/05/91	1.110	0.06 >	1.00 >	15.93 >	44.63
AM612	25A	49O	CEM	02/05/91	1.380	0.19 >	1.00 >	5.34 >	23.17
11933	82	2VL	CEM	09/27/91	0.845	1.95 >	100.00 >	51.33 >	69.02
5025	65	4CH	MT2	02/20/91	1.452	2.21	79.90	36.21 >	57.36
5025	65	4CS	CEM	02/20/91	0.803	7.90	7.73	0.98 >	33.75
4075	65	4CQ	CEM	02/20/91	0.779	3.73 >	100.00 >	26.84 >	49.66
4075	65	4CF	MT2	02/20/91	1.356	1.45	36.40	25.13 >	46.22
9827	79	4YW	CEM	08/23/91	0.941	0.20	3.70	18.73	44.98
9827	79	4XE	CEM	08/16/91	1.044 <	0.32	4.80 >	14.99 >	41.28
8334	76	4D2	MT2	02/21/91	1.252	5.61 >	100.00 >	17.83 >	45.67
8334	76	4DG	CEM	02/21/91	0.851	28.50	7.45	0.26 >	10.63
8375	76	499	MT2	02/05/91	1.080	5.00	74.90	14.99 >	44.86
8375	76	49R	CEM	02/05/91	1.306	11.10 >	100.00 >	9.02 >	35.27
8519	76	4AK	MT2	02/08/91	1.287	39 >	100.00 >	13.53 >	38.09
8519	76	4AU	CEM	02/08/91	0.887	39.90 >	100.00 >	2.51 >	19.94
AM242	25A	49P	CEM	02/05/91	1.317 <	3.20	42.80 >	13.38 >	37.12
AM242	25A	497	MT2	02/05/91	1.097 <	3.20 <	3.20 -	1.00	5.87
11916	82	2SZ	CEM	09/12/91	1.238	7.61 >	100.00 >	13.15 >	34.39
8720	27A	4D8	CEM	02/21/91	0.833	6.52	67.40	10.34	34.53
1315	67	4CD	MT2	02/20/91	1.446	10.00 >	100.00 >	10.00 >	31.19
8505	76	4AA	MT2	02/08/91	1.335	12.00 >	100.00 >	8.35 >	28.81
0709	68	4CD	MT2	02/20/91	1.446	13.70 >	100.00 >	7.29 >	33.98
0709	68	4CO	CEM	02/20/91	0.715	22.60 >	100.00 >	4.43 >	27.98
11932	82	2VK	CEM	09/27/91	0.884	0.92	6.42	6.99	15.15

Table 26 (Cont'd)

AVS No.	Shipment	Plt #	Cell Line	Test Date	Differntl.	IC 50	TC 25	SI	TAI
8514	76	4AR	CEM	02/08/91	1.016	15.00 >	100.00 >	6.69 >	31.18
8514	76	4AE	MT2	02/08/91	1.309	6.42	12.20	1.89	14.13
9698	79	4UK	CEM	08/06/91	0.565	8.21	49.00	5.97	20.43
0702	68	4CC	MT2	02/20/91	1.443	17.30 >	100.00 >	5.77 >	32.24
0702	68	4CN	CEM	02/20/91	0.787	20.10	0.32	0.02 >	26.25
9679	79	4UA	CEM	08/06/91	0.582	3.41	19.30	5.66	30.85
8380	76	49C	MT2	02/05/91	1.105	11.10	57.20	5.14 >	29.95
8380	76	49U	CEM	02/05/91	1.287	21.60	21.00	0.97 >	10.26
11934	82	2VL	CEM	09/27/91	0.845	10.90	53.60	4.90	18.79
4036	65	4CQ	CEM	02/20/91	0.779	16.00	58.30	3.64 >	18.43
4036	65	4CF	MT2	02/20/91	1.356	14.30	7.91	0.55	9.01
8815	27A	4DB	CEM	02/21/91	0.760	28.10	97.00	3.45 >	13.51
8815	27A	4DO	MT2	02/21/91	1.272	42.20 >	100.00 >	2.37 >	15.95
8382	76	49D	MT2	02/05/91	1.157	31.00 >	100.00 >	3.23 >	17.39
8722	27A	4D9	CEM	02/21/91	0.911	39.60 >	100.00 >	2.52 >	11.42
8378	76	49T	CEM	02/05/91	1.216	20.60	51.10	2.48	15.11
8372	76	498	MT2	02/05/91	1.127	43.30 >	100.00 >	2.31 >	18.06
8372	76	49Q	CEM	02/05/91	1.326	61.40	78.60	1.28 >	6.49
8336	76	4D3	MT2	02/21/91	1.330	45.20 >	100.00 >	2.21 >	14.26
8339	76	4D4	MT2	02/21/91	1.193	46.80 >	100.00 >	2.14 >	19.13
6196	74	4CK	MT2	02/20/91	1.429	52.70 >	100.00 >	1.90 >	12.14
6196	74	4CV	CEM	02/20/91	0.755	78.30	100.00	1.28 >	2.66
8386	76	49E	MT2	02/05/91	0.907	72.20 >	100.00 >	1.38 >	17.28
8335	76	4DG	CEM	02/21/91	0.851	71.60	93.80	1.31 >	3.55
8335	76	4D2	MT2	02/21/91	1.252	6.43	1.00	0.16 >	37.82
8397	76	4A1	CEM	02/05/91	0.900	100.00 >	100.00 >	1.00 >	6.07

4.4 Secondary Antiviral Testing:

SRI-USAMRIID Staff under the priorities set by the Sponsor, has performed secondary *in vivo* antiviral studies as well as *in vitro* cell culture work in the P3/P4 antiviral containment facilities located at Ft. Detrick.

As criteria for *in vivo* antiviral work, several antiviral studies were conducted using selected representatives of the following virus families: a) Filoviridae (Ebola and Marburg), b) Arenaviridae (Guanarito, Junin and Pichinde) and c) Bunyaviridae (Hantaan).

4.4.1. Background for Filoviridae Work

The filoviruses, Ebola and Marburg, represent a serious health threat not only to the indigenous population of West Africa but also to handlers of primates imported from these areas. Marburg virus was first identified in an outbreak during 1967 in which 7 deaths occurred among 31 laboratory workers in Germany and Yugoslavia and the source of infection was traced to direct contact with tissues from African green monkeys caught in Uganda. Outbreak of Ebola in Sudan and Zaire in 1976 resulted in over 500 cases with 400 deaths. Ebola and Marburg are clinically indistinguishable. They cause the most severe viral hemorrhagic fever known with disseminated intravascular coagulopathy (DIC) as a major clinical feature. Morphology of Ebola and Marburg are unique. The filamentous particles range from 130 to 14,000 nm in length but are more uniform in diameter (80 nm). Nucleocapsid is surrounded with a lipid envelope and contains a single-stranded RNA. Five different polypeptides are associated with the virion. Ebola and Marburg are serologically distinct.

4.4.1.1. Previous Antiviral Work with Filoviruses (*in vitro* and *in vivo*)

Ebola virus is not significantly inhibited by Ribavirin *in vitro* at least not in Vero, MRC-5 or FRL-103 cells. Also, Ribavirin prophylaxis (20 mg/kg) of Ebola in an immunosuppressed guinea pig model has been ineffective. Ribavirin prophylaxis in cynomolgus monkeys (an excellent model for human disease) has been ineffective when evaluated by three independent laboratories.

Thus, we have focused our attention to other possible drugs to prevent Ebola virus replication. The initial step in Anti-Filovirus drug discovery program was to establish proper *in vitro* antiviral screens and *in vivo* animal model systems, which we have completed. Secondly, to select compounds for initial testing based on morphological similarities (selected 20 VSV actives). Also 60 candidate compounds were selected from 8500 compounds tested in our large scale antiviral screening program. Several other compounds (28) were selected based upon literature search.

The best candidate compounds against Ebola from these studies as well as from literature and database searches proved to be S-AdoHcy Hydrolase Inhibitors (AVS-0303, 1654, 1655, 4275 and 1549, **Figure 68-A**). This lead to the probable mode of action hypothesis to be tested *in vitro* and *in vivo*.

S-Adenosylhomocysteine Hydrolase inhibitors are effective *in vitro* and *in vivo* against VSV, vaccinia and parainfluenza, tick-borne encephalitis, sindbis, but not HSV-1 or HSV-2. Compounds inhibit the cellular enzyme S-Adenosylhomocysteine (AdoHcy) hydrolase with accumulation of AdoHcy. AdoHcy is a competitive inhibitor of all known methyltransferases, including AdoMet-dependent methylation of the 5'-cap of some viral mRNA's. Inhibitors mimic the transition state intermediate and activity can be predicted by their inhibition constant (K_i) (**Figure 68-B**).

The method for *in vitro* antiviral testing of S-Adenosylhomocysteine Hydrolase inhibitors against Ebola is displayed in **Figure 69**.

S-Adenosylhomocysteine Hydrolase Inhibitors

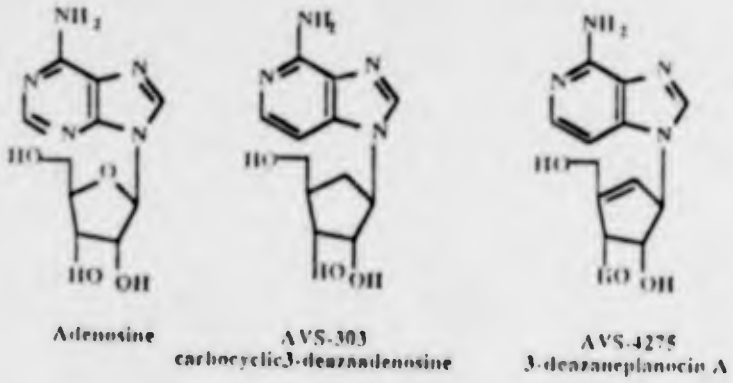


Figure 68-A

Probable Mechanism of Anti-Filovirus Activity of S-Adenosylhomocysteine Hydrolase Inhibitors

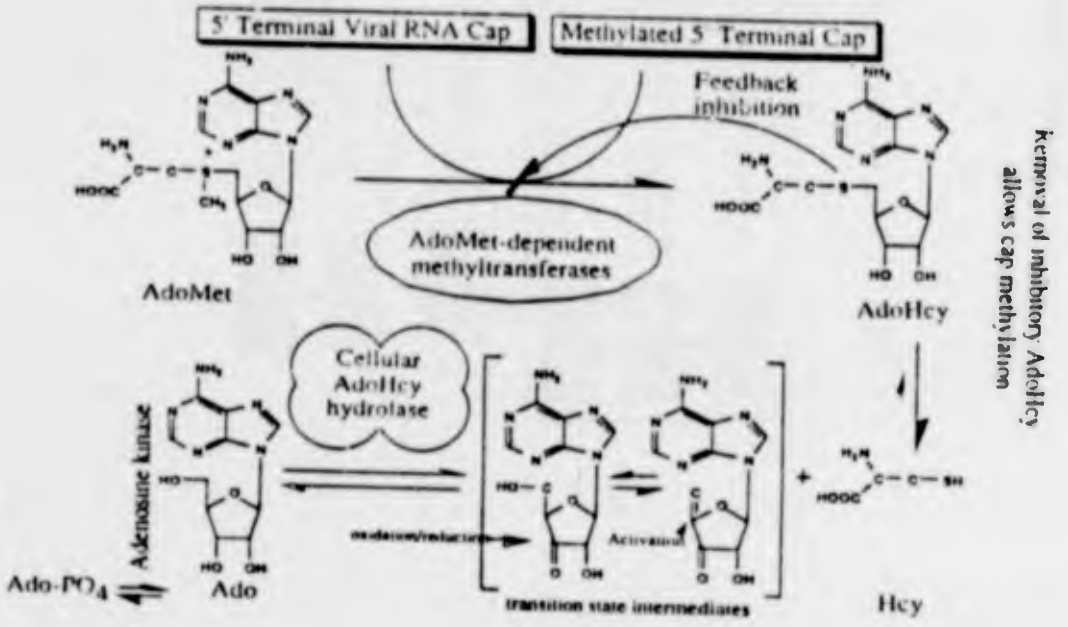


Figure 68-B

Methods for *In Vitro* Antiviral Testing of S-Adenosylhomocysteine Hydrolase Inhibitors Against Ebola

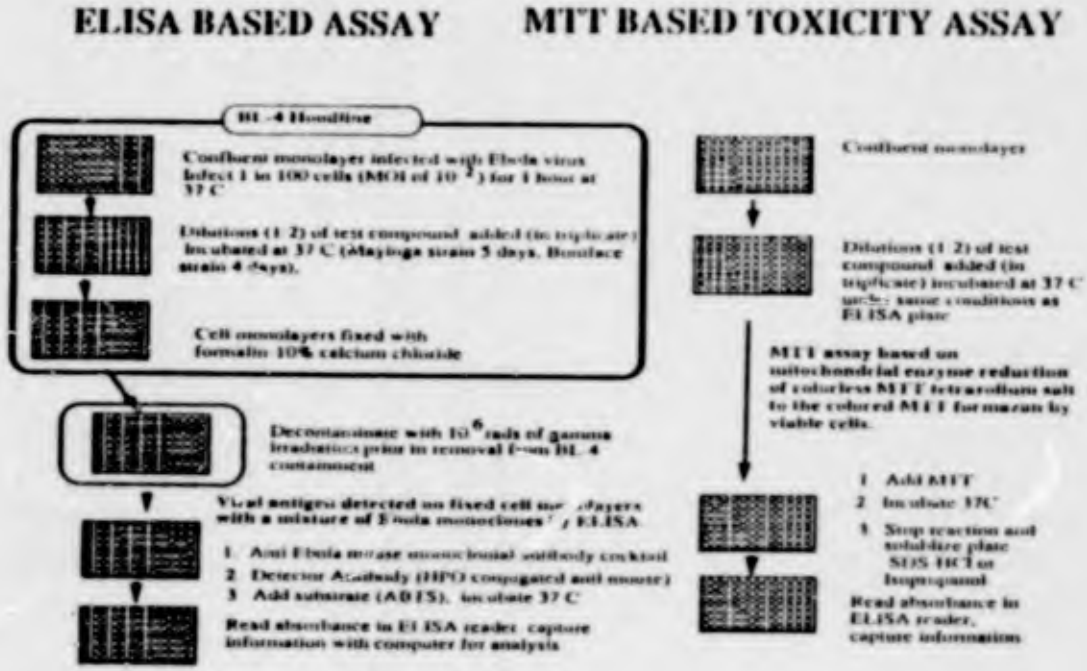


Figure 69

Results of antiviral and toxicity *in vitro* studies are displayed in Figure 70-A, Figure 70-B and Figure 70-C.

Inhibition by S-Adenosylhomocysteine Hydrolase Inhibitors of Ebola (Mayinga Strain) Replication in Vero E6 Cells

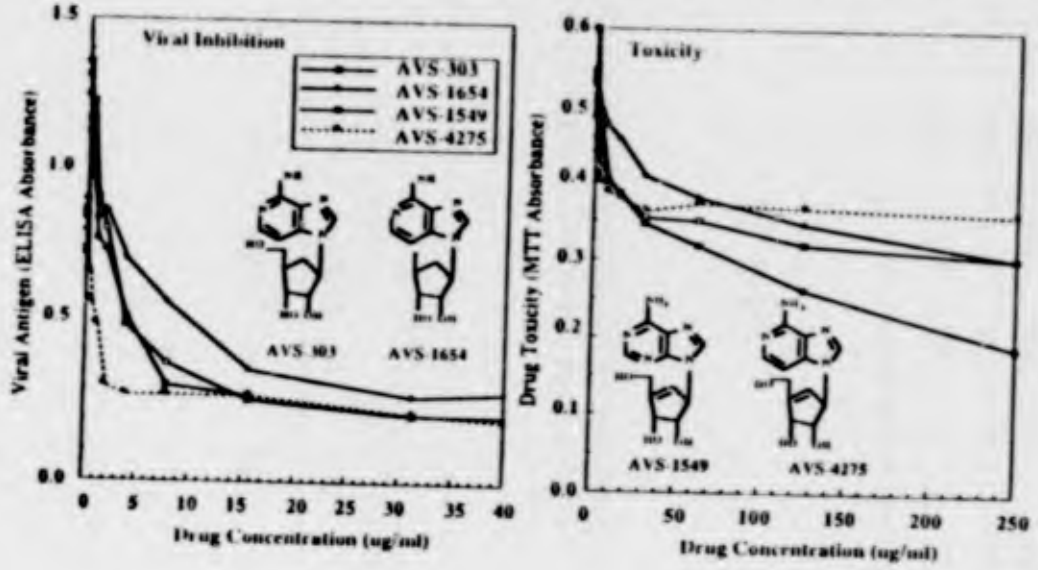


Figure 70-A

Cytotoxicity of SAH Hydrolase inhibitors

50% Effective Concentration (ug/ml)

Compound	PRK cells CPE	PRK cells Protein Synthesis	HeLa cells CPE	HeLa cells Protein Synthesis	Vero cells CPE	Vero cells Protein Synthesis	SAH Hydrolase *Ki*
AVS-303	> 400	88	>400	103	≥400	21	4 nm
AVS-1549	≥ 400	2.7	40	1.1	≥10	0.4	8 nm
AVS-4275	> 400	117	>400	≥129	>400	0.3	0.05 nm

From De Clercq et al. (1989) Antimicrob. Agents Chemother 33:1291-1297.

Figure 70-B

Comparison of Inhibitory Dose 50% (ug/ml) By Various Antiviral Assay Formats

AVS	Ebola Boniface Strain ELISA	Ebola Boniface Strain CPE	Ebola Boniface Strain PR	Ebola Mayinga Strain ELISA	Ebola Mayinga Strain CPE	Ebola Mayinga Strain PR	Vero E6 Cytotoxicity CPE	Vero E6 Cytotoxicity MTT
Ribavirin	>500	>500	>500	>500	>500	>500	500	500
303	4	2	8	2	4	12	>250	>250
1549	4	1.5	1	3	1	2	8	2
4275	0.3	1	0.5	0.5	0.5	1	125	125

Figure 70-C

Cumulative summary of the *in vitro* antiviral inhibitions results for AVS-303, AVS-1654, AVS-1655, AVS-4275 and AVS-1549 compounds against several Viral Hemorrhagic Fever Viruses (different cell lines) are summarized in Table 27.

Table 27

In Vitro Inhibition of Viral Hemorrhagic Fever Viruses by S-Adenosylhomocysteine Hyrdolase Inhibitors

		AVS 303		AVS 1654		AVS 1655		AVS 4275		AVS 1549	
Virus	Cell Line	MTC	MIC_{50}	MTC	MIC_{50}	MTC	MIC_{50}	MTC	MIC_{50}	MTC	MIC_{50}
Filoviridae											
Ebola	Vero E 6	64	16	125	32	>250	>50	32	1	16	2
Alphaviridae											
Venzuelan Equine Encephalitis	Vero	>250	NA	>250	NA	>250	NA	>250	NA	>250	NA
Flaviviridae											
Dengue 4	MK 2	>250	125	>250	NA	>250	NA	>250	NA	25	12 5
Yellow Fever	Vero	>250	100	>250	NA	>250	NA	>250	NA	250	NA
Japanese Encephalitis	Vero	>250	NA	>250	NA	>250	NA	>250	NA	250	NT
Bunyaviridae											
RFV	Vero	125	>125	>250	NA	>250	NA	>250	NA	25	12 5
Hantaan	Vero E 6	125	16	125	>125	>250	NA	64	4	12 5	6 4
CCHF	SW 13	>250	64	>250	NA	>250	NA	>250	2	250	6 4
Arenaviridae											
Lasse(Ar)	Vero	>250	>250	>250	>NA	>250	NA	>250	125	25	12 5
Lasse(Mo)	Vero	>250	>250	>250	NA	>250	NA	>250	125	25	12 5
Junin	Vero	>250	48	>250	64	>250	NA	>250	48	25	25
Machupo	Vero	>250	>250	250	>NA	>250	NA	>250	125	25	25 50
VHF	Vero	>250	>250	>250	125	>250	NA	>250	48	25	3 2 6 4
Amapari	Vero	>250	>250	>250	125	>250	NA	>250	64	25	25
Pichinde	Vero	>250	NA	>250	NA	>250	NA	NT	NT	NT	NT

NA= Not Acuve
NT=Not Tested

Ebola (Mayinga) infection in a series of inbred mouse strains were evaluated for their ability to cause lethal disease. Out of five different mouse strains, (C3H/HeJ, CBA/N, A/J, SCID cd-17 and Beige) only SCID cd-17 produced lethal disease. Clinical features consistent with wasting syndrome was observed and several of the histopathology findings were consistent with primate and human pathology. A lethal Model (100%) in adult SCID (>4 weeks) of either sex with mean time to death of 25 - 35 days, is dependent on the challenge virus dose. This SCID Mouse Model is useful for initial drug evaluation studies based on virus reduction in tissues and increased mean time to death. Virus distribution in SCID mice infected with 1000 pfu of Ebola are presented in **Figure 71**.

Virus Distribution in SCID Mice infected with 1000 pfu of Ebola

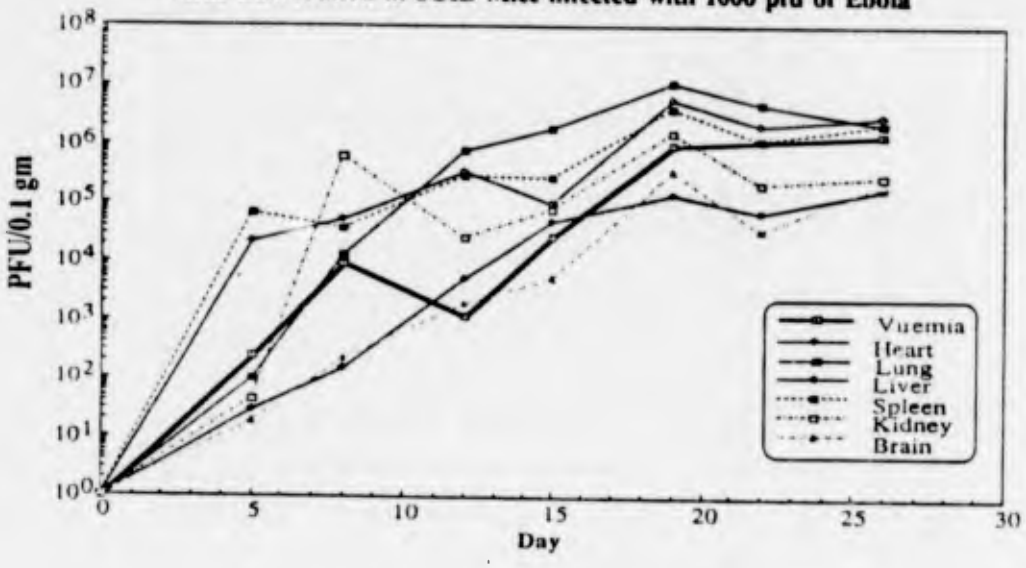
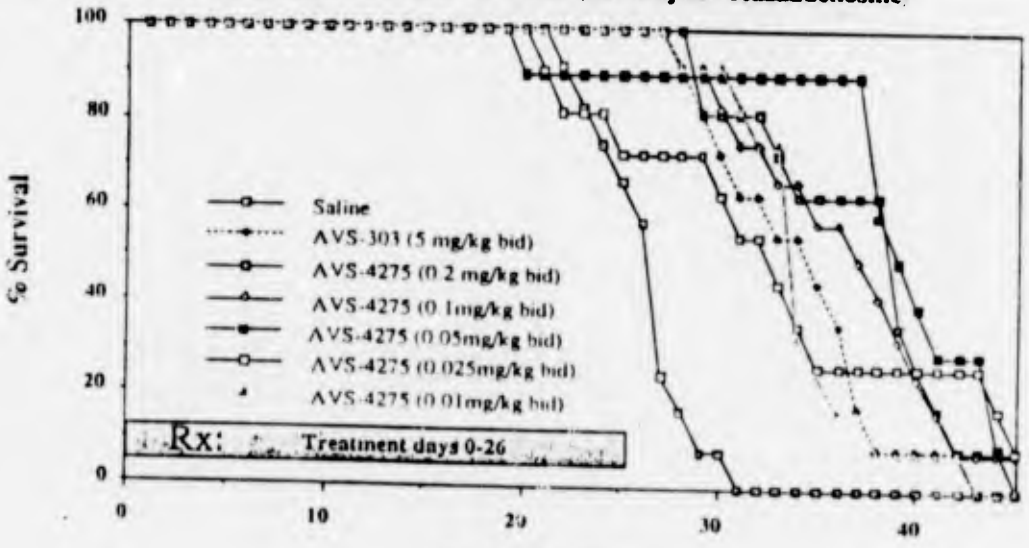


Figure 71

Antiviral survival of Ebola infected SCID mice prophylaxed with AVS-4275 (3-deazaneplanocin A) or AVS-303 (carbocyclic deazaadenosine) are shown in **Figure 72**.

Survival of Ebola Infected SCID Mice Prophylaxed with AVS-4275 (3-deazaneplanocin A) or AVS-303 (Carbocyclic deazaadenosine)



Day Post Infection

Figure 72

Survival of Ebola and inhibition of Viremia in Ebola infected SCID mice treated with AVS-0303 (carbocyclic 3-deazaadenosine) are displayed in **Figure 73-A** and inhibition of Viremia in Ebola infected SCID mice is shown in **Figure 73-B**. Virus distribution in Ebola infected SCID mice with prophylaxed AVS-0303 are in **Figure 73-C**.

Survival of Ebola Infected SCID Mice treated with AVS-0303 (carbocyclic 3-deazaadenosine)

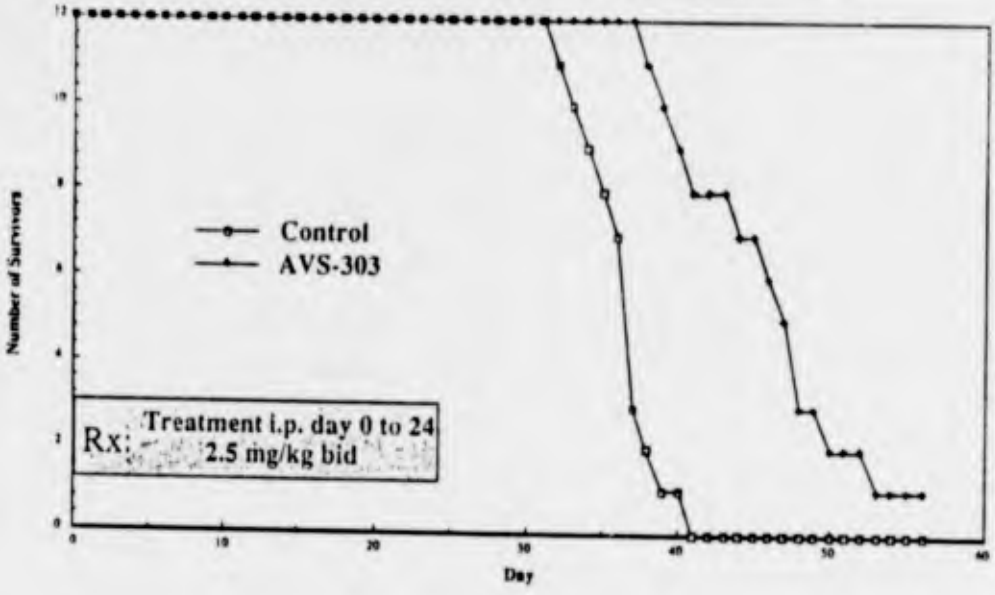


Figure 73-A

Inhibition of Viremia in Ebola Infected SCID Mice Prophylaxed with Carbocyclic 3-deazaadenosine (AVS-0303)

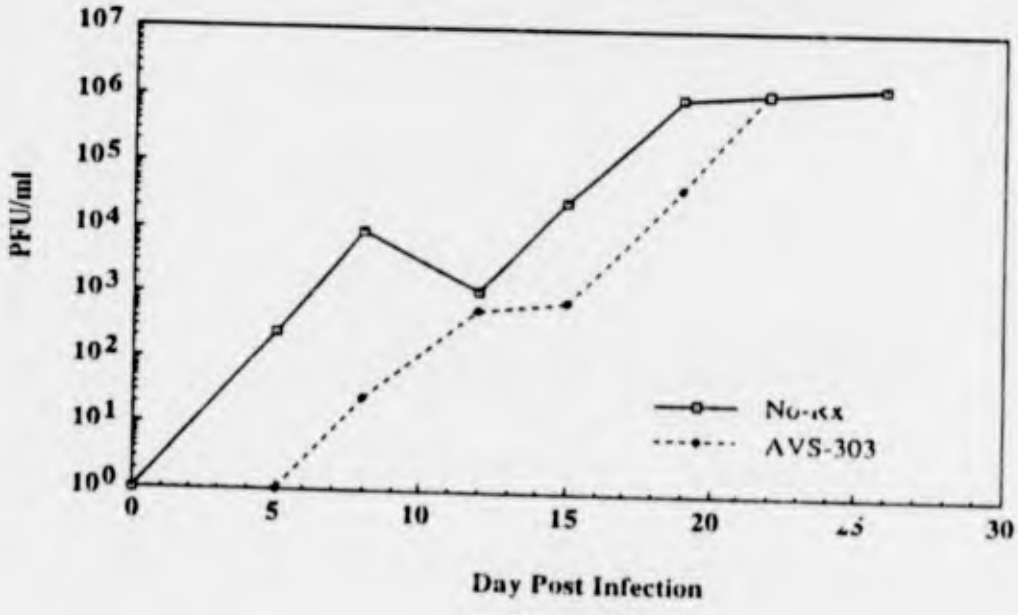


Figure 73-B

Virus Distribution in Ebola Infected SCID Mice Prophylaxed with carbocyclic 3-deazaadenosine (AVS-0303)

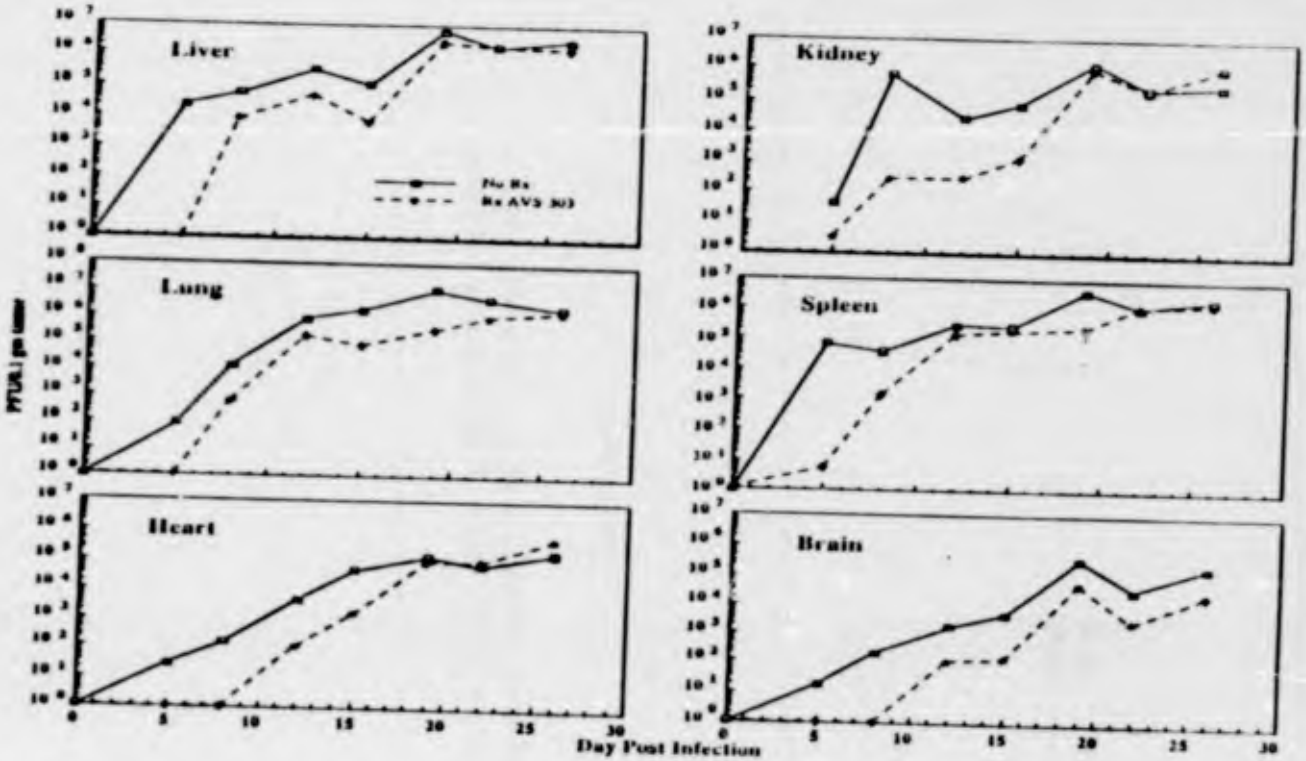


Figure 73-C

Survival of Ebola infected SCID mice treated with AVS-4275 (prophylaxed 3-deazaneplanocin A) are displayed in **Figure 74-A** and inhibition of Viremia in Ebola is in **Figure 74-B**. Virus distribution in Ebola infected SCID mice prophylaxed with AVS-4275 are in **Figure 74-C**, and the amount of virus in lung tissue after 14 days following therapy with AVS-4275 in **Figure 74-D**.

Survival of Ebola Infected SCID Mice Prophylaxed with 3-Deazaneplanocin A (AVS-4275)

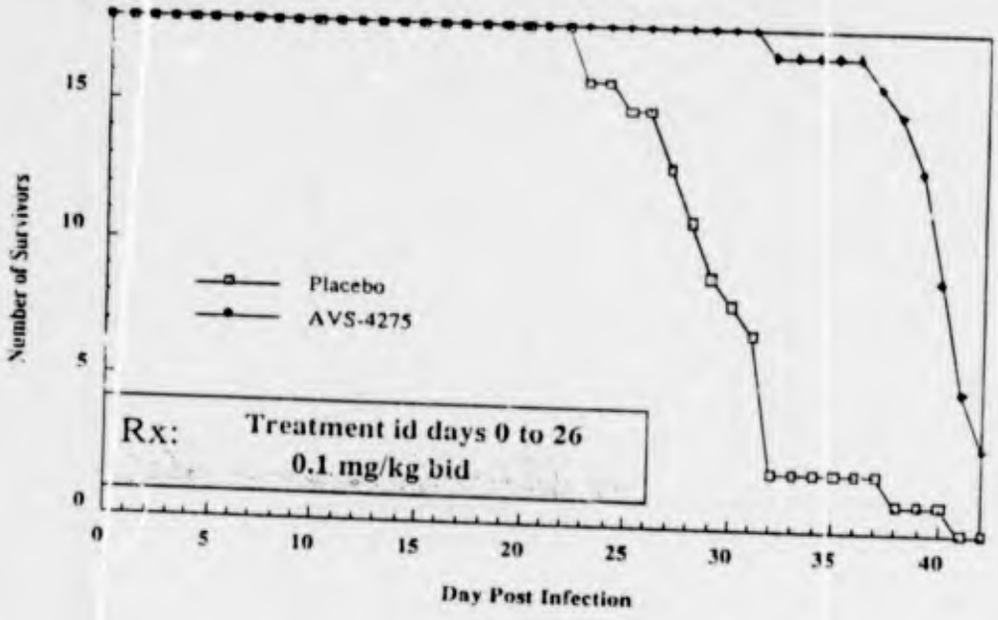


Figure 74-A

Inhibition of Viremia in Ebola Infected SCID Mice Prophylaxed with 3-deazaneplanocin A (AVS-4275)

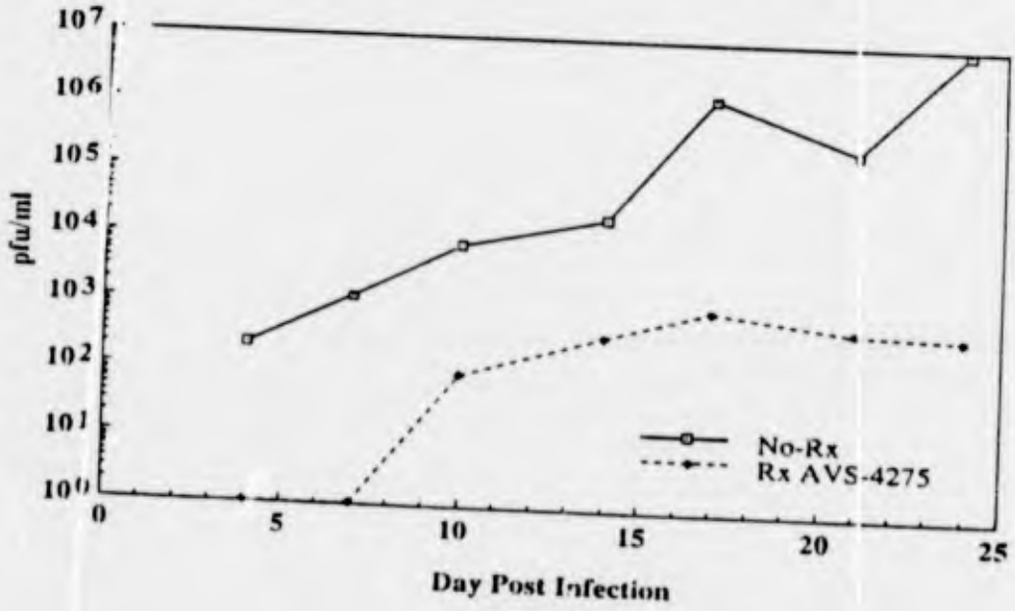


Figure 74-B

Virus Distribution in Ebola Infected SCID Mice Prophylaxed with 3-deazaneplanocin A (AVS-4275)

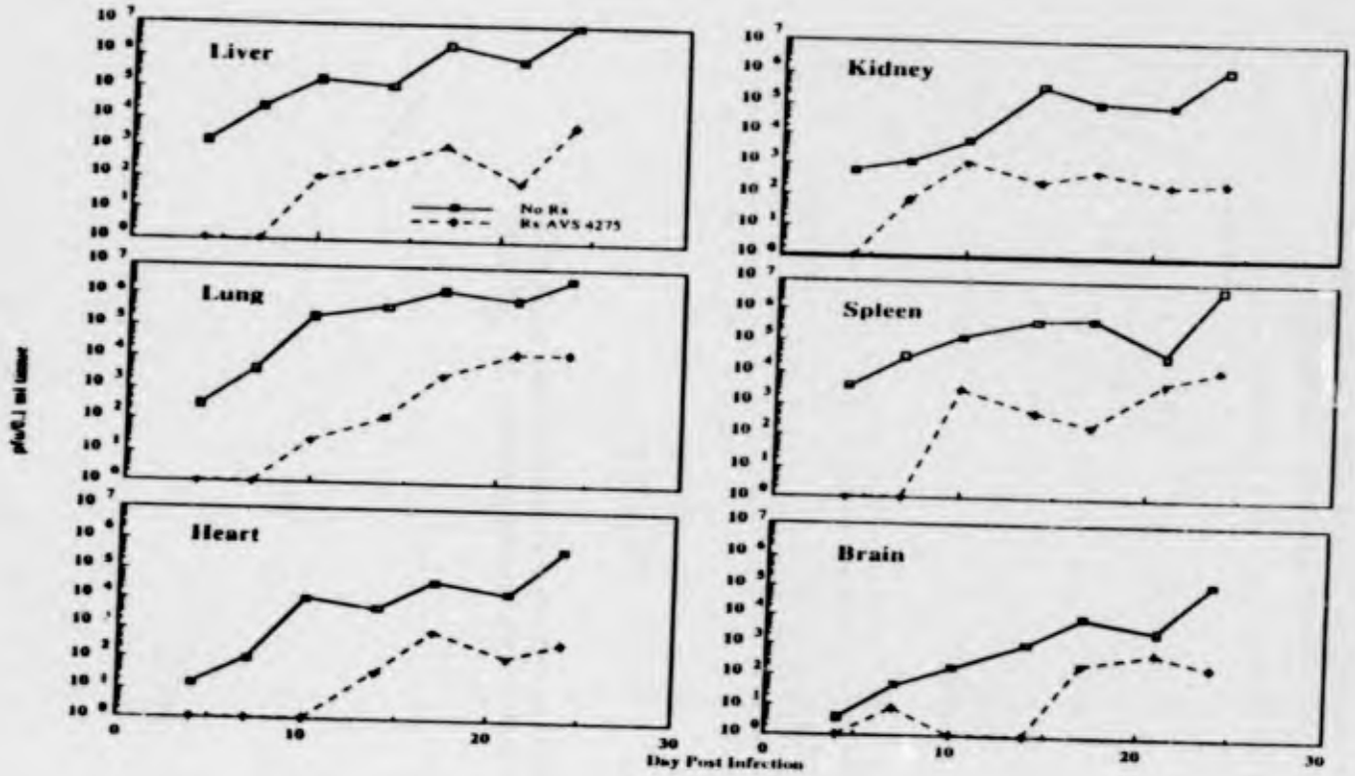


Figure 74-C

Virus in Lung Tissue at 14 Days Following Therapy with AVS-4275 1 to 4 Times Per Day

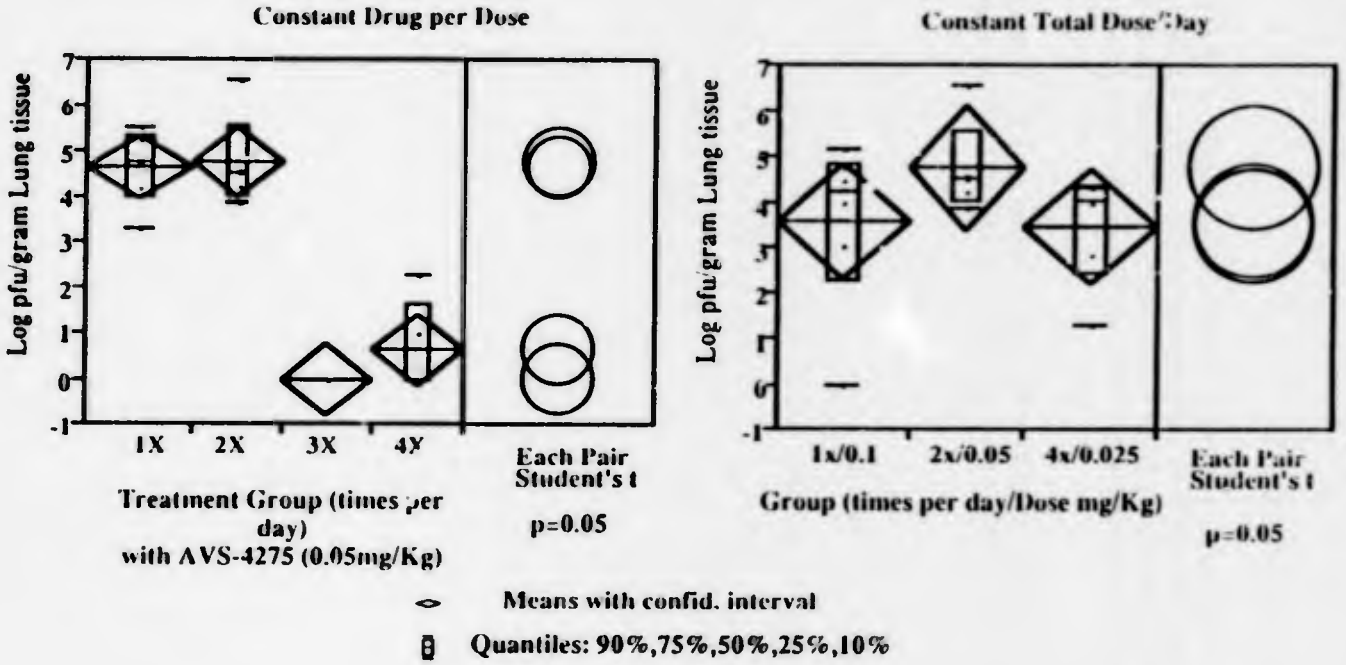


Figure 74-D

Ebola is inhibited *in vitro* by S-Adenosylhomocysteine hydrolase inhibitors in multiple cell lines. This suggests that cap methylation may be important in Ebola replication, but information is limited by difficulties on conducting studies under BL-4 conditions. AVS-0303 (carbocyclic 3-deazaadenosine) and AVS-4275 (3-deazaneplanocin A) are effective both *in vitro* and in a SCID mouse model at inhibiting viral replication. 3-deazaneplanocin A appears to be the most effective in reducing virus replication and will be evaluated further in an Ebola primates model.

4.4.1.3 Filovirus Work in Progress at Present Time

Several *in vitro* and *in vivo* studies were underway at the close of this contract to establish the best treatment regimen for AVS-0303 and AVS-4275 in SCID mice against Ebola virus. A plan to study the effect of AVS-0303 and AVS-4275 against Hemorrhagic Fever viruses are outlined in **Table 28** and the general *in vitro* effects of S-Homocystine Adenosine Hydrolase inhibitors on Hemorrhagic Fever are in **Table 29**.

Preliminary results of *in vivo* testing in progress at the close of the contract to verify the best treatment regimen in the SCID mouse model against Ebola by AVS-0303 and AVS-4275 are listed in **Table 30-A** and **30-B**.

Table 28

PLAN FOR AVS303 & AVS4275 EFFECACIES AGAINST VIRUSES IN VITRO SYSTEM

			DAYS		METHOD DETERMINING INFECTION / DRUG ACTIVITY				
VIRUS	STRAINS	CELL LINE	CPE	2ND OVERLAY	READ PLAQUES	CPE	ELISA	MTT-BAS	PLAQ VIR REDUCTION
Flavivirus									
YF		MK-2, VERO	-	-1	5	-	-	-	yes
JE		VERO							
Dengue	2	v e6	12	NA	NA	yes	-	12	yes
		vero	NA	5	6	no	-	-	yes
	4	vero	NA	5	6	NA	no	NA	yes
Togavirus									
VEE									
Bunyavirus									
RVF		vero	3	3	3-4	yes	NA	NA	yes
SF									
P.T.									
HTN		vero	no	must on d 7	10-12	no	yes/d7	no	yes
CCHF		sw-13	d3	3	3-4	3	no need	yes	yes
ARENAVIRUS									
PIC		VERO							
Lassa		vero/e6	7	7	8	yes	6-7	yes	yes
Junin		vero/e6	7	7	8	yes	6-7	yes	yes
UHF		vero/e6	7	7	8	yes	6-7	yes	yes
MACHUPO		VERO	7	7	8	yes	6-7	yes	yes
Filovirus									
EBOLA		vero-e6	7	7	8	yes	6-7	yes	yes
MARBURG		vero e6							

EFFECT OF S-HOMOCYSTINE ADENOSINE HYDROLASE INHIBITORS ON HEMORRHAGIC FEVER VIRUSES IN VITRO

VIRUS	CELL	AVS303		AVS4275		AVS1654		AVS1655		AVS1549	
		MIC	MIC50%	MIC	MIC50%	MIC	MIC50%	MIC	MIC50%	MIC	MIC50%
FLAVIVIRUS											
DENGUE 4 MK-2		250	125	250	250	250	250	250	250	25	12.5
BUNYAVIRUS											
RFV	Vero	125	>125	250	250	250	250	250	250	25	12.5
HANTAAN	Vero E 6	125	>125	64	16	125	>125	250	250	12.5	6.4
CCHF	SW-13	250	64	250	8-2	250	250	250	250	250	6.4
ARENAVIRUS											
LASSA(Ac)	Vero	250	250	250	125	250	250	250	250	25	12.5
LASSA(Mo)	Vero	250	250	250	125	250	250	250	250	25	12.5-3.2
JUNIN	Vero	250	48	250	48	250	64	250	250	25	25
VHF	Vero	250	250	250	48	250	125	250	250	25	3.2-6.4
MACHUPO	Vero	250	250	250	125	250	250	250	250	25	25-50
AMAPORI	Vero	250	250	250	64	250	125	250	250	25	6.4
FILOVIRUS											
EBOLA	Vero E 6	>64	16	32	1	125	32	250	>50	16	2

Table 30-A

TREATMENT OF AVS303 & 4275 ON EBOLA-SCID MICE SEPT.OF 1991
PURPOSE:DETERMINE BEST THERAPY REGIME--DOSE & INJECTION TIME

WEIGHT CHANGE

GROUP	MICE	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11
VIRUS	1	25.7	25.2	24.4	24.2	23.9	23.2	22.6	22.7	23.0	22.6
200pfu	2	23.7	23.4	22.6	22.9	23.3	23	22.7	22.6	22.9	
only	3	27.7	27.9	27.5	27.0	26.8	25.8	25.1	24.5	24.1	
C 1	4	28.3	28.1	27.2	26.6	26.1	24.8	23.9	23.5	23.3	
	5	27.6	28.2	27.1	26.2	26.4	26.5	27.0	26.9	26.8	
virus	1	20.7	20.8	20.9	21.3	20.8	20.4	20	20.1	20.2	
200pfu	2	23.5	23.3	22.6	22.4	21.9	20	19.5	20.3	20.1	
only	3	22.8	22.2	20.6	19.3	18.2	19'0	18.8	18.6	18.3	
C 2	4	25.2	24.8	24.2	24.0	22.7	22.4	22.6	21.8	21.2	
	5	22.1	21.9	22.4	22.0	21.8	20.7	20.3	20.1	19.6	
2.5mg	1	27.0	27.3	27.9	28.1	27.9	dead				
once	2	27.4	26.7	27.3	27.6	27.6	25.7	(postmorteum)			
a day	3	25.1	25.0	25.5	23.6	dead					
	4	25.5	25.1	25.0	25.1	25.5	24(postmorteum)				
avs303	5	26.3	26.4	27.2	28.1	27.7	25.6(postmorteum)				
c 3	6	26.8	26.5	26.4	26.8	27.0	dead				
avs303	1	27.5	27.3	26.2	25.6	dead					
2.5mg	2	33	32	30.8	30.5	dead					
2 x d	3	25.6	26.2	25.3	25.2	dead					
	4	26'7	26	25.2	24.9	24.4	dead				
c 4	5	29.5	30	29.2	27.2	dead					
	6	28.3	28	26.8	26.1	dead					
avs303	1	22.6	21.9	20.8	19.5	18.8	18.2	17.7	17.6	17.1	
2.5mg	2	22.5	21.6	21.4	19.7	dead					
3 x day	3	22.1	20.7	20.9	19.6	dead					
	4	22.6	22.6	21.2	21.2	dead					
c 5	5	22.8	22.4	22.1	20.8	dead					
	6	22.8	21.8	21.4	20.0	dead					
avs303	1	27.4	27.2	25.8	24.1	dead					
2.5mg	2	27.1	27.2	25.9	25.8	dead					
4 x day	3	25.8	25	24	23.4	dead					
	4	26	26.5	24.6	23.6	dead					
c 6	5	30.8	30.9	29.5	28.9	dead					
	6	23	23.9	23.9	22.5	dead					
avs303	1	27.3	27.4	26.9	26.6	26.5	27.5	25.3(postmorteum)			
5mg/kg	2	27.4	27.0	26.6	27.4	27.1	20.4	26.3(postmorteum)			
	3	28.6	29.2	30	30.5	29.5	29.8	28.1(postmorteum)			
one/day	4	30.4	30.9	29.2	29.2	dead					
c 7	5	24.9	25.2	24.8	25.3	25.2	25.8	23.7(postmorteum)			
	6	26.1	26.4	25.5	26.3	dead					
avs303	1	22.5	24.1	22.9	22.5	22.3	22.5	20.8	20.9	21.2	20.9
1.67mg/kg	2	28	27	26.3	25.1	25.5	23.8	dead			
x3/day	3	27.5	27.6	26.8	25.2	dead					
=5mg/kg	4	28.8	29.3	28.2	27.1	27.3	dead				
c 8	5	26.2	26.6	25.8	25.2	dead					
	6	25.7	25.8	25.2	24.8	24.1	23.2	21	dead		

Table 30-A (Cont'd)

WEIGHT CHANGE

GROUP	MICE	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11
avs303	1	22.1	20.6	20.1	19.6	19.2	dead				
1.25mg/kg	2	22.4	20.4	20.1	19.5	17.7	dead				
x4=5mg	3	22.6	21.4	21.1	20.7	20.2	dead				
c 9	4	23.8	22.4	21.8	21.2	20.2	dead				
	5	22.5	21.8	21.4	20.8	20.4	dead				
	6	23.2	22	21.7	20.7	20.4	18.4	18.3	18	15.8	14.3
avs303	1	21.7	21.8	21.6	21.4	21.1	20.8	21.1	21.4	21.2	21.2
2.5mg/kg	2	18.6	17.6	17.3	17.2	16.8	17.2	17.6	18.1	18.2	18.5
x 1=2.5mg	3	21	20.5	20.2	19.7	19.5	20.1	20	20.4	20.7	20.9
c 11											
AVS303	1	20.7	20.5	21.8	21.6	21.2	21.1	21	20.8	20.8	21
2.5mg	2	22.4	21.6	21.3	20.1	dead					
x2=5mg/kg	3	23.3	22.1	21.9	20.2	dead					
c 12											
avs303	1	21.7	21.1	18.4	18.7	18.6	17.9	18.	18.1	17.4	18.7
2.5x3=7.52		20	19.3	dead							
mg/kg/d	3	20.9	20	18.4	18.4	17.8	17.1	17.4	17.7	18	19.2
c 13											
2.5x4=10	1	21.2	20.5	19	dead						
mg/kg/d	2	25.4	25.3	dead							
c 14	3	26.7	25.5	dead							
avs 05	1	22.6	21. .	19.6	19.1	18.7	17.2	14.6	13.9	13.8	13.5
5mg/kg	2	22.3	20.3	19.2	18.8	18.4	16.3	15.7	15.6	15.8	16.6
1x=5mg	3	22.7	21.8	20.1	20.5	20	19.1	18.1	17.2	16.8	16.2
c 15											
avs303	1	19'0	18.9	18.3	17.9	17.7	17	17.6	17.4	18	18.8
1.67x3=	2	16.9	16.2	15.4	16.2	16	15.7	15.9	15.8	16.6	16.8
5mg/kg/d	3	20.3	19.4	18.2	dead						
c 16											
avs303	1	22.8	21.6	20.7	21	19.5	19.7	20	20	19.2	19.1
1.25x4=	2	22.1	20.9	dead							
5mg/kg/d	3	22.4	21.8	20.3	20.2	19.5	17.6	15	16	16	16. .
c 17											
	1	27.7	28.2	29.8	28.6	28.3	29	29.5	29.7	29.4	29.5
avs4275	2	26.4	26	26.5	26.9	27.3	28.2	28.9	29.6	29.7	29.4
0.05mg/kg	3	28.1	28.2	29.3	28.9	28.8	29	29.6	30	30	30.1
x 1=0.05	4	27.1	27.6	27.9	28.3	28.1	28.9	28.8	29.2	29.6	29.7
c 18	5	28.7	27.9	27.9	28.4	28.6	29.2	29.2	29.7	29.7	29.4
	6	28.6	28.3	28.4	28.1	28.4	29.8	29.2	29	29.1	28.9
avs4275	1	29.4	29	28.5	28.2	28.4	28.6	28	28.4	28.6	28.8
0.05mg/kg	2	27.5	27.7	27.8	26.5	26.5	26.2	26	26.2	26.4	26.7
x2=0.1/d	3	24.7	24.7	25.3	25.6	25.4	25.5	25.2	25.7	25.3	25.2
c 19	4	26.3	25.6	24.9	24.8	25.7	26	26.1	26.2	26	26
	5	27.9	27.7	27.2	26.8	26.4	25.3	24.7	24.4	24.8	25.5
	6	28.8	28.2	27.5	27.3	25.9	25.6	25.6	25.4	25.7	26
avs4275	1	22	21.5	21.2	20.6	20.5	20.5	20.6	20.5	20.5	20.6
0.05mg/kg	2	21.4	20.9	20.7	20.6	20.6	20.1	19.7	20.4	19.7	19.9
0.15mg/kg/d	3	22.5	21.2	20.5	20.4	20.4	20.5	20.	20.9	20.3	20.8
c 20	4	22.4	21.7	21.9	21.4	21.3	21.3	21.3	21.1	21.2	21.3
	5	23.1	22.2	21.8	21.4	20.8	19.4	19.3	19.2	19.2	18.9
	6	23.4	21.2	22.1	21.9	21.2	20.6	21.1	21.1	21.5	21.5

WEIGHT CHANGE

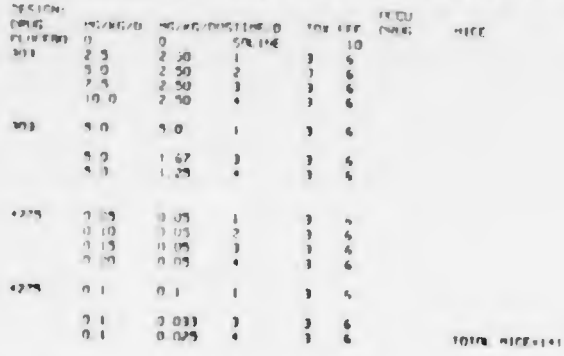
Table 30-A

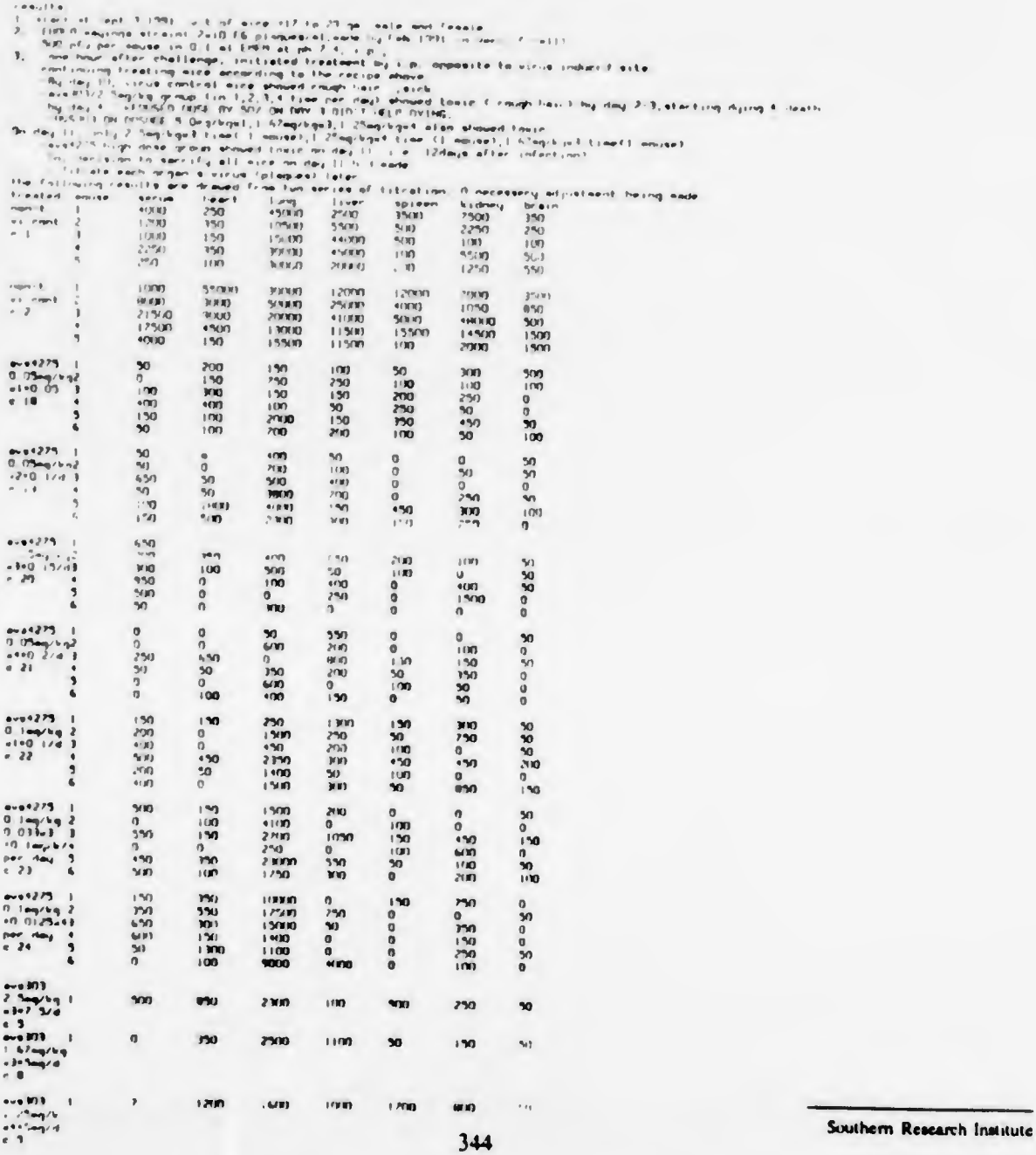
GROUP	MICE	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 7	DAY 8	DAY 9	DAY 10	DAY 11
avs4275	1	29	29	28.1	28.7	27.8	27.1	26.9	26.9	25.3	25.5
0.05x4=	2	25.1	24.8	24.4	24.4	24.4	24.6	25.2	24.9	25	24.4
0.2mg/kg	3	23.7	23.7	23.5	22.5	22	22	21.3	21.3	21.5	21.2
c 21	4	24.6	25.5	25.3	24.8	24.2	24.3	24.2	24.3	24.2	23.8
	5	24.3	24.7	24.1	22.4	21.6	22.5	22.6	23.2	23.4	21.7
	6	29.6	29.5	28.1	27.8	27.1	26.9	26.3	25.9	26.1	26.1
abs4275	1	28.4	28.3	28.6	28.7	28.9	28.5	28.8	28.9	28.5	28.1
0.1x1=	2	25.9	26	26.1	27	26.4	27.	28	28.2	28.2	28
0.1mg/kg	3	27.6	27.9	27.2	27.3	27.5	26.7	26.5	26	25.3	25.4
per day	4	28.1	28.2	28.1	29.3	28.4	29.4	29.8	29.5	29.6	29.7
c 22	5	26.5	26.6	27.2	26.8	26.4	25.2	26.8	27.1	27.4	27.2
	6	19.8	20.2	19.3	19.6	19.6	19.3	19.1	18.4	18.9	18.9
avs4275	1	20.9	20.6	20.4	20.9	20.4	19.6	19.9	19.1	19.4	19.7
0.033x3	2	17.9	17.7	16.8	17.4	16.9	16.7	17.6	17.4	17.7	17.8
=0.1mg/kg	3	21.9	21.7	20.9	21.6	20.9	20.3	20.4	20.7	20.7	20.6
c 23	4	19	18.8	18.2	18.1	17.5	17.9	18.3	18.2	18.3	17,9
	5	20.1	20.2	19.6	20.2	20.2	20.2	20.4	20.9	21	21,9
	6	20.7	20.2	20	20.2	19.9	20	20.9	21.3	21.5	21.4
avs4275	1	21.8	22.2	21.6	21.4	20.8	20.9	29.6	20.1	20.3	19.7
0.0125x4	2	24.3	23.4	22.9	22.0	22.3	22.4	22.1	23.	22.0	2.9
=0.1mg/k	3	23.5	22.9	22.7	22.4	21.7	21.4	21.5	22	21.9	21.6
c 24	4	22.9	22.4	22	21.6	20.9	20.9	21.1	21.1	21	20.6
	5	21.6	21.4	21.4	21	20,5	20.4	20.4	20.9	21.2	20.5
	6	24,6	24.6	23.8	23.7	23	22.6	22.6	22.9	22.3	21.5
avs4275	1	20.4	20.2	20.8	21.3	21	21.3	20.8	20,8	21.3	21.3
0.05mgx1	2	22.5	21.6	21.3	21.7	21.1	20.3	20.2	0.3	20	20.1
=0.05/d	3	21.3	20.6	20.3	20.8	20.6	20.4	20.2	20.5	20.8	20.7
c 25											
avs4275	1	23	22.6	22	20.8	20.5	21.4	20.7	20.9	20.1	21
0.05mgx2	2	22.3	22	21.8	21.9	21.7	21.7	21.2	19.7	20.4	19
=0.15/d	3	21.2	21.2	20.8	20.4	20.1	19.6	19	19.1	18.6	18.3
c 26											
avs4275	1	20.8	19.9	19.6	19.6	19.5	19.6	15.1	19.1	18.1	18,2
0.05mgx3	2	21.8	21.3	21.1	20.5	19.8	20.3	19	19.1	18.8	19.3
=0.15/d	3	23.3	23.3	23	22.9	23.2	23.7	23.6	23.7	23.6	23.8
c 27											
avs4275	1	22.8	22	21.8	21.9	22.4	22.4	22.5	22.3	21.7	20.9
0.05x4mg	2	29.6	28.2	28.4	28.4	27.9	28	28	26.6	25.6	24.4
=0.2/d	3	24.0	23.3	23.1	22.7	22.0	23,5	23.3	22.5	22.6	22.7
c 28											
avs4275	1	23	22.1	22.1	21.7	21.7	22.8	23.4	22.9	23.2	23.4
0.10x1=	2	23.2	22	22.6	22.6	22.1	21.9	22.9	22.4	22.6	22.3
0.1mg/kg	3	22.1	21.1	20.7	21	20.5	20.1	20.5	20.1	20.4	20.7
c 29											
avs4275	1	24.9	25.6	25.3	26	25.4	25.8	26.1	25.9	24.7	24.7
0.033x3=	2	29	27.9	27.6	29	27.9	28.4	28.5	28.4	28.6	28.4
0.1mg/kg	3	25	24.8	25.5	25.3	25.2	25.9	25.8	25.5	25.9	26.2
c 30											
avs4275	1	26.9	26.6	26.1	25.5	24.5	23.6	23.5	23.1	23.3	22.4
0.0125x4	2	21.6	21.6	21.5	21	20.4	19.3	19.4	19	19.2	18.7
=0.1mg/kg	3	14.5	14.8	15.8	16.1	15.7	16.5	16.2	16.1	16.2	16.2
c 31											

* c 1,2= virus only; [illegible] to c 9 avs [illegible] virus 500pfu/mouse in 0.1ml
[illegible] avs4275 virus [illegible] avs4275 only.

Table 30-B

Best Therapy Recipe of AVS-0303 and AVS-4275 on Ebola-SCID Mice Determination in Drug Dose Explored and Administrative Frequency per Day





Summary of Filovirus Program:

Primary human outbreaks of the filoviruses, Ebola and Marburg, cause the most severe viral hemorrhagic fever known, with mortality of 40 - 90% in sporadic outbreaks. S-adenosylhomocysteine hydrolase inhibitors (carbocyclic 3-deazaadenosine (AVS-0303) and 3-deazaneplanocin A (AVS-4275), which mimic the transition state intermediate, inhibit Ebola virus replication as assayed by reduction of viral antigen detected by monoclonal ELISA (effective concentration$_{50}$=4 μg/ml, toxic concentration$_{50}$= >250 μg/ml and effective concentration$_{50}$=0.3 μg/ml, toxic concentration$_{50}$=125 μg/ml respectively). Evaluation of antiviral compounds has been hampered by lack of an adequate small animal model. Infection of multiple adult mouse strains (C3H/HeJ, A/J, Beige, CBA/N, SCID-c.d.-17) with Mayinga strain of Ebola produced disease only in the adult SCID mice, where >0.1 pfu produces a uniformly lethal infection in 4 to 8 weeks old animals. After infection, virus can be recovered from all major organs (heart, lung, liver, spleen, kidney, brain) by day 5 post infection reaching peak titer by day 19 - 25. Clinical and pathological features are consistent with a wasting syndrome. Prophylaxis, with carbocyclic 3-deazaadenosine (minimum effective dose i.p. = 2.5 mg/kg, bid) or 3-deazaneplanocin A (minimum effective dose i.p. = 0.05 mg/kg, bid) begun at time of infection resulted in 15 and 10 day prolongation in the mean time to death over controls and reduction in viremia and virus replication in major organs of 3 or 1 logs respectively in heart, lung, liver, spleen, kidney, brain. The significant inhibition of viral replication in an immuno-compromised host strongly recommends continued evaluation in primates.

4.4.2 Background for Arenaviruses

A new agent has been identified to cause Venezuelan Hemorrhagic Fever, the agent being Guanarito Virus belonging to Arenaviridae family of viruses. From previous studies Ribavirin was known to be effective against Arenaviruses. The following studies were conducted to test the activity of Ribavirin against Venezuelan Hemorrhagic Fever.

4.4.2.1 *In Vitro* Studies

Initial *in vitro* studies were carried out with selected Arenaviruses treated with Ribavirin. Table 31 summarizes the inhibition of Arenaviruses (Guanarito (VHF), Junin (AHF), Machupo (BHF), Pichinde, Lassa (Acar), Lassa (Mozambique)) in Vero E6 cells by Ribavirin.

Table 31

Comparison of Inhibition by Ribavirin of Arenaviruses in Vero E6 Cells

Arenavirus	Days Incubation	IC_{50} (ug/ml)
Guanarito (VHF)	7	14
Junin (AHF)	7	20
Machupo (BHF)	7	6
Pichinde	6	18
Lassa (Acar)	6	8
Lassa (Mozambique)	6	6

Ribavirin Toxcity (TC_{50}) in Vero E6 cells by MTT = 250 ug/ml

4.4.2.2 *In Vivo* Studies

Since guinea pigs are a suitable model to study Arenaviruses, studies were conducted to verify that this model would be also suitable for the Venezuelan Hemorrhagic Fever. Results are shown in **Figure 75**.

Virus Distribution at Time of Death in Untreated Controls

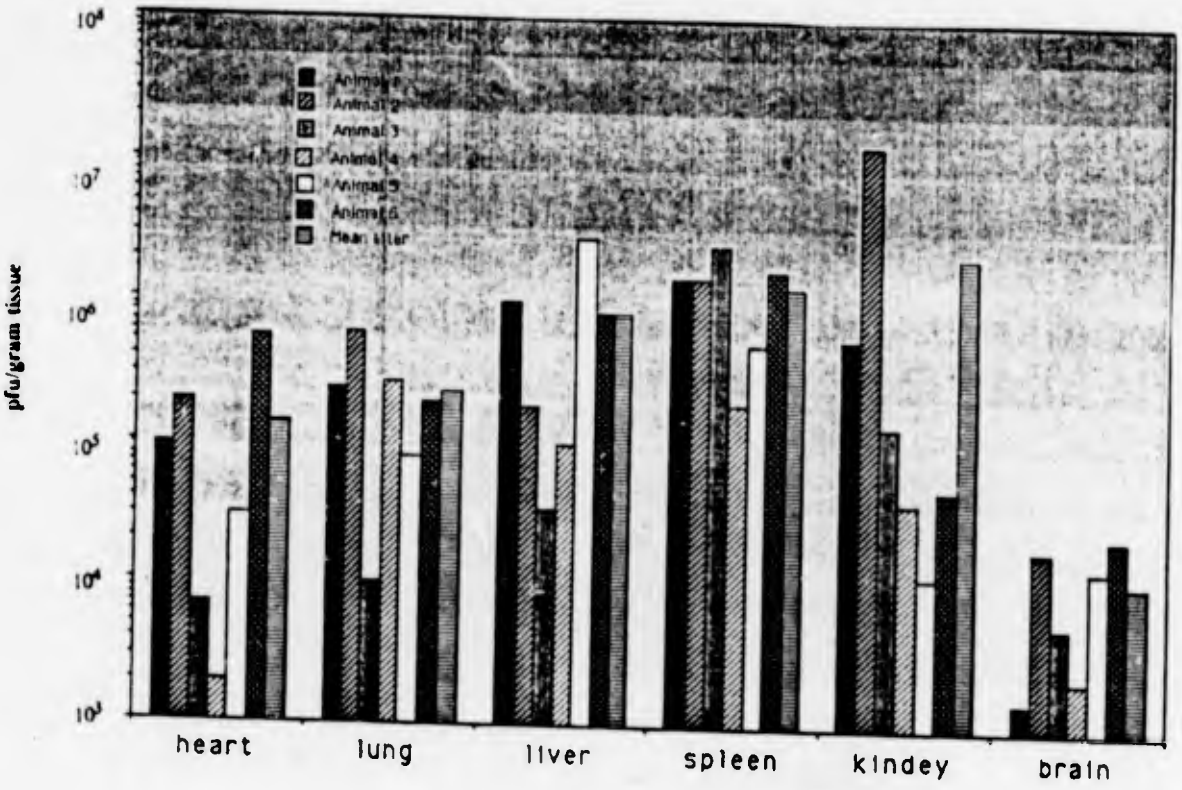


Figure 75

Results of the *in vivo* studies with Guinea Pigs models are shown in **Table 32**, **Figure 76-A** and **Figure 76-B** (high load of virus with dose 20 mg/kg of Ribavirin). Results of *in vivo* studies with Ribavirin at 20 or 30 mg/kg (low virus load) are presented in **Table 33**, **Figure 77-A** and **Figure 77-B**.

Table 32

Survival of VHF Infected Guinea Pigs Prophylaxed With Ribavirin (Days -1 to 26)

Ribavirin (mg/kg bid)	Virus (pfu)	Died	Total	% Survival	Fisher's Exact Test	MTD	Std Dev	R Students' t-Test
0	10,000	10	10	0%	-	14.6	1.3	-
20	10,000	6	10	40%	0.09	25.5	7.5	<0.001
0	1,000	10	10	0%	-	14.2	0.8	-
20	1,000	7	10	30%	0.21	30.4	6.2	<0.001

Survival of VHF Infected Guinea Pigs Prophylaxed with Ribavirin (Days -1 to 26 bid)

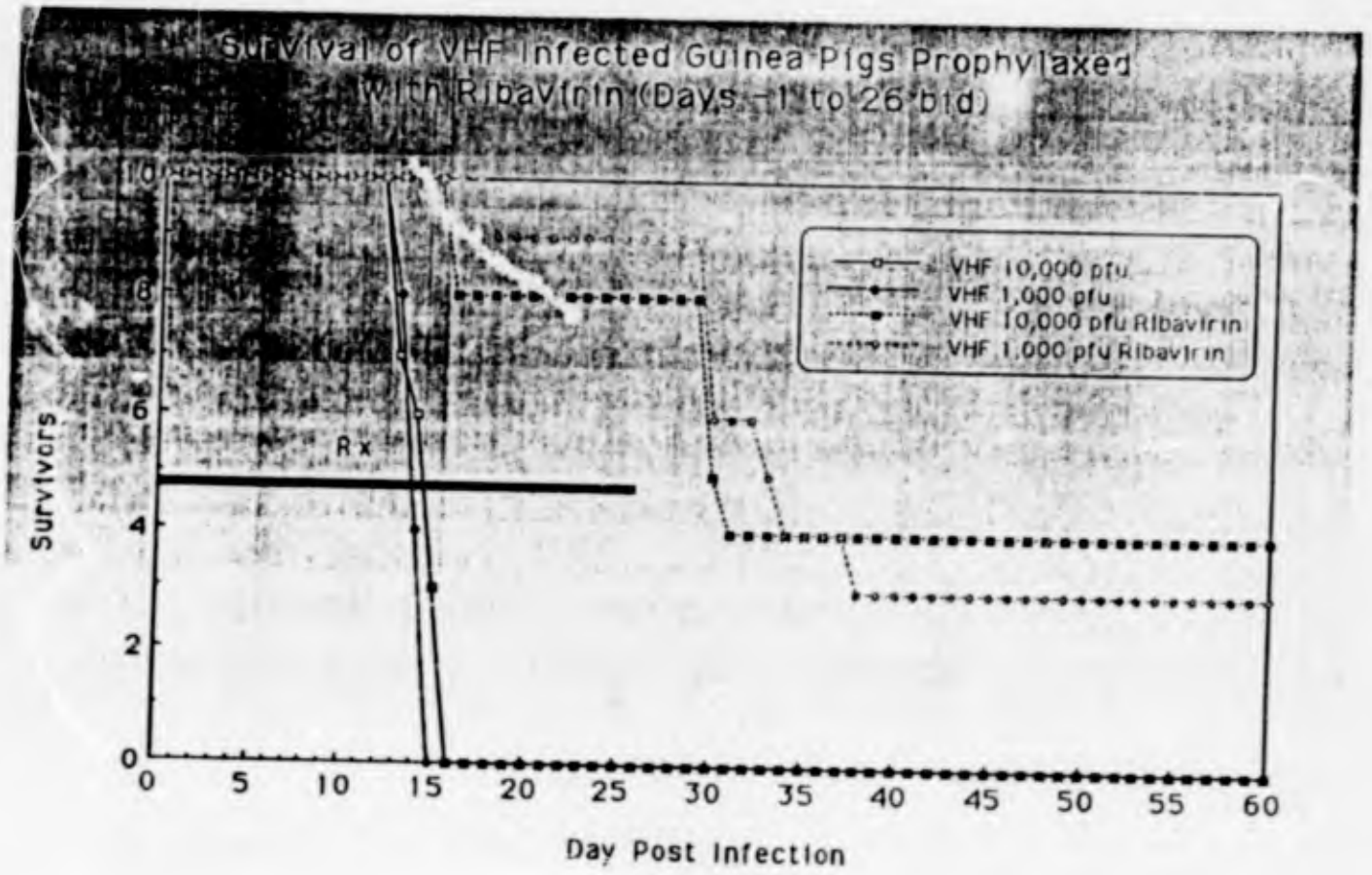


Figure 76-A

Weight Change of VHF Infected Guinea Pigs Prophylaxed with 20 mg/kg Ribavirin (Days -1 to 26 bid)

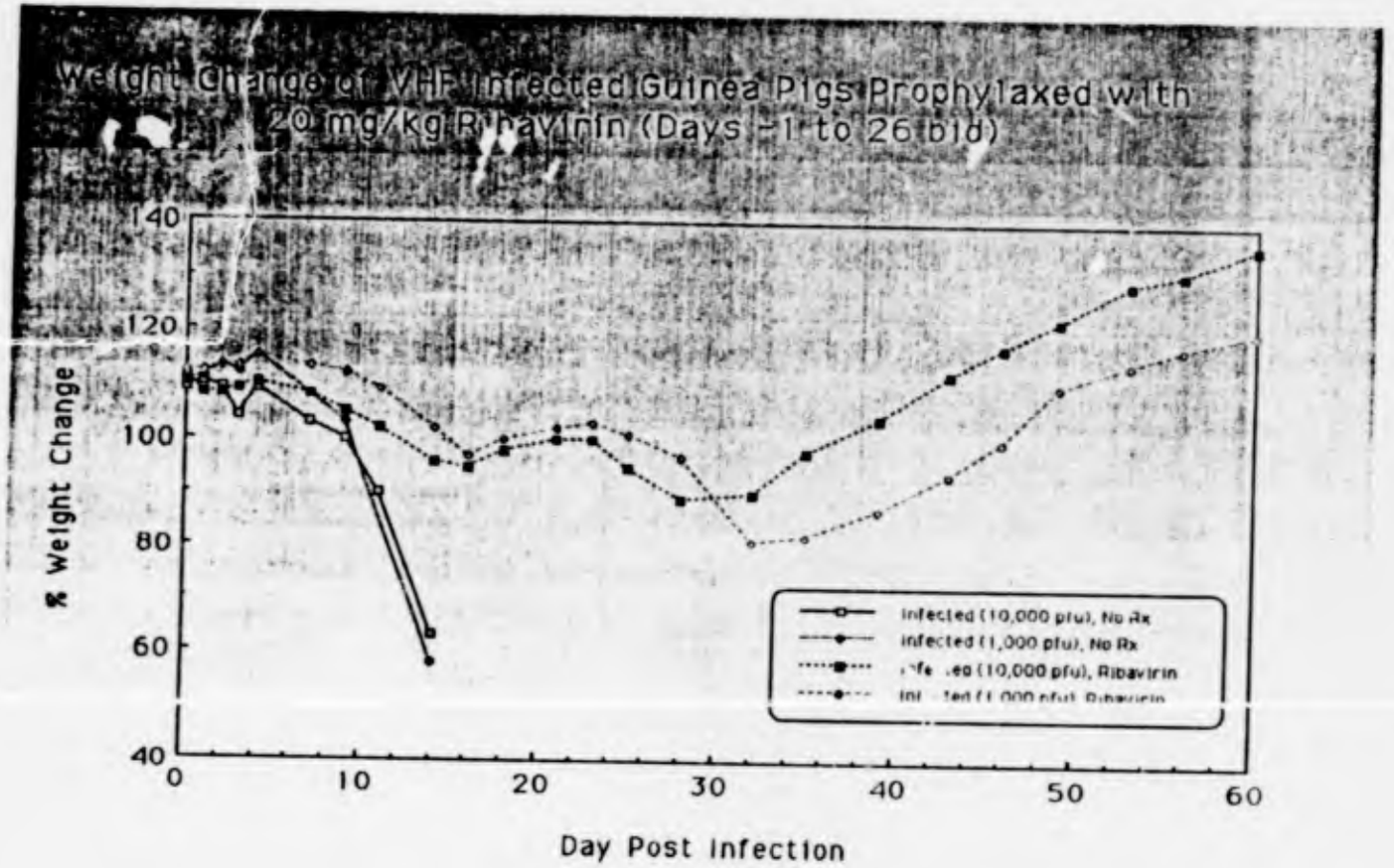


Figure 76-B

Table 33

Survival of VHF Infected Guinea Pigs Prophylaxed With Ribavirin (Days -1 to 38)

Ribavirin (mg/kg bid)	Virus (pfu)	Died	Total	% Survival	Fisher's Exact Test	MTD	Std Dev	p Students' t-Test
20	1000	2	6	67%	0.06	30.0	0.0	<0.01
30	1000	1	5	80%	0.01	34.0		<0.01
20	0	1	6	83%		81.0		
30	0	0	6	100%				
0	1000	6	6	0%	-	15.3	0.5	
0	100	6	6	0%		17.0	1.1	
0	10	3	6	50%		22.3	8.4	
0	1	0	6	100%				

Weight Change of VHF Infected Guinea Pigs Prophylaxed with Ribavirin (Days -1 to 38 bid)

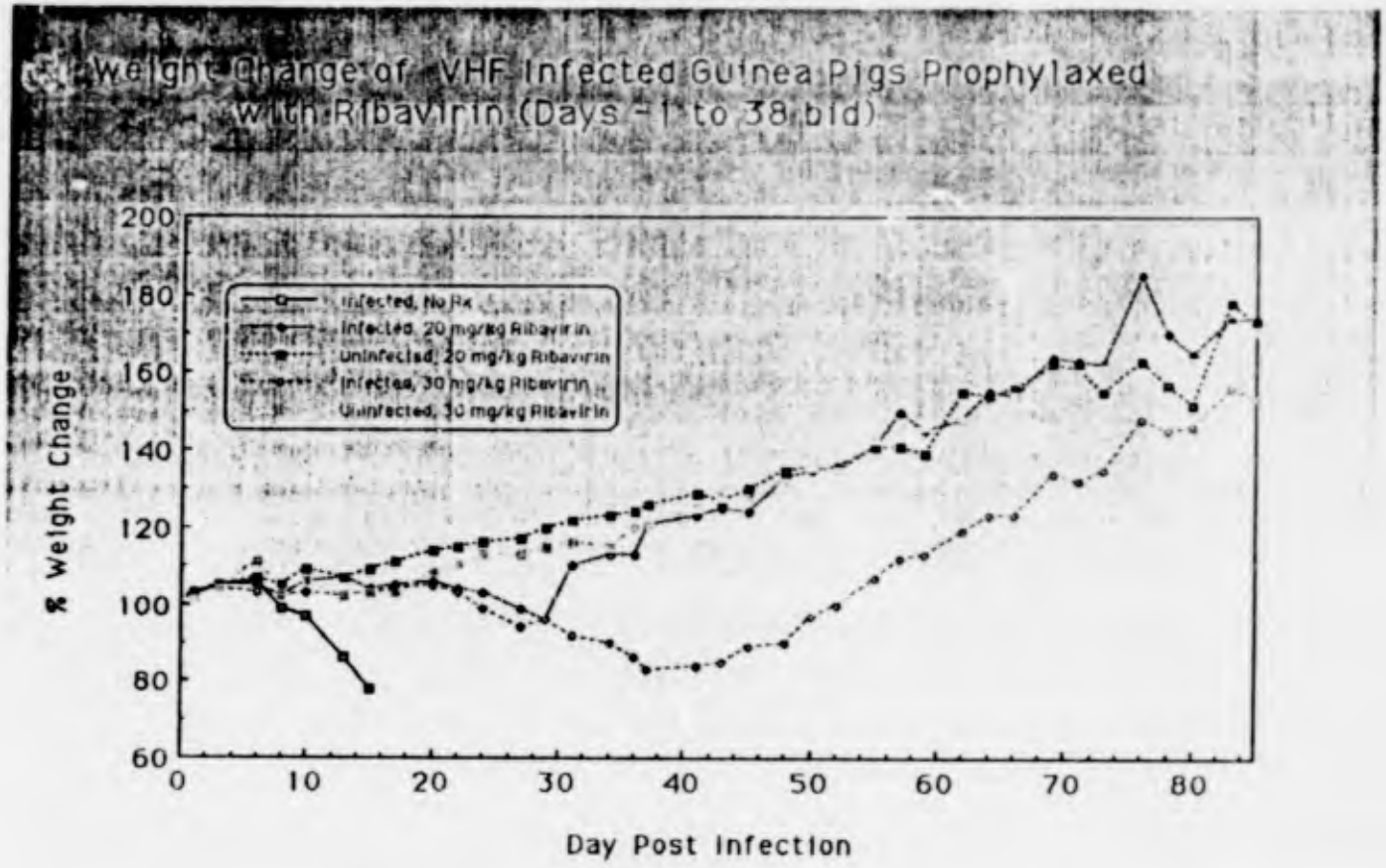


Figure 77-A

Survival of VHF Infected Guinea Pigs Prophylaxed with Ribavirin (Days -1 to 38 bid)

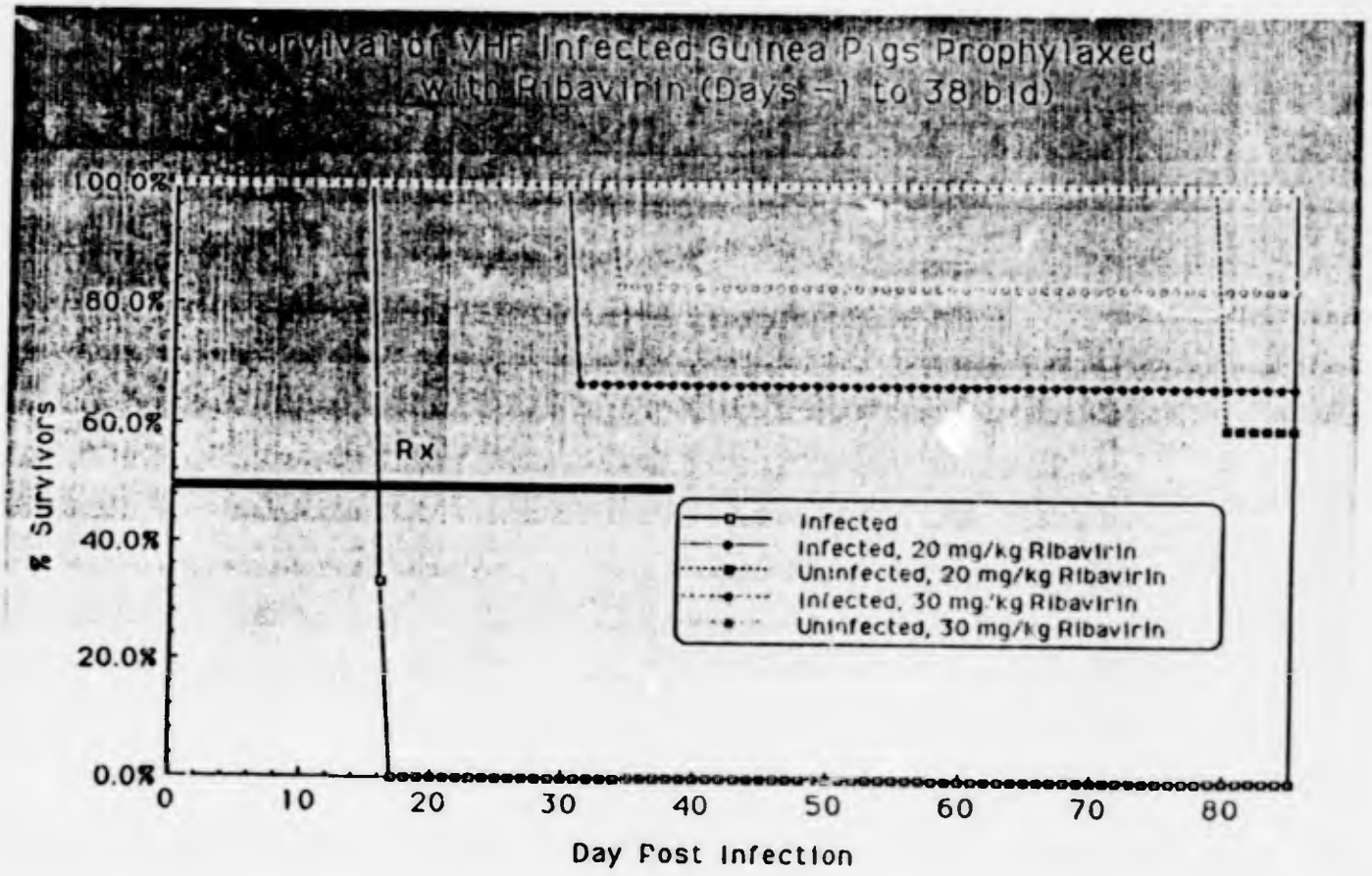


Figure 77-B

These studies indicate that Guinea Pig model is suitable to test antiviral against Guanarito virus, and that the Guinea Pig model represents a reasonable model for Venezuelan Hemorrhagic Fever. Ribavirin has moderate antiviral activity both *in vitro* and *in vivo* against Guanarito virus and could be possibly used against VHF.

4.4.3 Background for Bunyavirus

Studies were initiated against the Hantaan Virus as a representative of the Bunyaviridae to verify the effectiveness of AVS-4275 (inhibitor in SCID mouse animal model). Preliminary *in vivo* raw data for AVS-4275 (toxicity data, virus distribution in mouse organs and mouse weight charge data) are listed in **Table 34**.

It is worthwhile to note that these studies were in progress at the termination of this project and will be completed by the Sponsor.

Table 34

Does AVS-4275 Inhibit Hantaan Virus Replication in SCID Mice?

PURPOSE. In vitro study AVS4275 has inhibited HTN replication by [illegible] [illegible] [illegible] [illegible] data [illegible].
To see the activity of AVS4275 on scid mice infected with HTN virus, we plan this experiment.

DESIGN:
scid mice: [illegible] to [illegible] (most in [illegible]) =74 mice
[illegible] to [illegible] (most in [illegible]) =47 mice
Hantaan virus seed: 76-118 strain, 10x E6 pfu/ml, 100pfu/mouse
in [illegible] ml EMEM w/ [illegible] serum inoculated by i.p.
AVS4275, in saline, keeping at +4 C, warm it before use.

1. Mortality

Virus only/ saline 15 mice	AVS4275 0.35mg/kg 1 time per day 15 mice	AVS4275/0.33mg/k at [illegible], 12 noon, 5:00pm 15 mice	Ribavirin [illegible] [illegible] 15 mice

total mice = [illegible]

2. Distribution of HTN in 5 organs and serum.

sacrificed on	virus control	AVS4275/0.35mg/kg
day 7 (Nov [illegible])	5	5
day 9 (Nov [illegible])	5	5
day 11 (Nov [illegible])	5	5
day 14 (Nov [illegible])	5	5
day 16 (Nov [illegible])	5	5
day 18 (Nov [illegible])	5	5
day 21 (Nov [illegible])	5	5
day 23 (Nov [illegible])	5	5
day 25 (Nov [illegible])	5	5
	45	45

total=90

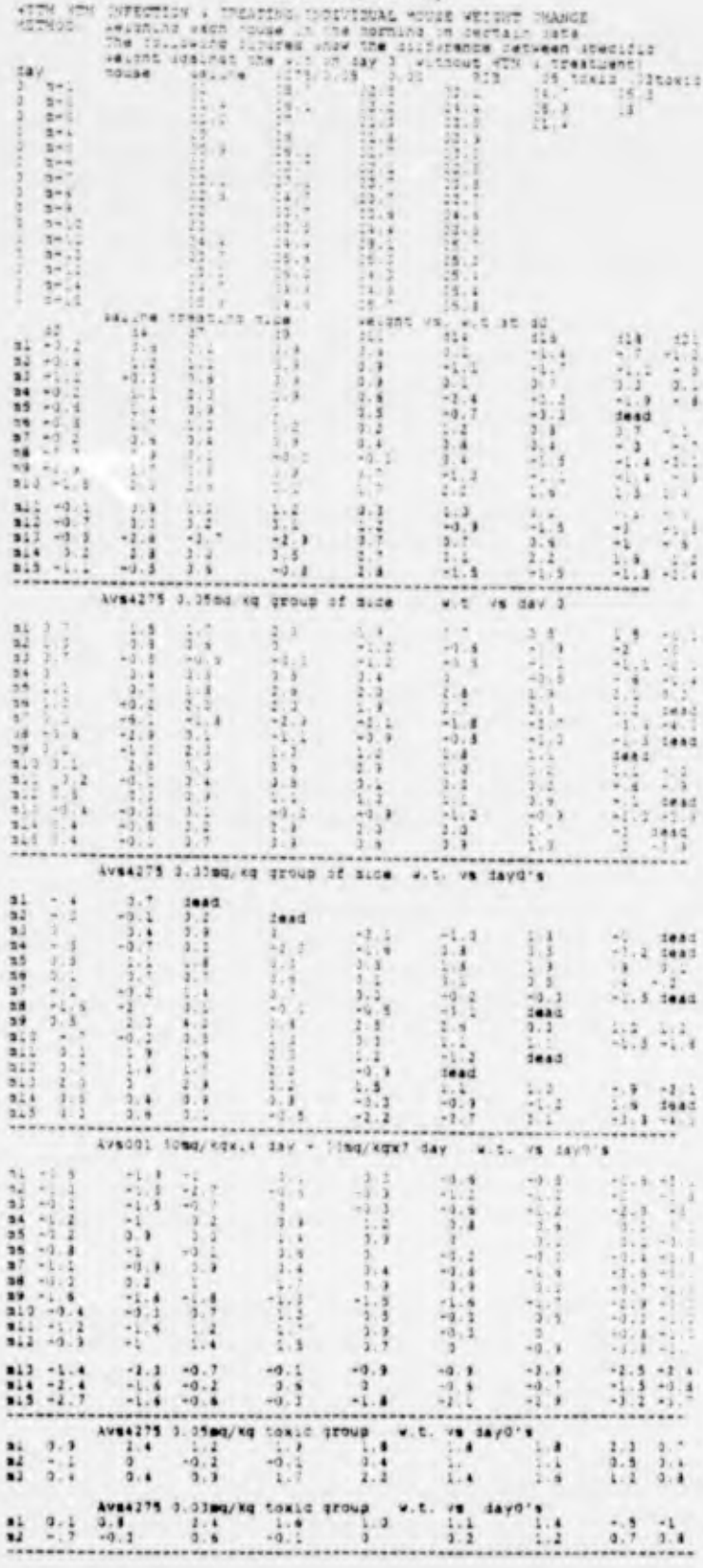
3. Toxicity of AVS4275
0.35mg/kg x 3 day 3 mice
0.33mg/kg x 3 day 3 mice

4. Sensitivity of HTN to new strain of scid mice
1000 pfu/mouse 5 mice

RESULT: SURVIVAL OF MOUSE (%)

DAYS	VIRUS	4275/0.35	0.33	RIB	D/0.35	D/0.33
4	15	15	15	15	3	3
[illegible]	15	15	14	15	3	3
6	15	15	14	15	3	3
7	15	15	14	15	3	3
10	15	15	[illegible]	15	3	3
[illegible]	15	15	[illegible]	15	3	3
[illegible]	15	15	[illegible]	15	3	3
[illegible]	15	15	12	15	3	3
14	15	15	12	15	3	3
15	15	15	[illegible]	15	3	3
[illegible]	15	15	13	15	3	3
[illegible]	14	15	13	15	3	3
18	14	14	13	15	3	3
19	14	[illegible]	10	15	3	3
20	14	[illegible]	10	15	3	3
21	14	[illegible]	8	15	3	3
22	14	[illegible]	8	15	3	3
23	13	13	5	15	3	3
24	13	13	5	15	3	3
25	13	13	5	14	3	3
26	13	13	5	14	3	3
27	13	13	5	14	3	3
28	[illegible]	[illegible]	4	14	3	3
29	8	8	[illegible]	14	3	3
30	4	3	[illegible]	14	3	3
31	4	3	[illegible]	14	3	1
32	[illegible]	5	[illegible]	14	3	1
33	5	[illegible]	3	14	3	1
34	5	3	3	14	3	1
35	3	3	3	14	3	1
36	3	3	3	14	3	1
37	3	3	[illegible]	[illegible]	3	1
38	3	3	3	[illegible]	3	1
39	3	[illegible]	3	[illegible]	2	1
40	3	[illegible]	3	8	2	1
41	3	3	3	5	2	1
42	3	3	3	4	2	1
43	3	3	3	3	[illegible]	1
44	3	3	3	3	0	1
45	3	3	3	2	0	1

Table 34 (Cont'd)



5. DISCUSSION AND CONCLUSION

We tested 11,555 *in vitro* prescreen antiviral assays during this reporting period. Out of these, the **prescreen protocol** successfully identified 455 potential active materials (~5%) for further confirmatory testing. Confirmatory testing of these potential active compounds were carried out in the **primary screen** against a broader range of more virulent viruses (VV, YF, JE, VE, PT or SF). Approximately seventy percent (1252/1802) of the prescreen compounds showed some degree of activity against one or more of these more virulent viruses.

We tested 1564 *in vitro* antiviral assays against the VV virus including the quality control test. Forty-one AVS compounds demonstrated antiviral activity at greater than 50% reduction levels against the Vaccinia Virus. Out of these, 8 AVS compounds appeared to have excellent *in vitro* antiviral potential with antiviral values that reached better than 95% reduction levels with TAI values that ranged from 19% to 51% and SI's from 2 - 26. The best of these active leads should be studied further.

We have performed 3928 *in vitro* antiviral assays against the YF Virus including the quality control tests. Around 144 compounds demonstrated antiviral activity at greater than 50% reduction levels. Out of these assays, 19 AVS compounds appeared to have excellent *in vitro* antiviral potential with antiviral values that reached better than 95% reduction levels with TAI values that ranged from 10% to 52%. These results warrant that the best of these active leads would be studied further.

In this contract period, we performed 3938 *in vitro* antiviral assays against the JE Virus including quality control tests. Around 82 compounds demonstrated antiviral activity at greater than 50% reduction levels. Out of these assays, 12 AVS compounds appeared to have excellent *in vitro* antiviral potential with antiviral values that reached better than 95% reduction levels with TAI values that ranged from 6% to 57%. The results warrant that the best of these active leads would be studied further.

Against VE virus, 3892 *in vitro* antiviral assays were performed during this contract period including the quality control tests. Around 174 compounds demonstrated antiviral activity at greater than 50% reduction levels. Out of these assays, 15 AVS compounds appeared to have excellent *in vitro* antiviral potential with antiviral values that reached better than 95% reduction levels with TAI values that ranged from 5% to 70%. These results warrant that the best of these active leads would be studied further.

During this contract period, we performed 4148 *in vitro* antiviral assays against the PT virus including the quality control tests. Around 161 compounds demonstrated antiviral activity at greater than 50% reduction levels. Out of these assays, 20 AVS compounds appeared to have excellent *in vitro* antiviral potential with antiviral values that reached better than 95% reduction levels with TAI values that ranged from 15% to 56%. These results definitely warrant that the best out of these active leads would be studied further.

We performed 3970 *in vitro* assays against the SF virus including the quality control tests. Around 230 compounds demonstrated antiviral activity at greater than 50% reduction levels. Out of these assays, 38 AVS compounds appeared to have excellent *in vitro* antiviral potential with antiviral values that reached better than 95% reduction levels with TAI values that ranged from 5 - 59%. These results warrant that the best of these active leads would be studied further.

During the time that we conducted primary HIV testing on this contract, we performed 856 *in vitro* assays including quality control tests. Out of these assays, 35 AVS compounds were found to be active (i.e., reduction in CPE of $\geq$50%) against HIV. Three of these compounds produced anti-HIV activity with Selectivity Indices (SI) of > 100.

The *in vitro* data indicates that the ELISA assay is suitable for performing *in vitro* antiviral studies against filoviruses. Similarly, the *in vivo* data indicates that the SCID mouse model is suitable for filovirus antiviral work. Several compounds which are S-Adenosylhomocysteine Hydrolase Inhibitors have shown good antiviral activity in both *in vitro* and *in vivo* assay systems. The most active compounds are AVS-0303 and AVS-4275. We recommend that these compounds be evaluated in primates.

Studies also confirm that the Guinea Pig model is suitable for testing Arenaviruses including Venezuelan Hemorrhagic Fever. Ribavirin has shown to be moderately effective both *in vitro* and *in vivo* against Venezuelan Hemorrhagic Fever.

Also, initial success has been obtained with *in vivo* studies against the Hantaan Virus with S-Adenosylhomocysteine Hydrolase Inhibitors. These findings should be pursued further.

6. ACKNOWLEDGMENTS

Dr. Jorma J. Kirsi served as Assistant Program Manager for the exotic RNA virus screen. Gussie Arnett served as Task Leader for *in vitro* antiviral testing using the VV virus. Dr. Lee J. Wilkoff served as Task Leader for the Centralized Cell Culture and Drug Preparation Laboratories. Ms. Elizabeth A. Dulmadge (Research Biologist) supervised the day-to-day operations of the Centralized Cell Culture and Drug Preparation Laboratories.

Ms. Connie Bryant (Assistant Biologist), Ms. Carrie Edwards (Biological Research Technician), Mr. James Gallaspy (Assistant Biologist), Ms. Jennifer Grant (Biological Technician), Ms. Allison Heald (Assistant Biologist), Ms. Diane Horton (Associate Biologist), Mr. John Hultquist (Assistant Biologist), Ms. Geraldine Jefferson (Assistant Biologist), Ms. Karen Shelton Keith (Assistant Biologist), Mr. Richard Kirkman (Associate Microbiologist), Mr. Byron Lambert (Associate Biologist), Mr. Timothy Lawson (Assistant Biologist), Ms. Sarah Pickett (Biological Research Technician), Mr. Robert Tubbs (Biological Technician) and Mr. James Willis (Biological Technician Trainee) performed the *in vitro* antiviral evaluations against the DNA and exotic RNA viruses.

Dr. Robert W. Buckheit, Jr. served as Assistant Program Manager for the anti-HIV screen. Mr. Richard Brown (Assistant Biologist), Mr. Michael Tucker (Assistant Biologist) and Mr. Steve Watkins (Assistant Biologist) performed the primary *in vitro* antiviral evaluations of AVS compounds against HIV.

Ms. Anne D. Brazier served as Task Leader for the Data Analysis and Reports Group. Ms. Santosh Niwas (Assistant Statistician) performed statistical analysis on the data. Ms. Kimberly Page (Biological Technician), and Ms. Rose Vizzinia (Data Technician) handled the data processing and reporting.

Mr. Martin Schulman and Mr. Steve Wideman (Programming Consultants) wrote the computer programs that enabled the MTT data to be automated from the plate reader in the laboratory to the printing of the "Antiviral MTT Assay" report (primary and prescreen) for submission to the sponsor.

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8. ABSTRACTS/PUBLICATIONS

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