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RESEARCH IN DRUG DEVELOPMENT AGAINST VIRAL DISEASES OF MILITARY IMPORTANCE (BIOLOGICAL TESTING)

Annual Report

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M. G. Hollingshead, G. C. Lavelle, L. Westbrook,
and G. J. Williams

December 15, 1986

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5011

Contract No. DAMD17-86-C-6013

SOUTHERN RESEARCH INSTITUTE
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P.O. Box 55305
Birmingham, Alabama 35255-5305

UNCLASSIFIED
SECURITY CLASSIFICATION OF THIS PAGE

AD-B109905L

REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION UNCLASSIFIED	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION / AVAILABILITY OF REPORT Distribution limited to U.S. Government Agencies only; proprietary information, November 15, 1985
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION Southern Research Institute 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code) 2000 Ninth Avenue South P.O. Box 55305 Birmingham, Alabama 35255-5305	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING / SPONSORING ORGANIZATION U.S. Army Medical Research & Development Command 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Contract No. DAMD17-86-C-6013
8c. ADDRESS (City, State, and ZIP Code) Fort Detrick, Frederick, Maryland 21701-5011	10. SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO. 63763A; PROJECT NO. 3M263, 763D807; TASK NO. AD; WORK UNIT ACCESSION NO. 056

11. TITLE (Include Security Classification)
RESEARCH IN DRUG DEVELOPMENT AGAINST VIRAL DISEASES OF MILITARY IMPORTANCE (BIOLOGICAL TESTING)

12. PERSONAL AUTHOR(S) W.M. Shannon, L.E. Holland, G. Arnett, L.V. Brando, M.G. Hollingshead, G.C. Lavelle, L. Westbrook, G.J. Williams

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual Report	FROM 11/15/85 TO 11/14/86	1986 December 15	154

16. SUPPLEMENTARY NOTATION

17 COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	13		Antiviral Drugs
06	15		Exotic RNA Viruses
			AIDS

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The purpose of this program is to evaluate the efficacy of candidate antiviral compounds against a spectrum of viruses of military importance; e.g., vaccinia virus (VV) adenovirus (Ad), vesicular stomatitis virus (VSV), Punta Toro virus (PT), sandfly fever virus (SF), Yellow fever virus (YF), Venezuelan equine encephalitis virus (VEE), Japanese encephalitis virus (JE), Pichinde virus (PIC), and Korean hemorrhagic fever virus (KHF), and against human immunodeficiency virus (HIV) and related animal retroviruses including the feline leukemia virus (FeLV) FAIDS variant. The program involves (a) primary testing of chemical compounds and natural products for antiviral efficacy in vitro using cpe-inhibition or plaque-reduction assays, (b) primary testing of materials for antiviral efficacy in two animal model systems in vivo (Venezuelan equine encephalitis virus infections in mice and Pichinde virus infections in hamsters), and (c) secondary evaluation of candidate active antiviral compounds. During the first year of operation, the project has been almost fully staffed, an immunization and

20. DISTRIBUTION / AVAILABILITY OF ABSTRACT ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION UNCLASSIFIED
22a. NAME OF RESPONSIBLE INDIVIDUAL Mrs. Judy Pawlus	22b. TELEPHONE (Include Area Code) SGRD-RMI-S 22c. OFFICE SYMBOL 301-663-7325

DD FORM 1473, 84 MAR

83 APR edition may be used until exhausted.
All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

monitoring program was put into place, all modifications and renovations were completed at the BL-3 facility, and implementation of actual antiviral screening operations began. During the first year, antiviral screening against VV, Ad, VSV, PIC, PT, SF, and FeLV (FAIDS) commenced. During this reporting period, 348 compounds were submitted for primary antiviral evaluations in cell culture and over 1000 assays were completed. Of the compounds submitted, over 100 of the compounds were found to exhibit significant antiviral activity against one or more target viruses in vitro. Potent and selective new antiviral agents were found with significant activity against VV, PT, SF, Ad, PIC, and VSV. Several compounds were found to be active against FeLV (FAIDS), but these were not as effective as the positive control drug 2',3'-dideoxycytidine (ddC). The antiviral efficacy of Ribavirin in the treatment of lethal Pichinde virus infections in MHA strain hamsters was confirmed and the animal model was developed for antiviral screening in vivo.

SUMMARY

The purpose of this program is to evaluate the efficacy of candidate antiviral compounds against a spectrum of viruses of military importance; e.g., vaccinia virus (VV) adenovirus (Ad), vesicular stomatitis virus (VSV), Punta Toro virus (PT), sandfly fever virus (SF), Yellow fever virus (YF), Venezuelan equine encephalitis virus (VEE), Japanese encephalitis virus (JE), Pichinde virus (PIC), and Korean hemorrhagic fever virus (KHF), and against human immunodeficiency virus (HIV) and related animal retroviruses including the feline leukemia virus (FeLV) FAIDS variant. The program involves (a) primary testing of chemical compounds and natural products for antiviral efficacy in vitro using cpe-inhibition or plaque-reduction assays, (b) primary testing of materials for antiviral efficacy in two animal model systems in vivo (Venezuelan equine encephalitis virus infections in mice and Pichinde virus infections in hamsters), and (c) secondary evaluation of candidate active antiviral compounds. During the first year of operation, the project has been almost fully staffed, an immunization and monitoring program was put into place, all modifications and renovations were completed at the BL-3 facility, and implementation of actual antiviral screening operations began. During the first year, antiviral screening against VV, Ad, VSV, PIC, PT, SF, and FeLV (FAIDS) commenced. During this reporting period, 348 compounds were submitted for primary antiviral evaluations in cell culture and over 1000 assays were completed. Of the compounds submitted, over 100 of the compounds were found to exhibit significant antiviral activity against one or more target viruses in vitro. Potent and selective new antiviral agents were found with significant activity against VV, PT, SF, Ad, PIC, and VSV. Several compounds were found to be active against FeLV (FAIDS), but these were not as effective as the positive control drug 2',3'-dideoxycytidine (ddC). The antiviral efficacy of Ribavirin in the treatment of lethal Pichinde virus infections in MHA strain hamsters was confirmed and the animal model was developed for antiviral screening in vivo.

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FOREWORD

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals", DHEW Publication No. (NIH) 78-23, Revised 1978.

In conducting the research described in this report, the investigators adhered to all safety, security, and biocontainment requirements specified in the contract, including all U.S. Public Health Service biosafety guidelines contained in "Biosafety in Microbiological and Biomedical Laboratories", DHHS Publication No. (CDC) 84-8395 (March 1984).

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1. INTRODUCTION

This is the First Annual Progress Report on SoRI Project No. 5975, Contract No. DAMD17-86-C-6013. It covers the progress of the research program during the report period from November 15, 1985 to November 15, 1986.

The goal of this program is to implement testing systems in which to evaluate the efficacy of candidate antiviral compounds against a spectrum of viruses of military importance. The program consists of three major task areas: a) primary testing of chemical compounds and natural products for antiviral efficacy in vitro, b) primary testing of chemical compounds and immunopotentiators for antiviral efficacy in vivo, and c) secondary evaluation of compounds found active in the primary in vitro and in vivo screens. In addition, the basic antiviral screening program has been modified to provide for the primary in vitro testing of chemical compounds and biological response modifiers for antiviral efficacy against human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS).

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 and its prodrug derivatives. 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 Institute of Allergy and Infectious Diseases (NIAID).

Members of the Togaviridae family (alphaviruses and flaviviruses) are capable of producing serious hemorrhagic or encephalitic diseases in humans (1-3). 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 encephalitic 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 (4). 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 (4). 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 (5). 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.

Other viruses which cause sporadic but severe hemorrhagic fever in Africa are Marburg and Ebola viruses (6). 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 attack with those particular types 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 (7). The feasibility of inserting foreign genes into vaccinia virus and obtaining expression of those genes in the host cell has already been demonstrated (8). Recombinant DNA technology has made it possible to insert and express heterologous genes in a variety of different viruses (9). Effective defense against possible altered viruses used as BW agents may well depend upon the development of antiviral drugs active against the vector viruses.

In recent years, the U.S. Armed Forces has become interested in participating in the national effort to develop selective antiviral drugs for use in the treatment of AIDS. Compounds submitted to USAMRIID from various sources are therefore being evaluated for their ability to selectively inhibit the infectivity and cytopathic effect of human immunodeficiency virus (HIV), also referred to as human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV), using in vitro assay procedures which have been described previously (10-12).

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 1,500 compounds in its primary in vitro screen. Fewer compounds have been evaluated in vivo. Several hundred nucleoside and nucleotide analogs have been examined each year, but none of these materials has shown better efficacy as a therapeutic agent against the exotic RNA viruses in vivo than Ribavirin. A new antiviral agent, Selenazole, has been 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 (13). The activity of this compound against YF virus is most impressive with an ED_{50} of 0.005 g/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 (RVF, Punta Toro and KHF) and the arenaviruses (PIC, Junin, Machupo, and Lassa Fever) (14). Clinical trials with Ribavirin in patients with Lassa Fever virus infections have yielded good results. It is the arenaviruses which are most susceptible to the antiviral effects of Ribavirin. The studies

reported by Canonico et al. (14) indicate that Ribavirin treatment increases survival of Pichinde virus-infected hamsters or guinea pigs from 11% to 100%. With Junin virus, Ribavirin has been found to be effective in the treatment of the hemorrhagic disease, but not in the treatment of the subsequent encephalitis. Similar results have been obtained with Machupo virus. Combined therapy with Ribavirin and immune plasma has been shown to be highly effective in protecting rhesus monkeys from the lethal effects of Lassa Fever virus infection.

Ribavirin has also been shown to be effective in the treatment of Sandfly fever virus infections 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 diseases, 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 present contract at Southern Research Institute was established to enable USAMRIID to evaluate approximately 1,500 compounds per year for efficacy against 11 different target viruses in a primary in vitro screen, 96 compounds per year for in vivo efficacy against a representative togavirus and arenavirus in appropriate animal model systems, and approximately 5 compounds per year in detailed in vivo studies. The basic contract also includes secondary testing studies with candidate antiviral agents that demonstrate promising activity both in vitro and in vivo. This report summarizes our progress in implementing the research program and includes summaries of antiviral test data collected up to November 15, 1986.

2. PROGRAM ORGANIZATION AND LOGISTICAL MATTERS

2.1 Personnel and Program Management

The project organization chart is presented in Figure 1. SoRI has selected a balanced team of professional and technical staff to work on Project 5975. The project is assigned to the Microbiology-Virology Division (Dr. William M. Shannon, Head) in the Biochemistry Research Department (Dr. Donald L. Hill, Director), a component of the Kettering-Meyer Laboratory (Dr. John A. Montgomery, Director). The project has been staffed with skilled, experienced personnel with extensive background in antiviral drug research and development as well as highly trained new staff members. The project organization has been developed to provide strong project management capability and a flexible, responsive staff devoted to accomplishing all aspects of the research work. A Scientific Advisory Group, which serves to provide expert advice to program staff, has been assembled for the project and consists of local scientists with international reputations in the areas of antiviral drug design and synthesis, basic virology, and clinical assessment of antiviral drugs in human patients.

The Principal Investigator/Project Manager for this contract research program is Dr. William M. Shannon, Associate Director, Biochemistry Research Department and Head, Microbiology-Virology Division. Dr. Shannon is responsible for the overall management and technical performance of the research work and for coordinating all activities on the project. Dr. Louis E. Holland, Head, Molecular Virology Section, Microbiology-Virology Division, is the Assistant Project Manager for the antiviral research program. Dr. Holland assists the Project Manager in all aspects of task coordination and planning and serves as the Project Manager in his absence. Dr. Holland is primarily responsible for overseeing the daily operation of the BL-3 facility. Other task leaders for the project include Dr. Lorraine V. Brando (Research Virologist), Dr. Melinda G. Hollingshead (Research Immunologist), Dr. George C. Lavelle (Senior Virologist), Dr. Gary J. Williams (Research Virologist), Ms. Gussie Arnett (Research Biologist), and Ms. Louise Westbrook (Research Biologist). Regular weekly meetings of the Project Manager, Assistant Project Manager, and Task Leaders are held to coordinate and monitor the work on this research project. Nearly all professional, technical, and support staff for this entire antiviral research program for USAMRIID have been assigned or hired. The one remaining unfilled position is the Senior Ph.D. Retrovirologist who will ultimately be the Task Leader for the HIV work. At present, Drs. Shannon, Holland, Lavelle, and Brando have taken the responsibility for the antiviral research work with the retroviruses. We would hope to identify and hire the Senior Retrovirologist within the next few months. The project organization chart presented in Figure 1 shows the staffing for each of the tasks, for both SoRI home site laboratories and BL-3 facility laboratories, and shows the assignment of personnel for each of the target viruses in the program.

2.2 Immunization Program

In February, 1986 the Microbiology-Virology Division of Southern Research Institute (SoRI) began an immunization program for its employees on Project 5975. This program was modeled after similar programs in use at USAMRIID. Richard J. Whitley, M.D. of the University of Alabama at Birmingham was appointed by the Deputy for Product Development of USAMRIID as an associate investigator under provisions of Army Regulation 40-7 "Clinical Use of Investigational Drugs". Dr. Whitley was thus authorized to administer the Venezuelan Equine Encephalomyelitis (VEE) Vaccine, Attenuated, Live,

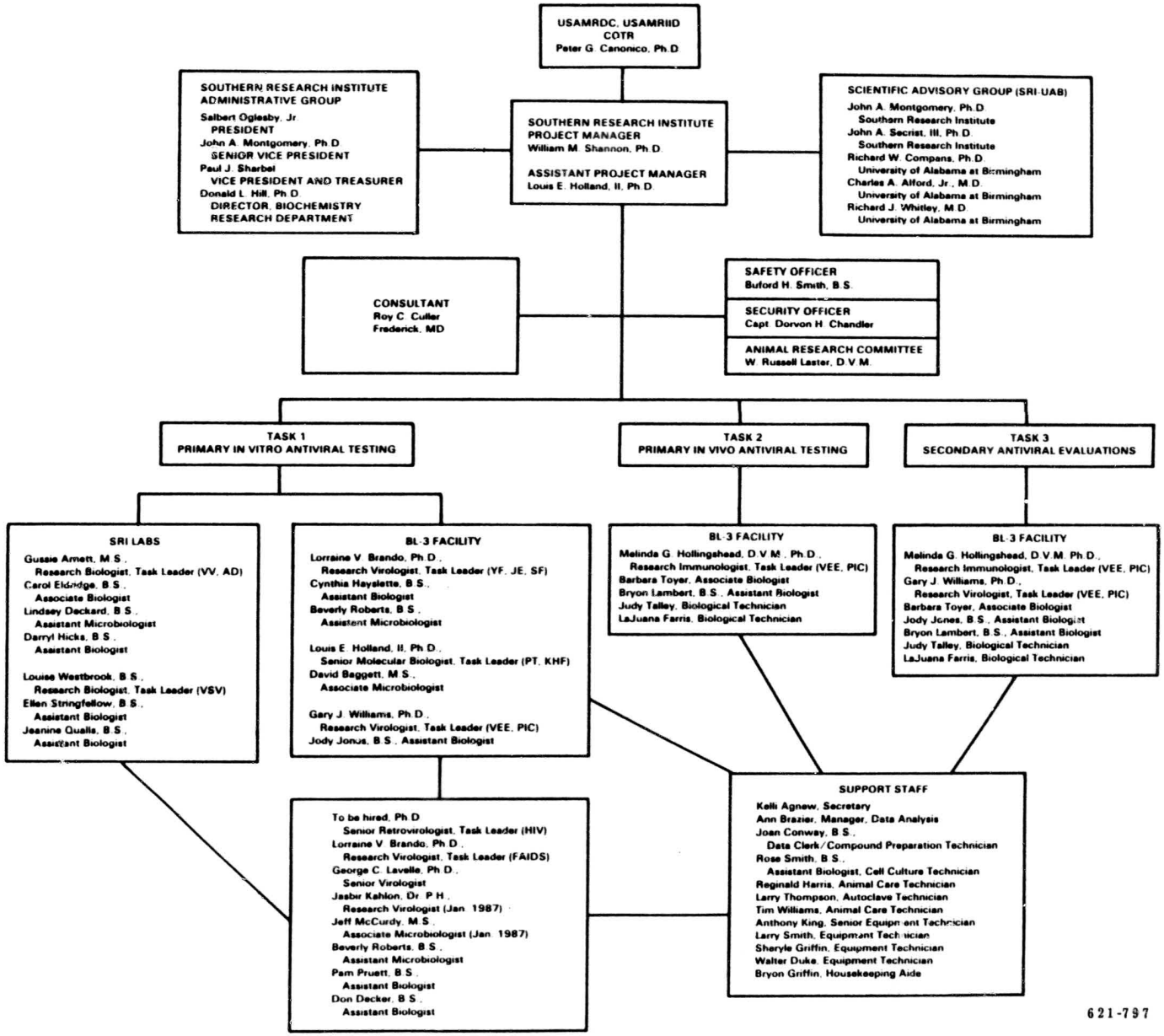

Figure 1. Project Organization Chart and Management Plan. U.S. Army Antiviral Drug Research and Development Project.

FIGURE 1. Project organization chart and management plan. The antiviral drug evaluations have been organized into three Tasks. Task 1 represents all the primary testing performed *in vitro*. This includes research done in laboratories at Southern Research Institute as well as in laboratories at the off-site BL-3 containment facility. The personnel assigned to each of the *in vitro* virus systems are indicated. Since the additional research with HIV and related viruses was a modification of our original contract, the personnel assigned to this portion of the project are listed separately. Task 2 represents all the primary testing performed *in vivo*. All of this work is being done at the BL-3 facility. Task 3 includes the secondary evaluations performed both *in vivo* and *in vitro*. the majority of this work is done at the BL-3 facility. The support staff participate at both locations and in all three Tasks of this project.

TC-83, and the Japanese Encephalitis (JE) Vaccine, Inactivated, Lyophilized. The Yellow Fever (YF) Vaccine was administered by the (local) Jefferson County Public Health Department.

Modified versions of the Volunteer Agreement Explanations and Affidavits (consent forms) in use at USAMRIID for both JE and VEE vaccines were developed for SoRI use. Such forms were neither necessary nor used for the commercially available YF vaccine. The immunization program and the nature of the vaccines were explained to all personnel and signed consent forms were required of each person before entry into the program.

The typical immunization protocol for male personnel was as follows:

Week 1:	Pre-immunization blood sample
	Live virus vaccine (YF or VEE)
Week 2:	JE #1
Week 3:	JE #2
Week 4:	-----
Week 5:	Live virus vaccine (YF or VEE)
Week 6:	JE #3
Week 9:	Post-immunization blood sample

Due to the requirement that female personnel not be pregnant when receiving vaccinations, they were required to have a pregnancy test before receiving the VEE vaccine. The pregnancy test was done the Friday or Monday following the last day of a female's menstrual cycle. If the test was negative (as it was in all cases to this date) the female began her immunizations with the VEE vaccine in Week 1 and continued the protocol as outlined for male personnel.

SoRI personnel were required to report to the Institute Safety Office each day for four days following the VEE and JE vaccines. Oral temperatures and inspections of vaccination sites as well as other post vaccination symptoms (if any) were recorded at this time. Post vaccination blood samples were taken three to four weeks after the last immunization.

Vaccinations were also administered to several people not employed by SoRI. This group included those individuals assigned to perform the building maintenance, members of various safety groups from The University of Alabama at Birmingham, and researchers from the University laboratories located within the BL-3 facility, but outside of our containment area.

In July 1986, in accordance with USAMRIID Policy and Procedure 86-01, SoRI began testing all personnel undergoing live virus immunizations for antibodies to Human Immunodeficiency Virus (HIV). Personnel were required to sign an information sheet similar to that used at USAMRIID. The testing was performed by International Clinical

Labs (Nashville, TN) through their local offices. All testing and results were kept confidential. All new employees scheduled to undergo the immunization protocol are tested for antibodies to HIV during a pre-employment physical.

Sera for antibody titer determination were sent to the Commander, USAMRIID, Medical Division. All titers reported here originate from the Research Serology Section of the Department of Epidemiology in the Disease Assessment Division of USAMRIID. The accompanying Table 1 outlines the antibody titer information available to date on all personnel that have completed the immunization program.

Some personnel, after having finished the immunization program, did not show sufficient serum antibody titers to either JE or VEE or both (see Table 1). After consultation with Dr. Gilcin Meadors at USAMRIID and Dr. Jack Poland at the Centers for Disease Control (CDC) in Fort Collins, CO, it was decided to administer booster immunizations for these viruses. Personnel with an antibody titer of 10 or less to JE virus, and those who were more likely to come into contact with the virus in the laboratories, were given a fourth dose of the inactivated JE vaccine. Blood samples were drawn three weeks after the booster. Sera were sent to both USAMRIID and CDC for antibody titer determinations. Results are not available at this writing.

Personnel with an antibody titer of 40 or less to VEE virus, and who were more likely to come into contact with the virus in the laboratories were given the Venezuelan Equine Encephalomyelitis vaccine, inactivated, dried MBLBR 109. Signed consent forms similar to those used at USAMRIID were obtained from all participating individuals. Blood samples were drawn two weeks post booster immunization and sera were sent to USAMRIID for antibody titer determinations. Results are not available at this writing.

2.3 BL-3 Facility Operations

The majority of this contract research project is being performed at an off-site facility designed especially for research requiring biosafety level 3 (BL-3) containment. A portion of this facility is being leased from The University of Alabama at Birmingham for the exclusive use by Southern Research Institute in conducting USAMRIID-sponsored research projects (Figure 2).

Numerous modifications, both minor and major, were made to the BL-3 containment facility prior to our occupancy. All suggestions made by Dr. John Huggins (USAMRIID scientific staff) during his pre-award site visit (March 27, 1985) by Mr. Ralph Kuehne (USAMRIID Safety Officer) during his two safety inspections (May 8, 1986 and August 7, 1986) and by Mr. Roy Culler (consulting facilities engineer) during his facility inspection (May 15, 1986) were incorporated into the building modifications. Included in the modifications were the installation of double-door, through-the-wall autoclaves and additional airlocks, an upgrading of the building security systems, and installation of an intercom system to provide a network for easy and rapid communication between laboratories.

We started operations at the BL-3 facility in phases. The initial phase concentrated on familiarization with the facility and start-up of new equipment. Southern Research Institute purchased for use on this project several items of equipment including: Nu-Aire model 425 6-ft. biological safety cabinets, a Dupont-Sorvall RC-5C superspeed centrifuge, a Dupont-Sorvall RT-6000B table-top centrifuge, Forma model 3326 double-door, water jacketed, CO_2 incubators, a Forma model 3033 Steri-Cult incubator, a Forma model 3956 large capacity, reach-in CO_2 incubator, a Gilford SBA 300 semi-automated clinical

TABLE 1. ANTIBODY TITERS

A. SRI Off Site Personnel[a]

In vitro[b]

Name	Sample[c] Date	Serum Antibody Titer[d] YF	JE	VEE
Baggett, D.	10/8/86			
"	11/26/86			
Brando, L.	2/11/86	<10	<10	<10
"	4/22/86	40	640	160
Conway, J.	2/4/86	<10	<10	<10
"	4/22/86	40	160	160
Decker, D.	6/24/86	<10	<10	<10
"	8/26/86	160	<10	640
" (JE B)[e]	11/12/86			
Hayslette, C.	10/2/86			
"	11/26/86			
Holland, L.	2/4/86	40	<10	<10
"	4/29/86	160	40	160
Jones, R.	2/4/86	<10	<10	<10
"	7/2/86	40	160	40
" (VEE B)	12/ 86			
Pruett, P.	6/24/86	<10	<10	<10
"	8/27/86	40	<10	160
Roberts, B.	5/30/86	<10	<10	<10
"	8/7/86	20	<10	<10
" (repeat)	9/17/86	20	<10	<10
" (JE B)	11/12/86			
" (VEE B)	12/5/86			
Smith, R.	2/4/86	<10	<10	<10
"	4/22/86	40	160	320
Williams, G.	2/4/86	<10	<10	<10
"	4/29/86	640	160	40
" (VEE B)	12/5/86			

TABLE 1 ANTIBODY TITERS (cont.)

In vivo[b]

Name	Sample Date	Serum Antibody Titer YF	JE	VEE
Campbell, S.[f]	2/4/86	<10	<10	<10
"	4/29/86	640	20	40
Harris, R.	4/23/86	<10	<10	<10
"	7/23/86	40	10	10
" (repeat)	9/17/86	160	<10	40
" (JE B)	11/12/86			
" (VEE B)	12/5/86			
Hollingshead, M.	7/7/86	<10	<10	<10
"	8/27/86	320	40	20
" (JE B)	11/12/86			
" (VEE B)	12/5/86			
Lambert, B.	8/26/86	<10	<10	<10
"	10/22/86	640	<10	80
" (VEE B)	12/5/86			
Means, F.[f]	4/30/86	<10	<10	<10
"	7/23/86	160	20	10
Thompson, L.	2/11/86	<10	<10	<10
"	4/29/86	40	10	20
" (VEE B)	12/5/86			
Toyer, B.	2/4/86	<10	<10	<10
"	4/22/86	320	80	160
Williams, T.	2/11/86	<10	<10	<10
"	4/29/86	640	160	160
Autoclaving/Glassware/ Housekeeping				
Griffin, B.	8/12/86	40	<10	<10
"	10/22/86	40	40	10
Griffin, S.	8/22/86	<10	<10	<10
"	10/22/86	20	<10	<10
King, A.	8/15/86	<10	<10	<10
"	10/22/86	160	<10	40

TABLE 1.ANTIBODY TITERS (cont.)

Autoclaving/Glassware Housekeeping (cont.)

Name	Sample Date	Serum Antibody Titer YF	JE	VEE
Smith, L.	8/15/86	40	<10	<10
"	10/22/86	80	40	10
Administrative				
Agnew, K.	9/12/86			
"	11/26/86			

TABLE 1 .ANTIBODY TITERS (cont.)

B. SRI Home Site Personnel [a]

In Vitro

Name	Sample Date	Serum Antibody Titer YF	JE	VEE
Arnett, G.	2/11/86	<10	<10	<10
"	4/22/86	160	40	40
" (VEE B)	12/5/86			
Deckard, L.	2/11/86	<10	<10	<10
"	4/29/86	320	160	10
Eldridge, C.[g]	2/4/86	160	<10	<10
"	4/29/86	160	<10	<10
Hicks, D.	2/4/86	<10	<10	<10
"	4/22/86	80	10	160
" (JE B)	11/22/86			
Paul, E.	5/23/86	<10	<10	<10
"	8/7/86	40	<10	<10
" (repeat)	9/17/86	80	<10	<10
" (JE B)	11/12/86			
Westbrook, L.	2/11/86 [h]			
"	6/11/86	<10	<10	<10
" (for anti HIV)	7/22/86	320	20	40
" (VEE B)	12/5/86			
Autoclaving/Glassware				
Duke, W.	7/1/86	<10	<10	<10
"	8/27/86	160	<10	10
Safety				
Smith, B.	2/10/86	<10	<10	<10
"	4/29/86	160	40	640
Administrative				
Shannon, W.	2/11/86	<10	<10	<10
"	4/29/86	640	80	320

TABLE 1. ANTIBODY TITERS (cont.)

C. Non-SRI Personnel

Maintenance Personnel

Name	Sample Date	Serum Antibody Titer YF	JE	VEE
Cleary, H.	6/24/86	<10	<10	<10
"	8/26/86	320	<10	80
Irvin, G.	6/24/86	<10	<10	<10
"	8/26/86	80	10	10
Irvin, J.D.	6/24/86	<10	<10	<10
"	8/26/86	80	<10	10
Rutledge, M.	6/24/86	<10	<10	<10
"	8/26/86	320	10	<10
Slocum, R.	6/24/86	80	<10	<10
"	8/26/86	160	<10	10
Smith, L.D.	7/16/86	<10	<10	<10
"	9/17/86	640	<10	20
" (JE B)	11/13/86			
" (VEE B)	12/5/86			

UAB Personnel

Bourne, J.	4/14/86	40	<10	<10
"	6/24/86	40	10	40
" (VEE B)	12/5/86			
Brown, R.	9/18/86			
"	11/26/86			
Cobbs, G.	8/12/86			
"	10/ /86			
Frances, B.	5/20/86	40	<10	<10
"	7/23/86	160	10	40
" (VEE B)	12/5/86			
King, G.	4/28/86	<10	<10	<10
"	7/23/86	160	<10	10
" (repeat)	9/17/86			
" (JE B)	11/12/86			
" (VEE B)	12/5/86			

TABLE 1 .ANTIBODY TITERS (cont.)

UAB Personnel (cont.)

Name	Sample Date	Serum Antibody Titer YF	JE	VEE
Richards, M.	5/20/86	<10	<10	<10
"	7/23/86	40	20	40
" (VEE B)	12/5/86			
Yates, J.	9/2/86			
"	10/29/86			

a. Off site indicates the BL-3 facility which is not on the main campus of SoRI. Home site indicates the main campus of SoRI.

b. Personnel are divided by tasks, i.e., in vitro antiviral testing, in vivo antiviral testing, and various support personnel.

c. The date each blood sample was drawn. The first date for each employee is the date of the pre-immunization blood sample. The second date is the post-immunization blood sample. Any further dates are explained below (e) and in the text.

d. Reciprocal of 80% plaque reduction neutralization test antibody titers to Yellow Fever Virus (YF), Japanese Encephalitis Virus (JE), and Venezuelan Equine Encephalitis Virus (VEE). Blank spaces indicate that the information is not available at time of writing.

e. Words or abbreviations in parentheses indicate (i) why another blood sample was drawn and, (ii) which, if any, booster vaccination was administered. Thus, (JE B) indicates a JE booster and (VEE B) indicates a VEE booster.

f. These people are no longer employed on Project 5975.

g. Due to an allergic reaction following the first JE immunization, the immunization protocol was discontinued for this employee.

h. Inadvertantly, the post-immunization sample for this employee was sent to and used by the Research Serology Department at USAMRIID as the pre-immunization sample. A subsequent blood sample taken for testing for antibodies to human immunodeficiency virus was sent and used as the post-immunization blood sample. Thus, both samples tested were obtained after completion of the immunization protocol. We have received no explanation for this obvious discrepancy.

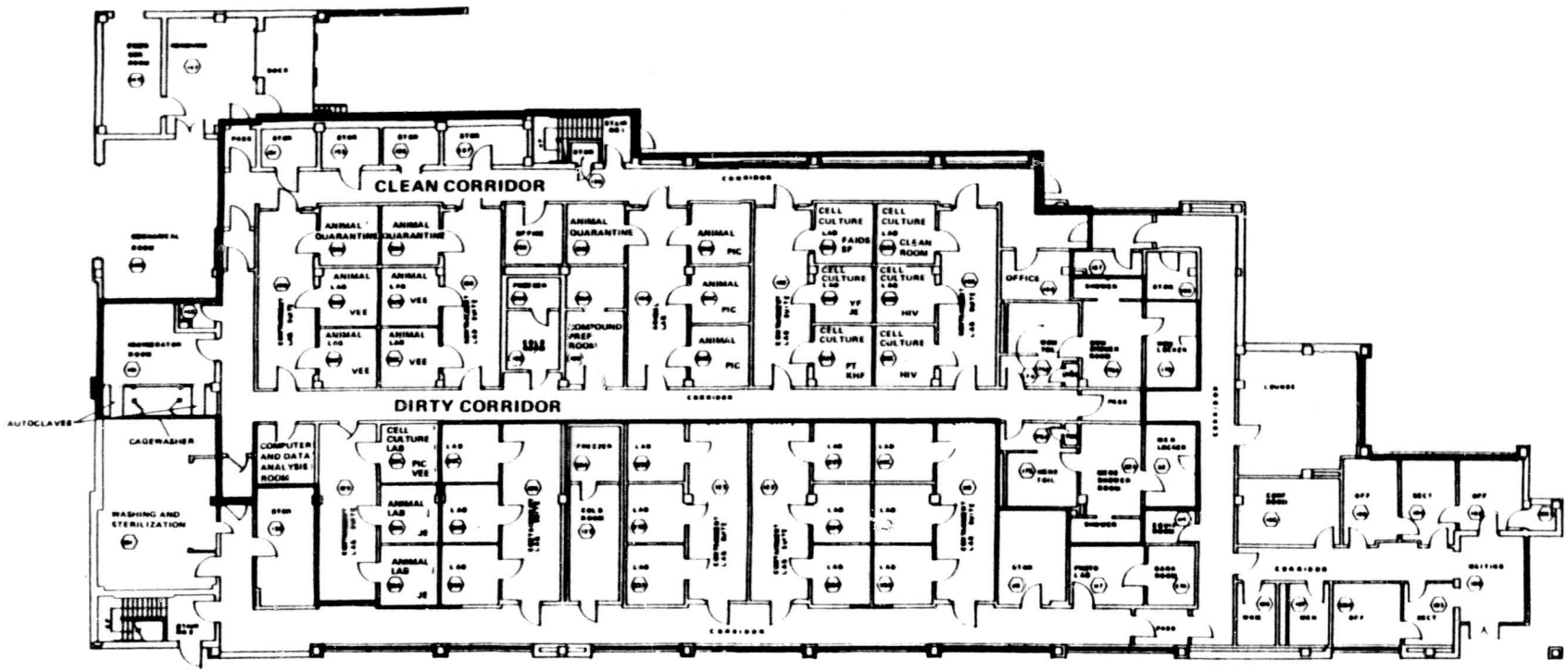

FIGURE 2. Floor plan of the SRI-UAB BL-3 containment facility. The portion of this building available for the exclusive performance of USAMRIID-sponsored research is enclosed by heavy outline. The assignment of individual laboratories for either cell culture or animal work is indicated, as is the specific virus assigned to each laboratory.

chemistry analyzer, a Forma model 8380 -120°C freezer, Olympus model CK-2 and Nikon model TMS inverted microscopes, balances, vortex mixers, magnetic stirrers, and water baths.

The second phase of start-up operations focused on training of personnel in the standard procedures and safety practices for working in the BL-3 containment laboratory (see Appendix A). During this phase, work was begun in vitro with class II infectious viruses, such as Pichinde virus, Punta Toro virus, Sandfly fever virus, human immunodeficiency virus, and the AIDS variant of feline leukemia virus, and in vivo with Pichinde virus. Approximately two months were dedicated to this phase of personnel training before we progressed to the final phase of start-up, which was the introduction of class III infectious viruses to the laboratory and development of methods for their use in antiviral evaluations.

Several features unique to the daily operation of our BL-3 facility should be mentioned. (i) All laboratory waste is decontaminated by autoclaving prior to removal from the building. In addition to autoclaving, all virus-contaminated waste (cell culture supplies, animals, etc.) is further decontaminated and disposed of by incinerator in the pathological incinerator at Southern Research Institute. (ii) Seventeen biological safety cabinets are located in our portion of the BL-3 containment facility. These cabinets are examined and recertified every six months. (iii) An insect and rodent control program is in place. This includes spraying of baseboards with insecticide and boric acid dusting twice monthly, perimeter poison baiting for wild rodents as needed, and the use of light traps to monitor the local mosquito population. (iv) A person responsible for providing routine and emergency building repairs, as well as performing preventive maintenance, is assigned to the BL-3 facility full-time and has been fully immunized. In addition, five more maintenance personnel are also immunized and available on-call for after-hours emergencies.

2.4 Other Laboratories Involved in the Project

Three of the target virus systems for antiviral drug development efforts included in this contract research program have been assigned to SoRI home site laboratories. These viruses are Vesicular Stomatitis Virus (VSV), Vaccinia Virus (VV), and Human Adenovirus (Ad). The floor plans for the SoRI home site laboratories involved in the project are presented in Figures 3 and 4. The ATH8 cell repository and some of the confirmatory and secondary testing specified in the contract modification workscope for the development of antivirals against AIDS and related retroviruses is also housed in the SoRI home site laboratories. Work on the project began in these facilities while renovations were being carried out in the BL-3 facility. Additional equipment items purchased for the home site laboratories for performance of work on this project included a Baker BioGard biological containment hood, a Queue system (Cryostar cryogenic preservation system) ultra-low temperature (-135 °C) freezer, Olympus CK-2 and Nikon TMS microscopes, a Forma double-door CO_2 incubator, water baths, vortex mixers, and magnetic stirrers.

2.5 Safety Program

The research activities of this project are under the review of several safety committees. The routine procedures and practices for biosafety are continually monitored by the SoRI Institutional Biosafety Committee (Louis E. Holland, Ph.D., Chairman). In addition, all work with animals is monitored by the SoRI Animal Research

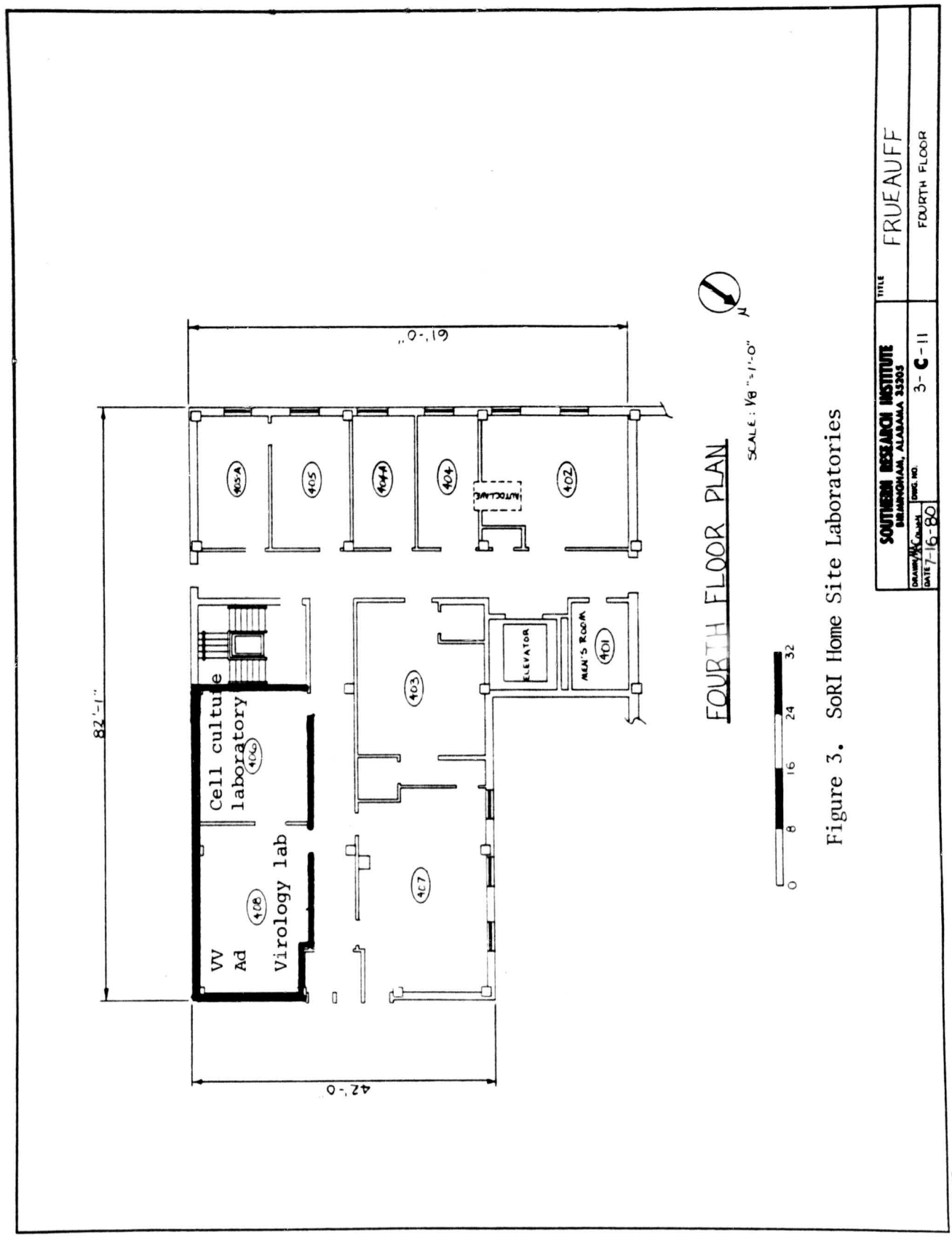

Figure 3. SoRI Home Site Laboratories

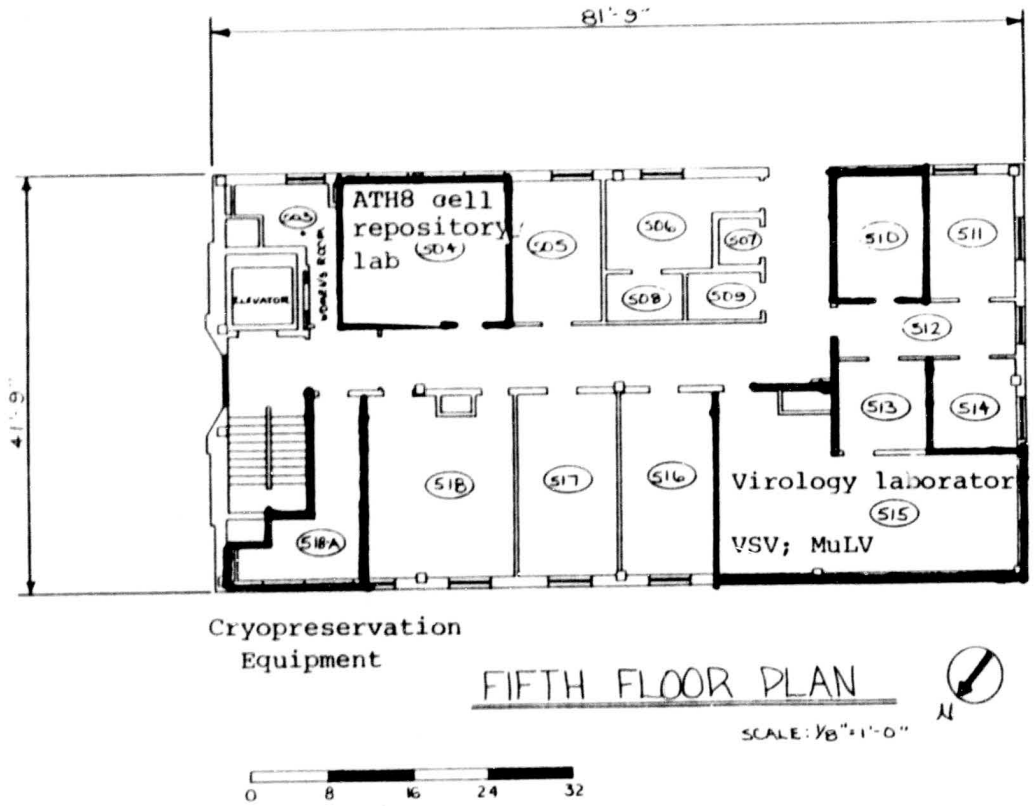

Figure 4. SoRI Home Site Laboratories, Cont'd

SOUTHERN RESEARCH INSTITUTE BIRMINGHAM, ALABAMA 35205		TITLE KM-I
DRAWN NC CHKD DATE 4-23-80	DWG. NO. 5-C-23	FIFTH FLOOR

Committee (W. Russell Laster, D.V.M., Chairman). Reports of all work related accidents, both major and minor, are filed with the SoRI Safety Office (Mr. Buford Smith, Safety Officer), and any necessary treatment is coordinated by this office.

In addition to the SoRI committees, all work and procedures related to this project which occur at the off-site facility have been reviewed and approved by the Institutional Biosafety Committee (C. Glenn Cobbs, M.D., Chairman) and the Occupational Health and Safety Office (Mr. Max L. Richard, Director) of The University of Alabama at Birmingham. This review included an on-site inspection on September 9, 1986 by Karl M. Johnson, M.D., Vice President of Medical Affairs, Viratek, Inc., Costa Mesa, CA, and Frederick A. Murphy, D.V.M. Ph.D., Director, Division of Viral Diseases, Centers for Disease Control, Atlanta, GA.

A manual of standard practices and procedures for working in the BL-3 containment facility was prepared and distributed to all project staff. This manual was adapted primarily from procedures described in the USAMRIID Standard Operating Procedures (SOP) "Hot Suite Operations" (24 September 1984) and in the CDC-NIH publication Biosafety in Microbiological and Biomedical Laboratories (First edition, March 1984). A copy of our procedures manual is included with this report as Appendix A.

3. CURRENT SCOPE OF WORK

This section describes the research objectives and the scope of work for each of the three main tasks being performed by Southern Research Institute (SoRI) on this contract. These tasks are: (a) Primary testing of compounds for antiviral efficacy in vitro, (b) Primary testing of compounds and immunopotentiators for antiviral efficacy in vivo, and (c) Secondary testing of compounds found active in primary testing.

3.1 Task 1: In Vitro Antiviral Evaluations

SoRI is conducting the primary screening of chemical compounds which are furnished by the Department of Antiviral Studies, USAMRIID, through its repository contractor (Technassociates, Inc., Rockville, MD) for antiviral efficacy in cell culture against representative viruses from the alpha-, flavi-, bunya-, arena-, rhabdo-, pox-, adeno-, and retrovirus groups. The test viruses consist of the following agents: (1) Venezuelan Equine Encephalomyelitis (VEE) Virus (2) Yellow Fever (YF) Virus, (3) Japanese Encephalitis (JE) Virus, (4) Sandfly Fever (SF) Virus, (5) Punta Toro (PT) Virus, (6) Korean Hemorrhagic Fever (KHF) Virus, (7) Pichinde (PIC) Virus, (8) Vesicular Stomatitis Virus (VSV), (9) Vaccinia Virus (VV), (10) Adenovirus (Ad), and (11) Human Immunodeficiency Virus (HIV). SoRI is scheduled to evaluate approximately 1500 compounds per year against each of these eleven viruses in vitro, using standard cpe-inhibition and quantitative plaque-reduction tests, to determine the virus rating and the 50% minimal inhibitory concentration (MIC_{50}), or median inhibition dose (ID_{50}), of active compounds, respectively. Determinations are also being made of the cytotoxicity of each candidate compound for the host cells, expressed in terms of the minimum cytotoxic concentration of the compound. We are calculating an in vitro therapeutic index for each active compound against each susceptible test virus. All screening data are being reported to Technassociates, Inc. and to USAMRIID essentially as it is obtained in hard-copy form or on diskettes.

3.2 Task 2: In Vivo Antiviral Evaluations

SoRI is conducting the primary testing of chemical compounds and immunopotentiators, being furnished by the Department of Antiviral Studies, USAMRIID, for antiviral efficacy in rodent models and is conducting a preliminary assessment of acute toxicity with each material submitted. SoRI is evaluating the compounds and immunopotentiators against representative viruses from the toga- and arenavirus groups in rodents. These challenge viruses consist of the following agents: (1) Venezuelan Equine Encephalomyelitis Virus in the mouse, and (2) Pichinde Virus in the hamster. SoRI is scheduled to evaluate approximately 96 compounds annually at the primary level against each of these viruses using the oral and/or parenteral routes of administration. Antiviral efficacy is being expressed in terms of the observed increase in the number of survivors or in the mean survival time in the treated group compared with that of the control untreated group. An in vivo virus rating is being determined.

3.3 Task 3: Secondary Evaluations

SoRI is conducting the secondary testing and further evaluation of compounds found active in the primary antiviral screen. Secondary testing includes an estimation of the in vivo therapeutic index of the candidate compound, the optimal dosage regimen, the optimal route of administration, the optimal treatment schedule, schedule dependency, the duration of antiviral effect, a determination of the utility of the candidate compound in combination with other drugs, and the influence of formulation on activity. SoRI is expecting to evaluate approximately 5 compounds per year in extensive secondary in vivo testing to include determinations of dose-response relationships and antiviral efficacy. Data will be reported to USAMRIID essentially as it is obtained.

In addition to the secondary in vivo testing of compounds active against exotic RNA viruses, secondary evaluations of compounds active against HIV in the primary screen are being performed. These compounds are being examined for their ability to inhibit HIV-induced p24 gag expression in permissive target cells by means of an indirect immunofluorescence assay, and for their ability to inhibit the production of HIV-induced reverse transcriptase activity from infected peripheral blood lymphocytes. Active compounds are also being tested for possible cytotoxic effects in uninfected human T-cells and for possible inhibition of normal T- and B-cell immunologic function. In addition, active compounds are being tested in vitro for efficacy against related murine, feline, and simian retroviruses.

4. EXPERIMENTAL METHODS

4.1 Test Compounds

4.1.1 Receipt, Cataloging, and Storage

The compounds submitted for testing in the antiviral screening program were shipped to the home site facility by Technassociates, Inc. The drugs were unpacked, checked against the enclosed shipping list, and recorded in a log book maintained in a bound SoRI laboratory notebook. The drugs were then stored in numerical order under the appropriate conditions according to the storage information listed on each drug data sheet. Drugs to be used in the BL-3 facility were delivered to the building after they were logged in at the home site. Copies of the shipping list and drug data sheets were distributed to each task leader.

One vial of each drug was provided in the first 8 shipments. Drugs which were supplied in the full amoung of 70 mg were tested in the 3 virus systems at the home site and then sent to the BL-3 facility. If less than 70 mg of drug was provided, it was sent directly to the BL-3 building for testing according to the priority screening list. Beginning with shipment 9, all drugs received had been split into separate amounts (25 and 45 mg) and the drugs were sent to the appropriate facility immediately upon receipt at SoRI. The 25 mg sample was used at the home site for drug solubility determination and assay against 3 virus systems (VV, Ad2, VSV), and the 45 mg sample was used for the 7 virus systems (PT, VEE, YF, KHF, PIC, JE, SF) in the BL-3 facility. Shipment 9 also contained the first compounds submitted for testing against HIV. A sample of each drug (usually 150 mg) was supplied in a separate vial which was sent to the BL-3 facility as soon as it had been logged in at the home site.

4.1.2 Determination of Solubilities

If drug solubility information was included on the drug data sheet, then that information was used. If no information was available, solubility was determined at the home site and the information distributed to each task leader.

To determine the solubility of a drug, a 1-mg sample was weighed, placed in a homogenizer vessel, and the first solvent on the priority list was added. If the drug did not dissolve in the solvent, it was heated to 40 °C (unless it was known to be heat-unstable). If the drug was not in solution after heating, it was homogenized with a hand homogenizer. If the drug still was not in solution, another sample was weighed and this procedure used with the next solvent on the priority list. The priority list of solvents was as follows: H_2O, 1N HCl or 1N NaOH (used only if the drug was known to be stable in the presence of acid or base), ETOH, DMSO or acetone. If a compound was not soluble in any of the above listed solvents, then it was suspended in cell culture medium with the aid of a hand homogenizer or a vortex mixer.

4.1.3 Compound Preparation for Testing

For use in an experiment, the drugs were weighed in advance and stored under the appropriate conditions. The drug solutions were prepared fresh on the day of drug addition to the experiment, using the solvent which had previously been determined to be optimal for that drug. The drug solution was then diluted in serial half-$\log_{10}$ dilutions in the appropriate cell culture medium.

Initial control experiments for each solvent in each virus, host-cell system were done to determine a noncytotoxic, inactive amount of solvent which could be used in each assay system. If a safe concentration could not be determined, then a solvent control was included with each experiment.

4.2 Primary Antiviral Screening In Vitro

Mammalian cells were pregrown as monolayer cultures in 96-well tissue culture plates using suitable cell culture medium. Stock viruses were pretitered according to the method of Reed and Muench, 1938 (15) and diluted in cell culture medium to yield 32 $CCID_{50}$ (cell culture infectious dose, 50%) units per 0.1 ml. Antiviral assays were designed to test a minimum of seven 0.5 $\log_{10}$ concentrations of each compound in triplicate against each of the challenge viruses. If drug cytotoxicity was provided, then drug concentrations were chosen to range from toxic to noncytotoxic. If no cytotoxicity information was available, testing began at 320 μg/ml. To each of the replicate host cell cultures were added 0.1 ml of the test drug solution and 0.1 ml of virus suspension. Cell controls containing medium alone, virus controls containing medium and virus, and drug cytotoxicity controls containing medium and each drug concentration were included on each plate. The covered plates were incubated at 37 °C until maximum virus-induced cytopathogenic effects (CPE) were observed in the untreated, virus-infected control cell cultures.

Positive control drugs were included in each antiviral assay to validate the test conditions used in the antiviral assays as follows:

Challenge Virus	Positive Control Antiviral Drugs
VEE	Selenazole
YF	Selenazole; Ribavirin
JE	Selenazole; Ribavirin
PT	Selenazole; Ribavirin
SF	Selenazole; Ribavirin
KHF	Selenazole; Ribavirin
PIC	Selenazole; Ribavirin
VSV	C-3-Deaza-AdO
VV	Selenazole; AraA
Ad	Selenazole; Ribavirin
HIV	2',3'-Dideoxycytidine (ddC)
FeLV	2',3'-Dideoxycytidine (ddC)

The degree of inhibition of virus-induced CPE and the degree of drug-induced cytotoxicity were observed microscopically. CPE were scored numerically from 0 (normal uninfected cells) to 4 (100% virus-induced cell destruction) for each individual culture in each well as follows:

4 = 100% of the cells affected by virus;
3 = 75% of the cells affected by virus;
2 = 50% of the cells affected by virus;
1 = 25% of the cells affected by virus;
0 = No CPE; normal cell monolayer;

u = Unsatisfactory test, e.g., contamination or leakage;
t = Drug is toxic to cells, CPE not discernible;
p = Drug is partially toxic to cells, cell monolayer is intact so that CPE may be discernible.

Antiviral activity was determined by calculating the degree of inhibition of virus-induced CPE in drug-treated, virus-infected cell cultures by means of a virus rating (VR). The VR is a standard weighted measurement of antiviral activity taking into account both the degree of CPE inhibition and drug cytotoxicity, and was determined by a modification of the method of Ehrlich et al., 1965 (16). The VR was calculated as 0.1 of the sum of the numerical differences between the recorded CPE grade of each test well and that of the corresponding virus control culture. Numerical differences between the reading of test wells containing a drug concentration which was partially cytotoxic (p) and their corresponding virus controls were halved. As requested by USAMRIID, the VR was also reported by the method of Sidwell and Huffman, 1971 (17). The VR calculated by the method of Ehrlich et al. was divided by 3 to obtain this value. The minimum inhibitory drug concentration which reduced the CPE by 50% (MIC_{50}) was calculated by using a regression analysis program for semilog curve fitting. A therapeutic index (TI) for each active test compound for each susceptible virus was determined by dividing the minimum cytotoxic concentration of the test compound by the MIC_{50}.

4.2.1 Vaccinia Virus (VV)

Vaccinia virus, strain Lederle CA, was obtained from Dr. Wilton Rightsel, at that time with Parke, Davis & Co., Detroit, Michigan. In our laboratories the virus was serially passaged in H.Ep-2 (continuous-passage human carcinoma of the larynx) and Vero (continuous-passage African green monkey kidney) cell cultures. For the in vitro antiviral screening against VV for USAMRIID, the virus was propagated and assayed in Vero cell monolayer cultures in Eagle's minimal essential medium (MEM) supplemented with 2% heat-inactivated fetal bovine serum (FBS) and 50 μg/ml of gentamicin.

Test compounds were screened for activity against VV by using a CPE-inhibition procedure as described above in Section 4.2. Replicate pregrown Vero cell monolayers were prepared by dispensing 0.2 ml aliquots of a suspension of 2×10^5 cells/ml in MEM + 5% heat-inactivated bovine calf serum (BCS) into wells of 96-well plates. The covered plates were incubated at 37 °C in a humidified atmosphere containing 2% CO_2 for 24 hours prior to use. The next day culture medium was removed from the cell monolayers, and 0.1 ml amounts of test compound and virus suspension (both diluted in MEM + 2% heat-inactivated FBS) were pipetted into the plate wells. The plates were returned to the CO_2 incubator. After four days incubation the cell monolayers were examined microscopically for virus-induced CPE and drug cytotoxicity.

4.2.2 Adenovirus Type 2 (Ad)

Adenovirus type 2, strain Adenoid 6, was obtained from the American Type Culture Collection and serially passaged in H.Ep-2 cell monolayer cultures in Eagle's MEM supplemented with 0.4% lactalbumin hydrolysate and 2% heat-inactivated FBS.

An early "cytotoxicity" is frequently observed in adenovirus-infected cell cultures. This "cytotoxicity", the result of penton toxin following an accumulation of unassembled viral components within the cell, can mask the later-appearing CPE. To prevent the

appearance of this early cytotoxicity virus stocks were treated as follows: Adenovirus-infected H.Ep-2 cell cultures were subjected to three cycles of freeze-thawing to disrupt the cell membranes. The material from the cultures was centrifuged, and the pooled supernatant fluid was treated with 100 μg/ml of purified trypsin (Worthington, TRL) for one hour at 37 °C. The enzyme action was then stopped by adding soybean trypsin inhibitor (Worthington, titrated for specific activity against trypsin) to the supernatant viral fluid. The trypsin-treated virus preparation, along with the untreated supernatant viral fluid was titrated for infectivity in H.Ep-2 cell cultures to make sure that the viral infectivity of the treated preparation was not diminished. Before the trypsin-treated stock adenovirus was used for antiviral screening, it was evaluated as the challenge virus against our positive control compounds Selenazole and Ribavirin.

Test compounds were screened for antiviral activity against adenovirus by the same procedure employed for the vaccinia virus antiviral assays.

4.2.3 Vesicular Stomatitis Virus (VSV).

The VSV (Indiana strain) was obtained from Dr. Robert Sidwell, Utah State University, Logan, Utah in 1973. Dr. Sidwell had originally obtained the virus from the ATCC. Since the virus has been in this laboratory, it has been passaged 3 times in L929 cells.

The L929 cells were obtained from the ATCC and have been carried in continuous passage or stored frozen in liquid nitrogen. The medium used to maintain the L929 cells was Eagle's minimum essential medium supplemented with 10 or 5% heat-inactivated fetal bovine serum, 100 units penicillin per ml, and 100 μg streptomycin per ml.

The preliminary in vitro CPE-inhibition assay described above was used to assay the drugs against VSV. The L929 cells were seeded into the 96 well plates at a concentration of 4×10^4 cells per well in 0.2 ml medium. The cells were pregrown for 20 hrs. before the addition of drug and virus. Virus-induced CPE was scored on Day 3 post-virus infection. carbocyclic 3-deaza-adenosine hydrochloride (C-3-deaza-AdO) was included in each VSV experiment as the positive control drug.

4.2.4 Punta Toro Virus (PT)

SoRI obtained 2 vials 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 moi ($\leq$ 0.1) in MEM + 10% inactivated fetal bovine serum (fbsi). Culture fluid was collected at five days post infection and clarified by centrifugation (5000 rpm, 15 minutes, 4 °C) in a Sorvall superspeed centrifuge. The supernate was dispensed into 0.5 ml aliquots, then frozen and stored at -120 °C. One aliquot was used to determine $CCID_{50}$ and PFU titers of the stock.

Antiviral screening of compounds for activity against PT virus was performed on Vero cells in 96 well trays (Corning). Cells were pre-treated for 30 minutes at 37 °C with DEAE-dextran (25 μg/ml) and 1% DMSO in 0.1 ml Hanks balanced salt solution. This was removed, then 0.1 ml containing 32 $CCID_{50}$ of PT virus was added and allowed to adsorb

for 30 minutes at 37 °C. 0.1 ml of test or control compound (Ribavirin or Selenazole) or medium was then added to appropriate wells. The test medium was MEM + 10% fbsi.

When virus control wells showed maximum CPE (5-7 days), the wells were rinsed with PBS and then fixed with methanol (4 min., 20 °C). Fixed cells were stained with 10% Giemsa stain (Sigma Chemicals, St. Louis, MO) and observed microscopically for CPE.

4.2.5 Sandfly Fever Virus (SF)

SoRI obtained vials of Sandfly fever virus, Sicilian strain (TC adapted) from Dr. George R. French of the Salk Institute, Government Services Division, Swiftwater, PA.

To grow virus stocks, Vero-76 cells (Dr. French, Salk Inst.) or Vero cells (ATCC), depending on availability, were pre-treated with DEAE-dextran (25 μg/ml) and 1% DMSO in growth medium (MEM + 10% fbsi) for 30 minutes at 37 °C. This was removed and then the cells were infected with SF virus at an moi of 0.1 in growth medium. 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 -120 °C. One aliquot was used to determine $CCID_{50}$ and PFU titers of the stock.

Antiviral screening of compounds for activity against SF virus were performed on Vero cells in 96 well trays (Corning). Cells were pre-treated for 30 minutes at 37 °C with DEAE-dextran (25 μg/ml) and 1% DMSO in 0.1 ml Hanks balanced salt solution. This was removed and then 0.1 ml growth medium containing 32 $CCID_{50}$ of SF virus was added and allowed to adsorb for 30 minutes at 37 °C. 0.1 ml of test compound, control compound (Ribavirin or Selenazole), or growth medium was then added to appropriate wells.

When virus control wells showed maximum CPE (5-6 days), the wells were rinsed with PBS and the cells fixed with methanol (4 min., 20 °C). Fixed cells were stained with 10% Giemsa stain (Sigma) and observed microscopically for CPE.

4.2.6 Pichinde Virus (PIC)

Vero cells obtained from Dr. George French of the Salk Institute, Swiftwater, PA were maintained in Eagle's Minimal Essential Medium (MEM) containing 10% fetal bovine serum. Baby hamster kidney (BHK-21) cells were obtained from the American Type Culture Collection and maintained in MEM containing 10% newborn calf serum and 10% tryptose phosphate broth.

Pichinde virus strain 4763 was obtained from Dr. J. Gangemi of the University of South Carolina, School of Medicine. The virus preparation received was that of a guinea pig spleen homogenate from pichinde virus infected guinea pigs. We passaged the virus four times in BHK-21 cells using input multiplicities of 10, 10, 1, and 0.1 pfu/cell. Virus containing culture fluids were collected and centrifuged 600 x g for 5 min. The debris-free supernantants were collected, aliquoted into 0.5 ml volumes and rapidly frozen in a dry-ice/ethanol bath. Ampules of virus were stored at -120°C. From the third passage of virus, a three-time plaque purified preparation of virus was generated in Vero cells and amplified twice in BHK-21 cell cultures. This preparation of virus, containing 6.4×10^7 plaque forming units (pfu) per ml, was used in the plaque reduction assay described below.

The agar overlay used in the assay contains Eagle's MEM, fetal bovine serum, glutamine, non-essential amino acids, sodium bicarbonate, and Noble agar in final concentrations of 1x, 2%, 1 mM, 1 mM, 2.2 mg/ml and 1% respectively.

Vero cells, seeded into six well cell culture trays at a density of 8×10^5 cells per well, were incubated at 37 °C in a humidified 5% CO_2 incubator. The following day the cultures were examined microscopically for confluency. Ribavirin, Selenazole, and experimental antiviral drugs were serially diluted in half-log increments in MEM containing 10% fetal calf serum (final concentration used were 320 μg/ml to 0.1 μg/ml). Culture fluids were removed by aspiration and to four of the six cultures of each tray was added a 0.2 ml inocula of pichinde virus diluted in MEM with 10% fetal bovine serum so as to contain 40 to 80 pfu. To the remaining cultures of each tray, 0.2 ml of MEM with 10% fetal bovine serum was added. The cultures were reincubated as above and periodically shaken so that the inocula was dispensed over the entire monolayer.

One hour later, 0.2 ml of the appropriate dilution of drug was added to three of the virus infected cultures (virus infected, treated) and to one of the mock infected cultures (toxicity control) on each tray. To the remaining mock infected (cell control) and virus infected (virus control) cultures was added 0.2 ml of MEM with 10% fetal bovine serum. The culture fluids were mixed by shaking the culture trays, then 1.6 ml of agar overlay was added and each culture was thoroughly mixed as above. The cultures were set on a level surface until the agar overlay solidified and were then reincubated at 37 °C.

Three days later, 2 ml of a sterilely filtered 0.1% neutral red solution (in phosphate buffered saline) was added to each culture. Cultures were reincubated; then six to eight hours later they were examined for pichinde virus plaques and the plaques were counted. With each daily assay, Ribavirin or Selenazole (positive antiviral drugs) was included in a parallel assay.

4.2.7 Yellow Fever Virus (YF)

SoRI obtained 1 vial of Yellow Fever Virus, Asibi strain, from Dr. Andrew J. Main, Jr., of the Yale Arbovirus Research Unit, New Haven, CT.

Currently, we are attempting to grow virus stocks in Vero cells. Antiviral screening has not yet begun.

4.2.8 Venezuelan Equine Encephalitis Virus (VEE)

SoRI obtained vials of Venezuelan Equine Encephalitis Virus, Trinidad donkey strain, from Dr. George R. French of The Salk Institute, Swiftwater, PA. Work has not yet begun with this virus due to insufficient immune response to the initial vaccine by several members of our technical staff (see Section 2.2). We are currently reorganizing staff and laboratory space so that *in vitro* work with this virus can begin.

4.2.9 Japanese Encephalitis Virus (JE)

SoRI obtained vials of Japanese Encephalitis Virus, Nakayama strain, from Dr. George R. French of the Salk Institute, Swiftwater, PA.

Due to low antibody levels to JE Virus in some personnel, we have not yet begun antiviral screening against this virus. We are currently reorganizing staff and isolation laboratory space to begin work with this virus in vitro.

4.2.10 Korean Hemorrhagic Fever Virus (KHF)

SoRI obtained several vials of Korean Hemorrhagic Fever (Hantaan) virus, strain 76-118, from Dr. John W. Huggins and Mr. Orville Brand of USAMRIID. Work with this virus is currently in progress.

4.2.11 Human Immunodeficiency Virus (HIV)

The human T-cell line H9, H9 cells infected with the HTLV-III_B strain of HIV, and H9 cells infected with the RF-II Haitian variant of HIV were obtained from Dr. Robert C. Gallo's laboratory (NCI) through Dr. Howard Streicher. The cells have been propagated in RPMI 1640 medium supplemented with 4 mM L-glutamine, 50 μM 2-mercaptoethanol, 15% heat-inactivated fetal calf serum, and antibiotics (penicillin/streptomycin). The human T-cell line ATH8 was obtained from Dr. Samuel Broder's laboratory (NCI) through Dr. Hiroaki Mitsuya. This cell line has been propagated in RPMI 1640 medium supplemented with 4 mM L-glutamine, 50 μM 2-mercaptoethanol, and antibiotics (penicillin/streptomycin), and containing 15% heat-inactivated fetal calf serum and either 50 units recombinant human interleukin-2 (ala-125; AMGen Biologicals) per ml or 15% natural human interleukin-2 (lectin-depleted; Advanced Biotechnologies, Inc.) with 20 units of the recombinant interleukin-2 per ml.

The initial screening of compounds for antiviral activity employs the CPE-inhibition assay developed by Broder and coworkers (11,12). This assay is based on the ability of uninfected AHT8 cells to grow and form a pellet at the bottom of a culture tube. Starting about 4 days after HIV addition, infected ATH8 cells begin to die and the pellet starts to break up. The cell pellet is completely destroyed within 10 days. The protective effect of test compounds can be assessed by adding them at varying concentrations to the cultured cells at the time of virus infection, then monitoring the status of the cell pellet.

ATH8 cells were treated with polybrene (1 μg/ml) for 30 min at 37°C, pelleted, then resuspended in clarified supernate freshly harvested from HTLV-III_B-infected H9 cells. Following a 60 minute adsorption period at 37°C, the cells were dispensed into either 15 ml round-bottom culture tubes (1.0 ml containing 2×10^5 cells per tube) or individual U-bottom wells of 96-well trays (0.1 ml containing 1×10^4 cells per well). To each tube, or well, was added an equal volume of supplemented RPMI 1640 medium containing test compound and twice the normal concentration of interleukin-2. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Periodically, aliquots were taken from individual samples and the total cell number and viability (based on trypan-blue dye exclusion) were determined. Each cell pellet was observed on day 10 post-infection, and its size was compared to the size of the infected and uninfected cell controls.

4.2.12 Feline Leukemia Virus - FAIDS Variant (FeLV)

SoRI obtained a frozen sample of Feline Leukemia Virus, FAIDS Variant from Dr. Ed Hoover of Colorado State University.

Following the procedure for growing stock virus established by Dr. Hoover, feline embryonic fibroblasts, AH-927 (Dr. Hoover), were grown in MEM + 10% fbsi + 1% non-essential amino acids. Twenty-four hours prior to infecting with virus, the cells were subcultured at a ratio of 1:4 and 0.2 ml/100 ml growth medium of Polybrene (2 mg/ml stock) was added to the medium. On the day of infection, the growth medium was removed and the cells were washed twice with PBS. The virus inoculum was allowed to adsorb for 1 hour at 37 °C. The virus inoculum was removed, the cells washed twice with PBS, and then a minimal volume of growth medium containing Polybrene was added. After five days incubation the culture fluid was collected and clarified by centrifugation (5000 rpm x 15 min). The supernate was dispensed into 1.0 ml aliquots, frozen, and then stored at -120 °C. One aliquot was used to determine the $CCID_{50}$ and the focus forming units (FFU) titers of the stock.

Antiviral screening of compounds for activity against FeLV-FAIDS was performed in 96 well trays (Corning). This screening procedure is a modification of the FeLV infectivity assay established bv Fischinger et al. (22). Forty-eight hours prior to the assay, the indicator cells, 81C (obtained from D. Graves, University of Oklahoma, Oklahoma City, OK) were subcultured at a ratio of 1:2. Twenty hours prior to the assay, 96 well trays were seeded with the 81C cells at 5×10^3 cells/well. On the day of the assay, the cells were pre-treated for 30 minutes at 37 °C with DEAE-dextran (25 μg/ml) in 0.1 ml Hanks balanced salt solution. This was removed and then 0.1 ml of growth medium (MEM + 10% fbsi) containing 32 $CCID_{50}$ of FeLV-FAIDS or 0.1 ml of growth medium was added to each well. The virus was allowed to adsorb for 1 hour then 0.1 ml of test or control compound (dideoxycytidine or 3'-azidothymidine), or growth medium was added. Plates were incubated at 37 °C. Cells were fed fresh growth medium containing compound on day 4 post-infection. Culture media were completely changed and replaced with fresh media containing compound on day 7 post infection. On day 10 post infection the cells were rinsed with PBS and then fixed with methanol (4 min., 20 °C). The fixed cells were stained with 10% Giemsa (Sigma) and examined microscopically for CPE.

4.3 Confirmatory Tests with Active Compounds

Active compounds from the primary in vitro screen will be tested for efficacy in confirmatory in vitro assays. The compounds listed in the summary table of active compounds (Table 13; Discussion, Section 6) are therefore scheduled to be evaluated further for their antiviral potential in confirmatory tests.

4.3.1 Active Compounds from the Basic Screen

The 50% minimum inhibitory concentration (MIC_{50}), in μg/ml, of the most active compounds from the primary in vitro screen will be confirmed by plaque-reduction assay.

Cells are seeded into 6-well or 24-well plastic tissue culture plates and grown for 24 hours at 37 °C in a humidified atmosphere of 5% CO_2 in air. The cultures are infected with an appropriate concentration of virus to yield a countable number of plaques. Following adsorption of virus to the cells, the test compounds are added to duplicate cultures in seven half-log_{10} dilutions, i.e., at concentrations that bracket the MIC_{50}. The cell cultures are overlaid with agarose or methylcellulose in growth medium and incubated at 37 °C for 3 to 6 days, depending on the virus, to allow for plaque formation. Cultures are fixed with 10% formalin and stained. Plaques are then counted and the MIC_{50} values are determined from best-fit curves obtained by regression analysis. The minimum toxic

concentration (MTC) and the in vitro therapeutic index (TI) will also be confirmed in these assays.

4.3.2 Active Compounds from the AIDS Screen

Candidate active antiviral compounds from the primary anti-HIV screen (ATH8 cell cytopathic assay) are to be tested in confirmatory assays for antiviral efficacy in vitro. These assays consist of a quantitative virus yield-reduction assay based on viral reverse transcriptase levels in infected H9 cells and a virus yield-reduction assay based on viral p24 gag protein expression in uninfected H9 cells.

4.3.2.1 Reverse Transcriptase Assay

H9 cells are exposed to HTLV-III/LAV (HIV) and cultured in the presence and absence of various concentrations of active test compound. After 24 hours, the cells are washed, resuspended in fresh growth medium, and cultured further with the same concentrations of test compound; or, for controls, with fresh growth medium alone (no test compound). On Day 5 postinfection, the cells are centrifuged and the supernatant fluids are harvested and assayed for reverse transcriptase activity according to previously described methods (18, 19). Results are expressed as counts per minute of deoxy(methyl-^{3}H)thymidine monophosphate (40-60 Ci/mmol) incorporated per 10 μl of concentrated (25-fold) culture supernatants. Antiviral efficacy is measured by the percent reduction in reverse transcriptase activity observed in treated, virus-infected cultures when compared with the enzyme activity in the control (untreated), virus-infected cultures.

4.3.2.2 Immunofluorescence Assay for p24 gag Protein Expression

The details of the indirect immunofluorescence assay (IFA) for HTLV-III/LAV (HIV) based on intracellular expression of viral p24 have been worked out using virus-infected H9 cells and monoclonal antibody to p24 (20) which was obtained from Drs. Veronese and Gallo through Dr. Howard Streicher, National Cancer Institute, Bethesda, MD. The dilution of antibody for use under routine conditions was determined by titration of the antibody on acetone-fixed, virus-infected cells. Fluorescein-conjugated goat anti-mouse IgG was obtained commercially. In the staining procedure, virus-infected cells at a density of approximately 1 x 10^6 cells/ml were spotted on glass slides, air-dried, and fixed in cold acetone (-20 °C). Fixed cells were incubated with dilutions of monoclonal antibody in PBS for 30 minutes at 37 °C, washed in three changes of PBS for a total of about 10 minutes, then incubated with a 1:20 dilution of fluorescein-conjugated antiserum for 30 minutes at 37 °C, washed again, and mounted in phosphate-buffered glycerol. Cytoplasmic fluorescence was clearly observable at antibody dilutions up to 1:2000. Staining was progressively weaker at dilutions of 1:4000 and 1:8000.

In confirmatory tests with candidate active antiviral agents, reductions in virus yields are measured based on IFA determinations of p24 gag protein expression in treated and untreated infected H9 cells in vitro. Replicate cultures of H9 cells (10^5 cells per culture) are exposed to various concentrations of active test compounds for 4 hours and then to polybrene (2 μg/ml) for 30 minutes. The cells are pelleted and exposed to HIV (3,000 virus particles per cell) for 1.5 to 2.0 hours. The cells are then resuspended in fresh growth medium containing test compound or, for control, in medium alone (without test compound) and are then incubated at 37 °C in an atmosphere of 5% CO_2 in air. The percentage of target H9 cells expressing HIV p24 gag protein is determined on Days 8, 9,

and 10 postinfection by IFA using the anti-HIV p24 monoclonal antibody provided by NCI. The antiviral efficacy of the test compound is measured by the percent reduction in the number of cells exhibiting specific immunofluorescence in the cultures after treatment when compared with the number of cells expressing p24 in the control (untreated), virus-infected cultures.

4.4 Antiviral Evaluations In Vivo

4.4.1 Pichinde Virus in MHA Strain Hamsters

The in vivo arenavirus model is Pichinde virus (strain An 4763) infection of inbred MHA strain hamsters. Hamsters (Charles River Labs) are received at 3 weeks of age and are quarantined for 1 week prior to use. The hamsters are positive for antibodies to Sendai virus and Pneumonia Virus of Mice (PVM). Animals are housed in microisolator cages (Lab Products) at 5 hamsters/cage and are fed Wayne Autoclavable Lab Rodent Chow ad libitum. All cage manipulations including feeding and watering in animal rooms holding infected animals are performed in biosafety cabinets. Cages and water bottles are decontaminated by autoclaving prior to cleaning.

4.4.1.1 Assessment of Infectivity and Lethality.

In order to assess the infectivity and lethality of Pichinde virus (strain An 4763) in MHA strain hamsters, a preliminary challenge experiment was performed with BHK cell passage 1 Pichinde virus (PIC) (see Section 4.2.6). The stock virus (1.8×10^7 pfu/ml) was diluted in Minimal Essential Medium (MEM) containing 2% fetal calf serum (FCS) to provide challenge doses of (1) 1×10^5 pfu; (2) 5×10^4 pfu; (3) 1×10^4 pfu; (4) 5×10^3 pfu; (5) 1×10^3 pfu and (6) 5×10^2 pfu in an inoculum volume of 0.5 ml. Groups of ten animals each (5 male, 5 female) were inoculated by the intraperitoneal (IP) or subcutaneous (SC) routes at each challenge level. Virus diluent (MEM with 2% FCS) was administered SC and IP to ten animals each. Ten uninoculated animals were held as normal controls. Animals were monitored for 21 days post-inoculation and daily animal mortalities were recorded. The percent mortality and the average day of death (ADD) were calculated for each group using the formulae:

$$\% \text{ mortality} = (\# \text{ dead}/\text{total } \# \text{ inoculated}) \times 100$$

$$\text{ADD} = [\Sigma(\text{day of death}) \times (\# \text{ dead that day})] / \text{total } \# \text{ dead}$$

4.4.1.2 Determination of LD_{50}

The 50 percent lethal dose (LD_{50}) of our passage 4 PIC stock for 4 week old MHA strain hamsters was determined by titrating serial two-fold dilutions of virus from a high dose of 3×10^4 pfu/hamster to a low dose of 1.8 pfu/hamster. Groups of 10 hamsters (5 male, 5 female) were inoculated IP with each challenge dose in 0.5 ml of MEM with 2% FCS. Controls included (1) 10 uninoculated hamsters and (2) 10 hamsters receiving 0.5 ml of virus diluent. Animals were monitored for 21 days post-inoculation. The percent mortality and ADD were calculated for each group. The LD_{50} was determined by the method of Reed and Meunch (15).

4.4.1.3 Protective Effect of Positive Control Compound

The efficacy of Ribavirin in decreasing mortality and increasing ADD was assessed in hamsters challenged with various numbers of 50% lethal doses of passage 4 PIC. Five groups of 10 hamsters (5 male, 5 female) each were challenged on day 0 with 100 LD_{50}, 32 LD_{50}, 10 LD_{50}, or no challenge. One group of animals from each challenge level was treated SC once daily for 7 days, starting 4 hours post-infection, with either 100 mg Ribavirin/kg of body weight, 56 mg Ribavirin/kg of body weight, 32 mg Ribavirin/kg of body weight, Ribavirin diluent (water), or no treatment. An additional control included 10 animals inoculated with virus diluent alone. The animals were monitored for 21 days post-infection and the percent mortality and ADD for each group were calculated.

4.4.2 Venezuelan Equine Encephalitis Virus (VEE) in Mice

Outbred female CD-1 mice are received at 3 weeks of age and quarantined for 1 week. The mice are virus-antibody free. Mice are housed in microisolator cages (Lab Products) and fed Wayne Autoclavable Lab Rodent Chow ad libitum. Upon notification of satisfactory VEE antibody titers in BL-3 personnel, the mice will be challenged with VEE (Trinidad Donkey Strain) and an LD_{50} determination performed.

4.5 Secondary Evaluations

Following confirmatory testing, candidate active antiviral agents are further evaluated in a battery of secondary evaluations, primarily to assess the selectivity of the compound, i.e., its potential for inhibiting virus replication without causing cytotoxic or immunotoxic effects.

4.5.1 Basic Screen

4.5.1.1 Compound Cytotoxicity Determinations

Vero, Vero-E6, L-929, and H.Ep-2 cells are used to more quantitatively determine the cytotoxicity of active compounds. Cells will be grown in 96-well plates until near confluency. The medium is replaced with fresh growth medium containing the test compound at four different concentrations (0.5-$\log_{10}$ dilutions: e.g., 32, 100, 320, and 1000 μg/ml) to bracket the minimal cytotoxic concentration estimated from the primary CPE-inhibition assays. Quadruplicate wells are used for each drug concentration along with an equal number of untreated controls. Cells are exposed to the test compound in a manner identical to that used for the antiviral experiments after which the cells are trypsinized, serially diluted in medium, and plated in Costar 12-well plates. After incubation in compound-free medium for 3 to 5 days, the cells are washed with PBS, fixed with 10% formalin, and stained with crystal violet. The number of cell colonies are counted in each well and the relative plating efficiency in terms of % survival is calculated by dividing the average number of untreated cell colonies by the average number of treated cell colonies x 100. The minimum toxic concentration is the lowest concentration of test compound that results in a 50% reduction in % survival of viable host cells under the test conditions.

4.5.1.2 Determination of Selectivity Ratio or In Vitro Therapeutic Index

A selectivity ratio or in vitro therapeutic index is calculated for each active test compound for each susceptible virus in the screen. The therapeutic index is determined by dividing the minimum cytotoxic concentration of the test compound by the median inhibitory dose (ID_{50}) or minimum inhibitory concentration, 50% (MIC_{50}).

4.5.2 AIDS Screen

4.5.2.1 Effects on Normal T-Cells

The viability of normal H9 or TM3 cells after exposure to various concentrations of the candidate antiviral drugs in vitro for varying periods of time is assessed by hemocytometer cell counts in conjunction with the trypan blue dye-exclusion test. Assays are performed in triplicate and the data are expressed in terms of the mean numbers of viable cells per ml + the standard deviation.

4.5.2.2 Effects on Normal B-Cell and T-Cell Immunologic Function

Any active antiviral compounds uncovered in the primary HTLV-III/LAV CPE-inhibition screen in ATH8 cells will be assessed for their effects on normal T-cell function in vitro. Responder TM3 cells (5×10^4 cells) will be washed and cultured for 3 days with tetanus toxoid and with irradiated autologous peripheral blood mononuclear (PBM) cells (10^5 cells) in round-bottom microtiter plate wells using cell culture medium containing 15% fetal calf serum (160 μl per well) in the presence and absence of the candidate compounds in varying concentrations. In other assays, 10^5 fresh PBM cells will be cultured with or without mitogens (phytohemagglutinin or pokeweed mitogen) for 3 days in flat-bottom microtiter plate wells in the presence and absence of inhibitors. All cultures will be exposed to either 0.5 μCi of methyl-^{3}H -thymidine of 1.0 μCi of [3-^{3}H] -uridine (depending on the nucleoside analog being evaluated) for the last 5 hours of incubation prior to harvest onto glass fiber filters. The amount of incorporated radioactivity will then be determined in a Packard liquid scintillation spectrometer. Data will be expressed as the arithmetic mean counts per minute (cpm) x 10^3 + the standard deviation of triplicate determinations. Incorporation of ^{3}H-thymidine into DNA and incorporation of ^{3}H-uridine into RNA will be employed for adenine- and guanine-containing nucleoside analogs, whereas ^{3}H-uridine incorporation into RNA alone will be employed for the thyminidine- and cytosine-containing nucleoside analogs.

The effects of candidate antiviral compounds on the helper activity of TM3 cells will be assessed by IgG production in indicator B cells in vitro. Indicator B cells (10^5 cells) will be exposed to tetanus toxoid for 4 hours, extensively washed, and then co-cultured with 5×10^4 TM3 cells in the presence and absence of the candidate antiviral drugs at varying concentrations. After 8 days, the culture supernatants will be collected and assessed for IgG production using an enzyme-linked immunosorbent assay (ELISA assay).

4.5.2.3. Other Animal Retroviruses

In addition to the FeLV (FAIDS) assays, active compounds from the primary HIV screen will be tested for selective inhibitory activity against murine leukemia viruses

(strain CAS-BR-M, strain N-6 derivative, and strain 2713) and against simian AIDS type D/Washington virus in vitro.

The MuLV strains will be provided by Dr. John Bilello, VA Medical Center, Baltimore, MD. The CAS-BR-M and N-6 strains of MuLV will be assayed in SC-1 feral mouse embryo cells using Eagle's minimum essential medium (MEM) with Earle's balanced salt solution containing 5 to 10% heat-inactivated fetal bovine serum as the growth medium. The UV-XC plaque-reduction assay procedure will be employed in the evaluation of candidate antiviral agents for efficacy. The 2713 strain of MuLV will be assayed in normal rat kidney (NRK) cells by means of a focus-inhibition assay similar to that employed with the FAIDS virus.

For the UV-XC plaque-reduction assays, SC-1 cells are grown as monolayers in Falcon 6-well tissue culture plates. The test compound is added to the cultures at the time of virus inoculation and is present for three days until the cultures are irradiated with UV light and rat XC cells are added. Five concentrations of test compound in serial half-$\log_{10}$ dilutions, ranging from cytotoxic to noncytotoxic, are tested against two different concentrations of MuLV. The test cultures (triplicate cultures per drug concentration) each contain 2 ml of test compound dilution in growth medium and 0.5 ml of the appropriate dilution of the virus suspension. Virus control cultures (6 cultures per virus dilution) contain 2 ml of growth medium (without test compound) and 0.5 ml of virus suspension. Drug cytotoxicity control cultures (containing test compound but no virus) and cell control cultures (containing no test compound or virus) are included in each assay. On Day 3 after virus inoculation, the cultures are irradiated and XC cells are added. Three days after UV irradiation, the cell cultures are stained with hematoxylin and the plaques are counted. Antiviral activity is expressed in terms of the reduction in the mean number of plaques counted in treated, virus-infected cultures compared with the mean number of plaques counted in the untreated, virus-infected control cultures. Hemacytometer cell counts using trypan blue dye-exclusion to determine cell viability are performed on Day 3, at the end of drug treatment, on duplicate treated and control (untreated) cultures.

The simian AIDS type D/Washington virus will be provided by Dr. Che-Chung Tsai, Regional Primate Research Center, University of Washington, Medical Lake, WA. Attempts will be made to develop an antiviral assay based on CPE-inhibition in A549 human adenocarcinoma cells, or in various T-cell lines. If reproducible cytopathology cannot be obtained, we plan to perform virus yield-reduction assays based on observed reductions in reverse transcriptase activity in infected cells after treatment with candidate antiviral drugs. The possibility of a focus-inhibition assay in NIH 3T3 cells will also be examined.

4.6 Data Analysis and Processing

Data collected in the laboratories are submitted to and processed from a central office located at the SoRI homesite facilities. Screening data collected in the BL-3 containment area are telecommunicated to the homesite using Compac II personal computers with Hayes Internal Modems.

Copies of screening raw data are presently submitted to Technassociates, Inc. on a regular weekly basis for input into the VAX computer. Once the Rbase-Program Interface (PI) computer is appropriately modified and all desired information is included on the data

record forms, we will be able to input the data directly on our computers, down-load onto floppy diskettes, and send these to Technassociates already formatted to transfer to their Vax computer. Also, these data will be stored on our DECSYSTEM 2040 Mainframe computer as a back-up database as well as a means of generating various types of summaries for report purposes.

5. RESULTS

5.1 Primary Antiviral Screening In Vitro

During the twelve-month reporting period from November 15, 1985 to November 15, 1986, a total of 348 test compounds were submitted to SoRI for evaluation in the primary antiviral screen in vitro. A number of these compounds were shipped to SoRI in amounts that were too small for appropriate evaluation in the entire screen and priorities were placed on the spectrum of target viruses against which those particular compounds were tested. Some of the compounds were shipped to SoRI for screening against human immunodeficiency virus (HIV) alone. During this reporting period, results were obtained for the majority of the submitted compounds which were assayed for activity against vaccinia virus (VV), adenovirus (Ad), vesicular stomatitis virus (VSV), Punta Toro (PT) virus, sandfly fever (SF) virus, Pichinde (PIC) virus, and feline AIDS (FAIDS) virus. Screening operations with the four BL-3 level virus, namely yellow fever (YF) virus, Venezuelan equine encephalitis (VEE) virus, Japanese encephalitis (JE) virus, and Korean hemorrhagic fever (KHF) virus, had not yet begun, nor had routine screening of compounds for activity against HIV yet commenced. The results presented in this section of the report represent a summary of the active compounds found among those submitted and evaluated in over 1,000 antiviral assays. Summary tables which present all of the in vitro antiviral test results (both positive and negative results) are included in Appendix B.

5.1.1. Vaccinia Virus (VV)

The data summarized in Table 2 indicate that 32 of the compounds tested for activity against vaccinia virus (VV) in vitro exhibited significant antiviral efficacy with VR's $\geq$1.0 and TI values >1.0. The compounds are listed in descending order of selective antiviral activity, with those compounds with the highest TI values at the top of the list. Compounds AVS-1986, AVS-1985, AVS-1987, AVS-1160, and AVS-148 all had TI ratios $\geq$100 and VR's >5.0. It can be seen that compound AVS-1986 was the most active compound screened against VV in vitro. It had a VR $\geq$7.4 and a TI of >533. In concomitant assays, the positive control drug (araA) yielded VR's in the range of 2.4 to 3.1 and TI values in the range of 3.1 to 33. Thus, these new antiviral agents are much more selective in their inhibition of vaccinia virus replication in cell culture (Vero cells) than araA. With MIC_{50}'s in the range of <0.32 to 1.7 μg/ml, these top five compounds are also more potent than araA which has an MIC_{50} in the range of 3.2 to 9.8 μg/ml. These compounds are easily among the most active materials yet found with anti-poxvirus potential and they should be virogously pursued in confirmatory in vitro assays and in animal model chemotherapy studies in vivo.

5.1.2. Adenovirus Type 2 (Ad)

The compounds active against human adenovirus type 2 (Ad) are listed in Table 3. Fifteen of the submitted compounds tested for activity against Ad were significantly effective in inhibiting virus-induced cpe in HEp-2 cells. The VR's of these active test materials ranged from 1.0 to 2.4 and the TI values ranged from 1.0 to 5.3. The most active compounds in terms of selectivity appeared to be compounds AVS-70, AVS-79, and AVS-253. These compounds had ID_{50} values in the range of 0.06 to 8.1 μg/ml. Compounds AVS-349 and AVS-1089 were also potent inhibitors of adenovirus in vitro, with ID_{50} values of 0.1 and 3.2 μg/ml and TI values of 3.2 and 3.1, respectively.

TABLE 2. COMPOUNDS ACTIVE AGAINST VACCINIA VIRUS [a]

AVS #	Shipment	VR	VR *	ID_{50}	MTC	TI
1986	2	≥7 4	≥2.5	0.6	>320	>533
1985	2	≥7.2	≥2.4	<0.32	100	>312
1987	2	6.8	2.3	1.7	>320	>188
1160	5	≥5.6	≥1.9	0.6	100	166.7
148	2	5.4	1.8	1.0	100	100
1988	2	6.4	2.1	3.6	>320	> 89
138	1	3.8	1.3	19.9	>320	> 16
138[b]	1	3.1	1.0	8.8	100	11.4
303	1	5.2	1.7	0.6	10	15.9
303[b]	1	≥3.5	≥1.2	0.4	10	25
2170	8	4.7	1.6	1.3	10	7.7
2290	11	3.2	1.1	5.7	32	5.6
272	1	4.7	1.6	0.7	3.2	4.6
272[b]	1	≥3.4	≥1.1	0.5	3.2	6.4
215[b]	1	>4.2	>1.4	0.7	3.2	4.6
215	1	3.0	1.0	2.8	10	3.6
71	2	1.7	0.6	81.4	320	3.9
1089	5	1.7	0.6	81.4	320	3.9
122	4	2.1	0.7	10	32	3.2
79	1	1.7	0.6	32	100	3.1
79[b]	1	1.4	0.5	1.7	3.2	1.9
1972	1	0	0	---	32	---
1972[b]	1	1.2	0.4	32	100	3.1
2136	5	1.7	0.6	144.2	>320	> 2.2
1	4	2.1	0.7	150.7	>320	2.1
2291	11	1.9	0.6	4.7	10	2.1
200	2	1.0	0.3	52.4	100	1.9
257	5	1.4	0.5	174.1	320	1.8
139	1	2.7	0.9	1.9	1.0	0.5
139[b]	1	1.3	0.4	1.8	3.2	1.8
646	2	≥4.0	≥1.3	0.6	1.0	1.7
206	5	1.2	0.4	85.3	100	1.2

a. Compounds identified by their AVS number are listed in descending order from the most active to the least active. Assays were done in Vero cells. An active compound is defined here as having a therapeutic index (TI) of greater than 1.0 and a virus rating (VR) of 1.0 or greater. The VR is a measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced CPE and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (16). VR * is the designation for the virus rating calculated by the method of Sidwell and Huffman (17). The drug concentration which reduced the CPE by 50% (50% inhibitory dose, ID_{50}) was calculated using a regression analysis program for semilog curve fitting and is shown here in units of µg/ml. The minimum cytotoxic drug concentration (MTC) is also in units of µg/ml. The therapeutic index of a test compound was determined by dividing the MTC by the ID_{50}.

b. This assay was done in L929 cells.

TABLE 3. COMPOUNDS ACTIVE AGAINST ADENOVIRUS[a]

AVS #	Shipment	VR	VR*	ID_{50}	MTC	TI
70	1	2.4	0.8	0.06	0.32	5.3
79	1	1.3	0.4	2.3	10	4.4
253	1	1.6	0.5	8.1	32	4.0
1984	2	1.9	0.6	88	>320	3.6
2173	8	1.1	0.4	27.3	100	3.6
2229	10	1.0	0.3	28	100	3.6
136	4	1.3	0.4	96.9	320	3.3
2277	11	1.1	0.4	100	320	3.2
349	2	1.0	0.3	0.1	0.32	3.2
1089	5	1.0	0.3	3.2	10	3.1
1	4	1.4	0.5	57.4	100	1.7
195	4	1.4	0.5	217	>320	>1.5
94	1	1.0	0.3	231.1	320	1.4
2290	11	1.0	0.3	25.4	32	1.3
215	1	2.3	0.8	9.5	10	1.1

a. Compounds identified by their AVS number are listed in descending order from the most active to the least active. Assays were done in HEp-2 cells. An active compound is defined here as having a therapeutic index (TI) of greater than 1.0 and a virus rating (VR) of 1.0 or greater. The VR is a measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced CPE and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (16). VR* is the designation for the virus rating calculated by the method of Sidwell and Huffman (17). The drug concentration which reduced the CPE by 50% (50% inhibitory dose, ID_{50}) was calculated using a regression analysis program for semilog curve fitting and is shown here in units of µg/ml. The minimum cytotoxic drug concentration (MTC) is also in units of µg/ml. The therapeutic index of a test compound was determined by dividing the MTC by the ID_{50}.

5.1.3. Vesicular Stomatitis Virus (VSV)

From the data presented in Table 4, it can be seen that only 3 compounds: AVS-2127, AVS-303, and AVS-1160 exhibited significant antiviral efficacy against VSV in L-929 cells. These compounds exhibited VR's in the range of 1.0 to 3.2, ID_{50}'s in the range of <0.32 to 23.4 μg/ml, and TI values of 3.1 to 4.3. The positive control drug (the carbocyclic analog of 3-deazaadenosine) yielded VR's in the range of 2.1 to 3.4, ID_{50}'s in the range of 0.8-4.9 μg/ml, and TI values of 3.2 to 10.3. Compound AVS-1160 appeared to be the most potent anti-VSV compound in these studies with an ID_{50} in the range of <0.32 to 1.0 μg/ml and TI values of 3.1 to 3.2.

5.1.4 Punta Toro Virus (PT)

A total of 35 compounds exhibited significant antiviral efficacy against Punta Toro (PT) virus in Vero cells (Table 5). By far, the most active and most potent compound against this particular virus was compound AVS-148 with a VR of 6.3, an ID_{50} of <0.32 μg/ml, a minimum toxic concentration (MTC) of 320 μg/ml, and a calculated TI of >1000. Ribavirin, the positive control drug, yielded VR's in the range of 1.5 to 3.8, ID_{50}'s in the range of 8.2 to 41.8 μg/ml, and TI values of 2.1 to >12.2 in this assay system. Compound AVS-148 is therefore an excellent candidate for follow-up in vitro and in vivo studies as an antiviral agent with activity against PT virus.

5.1.5. Sandfly Fever Virus (SF)

Approximately 38 of the submitted compounds tested in the antiviral screen for efficacy against sandfly fever (SF) virus in Vero cells demonstrated inhibition of virus-induced cpe (Table 6). The most active compounds, in terms of antiviral selectivity, were compounds AVS-2159, AVS-139, AVS-253, AVS-94, AVS-84, AVS-79, AVS-1089, AVS-2220, AVS-2160, AVS-2188, and AVS-1983. All 11 of these materials had TI values ≥ 100. AVS-2159 and AVS-139 appeared to be the most selective, with TI values >1000. The most potent inhibitor of SF virus in vitro appeared to be AVS-139 with an ID_{50} of <0.001 μg/ml. AVS-253, however, consistently yielded the highest VR values and appeared to be as active as the positive control drugs in its inhibition of SF virus in vitro.

5.1.6. Pichinde Virus (PIC)

Initial work was started using Pichinde virus strain 3739 as had been indicated in our original proposal. It was later decided that strain 4763 be used since it was being used by other contractors in the program. During the early stages of our Pichinde virus work, a great deal of effort was spent on the development of a CPE-inhibition assay for use in the drug screen. It was felt that this type of assay would be the most economical to use, both in supplies and labor.

Previously published work indicated that high titered Pichinde virus preparations could be obtained by infecting BHK-21 cell cultures at multiplicities of infection (m.o.i.) of greater than 10 pfu per cell. After receiving the homogenized guinea pig spleen preparation of strain 4763, we prepared virus stocks in BHK-21 cell cultures using input m.o.i.s of 10 pfu/cell or greater. When these stocks of virus were tested for their ability to cause viral cpe in Vero cell cultures, minimal amounts of cpe were detectable microscopically. Even when Vero cell cultures were inoculated with undiluted preparations of these stocks containing up to 10^7 pfu, only about 50% viral cpe was evident.

TABLE 4. COMPOUNDS ACTIVE AGAINST VESICULAR STOMATITIS VIRUS[a]

AVS #	Shipment	VR	VR*	ID_{50}	MTC	TI
2127	7	1.0	0.3	23.4	100	4.3
303	1	2.8	0.9	3.1	10	3.2
1160	5	3.2,2.6	1.1,0.9	<0.32,1.0	1.0,3.2	3.1,3.2

a. Compounds identified by their AVS number are listed in descending order from the most active to the least active. Assays were done in L929 cells. An active compound is defined here as having a therapeutic index (TI) of greater than 1.0 and a virus rating (VR) of 1.0 or greater. The VR is a measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced CPE and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (16). VR* is the designation for the virus rating calculated by the method of Sidwell and Huffman (17). The drug concentration which reduced the CPE by 50% (50% inhibitory dose, ID_{50}) was calculated using a regression analysis program for semilog curve fitting and is shown here in units of µg/ml. The minimum cytotoxic drug concentration (MTC) is also in units of µg/ml. The therapeutic index of a test compound was determined by dividing the MTC by the ID_{50}.

TABLE 5. COMPOUNDS ACTIVE AGAINST PUNTA TORO VIRUS [a]

AVS #	Shipment	VR	VR*	ID_{50}	MTC	TI
148	2	6.3	2.1	<0.32	320	>1000
94	1	3.0	1.0	10	>320	> 32
1975	1	2.7	0.9	10.5	320	30.5
15	1	>4.3	>1.4	18.2	>320	> 17.6
68	1	2.4	0.8	2.4	32	13.3
253	1	3.4,1.6,4.3	1.1,0.5,1.4	3.2,18.3,2.3	32,32,32	10,1.8,13.9
113	1	2.0	0.7	3.2	32	10
1974	1	1.7	0.6	14.2	100	7
200	2	2.1	0.7	15.6	100	6.4
2026	9	1.8	0.6	17.3	100	5.8
1	4	3.2	1.1	18	>100	> 5.6
1973	1	1.6	0.5	19.2	100	5.2
360	2	2.0	0.7	0.2	1.0	5
206	4,5	2.7,1.5	0.9,0.5	67,73	>320,320	>4.8,4.4
84	1	1.9	0.6	66.4	320	4.8
64	1	2.0	0.7	68	320	4.7
197	5	1.7	0.6	73	>320	> 4.4
136	4	1.5	0.5	73	>320	> 4.4
95	2	1.9	0.6	22.7	100	4.4
1089	5	3.4	1.1	0.9	3.2	3.6
199	2	1.5	0.5	30.7	100	3.3
272	1	2.3	0.8	1.0	3.2	3.2
2018	3	1.3	0.4	31	100	3.2
33	2	1.2	0.4	32	100	3.1
202	4	1.9	0.6	141	320	2.3
87	2	1.1	0.4	47	100	2.1
2006	3	1.2	0.4	56	100	1.8
2005	3	1.0	0.3	64	100	1.6
215	1	2.7	0.9	2.2	3.2	1.5
2034	9	2.1	0.7	6.8	10	1.5
2020	3	1.6	0.5	21	32	1.5
1992	3	1.5	0.5	66	100	1.5
1980	1	1.7	0.6	227	>320	> 1.4
2138	5	1.4	0.5	236.3	>320	> 1.4
1995	3	1.1	0.4	69	100	1.4

a. Compounds identified by their AVS number are listed in descending order from the most active to the least active. Assays were done in Vero cells. An active compound is defined here as having a therapeutic index (TI) of greater than 1.0 and a virus rating (VR) of 1.0 or greater. The VR is a measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced CPE and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (16). VR* is the designation for the virus rating calculated by the method of Sidwell and Huffman (17). The drug concentration which reduced the CPE by 50% (50% inhibitory dose, ID_{50}) was calculated using a regression analysis program for semilog curve fitting and is shown here in units of µg/ml. The minimum cytotoxic drug concentration (MTC) is also in units of µg/ml. The therapeutic index of a test compound was determined by dividing the MTC by the ID_{50}.

TABLE 6. COMPOUNDS ACTIVE AGAINST SANDFLY FEVER VIRUS [a]

AVS #	Shipment	VR	VR*	ID_{50}	MTC	TI
2159	9	3.8	1.3	<0.32	>320	>1000
139	1	1.9	0.6	<0.001	1.0	>1000
253	1	>6.1,4.4,6.0	>2.0,1.5,2.0	1.0,0.14,1.0	>320,>100,>100	>320,>714,>100
94	1	>4.7	>1.6	1.3	>320	> 246
84	1	4.4	1.5	1.3	320	246
79	1	>5.5	>1.8	0.65	>100	> 154
1089	5	5.2	1.7	1.0	>100	> 100
2220	10	3.2	1.1	<0.32	32	> 100
2160	9	3.1	1.0	<0.32	32	> 100
2188	9	2.6	0.9	<0.32	32	> 100
1983	2	3.0	1.0	0.32	32	100
1984	2	2.9	1.0	1.0	32	32
646	2	4.2,4.3	1.4,1.4	<0.1,0.6	3.2,10	>32,16.7
1	4	4.5	1.5	5.2	>100	> 19.2
1985	2	3.7	1.2	6.1	>100	> 16.4
360	2	2.5,1.8	0.8,0.6	0.02,0.32	0.32,1.0	16,3.1
206	4	3.7	1.2	22.6	320	14.2
148	2	3.6	1.2	2.3	32	13.9
2219	10	2.5	0.8	2.5	32	12.8
197	5	3.4	1.1	49.7	>320	> 6.4
71	2	3.7	1.2	16.4	>100	> 6.1
2013	3	3.1	1.0	52.3	>320	> 6.1
1976	1	2.4	0.8	18.2	>100	> 5.5
1987	2	3.1	1.0	19	100	5.3
2226	10	1.4	0.5	8.8	32	3.6
2136	5	1.4	0.5	88	320	3.6
1997	3	2.1	0.7	96.3	320	3.3
33	2	3.4	1.1	10	32	3.2
272	1	2.2	0.7	1.0	3.2	3.2
65	2	2.6	0.9	1.6	3.2	2.0
347	2	1.5	0.5	5.1	10	2.0
230	1	2.3	0.8	53.5	100	1.9
2221	10	1.2	0.4	1.8	3.2	1.8
2217	10	1.3	0.4	1.9	3.2	1.7
1160	5	1.0	0.3	70.4	100	1.4
349	2	1.7	0.6	2.5	3.2	1.3
2223	10	1.1	0.4	79.8	100	1.3
345	5	1.0	0.3	88	100	1.1

a. Compounds identified by their AVS number are listed in descending order from the most active to the least active. Assays were done in Vero cells. An active compound is defined here as having a therapeutic index (TI) of greater than 1.0 and a virus rating (VR) of 1.0 or greater. The VR is a measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced CPE and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (16). VR* is the designation for the virus rating calculated by the method of Sidwell and Huffman (17). The drug concentration which reduced the CPE by 50% (50% inhibitory dose, ID_{50}) was calculated using a regression analysis program for semilog curve fitting and is shown here in units of µg/ml. The minimum cytotoxic drug concentration (MTC) is also in units of µg/ml. The therapeutic index of a test compound was determined by dividing the MTC by the ID_{50}.

Since Pichinde virus, like other arenaviruses, has been shown to generate defective interfering (DI) particles readily, it was possible that our stock preparations of strain 4763 contained sufficient numbers of DI particles so as to prevent cpe in vitro. In order to prevent the amplification of Pichinde virus DI particles, new stocks of strain 4763 were made by infecting BHK-21 cell cultures at a low m.o.i. (0.1 pfu per cell). When this new virus preparation was tested for it's ability to cause viral cpe in Vero cell cultures, little cpe was evident. Plaque purified Pichinde virus stocks were made but those preparations of virus also produced little cpe in vitro.

While we are still attempting to develop a usable cpe-inhibition assay, a plaque reduction assay has been developed and compounds are being screened using this method. Our results to date are shown in Table 7. Only a few of the submitted antiviral drugs along with Ribavirin and Selenazole, have been tested in this assay. It should be noted however, that the plaque reduction assay, used for the screening of anti-Pichinde virus drugs, is labor intensive and therefore will require more time for testing antiviral drugs than the conventional cpe-inhibition assay used in the other antiviral screens.

The data summarized in Table 7 represent the results with compounds found to be active against Pichinde virus replication in Vero cells using the plaque-reduction assay procedure. Nine of the test compounds submitted for antiviral screening demonstrated significant antiviral efficacy against PIC in vitro. Five of the compounds had TI values >10. The compound that exhibited the greatest selectivity for inhibition of PIC was AVS-215, followed by AVS-230. These top five compounds are good candidates for evaluation as chemotherapeutic agents against PIC infections in MHA strain hamsters.

5.1.7. Yellow Fever Virus (YF)

No antiviral screening results are available for YF virus.

5.1.8. Venezuelan Equine Encephalitis Virus (VEE)

No antiviral screening results are available for VEE virus.

5.1.9. Japanese Encephalitis Virus (JE)

No antiviral screening results are available for JE virus.

5.1.10. Korean Hemorrhagic Fever Virus (KHF)

No antiviral screening results are available for KHF virus.

5.1.11. Human Immunodeficiency Virus (HIV)

No antiviral screening results are available for HIV virus.

5.1.12. Feline Leukemia Virus-FAIDS Variant (FeLV)

The 9 compounds found active against the FAIDS variant of feline leukemia virus (FeLV) in feline embryo clone 81C cells using a cpe-inhibition assay are listed in Table 8. Of these active compounds, AVS-999 with a VR of 2.7 and a TI of 6.8 was the most active of the series. However, none of the compounds were as active as the positive control drug

TABLE 7. COMPOUNDS ACTIVE AGAINST PICHINDE VIRUS[a]

AVS Number	Shipment	ID_{50}	MTC	TI
215	1	2.2	>100	>45.4
230	1	9.8	320	32.7
139	1	15.4	>320	>20.8
253	1	2.0	32	16
272	1	2.0	32	16
1972	1	79.3	100	1.3
1973	1	25	32	1.3
228	1	28.4	32	1.1
302	1	0.3	0.32	1.1

Note: AVS numbers 64, 138 and 303 have also been tested against Pichinde Virus but were not considered active.

[a]Compounds identified by their AVS numbers are listed in decending order from the most active to the least active. Assays were done in Vero cells. An active compound is defined here as having a therapeutic index (TI) of greater than 1.0. The drug concentration which reduced the mean plaque number by 50% (50% inhibitory dose, ID_{50}) was calculated using a regression analysis program for semilog curve fitting and is shown here in units of µg/ml. The minimum cytotoxic drug concentration (MTC) is the lowest drug concentration at which toxicity was observed. This is also in units of µg/ml. The therapeutic index of a test compound was determined by dividing the MTC by the ID_{50}.

TABLE 8. COMPOUNDS ACTIVE AGAINST FELINE LEUKEMIA VIRUS - FAIDS VARIANT (FeLV)[a]

AVS Number	Shipment	VR	VR*	ID_{50}	MTC	TI
999	9	2.7	0.9	14.8	100	6.8
215	9	2.4	0.8	11.1	32	2.9
79	9	1.5	0.5	0.47	1	2.1
206	9	1.9	0.6	15.8	32	2.0
345	9	1.3	0.4	17.8	32	1.8
200	9	1.3	0.4	27.5	32	1.2
245	9	2.1	0.7	80.8	100	1.2
646	9	1.0	0.3	2.58	3.2	1.2

[a]Compounds identified by their AVS number are listed in descending order from the most active to the least active. Assays were done in 81C cells. An active compound is defined here as having a therapeutic index (TI) of greater than 1.0 and a virus rating (VR) of 1.0 or greater. The VR is a measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced CPE and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (16). VR* is the designation for the virus rating calculated by the method of Sidwell and Huffman (17). The drug concentration which reduced the CPE by 50% (50% inhibitory dose, ID_{50}) was calculated using a regression analysis program for semilog curve fitting and is shown here in units of µg/ml. The minimum cytotoxic drug concentration (MTC) is also in units of µg/ml. The TI of a test compound was determined by dividing the MTC by the ID_{50}.

2'3'-dideoxycytidine (ddC) against FAIDS virus in vitro (VR = 4.0 and TI = 25). AVS-1 (Ribavirin), which is being evaluated in clinical trials for the treatment of AIDS, was active with a VR of 2.3, but the TI (calculated as the quotient of the MTC/MIC_{50}) was 1.0, indicating that there was little if any selectivity involved in the antiviral action of this compound against the feline virus.

5.2 Antiviral Evaluations In Vivo

5.2.1 Pichinde Virus in MHA Hamsters

Our passage 1 Pichinde virus stock was uniformly fatal in hamsters at all challenge levels tested. The average day of death (ADD) for intraperitoneally (IP) inoculated animals was Day 10 while the ADD for subcutaneously (SC) inoculated animals was Day 11-12. As shown in Table 9, the ADD for IP inoculated animals was 1.3 to 3 days shorter than for animals receiving the same challenge dose subcutaneously. Since IP inoculation also affords greater safety to technicians, we have chosen this challenge route for routine use in the primary in vivo evaluation of test compounds against PIC. Clinical signs in infected hamsters included generalized body petechiation, ruffled hair coat, weight loss, ocular and nasal hemorrhage, dark tarry intestinal contents, fatty change in the liver (gross necropsy only) and splenic enlargement. In addition, several infected animals had matter eyes and yellow fluid diarrhea.

In order to more effectively standardize virus challenges, the LD_{50} was determined for our virus stock. Since we had a large supply available of our passage 4 Pichinde virus preparation for use in future experiments, we chose to use this stock for the LD_{50} determination. The percentage mortality and ADD for each challenge level in the LD_{50} determination is shown in Table 10. Viral challenges of 3.8×10^3 pfu/hamster or greater were uniformly fatal while as few as 1.8 pfu/hamster were lethal for 20% of challenged animals. The LD_{50} for the PIC passage 4 stock was determined to be 32 pfu/hamster.

5.2.1.1 Ribavirin vs. Pichinde Virus

The efficacy of Ribavirin in decreasing the percentage mortality and increasing the ADD was initially evaluated in 4-week-old hamsters challenged IP with 3×10^4 pfu of passage 1 PIC. Ribavirin was administered SC once daily for 7 days with the first treatment occurring 4 hours after virus inoculation. Ribavirin was administered at two dosage levels (1) 25 mg/kg of body weight and (2) 50 mg/kg of body weight. Each Ribavirin dose was administered to ten infected hamsters and to ten uninfected hamsters. Controls included: (1) untreated, uninfected hamsters; (2) untreated, infected hamsters; (3) hamsters receiving virus diluent, and (4) uninfected hamsters receiving Ribavirin diluent once daily for 7 days. Animals were monitored for 21 days post infection and the percentage mortality and ADD for each group were calculated. The results of this initial evaluation are shown in Table 11A. Ribavirin at 25 mg/kg of body weight and at 50 mg/kg of body weight was unsuccessful in decreasing mortality; however, an increase in the ADD was obtained at both dosages. The ADD in untreated infected controls was 10.6 days while that for Ribavirin treated animals at 25 mg/kg and 50 mg/kg were 12.5 and 12.9 days, respectively.

Ribavirin treatment of hamsters inoculated with known numbers of 50% lethal doses of PIC was effective in both decreasing mortality and increasing the average day of death. As shown in Table 11B, 100 LD_{50} resulted in 100% mortality in untreated controls

TABLE 9. MHA HAMSTER CHALLENGE WITH PICHINDE 4763 PASSAGE 1[a]

Challenge	Challenge Route	No. Dead/Total	% Mortality	ADD
1×10^5 pfu	IP	10/10	100	10.6
5×10^4 pfu	IP	10/10	100	10.0
1×10^4 pfu	IP	10/10	100	9.9
5×10^3 pfu	IP	10/10	100	9.9
1×10^3 pfu	IP	10/10	100	9.9
5×10^2 pfu	IP	10/10	100	10.1
Virus diluent	IP	0/10	0	N/A
1×10^5 pfu	SC	10/10	100	12.3
5×10^4 pfu	SC	10/10	100	11.3
1×10^4 pfu	SC	10/10	100	11.2
5×10^3 pfu	SC	10/10	100	10.9
1×10^3 pfu	SC	10/10	100	12.9
5×10^2 pfu	SC	10/10	100	11.6
Virus diluent	SC	0/10	0	N/A
Untreated, uninfected	-	0/10	0	N/A

[a]Four-week-old MHA hamsters were administered various dosages of virus challenge in 0.5 ml by either intraperitoneal (IP) or subcutaneous (SC) inoculation. Surviving animals were sacrificed on Day 21, and the percentage (%) mortality and average day of death (ADD) were determined as described in Section 4.4.1.1. pfu = plaque forming unit; N/A = not applicable.

TABLE 10. LD_{50} TITRATION WITH PICHINDE PASSAGE 4[a]

Challenge	No. Dead/Total	% Mortality	ADD
3.0×10^4 pfu	10/10	100	10.2
1.5×10^4 pfu	10/10	100	10.7
7.5×10^3 pfu	10/10	100	10.1
3.8×10^3 pfu	10/10	100	11.6
1.9×10^3 pfu	9/10	90	11.1
9.4×10^2 pfu	8/10	80	10.9
4.7×10^2 pfu	6/10	60	11.7
2.3×10^2 pfu	4/10	40	14.3
1.2×10^2 pfu	4/10	40	9.8
58.6 pfu	6/10	60	10.5
29.3 pfu	7/10	70	10.0
14.7 pfu	4/10	40	10.3
7.3 pfu	4/10	40	11.0
3.7 pfu	8/10	80	13.8
1.8 pfu	2/10	20	10.5
Virus diluent	0/10	0	N/A
Uninfected Control	0/10	0	N/A

[a]Four-week-old MHA hamsters were administered various dosages of virus challenge in 0.5 ml by intraperitoneal inoculation. Surviving animals were sacrificed on Day 21, and the percentage (%) mortality and average day of death (ADD) were determined as described in Sections 4.4.1.1. pfu = plaque forming unit; N/A = not applicable.

TABLE 11. RIBAVIRIN TREATMENT OF PICHINDE-INFECTED HAMSTERS[a]

A. Passage 1 Virus

Treatment	Challenge	No. Dead/Total	% Mortality	ADD
Untreated	None	0/10	0	N/A
Virus diluent	None	0/10	0	N/A
Rbvn diluent	None	0/10	0	N/A
50 mg Rbvn/kg	None	0/10	0	N/A
25 mg Rbvn/kg	None	0/10	0	N/A
50 mg Rbvn/kg	3×10^4 pfu	8/8	100	12.9
25 mg Rbvn/kg	3×10^4 pfu	10/10	100	12.5
Untreated	3×10^4 pfu	9/9	100	10.6

B. Passage 4 Virus

Treatment	Challenge	No. Dead/Total	% Mortality	ADD
100 mg Rbvn/kg	100 LD_{50}	5/10	50	15.6
100 mg Rbvn/kg	32 LD_{50}	4/10	40	16.5
100 mg Rbvn/kg	10 LD_{50}	3/10	30	14.0
100 mg Rbvn/kg	None	0/10	0	N/A
56 mg Rbvn/kg	100 LD_{50}	8/10	80	15.1
56 mg Rbvn/kg	32 LD_{50}	5/10	50	13.2
56 mg Rbvn/kg	10 LD_{50}	5/10	50	14.4
56 mg Rbvn/kg	None	0/10	0	N/A
32 mg Rbvn/kg	100 LD_{50}	5/10	50	16.2
32 mg Rbvn/kg	32 LD_{50}	6/10	60	15.5
32 mg Rbvn/kg	10 LD_{50}	8/10	80	13.6
32 mg Rbvn/kg	None	0/10	0	N/A

(Continued)

TABLE 11, CONT'D

B. Passage 4 Virus, Cont'd

Treatment	Challenge	No. Dead/Total	% Mortality	ADD
Untreated	100 LD_{50}	10/10	100	12.7
Untreated	32 LD_{50}	9/10	90	11.1
Untreated	10 LD_{50}	8/10	80	13.6
Untreated	None	0/10	0	N/A
Rbvn diluent	100 LD_{50}	5/10	50	11.0
Rbvn diluent	32 LD_{50}	10/10	100	10.6
Rbvn diluent	10 LD_{50}	7/10	70	11.3
Rbvn diluent	None	0/10	0	N/A
Virus diluent	None	0/10	0	N/A

[a]Four-week-old MHA hamsters were administered the indicated challenge dosage of either passage 1 or passage 4 PIC virus stock in 0.5 ml by intraperitoneal inoculation. Animals were administered the indicated drug dosages subcutaneously once daily for seven days. Surviving animals were sacrificed on Day 21, and the percentage (%) mortality and average day of death (ADD) were determined as described in Section 4.4.1.1. Rbvn = Ribavirin; N/A = not applicable.

with an ADD of 12.7 days. In contrast, treatment with 100 mg Ribavirin/kg of body weight reduced mortality to 50% with an ADD at 15.6 days. Mortality rates in untreated animals challenged with 32 LD_{50} or with 10 LD_{50} were 90% and 80% while treatment with 100 mg Ribavirin/kg reduced the mortality rates to 40% and 30% respectively. Two discrepancies were noted in the experimental results: (1) A group of 10 animals challenged with 100 LD_{50} of PIC and treated for 7 days with Ribavirin diluent had a mortality rate of 50%, and (2) the mortality rate in the 32 mg/kg treated animals challenged with 100 LD_{50} was only 50% while similarly treated animals challenged with 10 LD_{50} had a mortality rate of 80%. In the first case, one cage of 5 animals is believed to have not received the viral inoculum. In the second case, a plausible explanation for the discrepancy may be that the two experimental groups were inadvertently injected with inoculum intended for the opposite groups.

5.2.1.2 Other Studies

During the virus challenge experiments we observed two clinical signs in the PIC-infected hamsters which were not characteristic of normal PIC pathogenesis. Many infected animals developed matted eyes, characteristic of Sendai virus infection, and a yellow fluid diarrhea, most likely caused by an opportunistic intestinal bacterium. We wanted to determine to what extent the cause of either of these clinical signs was contributing to the lethality of the the PIC infection.

The effects of pretreatment with antiviral and/or antibacterial agents on the percentage mortality and ADD in PIC infected hamsters were investigated using Ribavirin and tetracycline. Two groups of 10 hamsters each (5 male, 5 female) were pretreated with one of the following: (1) 100 mg Ribavirin/kg body weight, SC, once daily on Days -14 through -8; (2) 100 mg Ribavirin/kg body weight, SC, once daily on Days -14 through -8, and 5 mg tetracycline/ml of drinking water on Days -7 through 21; (3) 5 mg tetracycline/ml of drinking water on Days -7 through 21; (4) Ribavirin diluent, SC, once daily on Days -14 through -8; (5) Ribavirin diluent, SC, once daily on Days -14 through -8, and 5 mg tetracycline/ml of drinking water on Days -7 through 21; (6) no treatment. One group of hamsters from each treatment was challenged on Day 0 with 100 LD_{50} of passage 4 PIC and the second group from each treatment was maintained as uninfected controls. The percentage mortality and ADD were calculated for each group after monitoring for 21 days post-inoculation.

Attempts to alter the disease course and clinical presentation in PIC infected hamsters by pretreatment with Ribavirin and tetracycline were ineffectual. As shown in Table 12, no decrease in the percentage mortality or ADD in treated versus untreated animals was obtained. Thus, we feel that the mortality we are observing as the result of PIC virus challenge is due to pathogenesis of the PIC infection.

In another experiment we examined whether plaque purification of PIC causes an alteration in viral pathogenesis. Vezza et al. (21) reported that clonal isolates of PIC were no longer lethal in adult MHA hamsters. We plaque-purified virus from a well isolated PIC strain 4763 virus plaque three times. This virus was assessed for infectivity and lethality in four-week-old MHA hamsters inoculated IP. Mortality was monitored for 21 days post-infection. Our plaque-purified isolate was uniformly lethal in MHA hamsters and produced an ADD of Day 9 to 10. Clinical signs suggested that there was actually enhanced pathogenicity, and the LD_{50} was 5.7 pfu. Thus, plaque purification of PIC virus does not necessarily result in a loss of lethality.

TABLE 12. EFFECTS OF ANTIVIRAL/ANTIBACTERIAL TREATMENT ON PICHINDE INFECTION[a]

Treatment	Challenge	No. Dead/Total	% Mortality	ADD
Rbvn[b]	100 LD_{50}	10/10	100	9.6
Rbvn + Tet[b,c]	100 LD_{50}	10/10	100	10.0
Tet[c]	100 LD_{50}	10/10	100	9.4
Rbvn diluent + Tet[c,d]	100 LD_{50}	10/10	100	9.4
Rbvn diluent[d]	100 LD_{50}	10/10	100	9.9
Untreated	100 LD_{50}	9/10	90	10.4
Rbvn[b]	None	0/10	0	N/A
Rbvn + Tet[b,c]	None	0/10	0	N/A
Tet[c]	None	0/10	0	N/A
Rbvn diluent + Tet[c,d]	None	0/10	0	N/A
Rbvn diluent[d]	None	0/10	0	N/A
Untreated	None	0/10	0	N/A

[a]Four-week-old MHA hamsters were treated as indicated beginning 14 days before virus challenge. Animals were challenged on Day 0 by intraperitoneal inoculation with PIC passage 4 virus. Surviving animals were sacrificed on Day 21 post-challenge, and the percentage (%) mortality and average day of death (ADD) were determined as described in Section 4.4.1.1. N/A = not applicable; Rbvn = Ribavirin; Tet = Tetracycline.

[b]Treatment with Ribavirin was with 100 mg drug/kg body weight, subcutaneously once daily, on Days -14 through -8.

[c]Treatment with tetracycline was with 5 mg drug/ml of drinking water, on Days -7 through 21.

[d]Ribavirin diluent was administered subcutaneously, once daily, on Days -14 through -8.

6. DISCUSSION AND CONCLUSIONS

Over 100 of the 348 compounds submitted for primary antiviral screening in cell culture were found to exhibit significant antiviral efficacy against one or more of the target viruses in vitro (Table 13). The data that are summarized in Table 13 consist of the antiviral test results for each active compound by shipment number and by AVS number and includes the data for all of the viruses that are inhibited by each compound. The complete data, including negative results, for each compound may be found in the summary tables in Appendix B.

A number of compounds were found to have potent, broad-spectrum antiviral activity against both RNA-containing and DNA-containing viruses in cell culture. These included AVS-1, AVS-79, AVS-94, AVS-139, AVS-148, AVS-215, AVS-253, AVS-272, AVS-303, AVS-646, AVS-1089, AVS-1160, AVS-1985, and AVS-1987. Several compounds exhibited highly selective and potent antiviral efficacy against a challenge virus with a TI >1000. These highly active compounds included AVS-139 vs. SF virus (MIC_{50} 0.001 μg/ml), AVS-148 vs. PT virus (MIC_{50} 0.32 μg/ml), and AVS-2159 vs. SF virus (MIC_{50} 0.32 μg/ml).

A number of compounds were found to have highly selective antiviral efficacy against vaccinia virus in Vero cells. These included AVS-148, AVS-303, AVS-1160, AVS-1985, AVS-1986, AVS-1987, and AVS-1988. Some of these compounds represented the most active anti-poxvirus antiviral agents yet uncovered in these laboratories, with six compounds yielding VR's in the range of 5.4 to ≥7.4. It is obvious that these in vitro activities should be immediately confirmed with quantitative plaque-reduction assays and that in vivo chemotherapy trials in a suitable animal model system should be pursued. The compounds are relatively noncytotoxic and are excellent candidates for further development as anti-poxvirus drugs. Such drugs might be useful in the treatment of monkey pox or Tanapox virus infections in man, or in the defense against BW attack with either small pox virus or an altered vaccinia virus carrying genetic information for various toxins or other toxic products.

Several compounds were found to have significant activity against human adenovirus type 2 in H.Ep-2 cells. These included AVS-70, AVS-79, AVS-253, AVS-349, AVS-1089, and AVS-1984. Such compounds might be found useful in the treatment of adenovirus respiratory infections and epidemic keratoconjunctivitis among military recruits. Adenoviruses have also been isolated as the causative agents of secondary infections in immunosuppressed patients, including AIDS patients. It has recently been reported (Klein et al., Abstr. Int. AIDS Mtg., Paris, 1986; Eron et al., ibid.) that in AIDS patients treated with 9-11,3-dihydroxy-2-propoxymethyl)guanine (DHPG) for severe cytomegalovirus (CMV) infections, apparently latent adenovirus infections have been reactivated.

Several compounds (AVS-303, AVS-1160, and AVS-2127) were active against VSV in vitro. AVS-1160 had activity comparable to the positive control drug, carbocyclic 3-deazaadenosine, and should therefore be pursued as a potential antiviral agent against rhabdovirus infections, including rabies virus infections.

Compound AVS-148 exhibited marked antiviral efficacy against Punta Toro (PT) virus, a representative bunyavirus in Vero cells. This compound should be examined against other bunyaviruses, including Rift Valley Fever virus in vitro and in vivo.

Several compounds demonstrated significant activity against Pichinde (PIC) virus in plaque-reduction tests in Vero cells. These compounds included AVS-1 (ribavirin), AVS-139, AVS-215, AVS-230, AVS-253, and AVS-272. The latter five compounds exhibited good selectivity in their inhibition of Pichinde virus replication in vitro and should therefore be examined for in vivo therapeutic efficacy in the treatment of lethal Pichinde virus infections in our MHA strain hamster model. These compounds should also be examined for potential efficacy in the treatment of other arenavirus infections.

While a number of compounds showed inhibitory effects against feline AIDS (FAIDS) virus in cell culture, none were as active as the positive control drug, 2',3'-dideoxycytidine (ddC). These compounds will now be evaluated against HIV in vitro.

The efficacy of the positive control drug ribavirin in decreasing the mortality and increasing the mean survival time of Pichinde virus-infected MHA strain hamsters was readily demonstrated. This animal model system is now in place and we are ready to evaluate compounds for antiviral efficacy against Pichinde virus infections in vivo.

TABLE 13

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #1

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
15	PT	>4.3	>1.4	18.2	Vero	>320.0	17.6
64	PT	2.0	0.7	68.0	Vero	320.0	4.7
	SF	2.7	0.9	-	Vero	320.0	-
68	PT	2.4	0.8	2.4	Vero	32.0	13.3
	SF	2.7	0.9	-	Vero	320.0	-
70	Ad	2.4	0.8	0.06	H.Ep-2	0.32	5.3
79	VV	1.4	0.5	1.7	L929	3.2	1.9
	VV	1.7	0.6	32.0	Vero	100.0	3.1
	Ad	1.3	0.4	2.3	H.Ep-2	10.0	4.4
	SF	>5.5	>1.8	0.65	Vero	>100.0	>154.0
	PT	1.7	0.6	-	Vero	>100.0	-
84	SF	4.4	1.5	1.3	Vero	320.0	246.0
	PT	1.9	0.6	66.4	Vero	320.0	4.8
94	Ad	1.0	0.3	231.1	H.Ep-2	320.0	1.4
	SF	>4.7	>1.6	1.3	Vero	>320.0	>246.0
	PT	3.0	1.0	10.0	Vero	>320.0	32.0
113	PT	2.0	0.7	3.2	Vero	32.0	10.0
138	VV	3.1	1.0	8.8	L929	100.0	11.4
	VV	3.8	1.3	19.9	Vero	>320.0	16.1
	PT	1.1	0.4	-	Vero	>320.0	-
139	VV	1.3	0.4	1.8	L929	3.2	1.8
	SF	1.9	0.6	<0.001	Vero	1.0	>1000.0
	PIC	-	-	15.4	Vero	>320	>20.8

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
215	VV	3.0	1.0	2.8	L929	10.0	3.6
	VV	>4.2	>1.4	0.7	Vero	3.2	4.6
	Ad	2.3	0.8	9.5	H.Ep-2	10.0	1.1
	PT	2.7	0.9	2.2	Vero	3.2	1.5
	PIC	-	-	2.2	Vero	>100	>45.4
228	PIC	-	-	28.4	Vero	32.0	1.1
230	Ad[a]	1.3	0.4	66.4	H.Ep-2	>100.0	>1.5
	SF	2.3	0.8	53.5	Vero	100.0	1.9
	PIC	-	-	9.8	Vero	320.0	32.7
253	Ad	1.6	0.5	8.1	H.Ep-2	32.0	4.0
	SF	>6.1	2.0	1.0	Vero	>320.0	320.0
	SF	4.4	1.5	0.14	Vero	>100.0	>714.0
	SF	6.0	2.0	1.0	Vero	>100.0	>100.0
	PT	3.4	1.1	3.2	Vero	32.0	10.0
	PT	1.6	0.5	18.3	Vero	32.0	1.8
	PT	4.3	1.4	2.3	Vero	32.0	13.9
	PIC	-	-	2.0	Vero	32.0	16.0
272	VV	≥3.4	≥1.1	0.5	L929	3.2	6.4
	VV	4.7	1.6	0.7	Vero	3.2	4.6
	SF	2.2	0.7	1.0	Vero	3.2	3.2
	PT	2.3	0.8	1.0	Vero	3.2	3.2
	PIC	-	-	2.0	Vero	32.0	16.0
302	PIC	-	-	0.3	Vero	0.32	1.1
303	VV	≥3.5	≥1.2	0.4	L929	10.0	25.0
	VV	5.2	1.7	0.6	Vero	10.0	15.9
	VSV	2.8	0.9	3.1	L929	10.0	3.2
	Ad	1.2	0.4	-	H.Ep-2	>320.0	-
1972	VV	1.2	0.4	32.0	L929	100.0	3.1
	PT[a]	2.5	0.5	6.2	Vero	10.0	1.6
	PIC	-	-	79.3	Vero	100.0	1.3

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
1973	PT	1.6	0.5	25.0	Vero	32.0	5.2
	PIC	-	-	25.0	Vero	32.0	1.3
1974	PT	1.7	0.6	14.2	Vero	100.0	7.0
1975	PT	2.7	0.9	10.5	Vero	320.0	30.5
1976	SF	2.4	0.8	18.2	Vero	>100.0	>5.5
1980	PT	1.7	0.6	227.0	Vero	>320.0	>1.4
Positive Controls:							
Ara-A	VV	2.6	0.9	1.9	L929	32.0	16.8
	VV	3.6	1.2	10.0	Vero	320.0	32.0
C-3-deaza-Ad0	VSV	2.8	0.9	4.9	L929	32.0	6.5
	VSV	3.4	1.1	0.8	L929	3.2	4.0
Ribavirin	SF	>6.0	>2.0	1.0	Vero	>320.0	>320.0
	SF	2.7	0.9	22.7	Vero	>100.0	>4.4
	SF	3.0	1.0	19.1	Vero	>100.0	>5.2
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8
	PT	5.1	1.7	1.0	Vero	320.0	320.0
	PT	2.1	0.7	14.9	Vero	>100.0	>6.7
	PT	2.3	0.8	19.0	Vero	>100.0	>5.3
	PT	3.8	1.3	8.2	Vero	>100.0	>12.2
	PT	2.5	0.8	17.5	Vero	100.0	5.7
	PIC	-	-	4.3	Vero	>100.0	>23.3
	PIC	-	-	9.3	Vero	>100.0	>10.8
	PIC	-	-	8.9	Vero	>100.0	>11.2

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
Selenazole	SF	4.9	1.6	3.6	Vero	100.0	28.0
	SF	3.2	1.1	0.31	Vero	32.0	103.0
	SF	3.5	1.2	7.1	Vero	32.0	4.5
	PT	1.6	0.5	6.5	Vero	10.0	1.5
	PT	2.1	0.7	3.2	Vero	10.0	3.1
	PT	1.2	0.4	32.0	Vero	100.0	3.1
	PT	1.1	0.4	54.7	Vero	32.0	-

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$ 1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

[a]A second assay of this compound with this virus did not show antiviral activity.

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #2

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
33	SF	3.4	1.1	10.0	Vero	32.0	3.2
	PT	1.2	0.4	32.0	Vero	100.0	3.0
65	SF	2.6	0.9	1.6	Vero	3.2	2.0
71	VV	1.7	0.6	81.4	Vero	320.0	3.9
	SF	3.7	1.2	16.4	Vero	>100.0	>6.1
78	SF	1.8	0.6	-	Vero	1.0	-
87	PT	1.1	0.4	47.0	Vero	100.0	2.1
95	PT	1.9	0.6	22.7	Vero	100.0	4.4
96	PT	1.0	0.3	-	Vero	32.0	-
148	VV	5.4	1.8	1.0	Vero	100.0	100.0
	PT	6.3	2.1	<0.32	Vero	320.0	>1000.0
	SF	3.6	1.2	2.3	Vero	32.0	13.9
181	SF	1.4	0.5	-	Vero	>320.0	-
199	PT	1.5	0.5	30.7	Vero	100.0	3.3
200	VV	1.0	0.3	52.4	Vero	100.0	1.9
	PT	2.1	0.7	15.6	Vero	100.0	6.4
347	SF	1.5	0.5	5.1	Vero	10.0	2.0
349	Ad	1.0	0.3	0.1	H.Ep-2	0.3	3.2
	SF	1.7	0.6	2.5	Vero	3.2	1.3

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
360	PT	2.0	0.7	0.2	Vero	1.0	5.0
	SF	2.5	0.8	0.02	Vero	0.32	16.0
	SF	1.8	0.6	0.32	Vero	1.0	3.1
646	VV	≥4.0	1.3	0.6	Vero	1.0	1.7
	SF	4.2	1.4	<0.10	Vero	3.2	>32.0
	SF	4.3	1.4	0.6	Vero	10.0	16.7
1983	SF	3.0	1.0	0.32	Vero	32.0	100.0
1984	Ad	1.9	0.6	88.0	H.Ep-2	>320.0	3.6
	SF	2.9	1.0	1.0	Vero	32.0	32.0
1985	VV	≥7.2	≥2.4	<0.32	Vero	100.0	312.5
	SF	3.7	1.2	6.1	Vero	>100.0	>16.4
1986	VV	≥7.4	≥2.5	0.6	Vero	>320.0	533.3
	SF	2.5	0.8	-	Vero	320.0	-
1987	VV	6.8	2.3	1.7	Vero	>320.0	188.2
	SF	3.1	1.0	19.0	Vero	100.0	5.3
1988	VV	6.4	2.1	3.6	Vero	>320.0	88.9
Positive Controls:							
Ara-A	VV	3.1	1.0	9.8	Vero	320.0	32.7
	VV	2.4	0.8	3.2	Vero	3.2	3.1
C-3-deaza-AdO	VSV	3.2	1.1	3.0	L929	10.0	3.3
	VSV	2.5	0.8	2.6	L929	3.2	1.2

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
Ribavirin	Ad	1.7	0.6	21.2	H.Ep-2	32.0	1.5
	Ad	1.2	0.4	26.2	H.Ep-2	32.0	1.2
	PT	2.3	0.8	41.8	Vero	>100.0	>2.4
	PT	3.8	1.3	8.2	Vero	>100.0	>12.2
	PT	2.5	0.8	17.5	Vero	100.0	5.7
	PT	1.5	0.5	47.0	Vero	100.0	2.1
	PT	2.5	0.8	13.8	Vero	>100.0	>7.2
	PT	1.8	0.6	32.0	Vero	>100.0	>3.1
	SF	3.0	1.0	0.32	Vero	32.0	100.0
	SF	5.2	1.7	1.9	Vero	>100.0	>52.6
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8
	SF	2.9	1.0	14.4	Vero	>100.0	>6.9
	SF	2.5	0.8	24.1	Vero	>100.0	>30.1
	SF	3.0	1.1	20.8	Vero	>100.0	>4.8
Selenazole	Ad	1.4	0.5	7.5	H.Ep-2	10.0	1.3
	PT	1.2	0.4	32.0	Vero	100.0	3.1
	PT	2.7	0.9	5.5	Vero	32.0	5.8
	PT	2.1	0.7	19.8	Vero	100.0	5.1
	SF	5.3	1.8	0.19	Vero	100.0	526.0

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$ 1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #3

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
1991	SF[a]	1.2	0.4	0.89	Vero	3.2	3.6
1992	PT	1.5	0.5	66.0	Vero	100.0	1.5
1995	PT	1.1	0.4	69.0	Vero	100.0	1.4
	SF[a]	2.9	1.0	7.8	Vero	100.0	12.8
1996	SF	1.9	0.6	-	Vero	100.0	-
1997	SF	2.1	0.7	96.3	Vero	320.0	3.3
2002	SF	1.4	0.5	-	Vero	320.0	-
2003	SF[a]	2.1	0.7	95.1	Vero	100.0	1.1
2004	SF	1.5	0.5	-	Vero	>320.0	-
2005	PT	1.0	0.3	64.0	Vero	100.0	1.6
2006	PT	1.2	0.4	56.0	Vero	100.0	1.8
2007	SF	2.6	0.9	-	Vero	100.0	-
2013	SF	3.1	1.0	52.3	Vero	>320.0	>6.1
2018	PT	1.3	0.4	31.0	Vero	100.0	3.2
2020	PT	1.6	0.5	21.0	Vero	32.0	1.5

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
Positive Controls:							
Ribavirin	PT	2.1	0.7	14.9	Vero	>100.0	>6.7
	PT	2.8	0.9	10.0	Vero	>100.0	>10.0
	PT	2.3	0.8	19.0	Vero	>100.0	>5.3
	PT	3.8	1.3	8.2	Vero	>100.0	>12.2
	PT	2.5	0.8	17.5	Vero	100.0	5.7
	PT	2.7	0.9	10.0	Vero	>100.0	>10.0
	PT	2.4	0.8	14.7	Vero	>100.0	>6.8
	SF	4.4	1.5	2.4	Vero	>100.0	>41.7
	SF	3.8	1.3	12.6	Vero	>100.0	>7.9
	SF	2.7	0.9	22.7	Vero	>100.0	>4.4
	SF	3.0	1.0	19.1	Vero	>100.0	>5.2
	SF	5.2	1.7	1.9	Vero	>100.0	>52.6
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8
	SF	2.9	1.0	14.4	Vero	>100.0	>6.9
	SF	2.5	0.8	24.1	Vero	>100.0	>30.1
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8
	SF	4.4	1.5	2.3	Vero	>100.0	>43.5
	SF	3.4	1.1	14.1	Vero	>100.0	>7.1
	SF	4.0	1.3	0.92	Vero	>100.0	>109.0
	SF	4.0	1.3	6.1	Vero	>100.0	>16.4
	SF	4.4	1.5	2.9	Vero	>100.0	>34.5
	SF	3.0	1.0	10.0	Vero	>100.0	>10.0
Selenazole	PT	1.2	0.4	32.0	Vero	100.0	3.1
	PT	2.1	0.7	19.8	Vero	100.0	5.1
	SF	5.3	1.8	0.19	Vero	100.0	526.0
	SF	3.5	1.2	7.1	Vero	32.0	4.5

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$ 1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

[a]A second assay of this compound with this virus did not show antiviral activity.

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #4

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC50)
1	VV	2.1	0.7	150.7	Vero	>320.0	2.1
	Ad	1.4	0.5	57.4	H.Ep-2	100.0	1.7
	PT	3.2	1.1	18.0	Vero	>100.0	>5.6
	SF	4.5	1.5	5.2	Vero	>100.0	>19.2
122	VV	2.1	0.7	10.0	Vero	32.0	3.2
136	Ad	1.3	0.4	96.9	H.Ep-2	320.0	3.3
	PT	1.5	0.5	73.0	Vero	>320.0	>4.4
195	Ad	1.4	0.5	217.0	H.Ep-2	>320.0	>1.5
202	PT	1.9	0.6	141.0	Vero	320.0	2.3
	SF	2.0	0.7	-	Vero	320.0	-
206	PT	2.7	0.9	67.0	Vero	>320.0	>4.8
	SF	3.7	1.2	22.6	Vero	320.0	14.2
2039	SF	1.1	0.4	-	Vero	32.0	-
2040	SF	1.6	0.5	-	Vero	320.0	-
2041	SF	1.1	0.4	-	Vero	32.0	-
Positive Controls:							
Ara-A	VV	2.4	0.8	10.0	Vero	32.0	3.2
Selenazole	VV	2.6	0.9	24.0	Vero	32.0	1.3
	Ad	1.7	0.6	9.7	H.Ep-2	32.0	3.3

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
Ribavirin	PT	2.3	0.8	19.0	Vero	>100.0	>5.3
	PT	3.8	1.3	8.2	Vero	>100.0	>12.2
	PT	2.5	0.8	17.5	Vero	100.0	5.7
	PT	2.7	0.9	10.0	Vero	>100.0	>10.0
	PT	3.0	0.8	14.7	Vero	>100.0	>6.8
	SF	2.6	0.9	25.9	Vero	>100.0	>3.9
	SF	2.8	0.9	14.9	Vero	>100.0	>6.7
	SF	3.3	1.1	17.5	Vero	>100.0	>5.7
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$ 1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #5

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
197	PT	1.7	0.6	73.0	Vero	>320.0	>4.4
	SF	3.4	1.1	49.7	Vero	>320.0	>6.4
206	VV	1.2	0.4	85.3	Vero	100.0	1.2
	PT	1.5	0.5	73.0	Vero	320.0	4.4
245	SF	1.4	0.5	-	Vero	>100.0	-
257	VV	1.4	0.5	174.1	Vero	320.0	1.8
286	VV	1.5	0.5	-	Vero	0.1	-
345	SF	1.0	0.3	80.0	Vero	100.0	1.1
1089	VV	1.7	0.6	81.4	Vero	320.0	3.9
	Ad	1.0	0.3	3.2	H.Ep-2	10.0	3.1
	PT	3.4	1.1	0.9	Vero	3.2	3.6
	SF	5.2	1.7	1.0	Vero	>100.0	>100.0
1160	VV	≥5.6	≥1.9	0.6	Vero	100.0	166.7
	VSV	3.2	1.1	<0.32	L929	1.0	>3.1
	VSV	2.6	0.9	1.0	L929	3.2	3.2
	SF	1.0	0.3	70.4	Vero	100.0	1.4
1250	Ad[a]	≥2.0	≥0.7	1.3	H.Ep-2	3.2	2.5
2136	VV	1.7	0.6	144.2	Vero	>320.0	>2.2
	SF	1.4	0.5	88.0	Vero	320.0	3.6

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
2138	PT	1.4	0.5	236.3	Vero	>320.0	>1.4
Positive Controls:							
Ara-A	VV	2.6	0.9	17.0	Vero	100.0	5.9
	VV	3.2	1.1	2.7	Vero	32.0	11.9
C-3-Deaza-AdO	VSV	3.0	1.0	3.1	L929	10.0	3.2
Ribavirin	Ad	1.7	0.6	32.0	H.Ep-2	100.0	3.1
	PT	1.5	0.5	47.0	Vero	100.0	2.1
	PT	2.5	0.8	13.8	Vero	>100.0	>7.2
	PT	2.4	0.8	33.9	Vero	100.0	2.9
	PT	2.4	0.8	9.2	Vero	>100.0	>10.9
	PT	2.4	0.8	18.8	Vero	100.0	5.3
	PT	1.5	0.5	47.3	Vero	100.0	2.1
	SF	4.5	1.5	5.2	Vero	>100.0	19.2
	SF	2.6	0.9	25.9	Vero	>100.0	3.9
	SF	2.8	0.9	14.9	Vero	>100.0	>6.7
	SF	3.3	1.1	17.5	Vero	>100.0	>5.7
	SF	2.3	0.8	20.0	Vero	100.0	5.0
	SF	2.6	0.9	8.9	Vero	>100.0	11.2
	SF	3.0	1.0	9.8	Vero	>100.0	10.2
	SF	2.9	1.0	14.4	Vero	>100.0	>6.9
	SF	2.5	0.8	24.1	Vero	>100.0	>30.1
Selenazole	Ad	1.7	0.6	9.7	H.Ep-2	32.0	3.3
	Ad	2.0	0.7	8.1	H.Ep-2	32.0	4.0

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

[a]A second assay of this compound with this virus did not show antiviral activity.

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #6 (No active compounds)

COMPOUND SHIPMENT #7

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
2127	VSV	1.0	0.3	23.4	L929	100.0	4.3
Positive Control:							
C-3-Deaza-	VSV	2.1	0.7	3.1	L929	10.0	3.2
AdO	VSV	3.3	1.1	3.1	L929	32.0	10.3

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #8

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
2170	VV	4.7	1.6	1.3	Vero	10.0	7.7
2173	Ad	1.1	0.4	27.3	H.Ep-2	100.0	3.6
Positive Controls:							
Ara-A	VV	2.5	0.8	5.7	Vero	10.0	1.7
Ribavirin	Ad	1.7	0.6	32.0	H.Ep-2	100.0	3.1
	Ad	1.9	0.7	30.7	H.Ep-2	100.0	3.2
	Ad	1.9	0.7	19.7	H.Ep-2	100.0	5.1
Selenazole	VV	2.5	0.8	7.5	Vero	10.0	1.3
	Ad	2.0	0.7	8.1	H.Ep-2	32.0	4.0
	Ad	1.3	0.4	9.7	H.Ep-2	32.0	3.3
	Ad	1.6	0.5	7.2	H.Ep-2	32.0	4.4

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #9

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
79	FeLV	1.5	0.5	0.47	81C	1.0	2.1
136	FeLV	1.2	0.4	-	81C	32.0	-
200	FeLV	1.3	0.4	27.5	81C	32.0	1.2
202	FeLV	1.5	0.5	-	81C	32.0	-
206	FeLV	1.9	0.6	15.8	81C	32.0	2.0
215	FeLV	2.4	0.8	11.1	81C	32.0	2.9
245	FeLV	2.1	0.7	80.8	81C	100.0	1.2
345	FeLV	1.3	0.4	17.8	81C	32.0	1.8
646	FeLV	1.0	0.3	2.58	81C	3.2	1.2
999	FeLV	2.7	0.9	14.8	81C	100.0	6.8
2026	PT	1.8	0.6	17.3	Vero	100.0	5.8
2028	PT	1.2	0.4	-	Vero	100.0	-
2034	PT	2.1	0.7	6.8	Vero	10.0	1.5
2159	SF	3.8	1.3	<0.32	Vero	>320.0	>1000.0
2160	SF	3.1	1.0	<0.32	Vero	32.0	>100.0

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
2161	SF	1.8	0.6	-	Vero	100.0	-
2162	PT	1.3	0.4	-	Vero	>100.0	-
2188	SF	2.6	0.9	<0.32	Vero	32.0	>100.0
Positive Controls:							
Ara-A	VV	2.5	0.8	5.7	Vero	10.0	1.7
	VV	2.4	0.8	3.2	Vero	10.0	3.1
Ribavirin	Ad	1.9	0.63	30.7	H.Ep-2	100.0	3.2
	Ad	1.2	0.4	26.2	H.Ep-2	32.0	1.2
	PT	2.6	0.7	18.5	Vero	>100.0	>5.4
	PT	2.4	0.8	33.9	Vero	100.0	2.9
	PT	2.4	0.8	9.2	Vero	>100.0	>10.9
	PT	2.8	0.9	32.9	Vero	>100.0	>3.0
	PT	2.7	0.9	22.2	Vero	>100.0	>4.5
	PT	1.8	0.6	23.9	Vero	>100.0	>4.2
	SF	3.4	1.1	3.2	Vero	>100.0	>31.3
	SF	2.6	0.9	8.9	Vero	>100.0	11.2
	SF	3.0	1.0	9.8	Vero	>100.0	10.2
	SF	2.9	1.0	14.4	Vero	>100.0	6.9
	SF	2.5	0.8	24.1	Vero	>100.0	>30.1
Selenazole	VV	2.5	0.8	7.5	Vero	10.0	1.3
	Ad	1.3	0.4	9.7	H.Ep-2	32.0	3.3
Azido-thymidine	FeLV	3.7	1.2	5.8	81C	>78.4	>13.5

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
Dideoxy-	FeLV	3.0	1.0	2.6	81C	7.3	2.8
cytidine	FeLV	2.0	0.7	0.76	81C	10.0	13.2
(ddC)	FeLV	4.0	1.3	0.4	81C	10.0	25.0
	FeLV	3.2	1.1	0.98	81C	10.0	10.2

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #10

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
2214	SF	1.4	0.5	-	Vero	10.0	-
2215	SF	1.3	0.4	-	Vero	320.0	-
2217	SF	1.3	0.4	1.9	Vero	3.2	1.7
2219	SF	2.5	0.8	2.5	Vero	32.0	12.8
2220	SF	3.2	1.1	<0.32	Vero	32.0	≥100.0
2221	SF	1.2	0.4	1.8	Vero	3.2	1.8
2223	SF	1.1	0.4	79.8	Vero	100.0	1.3
2226	SF	1.4	0.5	8.8	Vero	32.0	3.6
2229	Ad	1.0	0.3	28.0	H.Ep-2	100.0	3.6
Positive Controls:							
C-3-Deaza-AdO	VSV	2.8	0.9	3.1	L929	32.0	10.3
Ara-A	VV	2.4	0.8	3.2	Vero	10.0	3.1
	VV	3.3	1.1	2.7	Vero	32.0	11.9
Ribavirin	Ad	1.2	0.4	26.2	H.Ep-2	32.0	1.2
	Ad	1.9	0.6	19.7	H.Ep-2	100.0	5.1
	Ad	1.9	0.6	25.5	H.Ep-2	100.0	3.9

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
Ribavirin	SF	3.1	1.0	14.4	Vero	>100.0	>6.9
	SF	4.4	1.5	2.3	Vero	>100.0	>43.5
	SF	3.4	1.1	14.1	Vero	>100.0	>7.1
	SF	4.0	1.3	0.92	Vero	>100.0	>109.0
	SF	4.0	1.3	6.1	Vero	>100.0	>16.4
	SF	4.4	1.5	2.9	Vero	>100.0	>34.5
	SF	3.0	1.0	10.0	Vero	>100.0	>10.0
Selenazole	VV	2.7	0.9	7.6	Vero	10.0	1.3
	Ad	1.6	0.5	7.2	H.Ep-2	32.0	4.4

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR ≥1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

TABLE 13, Cont'd

Active Compounds from the Basic Screen
(In Vitro Antiviral Test Results)

COMPOUND SHIPMENT #11

AVS No.	Virus	VR*	VR**	MIC_{50} (µg/ml)	Cell	Minimum Toxic Concentration (µg/ml)	Therapeutic Index (MTC/MIC_{50})
2277	Ad	1.1	0.4	100.0	H.Ep-2	320.0	3.2
2290	VV	3.2	1.1	5.7	Vero	32.0	5.6
	Ad	1.0	0.3	25.4	H.Ep-2	32.0	1.3
2291	VV	1.9	0.6	4.7	Vero	10.0	2.1
Positive Controls:							
Ara-A	VV	3.3	1.1	2.7	Vero	32.0	11.8
C-3-Deaza-	VSV	2.5	0.8	3.2	L929	10.0	3.1
AdO	VSV	2.5	0.8	3.2	L929	10.0	3.1
Ribavirin	Ad	1.9	0.6	25.5	H.Ep-2	100.0	3.9

*VR = Virus rating: A measurement of selective antiviral activity which takes into account the degree of inhibition of virus-induced cytopathogenic effects (CPE) and the degree of cytotoxicity produced by the test compound, determined by a modification of the method of Ehrlich et al. (Ann. N.Y. Acad. Sci. 130: 5-16, 1965). In our experience, a VR $\geq$1.0 indicates definite (+) antiviral activity, a VR of 0.5-0.9 indicates marginal to moderate (±) antiviral activity, and a VR <0.5 usually indicates no (-) significant antiviral activity.

**VR = Virus rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

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APPENDIX A

Operating Procedures for the Biosafety Level 3 Containment Facility

OPERATING PROCEDURES FOR THE BIOSAFETY LEVEL 3 CONTAINMENT FACILITY

I. Laboratory Access

A. General

1. Access to the Biosafety Level 3 (BL3) containment area is restricted when work with infectious agents is in progress. Persons are authorized to enter into the laboratories only if their presence is required on the basis of program or support need. Such persons shall be advised of the potential hazards and shall comply with all entry and exit procedures as described in Section III. The Program Manager has the final responsibility for assessing each circumstance and determining who may enter or work in the BL3 containment area.

2. Persons at increased risk of acquiring infection or for whom infection may be unusually hazardous will not be allowed in the laboratories. Included in this category are children, individuals who are immunodeficient, immunosuppressed, or undergoing immunosuppressive therapy, pregnant women, and people having recently had surgical procedures or injuries involving significant alteration to the normal integrity of the skin.

3. Women assigned to the containment area are directed to notify their immediate supervisor and the Program Manager as soon as pregnancy is suspected so that any risk can be avoided. Work in the BL3 area will discontinue as soon as pregnancy seems reasonably certain. Transfer to another laboratory within the Microbiology/Virology Division will be arranged by the Division Head.

B. Staff Personnel

1. At risk staff personnel will be immunized against agents for which immune prophylaxis is available prior to entry into the BL3 containment area. Currently, this includes yellow fever virus, Japanese encephalitis virus, and Venezuelan equine encephalomyelitis virus. Serum samples will be collected three to four weeks after the last immunization and at six-month intervals thereafter for evaluation of protective immunity. Booster immunizations will be given when antibody levels are judged to no longer be protective.

C. Other Personnel

1. Visitors are permitted only in the "clean" public areas near the front of the building, including the lounge.

2. Admission into the BL3 containment areas of service, contractor, or consulting personnel may occasionally be needed. They will be allowed to enter only with the prior approval of the Program Manager and once precautionary measures have been taken to adequately protect them from exposure to infectious substances.

   a. Admission may be allowed if one of the following conditions is satisfied:

      (1) The individual can provide documentation of immunization and protective seroconversion for the agents in use.

(2) The individual uses a full face mask, or hood, positive air pressure respirator at all times while within the containment area.
(3) Infectious work is suspended, decontamination performed, and the area declared "clean".

b. All entry and exit procedures, as described in Section III, must be followed.

c. Non-staff individuals will be accompanied at all times by a staff member while within the BL3 containment area. Exposure to infectious agents will be kept to a minimum, regardless of whether immunization has been accomplished.

d. Maintenance personnel will be given warning of any unusual situation, such as spills or leaks, which may be hazardous.

II. Signs

A. A hazard warning sign, incorporating the universal biohazard symbol, will be placed on airlock doors, changing room doors, and all laboratory suite and laboratory doors. These signs will indicate the infectious agents in use in that area, list the name and telephone number for the responsible Task Leader and other responsible people, and indicate the special requirements for entrance to that area.

B. A hazard warning sign, incorporating the universal biohazard symbol, will be placed on all freezers, refrigerators, and incubators used for storage of infectious agents. These signs will indicate the infectious agents being stored and the name and telephone number for the responsible Task Leader.

III. Entry and Exit Procedures

A. Personnel must always enter and exit the containment area, by way of the restroom/locker room doors, into the central corridor except in an absolute emergency. Entrance or exit will not be allowed through any of the airlocks except in absolute emergencies.

B. Upon entering the outer change room, all clothing, including underwear, must be removed and stored in the available lockers. Personnel will then dress into laboratory clothing consisting of scrub shirts and scrub pants. The wearing of socks, underwear and head covers in the containment area is optional.

C. Only individually-assigned work shoes or tennis shoes will be worn inside the containment area. These shoes will be specially marked to identify them for use only in the containment area and will be left in the restroom closet when not being used. Shower shoes will not be worn beyond the shower room door into the restroom, nor will tennis shoes or work shoes be worn beyond the restroom door into the shower room. Extra shoes will be provided for use by authorized visitors and maintenance personnel.

D. Personnel will wear a long-sleeved, wrap-around surgical gown (not a button-front laboratory coat) over their laboratory clothing whenever working with animals or infectious materials.

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1. Cell culture suites. The wrap-around gown must be worn whenever working in one of the individual cell culture laboratories (i.e., one of the A, B, or C rooms). This gown can be hung on a wall hook within that room and reused as needed throughout the day. Gowns need not be worn in the outer suite area of the cell culture laboratories unless infectious materials are present. Gowns are not to be worn in more than one suite, nor in more than one laboratory within a suite. A gown is not to be used for more than one day. At the end of each work day all used gowns should be placed into the laundry bag located in the suite.

2. Animal suites. A wrap-around gown must be put on whenever entering one of the animal suites. This gown is not to be worn in more than one suite. However, it may be worn in all the laboratories within a suite for which the same virus is used. Gowns can be hung on wall hooks within suites and reused within that suite throughout the day. A separate gown must be used when working in laboratories housing quarantine animals or other uninfected animals. A gown is not to be used for more than one day. At the end of each work day all used gowns should be placed into the laundry bag of that suite.

E. Since tennis shoes are considered "dirty" items, disposable shoe covers only need to be worn in certain areas. The purpose of wearing the shoe covers is to minimize the tracking of potentially contaminated dirt from one area to another.

1. Shoe covers should not be worn in the toilet area, central corridor, data room, compound preparation room, or the autoclave area.

2. Shoe covers must be worn whenever working in one of the individual cell culture laboratories. Shoe covers need not be worn in the outer suite area of the cell culture laboratories unless infectious materials are present. Shoe covers must be removed before exiting from one of the individual cell culture laboratories into the suite area. These shoe covers must be discarded and not reused.

3. Shoe covers must be put on whenever entering one of the animal suites, and must be removed and discarded before leaving that suite. These shoe covers may be worn in all the laboratories within the suite for which the same virus is used. An additional pair of shoe covers must be worn when entering a laboratory housing quarantine animals or other uninfected animals.

F. In order to access the offices, storage rooms, loading dock airlock, and water fountain located in the rear corridor you must first remove your wrap-around gown, wash your hands, and put on a clean pair of shoe covers. You may then exit into the rear corridor from one of the containment suite doors.

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G. When leaving the containment area, all objects should be removed from the laboratory clothing and left in the laboratory. Disposable clothing items (shoe covers, hair bonnets, etc.) should be placed in trash containers; scrub shirts and scrub pants should be placed in the laundry bags located in the restrooms. Tennis shoes must be stored in the restrooms.

H. Personnel must shower using germicidal soap before leaving the containment area. If eyeglasses are worn, they should be washed thoroughly.

I. Any jewelry worn in the containment area must be washed with disinfectant or worn during the exit shower.

J. The wearing of a hair bonnet is recommended, but not required. If a bonnet is not worn, then hair is to be washed during each exit shower. If a bonnet is worn at all times while in the containment area, then hair does not need to be washed.

IV. Laboratory Procedures.

A. General Procedures

1. The work surfaces of laboratory benches will be decontaminated with a liquid disinfectant solution at least once a day and after any spill of viable material.

2. Personal protection is partially dependent upon the carefully balanced directional airflows with the containment facility. Doors should be opened only long enough to allow passage in or out. Doors should never be needlessly held open and never propped open.

3. Mouth pipetting is strictly prohibited; mechanical pipetting devices will be used. Pipettes should have cotton-plugged tops.

4. Eating, drinking, and applying cosmetics are not permitted in the containment suites. Food should be stored and eaten in the Lounge located in the "clean" area of the facility.

5. Smoking in the containment area is only permitted in the data office (Room 199). Employees wishing to smoke will remove their rubber gloves, wrap-around gown, and shoe covers, and wash hands thoroughly before proceeding to the data office. Cigarettes, lighters, matches, etc. will not be carried while working in the laboratory; these items are to remain in the data office.

6. Incoming equipment and supplies should be uncrated in a "clean" area, such as the loading dock or lounge area, to avoid the need for decontamination of empty cartons, boxes, and packing materials.

7. An insect and rodent control program will be in effect. This program will include, but not be limited to, a regularly scheduled spraying of insecticide and the placement of traps and poison bait.

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8. Animals and plants not involved in the work being performed are not permitted in the containment area, except for small plants in the two offices.

9. Gas burners will be turned off at the valve nearest the incoming steel gas line before personnel leave the work area for any prolonged period. Open flames of any type should not be left unattended. Gas will not just be turned off at the burner, nor will pilot lights be left on overnight. Such practices could permit escape of gas through worn tubing with consequent hazard of fire or explosion.

10. Flammable liquids should be stored in refrigerators only if the flash point is above the refrigerator temperature and only if the internal wiring of the refrigerator has been modified to render it spark-free. These refrigerators will be clearly marked for storage of flammables.

B. Use of Biological Safety Cabinets

1. All work involving manipulations of virus-infected animals or cell cultures will be performed in a biological safety cabinet. No work involving infectious materials shall be conducted in open vessels on an open bench.

2. Work surfaces of biological safety cabinets will be wiped down with disinfectant solution at the start of work each day, whenever a different virus is to be used, upon completion of work, and after any spill of viable material.

3. When working in biological safety cabinets it is necessary to minimize the passage of items through the protective air barrier at the front opening of the cabinet. Careful planning and the placement of all required items within the hood prior to the start of work are important. Used pipettes should be disposed of into a durable leakproof container of disinfectant located within the cabinet. Tubes, paper towels, etc. should be disposed of into small, bench-top biohazard bags located within the cabinet.

C. Special Procedures for Handling Infectious Samples

1. All personnel will wash their hands with disinfectant soap after handling infectious materials and/or animals, and prior to leaving a containment suite.

2. The careful performance of certain laboratory procedures must be emphasized because they generate small particle aerosols. These procedures should be contained in biological safety cabinets or chemical hoods when infectious organisms are involved. Such procedures include, but are not limited to, these examples: (a) high speed blending, mixing, and grinding, (b) vortexing, (c) centrifugation with nonsealed table-top centrifuges, including microcentrifuges, (d) sonification, (e) opening vials of lyophilized cultures, and (f) thawing vials of frozen materials.

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3. Special care must be taken to avoid skin contamination with infectious materials. Gloves must be worn when handling infected animals or infectious materials. A squirt or spray bottle containing suitable disinfectant will be kept within the biological safety cabinets and used for surface decontamination of gloves and hands prior to their removal from the cabinet.

4. All employees working with infectious materials should adopt the habit of keeping hands away from mouth, nose, eyes, and face so as to minimize self-inoculation.

5. Safety glasses must be worn whenever working with infectious materials.

6. Lyophilized culture vials and sealed liquid nitrogen storage vials should be wrapped with disinfectant-wetted cotton or gauze before breaking.

7. Infectious materials will be stored only in designated refrigerators, incubators, or freezers (see paragraph II-B).

8. All infectious material stored in refrigerators or freezers should be properly labeled and stored in nonbreakable containers capable of withstanding the thermal shock of freezing and thawing.

D. Equipment

1. Equipment known to be (or suspected of being) faulty should not be operated. Mechanically unsafe equipment will be immediately tagged as such and reported to one of the Task Leaders. Should the equipment require servicing, it must be adequately decontaminated and removed from the containment area, if at all possible.

2. All personnel will be instructed in the operation, safety, and maintenance of centrifuges prior to being authorized to use such equipment. This is the direct responsibility of the supervising professional staff members.

3. Before centrifuging samples, check tubes for cracks, inspect the rotor or trunnion cup for rough walls caused by corrosion or adhering matter, and carefully remove bits of imbedded debris from rubber cushions. A small amount of germicidal solution added between the tube and trunnion cup will not only disinfect the surfaces of both, but also provides an excellent cushion against shocks that might otherwise break the tube. Be certain all opposite rotor positions are balanced by weight.

E. Special Procedures when Using Needles and Syringes

1. Hypodermic needles and syringes will be used only for parenteral injections and aspirations of fluids from laboratory animals and diaphragm bottles. Syringes should not be used for making dilutions. Dilutions of compounds or virus for infection, tissue culture inoculation, etc. should be made in capped plastic tubes, or in sterile serum bottles, using a pipettor.

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2. Only disposable needle-locking syringes or syringe-needle units will be used for the injection of infectious fluids and whenever possible for the aspiration of infectious fluids. Extreme caution must be used whenever handling needles and syringes to avoid autoinoculation and the generation of aerosols during use and disposal.

3. Needles should not be bent, sheared, replaced in the sheath or guard, or removed from the syringe following use. The used needles and syringes will be placed in a puncture-resistant container and autoclaved before disposal. If removal of the needle from the syringe is critical to the procedure, as in the transfer of blood samples for serum collection, then the needle will be held with a hemostat during removal.

4. It is advisable to use alcohol-soaked cotton or gauze around the needle and stopper when placing and removing a syringe and needle from a rubber-stoppered vaccine bottle.

F. Animal Receiving

1. Animals will be received on the loading dock in filter crates. A "clean side" person will receive the animals and log them in. The "clean side" person should wear gloves to handle the crates so that their hands do not become contaminated by anything that may be present on the outside of the crates. These gloves should be discarded on the loading dock before leaving.

2. The "clean side" person on the loading dock will spray all incoming crates with a 10% chlorox solution and pass them through the outer door of the airlock into the airlock. The fly fan must be turned on before opening the outer airlock door.

3. After the animal crates have been passed into the airlock, the "clean side" person will close the outer airlock door and turn off the fly fan. The "dirty side" person will open the inner airlock door and transfer the crates onto a cart in the corridor. The "dirty side" person should be wearing gloves when receiving crates through the airlock.

4. The crates will be transported to the appropriate quarantine room and be sprayed with 3% Lysol immediately prior to opening. Clean gloves should not be used to handle the crates until the crates have been disinfected the second time. Remember that the outsides of the crates are potentially contaminated until you disinfect them. Therefore, handling the crates prior to disinfection could contaminate your gloves, which could potentially contaminate the animals during transfer. Once the crates have been sprayed with Lysol, the crates should be opened whilewearing clean gloves and the animals transferred into cages. Cage tags should be completed and placed on the cages at this time.

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5. Gowns worn in the quarantine rooms will be left in the rooms on hangers provided there. Shoe covers and gloves used in the quarantine rooms should be removed and discarded in the room.

6. Because there are no hoods in the quarantine rooms all precautions possible must be taken not to contaminate experimental animals with anything which the quarantine animals may potentially be carrying. This is especially true with regard to the hamsters, which may be carrying viruses harmful to mice. Quarantine mice should be fed, watered and observed before quarantine hamsters so as not to cross-contaminate the mice. The same is true for experimental animals; as much as is possible the mice should be fed, watered and observed prior to the hamsters if interaction with both species in a single day is necessary. Where possible it would be best to deal with one species in the morning and the second in the afternoon after having showered out for lunch and dressing into a clean scrub suit.

V. Cleaning and Decontamination

A. General Laboratory Procedures

1. All areas of containment suites must be kept clean and orderly. A dirty, dusty, or cluttered laboratory is a safety hazard and not consistent with acceptible biological research.

2. Stock solutions of disinfectants will be maintained in each containment suite.

3. When vacuum lines are used, a disinfectant trap and a filter must be placed between the vacuum port and the aspiration flask to prevent pathogens from entering the system.

4. All waterbaths must contain a disinfectant.

5. All laundry from the containment area must be autoclaved before being sent out for washing.

6. Small hand tools and similar items can be decontaminated with a disinfectant solution when removing them from the containment area.

7. All infectious materials, contaminated glassware, and contaminated waste will be autoclaved prior to washing or disposal. Contaminated materials are to be placed in closed containers for subsequent autoclaving or placed directly into autoclaves.

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B. Suite Decontamination Procedures

1. Individual laboratories and suites should be thoroughly washed with a suitable disinfectant whenever a change in the organism under study occurs. Washing should include floors, counter tops, shelves, safety cabinets, ceilings, and walls. This procedure is sufficient provided: (a) the room continues to be used for work with infectious materials, and (b) no unimmunized personnel are to be assigned to the suite.

2. Decontamination with paraformaldehyde is required when: (a) the suite is to be designated "clean", or (b) unimmunized personnel are to be assigned to the suite.

C. Animal Rooms and Cages

1. Water bottles are to be removed from cages and placed into a leakproof container for autoclaving. Opening of the cage to remove the water bottle must be done within a biosafety cabinet, except in quarantine and acute toxicity rooms. Water bottles must be sterilized by autoclaving before being emptied.

2. Animal cages are to remain closed and be placed directly into the autoclave. If the autoclave is full or is in use, the cages should remain in the animal rooms until the autoclave is ready for the next load.

3. Cage racks too large for the autoclave can be removed via the rear airlock near the kitchen. Before cage racks are removed they must be thoroughly sprayed with a suitable disinfectant.

D. Use of Autoclaves

1. All personnel will be fully instructed as to the operational procedures, care, cleaning, and maintenance of autoclaves prior to receiving authorization for their use.

2. Never open the autoclave door when the warning light indicates that the door on the opposite side is open.

3. Conditions for autoclaving various materials are described in Appendix A.

4. Infectious materials should not be stored in autoclaves overnight for sterilization the next day.

5. Before opening the door at the completion of each autoclave cycle, the recording chart must be examined to confirm that the proper temperature and length of cycle were achieved.

6. Sterilization indicators are to be included in every load for autoclaving. Before the contents of the autoclave are removed, the indicators must be examined to confirm that appropriate autoclaving conditions were achieved.

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7. Charts from the recorders are to be changed each day. These charts should be marked with the date, day of the week, and the autoclave for which it was used. These charts should be maintained in a permanent file.

VI. Accidents, First Aid and Illness

A. General

1. It is the responsibility of each employee to notify his/her supervisor of any personal condition which might prevent him/her from temporarily entering the containment area, such as recent surgical procedures or injuries involving significant alteration to the normal integrity of the skin.

B. Accidental Exposure to an Infectious Agent

1. An employee who is accidentally exposed to an infectious agent should report the incident as soon as possible to his/her Task Leader and the Project Manager. In the event that a substance enters the mouth, eyes, or [illegible] or penetrates or comes in contact with the skin, disinfecting [illegible]dures should be started immediately. The supervisor will see that other necessary treatment or health monitoring is obtaining without delay.

2. In the case of an accident outside a biosafety cabinet, whereby a container holding an infectious substance breaks or spills, the following steps must be taken:

a. If clothing is contaminated, remove it, place it in a biosafety hood, and change into the emergency clothing that is maintained in the laboratory.

b. Leave the room and close the door.

c. Warn others of the hazard.

d. Take a shower.

e. Report the spill to your Task Leader.

f. Whoever cleans the room should wait at least 30 minutes after the spill before entering the room and must wear a surgical gown, shoe protectors, surgical mask or respirator, hair bonnet and rubber gloves.

g. Disinfectant should be appled liberally with sufficient contact time before other final clean-up measures are performed.

C. First Aid

1. If an individual is injured during work, he/she should (i) depart the suite and obtain necessary treatment, (ii) notify the

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Task Leader of the injury, and (iii) report the injury to the Division Head and the Safety Office so that proper forms can be initiated.

2. Persons requiring emergency care will seek it immediately; the preparation of paperwork will be secondary to obtaining prompt medical attention.

3. All needle punctures involving infectious materials will be reported promptly to the Task Leader, Project Manager, and Safety Office. The Safety Office will coordinate appropriate actions to obtain baseline and additional serum samples for laboratory diagnosis of possible resulting infection.

D. Suspected Occupational Illness

1. Employees assigned to containment suites, or who have performed duties during the past 21 days in an area containing infectious materials, who develop a fever greater than 100°F will attempt to notify their Task Leader and the Project Manager before seeking medical attention.

VII. Fire-Fighting Procedures

A. Laboratory Personnel

1. Upon notification of a possible fire condition within the containment facility, personnel will, if possible, turn off all gas burners, biosafety cabinets, electric motors, and other electrical equipment. All containers of infectious materials should be placed in autoclaves or in incubators, refrigerators, freezers, or other storage areas. Personnel will leave the area as quickly as possible, undergoing change room procedures if time permits.

2. Personnel should be trained by the Safety Office in the operation of fire extinguishers. Assist in fire-fighting measures as conditions permit; however, remember that personal safety is the primary concern.

B. Fire Department Personnel

1. All fire department personnel and other nonimmunized personnel needed within the containment area should wear coveralls and a self-contained breathing apparatus during the entire time they are within the suite.

2. Fire-fighting personnel should not open autoclaves, refrigerators, freezers, incubators or other storage areas displaying the biohazard symbol.

3. Upon completion of the mission, fire-fighting personnel will, if possible, remove equipment and clothing in the "dirty" change room area, take a shower, and dress in fresh clothing. If such action is not possible, our staff will direct emergency decontamination procedures. The specific procedures to be followed will depend on the agents currently in use, level of exposure, etc.

AUTOCLAVE TIME CHART

Type of Load	Steam Conditioning Cycle	Steam Cycle	Cycle Temperature (F°)	Drying Cycle	Description
Laundry	45 sec	60 min	260	10 min	Lab clothing
Biohazard Waste Bags	60 sec	60 min	260	10 min	Waste paper, disposable plastics
Animal cages	45 sec	45 min	260	5 min	Cages containing feed and bedding
Liquid material	45 sec	90 min	260	No	Animal water bottles
Cell Culture Pans	60 sec	60 min	250	5 min	Reusable glassware, disposable plastics
Animal Carcasses	60 min	90 min	260	10 min	Dead animals
Animal Feed/ Bedding	45 sec	30 min	250	10 min	Bags of fresh feed and bedding

REMARKS:

1. Use sterilization indicator strips (Castle Tech-test) in each load. These should be placed in animal cages and in the center of all laundry and waste bags.

2. Whenever possible do not mix dry material with liquid material in the same cycle. If necessary to do so, treat the load as liquid material.

3. Use Diack tubes in liquid samples.

APPENDIX B

Summary of Antiviral Test Results

SUMMARY OF ANTIVIRAL TEST RESULTS --> CONTRACT DAMD17-86-C-6013

(SOUTHERN RESEARCH INSTITUTE)

Shipment No. 1

AVS No.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
15	PT	>4.3	>1.4	18.2	Vero	>320.0	17.6	9/19/86
64	VV	0	0	--	L-929	>320.0	--	6/12/86
	VV	0	0	--	Vero	>320.0	--	7/17/86
	VSV	0	0	--	L-929	>320.0	--	6/13/86
	Ad	0.3	0.1	--	HEp-2	>320.0	--	9/11/86
	SF	>1.4	>0.5	--	Vero	>320.0	--	10/8/86
	SF	2.7	0.9	--	Vero	320.0	--	10/21/86
	PT	2.0	0.7	68.0	Vero	320.0	4.7	9/19/86
68	VV	0.6	0.2	82.1	L-929	100.0	1.2	6/12/86
	VV	0	0	--	Vero	320.0	--	7/17/86
	VSV	0	0	--	L-929	320.0	--	6/13/86
	Ad	0	0	--	HEp-2	100.0	--	9/11/86
	PT	2.4	0.8	2.4	Vero	32.0	13.3	9/19/86
	SF	2.7	0.9	--	Vero	320.0	--	10/21/86
70	VV	>0.6	>0.2	<0.32	L-929	≤0.32	1.0	6/12/86
	VV	0.3	0.1	--	Vero	100.0	--	7/17/86
	VSV	0	0	--	L-929	1.0	--	6/13/86
	Ad	≥1.3	≥0.4	<0.32	HEp-2	≤0.32	1.0	9/11/86
	Ad	2.4	0.8	0.06	HEp-2	0.32	5.3	9/25/86
79	VV	1.4	0.5	1.7	L-929	3.2	1.9	6/12/86
	VV	1.7	0.6	32.0	Vero	100.0	3.1	7/17/86
	VSV	0.8	0.3	9.8	L-929	10.0	1.0	6/13/86
	Ad	1.3	0.4	2.3	HEp-2	10.0	4.4	9/11/86
	SF	>5.5	>1.8	0.65	Vero	>100.0	>154.0	10/8/86
	PT	1.7	0.6	--	Vero	>100.0	--	9/19/86
84	VV	0.2	0.07	--	L-929	32.0	--	6/12/86
	VV	0.6	0.2	298.0	Vero	320.0	1.1	7/17/86
	VSV	0.4	0.1	--	L-929	100.0	--	6/13/86
	Ad	0	0	--	HEp-2	32.0	--	9/11/86
	SF	4.4	1.5	1.3	Vero	320.0	246.0	10/8/86
	PT	1.9	0.6	66.4	Vero	320.0	4.8	9/19/86
94	VV	1.3	0.4	--	L-929	>320.0	--	6/12/86
	VV	0	0	--	Vero	>320.0	--	7/17/86
	VSV	0	0	--	L-929	>320.0	--	6/13/86
	Ad	1.0	0.3	231.1	HEp-2	320.0	1.4	9/11/86
	SF	>4.7	>1.6	1.3	Vero	>320.0	>246.0	10/8/86
	PT	3.0	1.0	10.0	Vero	>320.0	32.0	9/19/86
113	VV	0.7	0.2	97.4	L-929	>320.0	3.3	6/12/86
	VV	0	0	--	Vero	100.0	--	7/17/86
	VSV	0	0	--	L-929	320.0	--	6/13/86
	Ad	0.2	0.07	--	HEp-2	100.0	--	9/11/86
	SF	2.2	0.7	39.8	Vero	10.0	0.3	10/21/86
	PT	2.0	0.7	3.2	Vero	32.0	10.0	9/19/86

138	VV	3.1	1.0	8.8	L-929	100.0	11.4	6/12/86
	VV	3.8	1.3	19.9	Vero	>320.0	16.1	7/17/86
	VSV	0	0	--	L-929	32.0	--	6/13/86
	Ad	1.1	0.4	95.8	HEp-2	100.0	1.0	9/11/86
	SF	0.2	0.07	--	Vero	32.0	--	10/15/86
	PT	1.1	0.4	--	Vero	>320.0	--	9/19/86
139	VV	1.3	0.4	1.8	L-929	3.2	1.8	6/12/86
	VV	2.7	0.9	1.9	Vero	1.0	0.5	7/17/86
	VSV	1.6	0.5	31.5	L-929	3.2	0.1	6/13/86
	Ad	0	0	--	HEp-2	0.3	--	9/11/86
	SF	1.9	0.6	<0.001	Vero	1.0	>1000	10/08/86
	PT	1.8	0.6	0.7	Vero	0.3	0.4	9/19/86
	PT	0	0	--	Vero	0.32	--	10/28/86
215	VV	3.0	1.0	2.8	L-929	10.0	3.6	6/12/86
	VV	>4.2	>1.4	0.7	Vero	3.2	4.6	7/17/86
	VSV	2.1	0.7	10.0	L-929	3.2	0.3	6/13/86
	Ad	2.3	0.8	9.5	HEp-2	10.0	1.1	9/11/86
	SF	2.1	0.7	1.7	Vero	1.0	0.6	10/10/86
	PT	2.7	0.9	2.2	Vero	3.2	1.5	10/3/86
228	VV	0.3	0.1	--	L-929	100.0	--	6/12/86
	VV	0	0	--	Vero	32.0	--	7/17/86
	VSV	0	0	--	L-929	32.0	--	6/13/86
	Ad	0.2	0.07	--	HEp-2	10.0	--	9/11/86
	SF	0.4	0.1	32.0	Vero	32.0	1.0	10/10/86
	PT	0	0	--	Vero	32.0	--	10/3/86
230	VV	0.4	0.1	--	L-929	1.0	--	6/12/86
	VV	0.9	0.3	66.4	Vero	100.0	1.5	7/17/86
	VSV	1.9	0.6	9.2	L-929	3.2	0.4	6/13/86
	Ad	1.3	0.4	66.4	HEp-2	>100.0	>1.5	9/11/86
	Ad	0.7	0.2	--	HEp-2	320.0	--	9/18/86
	SF	2.3	0.8	53.5	Vero	100.0	1.9	10/10/86
	PT	1.5	0.5	100.0	Vero	100.0	1.0	10/3/86
253	VV	≥0.5	≥0.2	--	L-929	≤0.32	--	6/12/86
	VV	2.6	0.9	6.9	Vero	3.2	0.5	7/17/86
	VSV	2.0	0.7	21.2	L-929	1.0	0.1	6/13/86
	Ad	1.6	0.5	8.1	HEp-2	32.0	4.0	9/11/86
	SF	>6.1	>2.0	1.0	Vero	>320.0	320.0	9/23/86
	SF	4.4	1.5	0.14	Vero	>100.0	>714.0	10/08/86
	SF	6.0	2.0	1.0	Vero	>100.0	>100.0	10/08/86
	PT	3.4	1.1	3.2	Vero	32.0	10.0	9/19/86
	PT	1.6	0.5	18.3	Vero	32.0	1.8	10/3/86
	PT	4.3	1.4	2.3	Vero	32.0	13.9	9/19/86
272	VV	≥3.4	≥1.1	0.5	L-929	3.2	6.4	6/12/86
	VV	4.7	1.6	0.7	Vero	3.2	4.6	7/17/86
	VSV	2.1	0.7	7.5	L-929	3.2	0.4	6/17/86
	Ad	2.2	0.7	8.1	HEp-2	3.2	0.4	9/11/86
	SF	2.2	0.7	1.0	Vero	3.2	3.2	10/10/86
	PT	2.3	0.8	1.0	Vero	3.2	3.2	10/3/86
302	VV	≥0.2	≥0.07	--	L-929	0.1	--	6/12/86
	VV	0.7	0.2	2.6	Vero	3.2	1.2	7/17/86
	VSV	0.1	0.03	--	L-929	0.32	--	6/17/86
	Ad	0	0	--	HEp-2	0.32	--	9/11/86
	SF	0.1	0.03	--	Vero	0.1	--	10/10/86

	PT	0.6	0.2	<0.01	Vero	≤0.01	1.0	10/3/86
	PT	0	0	--	Vero	≥0.01	--	10/15/86
303	VV	≥3.5	≥1.2	0.4	L-929	10.0	25.0	6/12/86
	VV	5.2	1.7	0.6	Vero	10.0	15.9	7/17/86
	VSV	2.8	0.9	3.1	L-929	10.0	3.6	6/17/86
	Ad	1.2	0.4	--	HEp-2	>320.0	--	9/11/86
	SF	0.1	0.03	--	Vero	320.0	--	10/15/86
	SF	0	0	--	Vero	32.0	--	11/07/86
	PT	0.8	0.3	--	Vero	320.0	--	10/09/86
1972	VV	1.2	0.4	32.0	L-929	100.0	3.1	6/12/86
	VV	0	0	--	Vero	32.0	--	7/17/86
	VSV	0	0	--	L-929	10.0	--	6/17/86
	Ad	0.6	0.2	>320.0	HEp-2	>320.0	--	9/11/86
	PT	1.5	0.5	6.2	Vero	10.0	1.6	10/09/86
	PT	0.2	0.1	--	Vero	>10.0	--	10/22/86
1973	VV	0.6	0.2	93.2	L-929	100.0	1.1	6/12/86
	VV	0	0	--	Vero	32.0	--	7/17/86
	VSV	0	0	--	L-929	32.0	--	6/17/86
	Ad	0.3	0.1	--	HEp-2	100.0	--	9/11/86
	PT	1.6	0.5	19.2	Vero	100.0	5.2	10/09/86
1974	VV	0.8	0.3	72.5	L-929	100.0	1.4	6/12/86
	VV	0	0	--	Vero	32.0	--	7/17/86
	VSV	0	0	--	L-929	32.0	--	6/17/86
	Ad	0	0	--	HEp-2	32.0	--	9/11/86
	PT	1.7	0.6	14.2	Vero	100.0	7.0	10/09/86
1975	VV	0	0	--	L-929	≥320.0	--	6/12/86
	VV	0	0	--	Vero	320.0	--	7/17/86
	VSV	0	0	--	L-929	32.0	--	6/17/86
	Ad	0.3	0.1	--	HEp-2	≥100.0	--	9/11/86
	PT	2.7	0.9	10.5	Vero	320.0	30.5	10/09/86
1976	VV	0.5	0.2	29.0	L-929	32.0	1.1	6/12/86
	VV	0	0	--	Vero	100.0	--	7/17/86
	VSV	0.6	0.2	93.2	L-929	100.0	1.1	6/17/86
	Ad	0.6	0.2	--	HEp-2	32.0	--	9/11/86
	SF	2.4	0.8	18.2	Vero	>100.0	>5.5	10/27/86
	PT	0.8	0.3	94.9	Vero	320.0	3.4	10/15/86
1977	VV	0.2	0.07	--	L-929	100.0	--	6/12/86
	VV	0	0	--	Vero	320.0	--	7/17/86
	VSV	0	0	--	L-929	32.0	--	6/17/86
	Ad	0.6	0.2	--	HEp-2	>320.0	--	9/11/86
	SF	0	0	--	Vero	>100.0	--	10/27/86
	PT	0	0	--	Vero	320.0	--	10/15/86
1978	VV	0	0	--	L-929	≥320.0	--	6/12/86
	VV	0	0	--	Vero	100.0	--	7/17/86
	VSV	0	0	--	L-929	320.0	--	6/17/86
	Ad	0.3	0.1	--	HEp-2	100.0	--	9/11/86
	SF	1.2	0.4	32.0	Vero	32.0	1.0	10/27/86
	PT	0.7	0.2	79.8	Vero	100.0	1.3	10/15/86
1979	VV	0.1	0.03	--	L-929	1.0	--	6/12/86
	VV	0	0	--	Vero	3.2	--	7/17/86
	VSV	0	0	--	L-929	1.0	--	6/17/86

	Ad	0	0	--	HEp-2	3.2	--	9/11/86
	SF	0	0	--	Vero	1.0	--	10/27/86
	PT	0	0	--	Vero	1.0	--	10/15/86
	PT	0	0	--	Vero	3.2	--	10/27/86
1980	PT	1.7	0.6	227.0	Vero	>320.0	>1.4	10/17/86
1981	PT	0	0	--	Vero	320.0	--	10/17/86
1982	VV	0.9	0.3	--	L-929	≥320.0	--	6/12/86
	VV	0	0	--	Vero	320.0	--	7/17/86
	VSV	0	0	--	L-929	320.0	--	6/17/86
	Ad	0	0	--	HEp-2	32.0	--	9/11/86
	SF	0.8	0.3	100.0	Vero	>100.0	>1.0	10/27/86
	PT	0	0	--	Vero	10.0	--	10/17/86
Ara-A	VV	2.6	0.9	1.9	L-929	32.0	16.8	6/12/86
	VV	3.6	1.2	10.0	Vero	320.0	32.0	7/17/86
	Ad	0	0	--	HEp-2	100.0	--	9/11/86
C-3-deaza-Ado	VSV	2.8	0.9	4.9	L-929	32.0	6.5	6/13/86
	VSV	3.4	1.1	0.8	L-929	3.2	4.0	6/17/86
Ribavirin	SF	>6.0	>2.0	1.0	Vero	>320.0	320.0	9/23/86
	SF	2.7	0.9	22.7	Vero	>100.0	>4.4	10/27/86
	SF	3.0	1.0	19.1	Vero	>100.0	>5.2	10/27/86
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8	11/07/86
	PT	5.1	1.7	1.0	Vero	320.0	320.0	9/19/86
	PT	2.1	0.7	14.9	Vero	>100.0	>6.7	10/22/86
	PT	2.3	0.8	19.0	Vero	>100.0	>5.3	10/27/86
	PT	3.8	1.3	8.2	Vero	>100.0	>12.2	10/28/86
	PT	2.5	0.8	17.5	Vero	100.0	5.7	10/28/86
Selenazole	SF	4.9	1.6	3.6	Vero	100.0	28.0	10/10/86
	SF	3.2	1.1	0.31	Vero	32.0	103.0	10/15/86
	SF	3.5	1.2	7.1	Vero	32.0	4.5	10/21/86
	PT	1.6	0.5	6.5	Vero	10.0	1.5	10/09/86
	PT	2.1	0.7	3.2	Vero	10.0	3.1	10/15/86
	PT	1.2	0.4	32.0	Vero	100.0	3.1	10/17/86
	PT	1.1	0.4	54.7	Vero	32.0	0.6	10/22/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 01 Positive Control (PC) _____

Date 12/5/86 PC MIC_{50} _____ µg/ml

Virus Pichinde 4763 Cell Type Vero

MIC_{50} 4.3 µg/ml

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
100	0	0	0	0	---	0	100
32	0	0	0	0	---	0	100
10	1	0	0	1	---	1	99
3.2	17	14	13	44	---	56	44
1	27	28	32	87	---	100	0
0.32	26	23	18	67	---	86	14
0.10	24	24	30	78	---	100	0
Cell Controls	0	0	0	0	---	0	n.a.
Virus Controls	27 32	20 23	29 15	31 X	177	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Jody Jones Date 12/5/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 64

Date 12/5/86

Virus Pichinde 4763

MIC_{50} >100 µg/ml

Positive Control (PC) AVS #01

PC MIC_{50} 4.3 µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
100	27	31	43	101	T	94	6
32	37	26	26	89	T	82	18
10	32	29	39	100	P	93	7
3.2	40	30	37	107	---	99	1
1	42	33	43	118	---	100	0
0.32	36	26	30	92	---	85	15
0.10	33	32	37	102	---	94	6
Cell Controls	0	0	0	0	---	0	n.a.
Virus Controls	29, 43	38, 37	35, 37	33 ⊠	252	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments: concentration of EtOH solvent in 100 µg/ml assays 0.5%. Plaques in cultures showing toxicity larger than those in cultures with no toxicity.

Signed: Jody Jones JJ.W Date 12/5/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 253 — Positive Control (PC) Ribavirin

Date 12/5/86 — PC MIC_{50} 4.3 µg/ml

Virus Pichinde 4763 — Cell Type Vero

MIC_{50} 2.0 µg/ml

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
100	0	0	0	0	p	0	100
32	0	0	0	0	p	0	100
10	0	0	0	0	---	0	100
3.2	0	0	0	0	---	KC	100
1	14	17	19	50	---	62	38
0.32	25	27	22	74	---	92	8
0.10	30	27	24	81	---	100	0
Cell Controls	0	0	0	0	---	0	n.a.
Virus Controls	18 21	31 26	28 28	31 X	183	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Jody Jones — Date 12/5/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 01

Date 12/12/86

Virus Pichinde 4763

MIC_{50} 9.3 µg/ml

Positive Control (PC) _____

PC MIC_{50} _____ µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320							
100	0	0	0	0		0	100
32	0	0	0	0		0	100
10	60	36	49	145		39	61
3.2	107	123	108	338		92	8
1	100	118	123	341		92	8
0.32	149	139	150	438		100	0
0.10	119	116	123	358		97	3
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	125 111	130 138	119 120	121 X	864	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Gary J. Williams Date 12/12/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 138

Date 12/12/86

Virus Pichinde 4763

MIC_{50} >320 µg/ml

Positive Control (PC) Ribavirin

PC MIC_{50} 9.3 µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	166	105	164	435		100	0
100	107	95	129	331		86	14
32	139	98	98	335		87	13
10	118	114	125	357		93	7
3.2	122	123	118	363		95	5
1	111	102	122	335		87	13
0.32	133	122	119	374		98	2
0.10	---	---	---	---	-------	---	----
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	155	139	135	126			
	105	110	128	X	896	n.a.	100	0

Key:
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Gary J. Williams Date 12/12/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 139 Positive Control (PC) Ribavirin

Date 12/12/86 PC MIC_{50} 9.3 µg/ml

Virus Pichinde 4763 Cell Type Vero

MIC_{50} 15.4 µg/ml

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	0	0	0	0		0	100
100	0	0	0	0		0	100
32	0	0	0	0		0	100
10	u	u	u			n.a.	n.a.
3.2	96	84	63	243		70	30
1	129	103	129	361		100	0
0.32	82	116	77	275		79	21
0.10	---	---	---	---		---	---
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	141 120	111 122	u 95	133 X	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Gary J Williams Date 12/12/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 215 — Positive Control (PC) Ribavirin

Date 12/12/86 — PC MIC_{50} 9.3 µg/ml

Virus Pichinde 4763 — Cell Type Vero

MIC_{50} 2.2 µg/ml

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320							
100	0	0	0	0		0	100
32	0	0	0	0		0	100
10	0	0	0	0		0	100
3.2	8	10	3	21		5	95
1	101	103	105	309		74	26
0.32	132	128	98	358		85	15
0.10	144	129	144	417		99	1
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	136 155	129 159	124 125	172 X	997	n.a.	100	0

Key:
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Gary J. Williams Date 12/12/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 228

Date 12/12/86

Virus Pichinde 4763

MIC_{50} 28.4 µg/ml

Positive Control (PC) Ribavirin

PC MIC_{50} 9.3 µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	----	----	----	---			
100	38	75	43	156	p	48	52
32	60	47	47	154	p	47	53
10	87	80	81	248		76	24
3.2	76	79	87	242		74	26
1	81	107	79	268		82	18
0.32	91	93	86	270		83	17
0.10	114	118	121	353		100	0
Cell Controls	0	0	0	0			n.a.
Virus Controls	127 106	113 112	94 95	119 X	766	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Gary J. Williams Date 12/12/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 230 — Positive Control (PC) Ribavirin

Date 12/12/86 — PC MIC_{50} 9.3 µg/ml

Virus Pichinde 4763 — Cell Type Vero

MIC_{50} 9.8 µg/ml

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	0	0	0	0	p	0	100
100	0	0	0	0		0	100
32	8	4	5	17		6	94
10	49	45	44	138		45	55
3.2	102	96	106	304		99	1
1	105	92	112	309		100	0
0.32	113	111	110	334		100	0
0.10	---	---	---	--	-----	---	---
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	118 90	102 95	100 113	102 X	n.a.	100	0

Key:
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Gary J. Williams Date 12/12/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 01

Date 12/12/86

Virus Pichinde (4763)

MIC_{50} 8.9 µg/ml

Positive Control (PC) n.a.

PC MIC_{50} n.a. µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320							
100	0	0	0	0		0	100
32	0	0	0	0		0	100
10	8	7	11	26		9	91
3.2	89	92	108	289		98	2
1	134	111	86	331		100	0
0.32	87	106	82	275		93	7
0.10	111	110	83	304		100	0
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	103 / 91	u / 108	97 / 104	u / X	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Gary J. Williams Date 12/15/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 272 | Positive Control (PC) Ribavirin

Date 12/12/86 | PC MIC_{50} 8.9 µg/ml

Virus Pichinde (4763) | Cell Type Vero

MIC_{50} 2.0 µg/ml

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320							
100	0	0	0	0	p	0	100
32	0	0	0	0	p	0	100
10	1	0	0	1		0	100
3.2	0	0	0	0		0	100
1	101	88	63	252		73	27
0.32	110	102	94	306		89	11
0.10	94	120	123	337		98	2
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	111	137	119	110	n.a.	100	0
	110	117	115	X	819		

Key:
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments: 100 µg/ml dilution contained 0.5% DMSO

Signed: Gary J. Williams Date 12/15/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 302

Date 12/12/86

Virus Pichinde (4763)

MIC_{50} 0.3 µg/ml

Positive Control (PC) Ribavirin

PC MIC_{50} 8.9 µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320							
100	0	0	0	0	T	0	100
32	0	0	0	0	T	0	100
10	0	0	0	0	T	0	100
3.2	0	0	0	0	P	0	100
1	12	23	28	63	P	19	81
0.32	0	0	0	0	P	0	100
0.10	121	126	120	367		100	0
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	95 100	124 98	137 100	124 X	778	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments: 100 µg/ml dilution contained 0.5% DMSO
MIC_{50} calculated from % Plaque Reduction found in 0.32 and 0.1 µg/ml assays

Signed: Gary J. Williams Date 12/15/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 303

Date 12/12/86

Virus Pichinde (4763)

MIC_{50} > 32 µg/ml

Positive Control (PC) Ribavirin

PC MIC_{50} 8.9 µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	u	u	u		n.a.	n.a.	n.a.
100	u	u	u		n.a.	n.a.	n.a.
32	113	108	101	322		85	15
10	125	117	136	378		99	1
3.2	125	96	111	332		87	13
1	153	135	140	428		100	0
0.32	138	142	134	414		100	0
0.10	---	---	---	---			
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	135	121	119	u			
	126	134	u	X	635	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments:

Signed: Gary J. Williams Date 12/15/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 1972

Date 12/12/86

Virus Pichinde (4763)

MIC_{50} 79.3 µg/ml

Positive Control (PC) Ribavirin

PC MIC_{50} 8.9 µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	0	0	0	0	T	0	100
100	30	36	49	115	P	34	66
32	93	108	92	293		85	15
10	97	117	127	341		99	1
3.2	116	115	106	337		98	2
1	133	113	124	370		100	0
0.32	90	116	127	333		97	3
0.10	----	----	----	----			
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	119	137	109	89			
	125	97	122	X	798	n.a.	100	0

Key
p partially toxic
t toxic
u unsatisfactory
n.a. not applicable

Comments: 320 µg/ml dilution contained 0.5% EtOH

Signed: Gary J. Williams Date 12/15/86

In Vitro Antiviral Screening Results
Plaque Reduction Assay

AVS# 1973

Date 12/12/86

Virus Pichinde (4763)

MIC_{50} 25.0 µg/ml

Positive Control (PC) Ribavirin

PC MIC_{50} 8.9 µg/ml

Cell Type Vero

Final [AVS] µg/ml	Plaque Forming Units			Σ	Toxicity	% Virus Control	% Plaque Reduction
320	0	0	0	0	P	0	100
100	0	0	0	0	P	0	100
32	0	0	0	0	P	0	100
10	116	108	102	326		88	12
3.2	117	119	120	356		97	3
1	u	u	u			n.a.	n.a.
0.32	122	142	129	393		100	0
0.10	----------						
Cell Controls	0	0	0	0		0	n.a.
Virus Controls	112 130	113 113	147 u	136 X	751	n.a.	100	0

Key

p partially toxic

t toxic

u unsatisfactory

n.a. not applicable

Comments: 320 µg/ml dilution contained 0.5% EtOH

Signed: Gary J. Williams Date 12/15/86

Shipment No. 2

AVS No.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
33	VV	0.6	0.2	100.0	Vero	320.0	3.2	7/24/86
	VSV	0	0	--	L-929	100.0	--	6/20/86
	Ad	0.3	0.1	--	HEp-2	100.0	--	9/18/86
	PT	1.2	0.4	32.0	Vero	100.0	3.1	10/17/86
	SF	3.4	1.1	10.0	Vero	32.0	3.2	10/28/86
65	VV	0.4	0.1	--	Vero	10.0	--	7/24/86
	VSV	0	0	--	L-929	320.0	--	6/20/86
	Ad	0.7	0.2	--	HEp-2	10.0	--	9/18/86
	PT	2.5	0.8	55.0	Vero	32.0	0.6	10/23/86
	SF	2.6	0.9	1.6	Vero	3.2	2.0	10/28/86
71	VV	1.7	0.6	81.4	Vero	320.0	3.9	7/24/86
	VSV	0	0	--	L-929	320.0	--	6/20/86
	Ad	0.3	0.1	--	HEp-2	100.0	--	9/18/86
	PT	0.6	0.2	32.0	Vero	100.0	3.1	10/23/86
	SF	3.7	1.2	16.4	Vero	>100.0	>6.1	10/28/86
78	VV	0.6	0.2	8.5	Vero	10.0	1.2	7/24/86
	VSV	0.6	0.2	29.8	L-929	32.0	1.1	6/20/86
	Ad	0	0	--	HEp-2	10.0	--	9/18/86
	PT	0.5	0.2	9.5	Vero	10.0	1.1	10/23/86
	SF	1.8	0.6	--	Vero	1.0	--	10/28/86
87	VV	0	0	--	Vero	320.0	--	7/24/86
	VSV	0	0	--	L-929	100.0	--	6/20/86
	Ad	0.3	0.1	--	HEp-2	320.0	--	9/18/86
	PT	1.1	0.4	47.0	Vero	100.0	2.1	10/23/86
	SF	0.6	0.2	9.3	Vero	10.0	1.1	10/28/86
95	VV	0	0	--	Vero	100.0	--	7/24/86
	VSV	0	0	--	L-929	>320.0	--	6/20/86
	Ad	0.8	0.3	--	HEp-2	320.0	--	9/18/86
	PT	1.9	0.6	22.7	Vero	100.0	4.4	10/23/86
	SF	(On Retest)						
96	VV	0	0	--	Vero	320.0	--	7/24/86
	VSV	0	0	--	L-929	100.0	--	6/20/86
	Ad	0	0	--	HEp-2	10.0	--	9/18/86
	PT	1.0	0.3	--	Vero	32.0	--	10/21/86
	SF	0.4	0.1	--	Vero	320.0	--	10/15/86
148	VV	5.4	1.8	1.0	Vero	100.0	100.0	7/24/86
	VSV	2.6	0.9	0.32	L-929	0.3	1.0	6/20/86
	VSV	2.6	0.9	2.2	L-929	1.0	0.5	8/15/86
	Ad	0.5	0.2	<0.32	HEp-2	≤0.3	≥1.0	9/18/86
	Ad	0.9	0.3	0.09	HEp-2	0.1	1.1	9/25/86
	PT	6.3	2.1	<0.32	Vero	320.0	>1000	10/21/86
	SF	3.6	1.2	2.3	Vero	32.0	13.9	10/14/86
181	VV	0	0	--	Vero	>320.0	--	7/24/86
	VSV	0	0	--	L-929	32.0	--	6/20/86
	Ad	0	0	--	HEp-2	>320.0	--	9/18/86
	PT	0.4	0.1	--	Vero	>320.0	--	10/21/86
	SF	1.4	0.5	--	Vero	>320.0	--	10/14/86

199	PT	1.5	0.5	30.7	Vero	100.0	3.3	10/21/86
200	VV	1.0	0.3	52.4	Vero	100.0	1.9	7/24/86
	VSV	0.1	0.03	--	L-929	100.0	--	6/20/86
	Ad	0.2	0.07	--	HEp-2	32.0	--	9/18/86
	PT	2.1	0.7	15.6	Vero	100.0	6.4	10/21/86
	SF	0.7	0.2	--	Vero	›100.0	--	10/14/86
346								
347	VV	0.6	0.2	7.5	Vero	3.2	0.4	7/24/86
	VSV	0.6	0.2	3.0	L-929	1.0	0.3	6/20/86
	Ad	0.8	0.3	0.1	HEp-2	0.3	3.2	9/18/86
	PT	1.5	0.5	1.0	Vero	1.0	1.0	10/28/86
	SF	1.5	0.5	5.1	Vero	10.0	2.0	10/17/86
349	VV	‹0.5	‹0.2	--	Vero	10.0	--	7/24/86
	VSV	0	0	--	L-929	1.0	--	6/20/86
	Ad	1.0	0.3	0.1	HEp-2	0.3	3.2	9/18/86
	PT	0.5	0.2	3.1	Vero	3.2	1.0	10/28/86
	SF	1.7	0.6	2.5	Vero	3.2	1.3	10/17/86
351	PT	0.6	0.2	9.3	Vero	10.0	1.1	10/28/86
352								
360	VV	0.6	0.2	3.0	Vero	1.0	0.3	7/24/86
	VSV	0.9	0.3	1.0	L-929	‹0.3	‹0.3	6/20/86
	Ad	1.5	0.5	0.1	HEp-2	0.1	1.0	9/18/86
	PT	2.0	0.7	0.2	Vero	1.0	5.0	10/31/86
	SF	2.5	0.8	0.02	Vero	0.32	16.0	10/17/86
	SF	1.8	0.6	0.32	Vero	1.0	3.1	11/05/86
361	VV	≥0.5	≥0.2	--	Vero	≤0.3	--	7/24/86
	VV	0.5	0.2	0.3	Vero	0.1	0.3	10/30/86
	VSV	›1.0	›0.3	‹0.3	L-929	≤0.3	1.0	6/20/86
	VSV	1.6	0.5	0.3	L-929	0.1	0.3	8/15/86
	Ad	1.3	0.4	0.1	HEp-2	0.1	1.0	9/18/86
	Ad	0.6	0.2	0.3	HEp-2	0.1	0.3	10/30/86
646	VV	≥4.0	1.3	0.6	Vero	1.0	1.7	7/24/86
	VSV	0	0	--	L-929	100.0	--	6/24/86
	Ad	1.5	0.5	3.2	HEp-2	3.2	1.0	9/18/86
	PT	1.6	0.5	13.2	Vero	0.32	0.02	10/31/86
	SF	4.2	1.4	‹0.10	Vero	3.2	≤32.0	10/17/86
	SF	4.3	1.4	0.6	Vero	10.0	16.7	11/05/86
1983	VV	0	0	--	Vero	320.0	--	7/24/86
	VSV	0.1	0.03	--	L-929	100.0	--	6/24/86
	Ad	0.9	0.3	100.0	HEp-2	320.0	3.2	9/18/86
	PT	0	0	--	Vero	›100.0	--	10/31/86
	SF	3.0	1.0	0.32	Vero	32.0	100.0	10/22/86
1984	VV	0	0	--	Vero	›320.0	--	7/24/86
	VSV	0	0	--	L-929	320.0	--	6/24/86
	Ad	1.9	0.6	88.0	HEp-2	›320.0	3.6	9/18/86
	PT	0.1	0.03	--	Vero	›100.0	--	10/31/86
	SF	2.9	1.0	1.0	Vero	32.0	32.0	10/22/86

1985	VV	≥7.2	2.4	<0.32	Vero	100.0	312.5	7/24/86
	VSV	0	0	--	L-929	3.2	--	6/24/86
	Ad	0.3	0.1	--	HEp-2	3.2	--	9/18/86
	PT	0.5	0.2	100.0	Vero	10.0	0.1	10/31/86
	SF	3.7	1.2	6.1	Vero	>100.0	>16.4	10/22/86
1986	VV	≥7.4	≥2.5	0.6	Vero	>320.0	533.3	7/24/86
	VSV	0	0	--	L-929	320.0	--	6/24/86
	Ad	0	0	--	HEp-2	>320.0	--	9/18/86
	PT	0	0	--	Vero	>320.0	--	10/30/86
	SF	2.5	0.8	--	Vero	320.0	--	10/22/86
	SF	0	0	--	Vero	>320.0	--	11/05/86
1987	VV	6.8	2.3	1.7	Vero	>320.0	188.2	7/24/86
	VSV	0	0	--	L-929	>320.0	--	6/24/86
	Ad	0	0	--	HEp-2	>320.0	--	9/18/86
	PT	0	0	--	Vero	>320.0	--	10/30/86
	SF	3.1	1.0	19.0	Vero	100.0	5.3	10/22/86
1988	VV	6.4	2.1	3.6	Vero	>320.0	88.9	7/24/86
	VSV	0	0	--	L-929	>320.0	--	6/24/86
	Ad	0	0	--	HEp-2	320.0	--	9/18/86
	PT	0	0	--	Vero	>320.0	--	10/30/86
	SF	0	0	--	Vero	>320.0	--	11/07/86
1989	VV	0	0	--	Vero	32.0	--	7/24/86
	VSV	0	0	--	L-929	10.0	--	6/24/86
	Ad	0	0	--	HEp-2	100.0	--	9/18/86
	PT	0.2	0.07	--	Vero	1.0	--	10/30/86
1990	VV	0	0	--	Vero	100.0	--	7/24/86
	VSV	0	0	--	L-929	100.0	--	6/24/86
	Ad	0	0	--	HEp-2	32.0	--	9/18/86
	PT	0.3	0.1	--	Vero	100.0	--	10/30/86
Ara-A	VV	3.1	1.0	9.8	Vero	320.0	32.7	7/24/86
	VV	2.4	0.8	3.2	Vero	3.2	3.1	10/30/86
C-3-deaza-Ado	VSV	3.2	1.1	3.0	L-929	10.0	3.3	6/20/86
	VSV	2.5	0.8	2.6	L-929	3.2	1.2	6/24/86
Ribavirin	Ad	1.7	0.6	21.2	HEp-2	32.0	1.5	9/18/86
	Ad	1.2	0.4	26.2	HEp-2	32.0	1.2	10/30/86
	PT	2.3	0.8	41.8	Vero	>100.0	>2.4	10/23/86
	PT	3.8	1.3	8.2	Vero	>100.0	>12.2	10/28/86
	PT	2.5	0.8	17.5	Vero	100.0	5.7	10/28/86
	PT	1.5	0.5	47.0	Vero	100.0	2.1	10/30/86
	PT	2.5	0.8	13.8	Vero	>100.0	>7.2	10/30/86
	PT	1.8	0.6	32.0	Vero	>100.0	>3.1	10/31/86
	SF	3.0	1.0	0.32	Vero	32.0	100.0	10/22/86
	SF	5.2	1.7	1.9	Vero	>100.0	>52.6	10/28/86
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8	10/28/86
	SF	2.9	1.0	14.4	Vero	>100.0	>6.9	11/05/86
	SF	2.5	0.8	24.1	Vero	>100.0	>30.1	11/05/86
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8	11/07/86
Selenazole	VV	2.5	0.8	13.8	Vero	10.0	0.7	7/24/86
	VV	2.2	0.7	17.4	Vero	10.0	0.6	10/30/86
	Ad	1.4	0.5	7.5	HEp-2	10.0	1.3	9/18/86
	Ad	1.4	0.5	50.7	HEp-2	32.0	0.6	10/30/86

PT	1.2	0.4	32.0	Vero	100.0	3.1	10/17/86
PT	2.1	0.7	19.8	Vero	100.0	5.1	10/21/86
PT	2.7	0.9	5.5	Vero	32.0	5.8	10/21/86
SF	0.6	0.2	93.2	Vero	100.0	1.1	10/14/86
SF	5.3	1.8	0.19	Vero	100.0	526.0	10/17/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

Shipment No. 3

AVS No.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
1991	PT	0.6	0.2	298.0	Vero	320.0	1.1	10/17/86
	SF	1.2	0.4	0.89	Vero	3.2	3.6	10/17/86
	SF	0	0	--	Vero	3.2	--	11/05/86
1992	PT	1.5	0.5	66.0	Vero	100.0	1.5	10/21/86
	SF	0.8	0.3	94.9	Vero	32.0	0.3	10/27/86
1993	PT	0.1	0.03	--	Vero	>320.0	--	10/21/86
	SF	0	0	--	Vero	10.0	--	10/27/86
1994	PT	0.8	0.3	66.0	Vero	32.0	0.5	10/21/86
	SF	0	0	--	Vero	100.0	--	10/27/86
1995	PT	1.1	0.4	69.0	Vero	100.0	1.4	10/21/86
	SF	2.9	1.0	7.8	Vero	100.0	12.8	10/21/86
	SF	0	0	--	Vero	100.0	--	10/27/86
1996	PT	0.7	0.2	81.0	Vero	32.0	0.4	10/21/86
	SF	1.9	0.6	--	Vero	100.0	--	10/21/86
1997	PT	0	0	--	Vero	>320.0	--	10/22/86
	SF	2.1	0.7	96.3	Vero	320.0	3.3	10/24/86
1998	PT	0.8	0.3	75.0	Vero	100.0	1.3	10/22/86
	SF	1.7	0.6	32.0	Vero	32.0	1.0	10/24/86
1999	PT	0	0	--	Vero	>320.0	--	10/22/86
	SF	0	0	--	Vero	>320.0	--	10/24/86
2001	PT	0.8	0.3	66.0	Vero	32.0	0.5	10/22/86
	SF	2.3	0.8	32.0	Vero	32.0	1.0	10/24/86
2002	PT	0.2	0.07	--	Vero	320.0	--	10/22/86
	SF	1.4	0.5	--	Vero	320.0	--	10/24/86
2003	PT	0.7	0.2	62.0	Vero	100.0	1.6	10/22/86
	SF	2.1	0.7	95.1	Vero	100.0	1.1	10/23/86
	SF	0.3	0.1	--	Vero	>100.0	--	11/10/86
2004	PT	0.3	0.1	--	Vero	>320.0	--	10/22/86
	SF	1.5	0.5	--	Vero	>320.0	--	10/23/86
	SF	0.9	0.3	--	Vero	>100.0	--	11/10/86
2005	PT	1.0	0.3	64.0	Vero	100.0	1.6	10/22/86
	SF	1.9	0.6	93.2	Vero	32.0	0.3	10/23/86
2006	PT	1.2	0.4	56.0	Vero	100.0	1.8	10/22/86
	SF	2.8	0.9	30.9	Vero	32.0	1.0	10/23/86
2007	PT	0.8	0.3	320.0	Vero	>320.0	>1.0	10/24/86
	SF	2.6	0.9	--	Vero	100.0	--	10/23/86
2008	PT	0.2	0.07	--	Vero	320.0	--	10/24/86
	SF	0.3	0.1	--	Vero	320.0	--	10/28/86

2009	PT	0.2	0.07	--	Vero	320.0	--	10/24/86
	SF	0.7	0.2	--	Vero	320.0	--	10/28/86
2010	PT	0.6	0.2	93.0	Vero	100.0	1.1	10/24/86
	SF	0.5	0.2	--	Vero	100.0	--	10/28/86
2011	PT	0.8	0.3	75.0	Vero	100.0	1.3	10/24/86
	SF	0	0	--	Vero	100.0	--	10/28/86
2012	SF	0.5	0.2	--	Vero	100.0	--	10/28/86
2013	SF	3.1	1.0	52.3	Vero	>320.0	6.1	11/12/86
2014	SF	0.2	0.07	--	Vero	320.0	--	11/12/86
2015	SF	0	0	--	Vero	>320.0	--	11/12/86
2016	SF	0	0	--	Vero	>320.0	--	11/07/86
2017	PT	1.5	0.5	53.0	Vero	32.0	0.6	10/27/86
	SF	0.4	0.1	--	Vero	320.0	--	11/12/86
2018	PT	1.3	0.4	31.0	Vero	100.0	3.2	10/27/86
	SF	0.4	0.1	--	Vero	320.0	--	11/12/86
2019	PT	0.7	0.2	2.4	Vero	3.2	1.3	10/27/86
	SF	0.2	0.07	--	Vero	3.2	--	11/13/86
2020	PT	1.6	0.5	21.0	Vero	32.0	1.5	10/27/86
	SF	0	0	--	Vero	>320.0	--	11/13/86
2021	PT	1.0	0.3	60.0	Vero	32.0	0.5	10/27/86
	SF	0	0	--	Vero	>320.0	--	11/13/86
2022	PT	0	0	--	Vero	320.0	--	10/28/86
	SF	0	0	--	Vero	>320.0	--	10/27/86
2023	PT	0	0	--	Vero	32.0	--	10/29/86
	SF	0.2	0.07	--	Vero	32.0	--	11/07/86
	VSV	0	0	--	L929	32.0	--	6/24/86
Selenazole	PT	1.2	0.4	32.0	Vero	100.0	3.1	10/17/86
	PT	2.1	0.7	19.8	Vero	100.0	5.1	10/21/86
	PT	1.1	0.4	54.7	Vero	32.0	0.6	10/22/86
	SF	5.3	1.8	0.19	Vero	100.0	526.0	10/17/86
	SF	3.5	1.2	7.1	Vero	32.0	4.5	10/21/86
Ribavirin	PT	2.1	0.7	14.9	Vero	>100.0	>6.7	10/22/86
	PT	2.8	0.9	10.0	Vero	>100.0	>10.0	10/24/86
	PT	2.3	0.8	19.0	Vero	>100.0	>5.3	10/27/86
	PT	3.8	1.3	8.2	Vero	>100.0	>12.2	10/28/86
	PT	2.5	0.8	17.5	Vero	100.0	5.7	10/28/86
	PT	2.7	0.9	10.0	Vero	>100.0	>10.0	10/29/86
	PT	2.4	0.8	14.7	Vero	>100.0	>6.8	10/29/86
	SF	4.4	1.5	2.4	Vero	>100.0	>41.7	10/23/86
	SF	3.8	1.3	12.6	Vero	>100.0	>7.9	10/24/86
	SF	2.7	0.9	22.7	Vero	>100.0	>4.4	10/27/86
	SF	3.0	1.0	19.1	Vero	>100.0	>5.2	10/27/86
	SF	5.2	1.7	1.9	Vero	>100.0	>52.6	10/28/86

SF	3.0	1.0	20.8	Vero	>100.0	>4.8	10/28/86
SF	2.9	1.0	14.4	Vero	>100.0	>6.9	11/05/86
SF	2.5	0.8	24.1	Vero	>100.0	>30.1	11/05/86
SF	3.0	1.0	20.8	Vero	>100.0	>4.8	11/07/86
SF	4.4	1.5	2.3	Vero	>100.0	>43.5	11/10/86
SF	3.4	1.1	14.1	Vero	>100.0	>7.1	11/12/86
SF	4.0	1.3	0.92	Vero	>100.0	>109.0	11/12/86
SF	4.0	1.3	6.1	Vero	>100.0	>16.4	11/13/86
SF	4.4	1.5	2.9	Vero	>100.0	>34.5	11/13/86
SF	3.0	1.0	10.0	Vero	>100.0	>10.0	11/13/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

Shipment No. 4

AVS No.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
1	VV	2.1	0.7	150.7	Vero	>320.0	2.1	7/31/86
	VSV	1.3	0.4	77.1	L-929	32.0	0.4	6/24/86
	Ad	1.4	0.5	57.4	HEp-2	100.0	1.7	9/25/86
	PT	3.2	1.1	18.0	Vero	>100.0	>5.6	10/27/86
	SF	4.5	1.5	5.2	Vero	>100.0	>19.2	10/29/86
52	VV	0.4	0.1	--	Vero	100.0	--	7/31/86
	VSV	0.4	0.1	--	L-929	32.0	--	6/24/86
	Ad	0.2	0.07	--	HEp-2	1.0	--	9/25/86
	PT	0.6	0.2	262.0	Vero	100.0	0.4	10/29/86
103	VV	0	0	--	Vero	>320.0	--	7/31/86
	VSV	0	0	--	L-929	100.0	--	6/24/86
	Ad	0	0	--	HEp-2	>320.0	--	9/25/86
	PT	0	0	--	Vero	>320.0	--	10/29/86
105	VV	0	0	--	Vero	320.0	--	7/31/86
	VSV	0.1	0.03	--	L-929	100.0	--	6/24/86
	Ad	0	0	--	HEp-2	3.2	--	9/25/86
	PT	0.8	0.3	66.0	Vero	32.0	0.5	10/29/86
122	VV	2.1	0.7	10.0	Vero	32.0	3.2	7/31/86
	VSV	1.2	0.4	75.1	L-929	3.2	0.1	6/24/86
	Ad	1.7	0.6	4.2	HEp-2	3.2	0.8	9/25/86
	PT	0.6	0.2	182.0	Vero	100.0	0.5	10/27/86
	SF	0	0	--	Vero	32.0	--	11/07/86
132	VV	0	0	--	Vero	320.0	--	7/31/86
	VSV	0	0	--	L-929	32.0	--	6/27/86
	Ad	0	0	--	HEp-2	100.0	--	9/25/86
	PT	0.4	0.1	--	Vero	>100.0	--	10/27/86
136	VV	0.2	0.07	--	Vero	320.0	--	7/31/86
	VSV	0.2	0.07	--	L-929	320.0	--	6/27/86
	Ad	1.3	0.43	96.9	HEp-2	320.0	3.3	9/25/86
	PT	1.5	0.5	73.0	Vero	>320.0	>4.4	10/27/86
	SF	0	0	--	Vero	>320.0	--	10/29/86
140	PT	0.3	0.1	1.0	Vero	1.0	1.0	10/27/86
	SF	0.5	0.2	0.08	Vero	1.0	12.5	10/29/86
142	VV	0	0	--	Vero	>320.0	--	7/31/86
	VSV	0	0	--	L-929	320.0	--	6/27/86
	Ad	0	0	--	HEp-2	320.0	--	9/25/86
	PT	0	0	--	Vero	>320.0	--	10/28/86
	SF	0.2	0.07	--	Vero	>320.0	--	10/29/86
147	VV	0.3	0.1	--	Vero	10.0	--	7/31/86
	VSV	0	0	--	L-929	10.0	--	6/27/86
	Ad	0	0	--	HEp-2	32.0	--	9/25/86
	PT	0	0	--	Vero	100.0	--	10/28/86
	SF	1.0	0.3	≥100.0	Vero	100.0	≤1.0	10/29/86
195	VV	0	0	--	Vero	100.0	--	7/31/86
	VSV	0	0	--	L-929	100.0	--	6/27/86

	Ad	1.4	0.5	217.0	HEp-2	>320.0	>1.5	9/25/86
	PT	0.5	0.2	--	Vero	>100.0	--	10/28/86
	SF	0	0	--	Vero	>100.0	--	10/29/86
202	VV	0.5	0.2	306.5	Vero	320.0	1.0	7/31/86
	VSV	0	0	--	L-929	100.0	--	6/27/86
	Ad	0	0	--	HEp-2	320.0	--	9/25/86
	PT	1.9	0.6	141.0	Vero	320.0	2.3	10/28/86
	SF	2.0	0.7	--	Vero	320.0	--	10/30/86
206	PT	2.7	0.9	67.0	Vero	>320.0	>4.8	10/28/86
	SF	3.7	1.2	22.6	Vero	320.0	14.2	10/29/86
332	PT	0.6	0.2	--	Vero	32.0	--	10/29/86
593	VV	0	0	--	Vero	320.0	--	7/31/86
	VSV	0	0	--	L-929	320.0	--	6/27/86
	Ad	0	0	--	HEp-2	>320.0	--	9/25/86
	PT	0	0	--	Vero	320.0	--	10/29/86
	SF	0.9	0.3	--	Vero	>320.0	--	10/30/86
2039	PT	0	0	--	Vero	32.0	--	10/29/86
	SF	1.1	0.4	--	Vero	32.0	--	10/30/86
2040	PT	0.5	0.2	298.0	Vero	32.0	0.1	10/29/86
	SF	1.6	0.5	--	Vero	320.0	--	10/30/86
2041	PT	0	0	--	Vero	32.0	--	10/29/86
	SF	1.1	0.4	--	Vero	32.0	--	10/30/86
2042	PT	0.2	0.07	--	Vero	3.2	--	10/29/86
	SF	0.6	0.2	--	Vero	1.0	--	10/29/86
Ara-A	VV	2.4	0.8	10.0	Vero	32.0	3.2	7/31/86
C-3-deaza-Ado	VSV	1.7	0.6	25.0	L-929	10.0	0.4	6/27/86
Selenazole	VV	2.6	0.9	24.0	Vero	32.0	1.3	7/31/86
	Ad	1.7	0.6	9.7	HEp-2	32.0	3.3	9/25/86
Ribavirin	PT	2.3	0.8	19.0	Vero	>100.0	>5.3	10/27/86
	PT	3.8	1.3	8.2	Vero	>100.0	>12.2	10/28/86
	PT	2.5	0.8	17.5	Vero	100.0	5.7	10/28/86
	PT	2.7	0.9	10.0	Vero	>100.0	>10.0	10/29/86
	PT	2.4	0.8	14.7	Vero	>100.0	>6.8	10/29/86
	SF	2.6	0.9	25.9	Vero	>100.0	>3.9	10/29/86
	SF	2.8	0.9	14.9	Vero	>100.0	>6.7	10/30/86
	SF	3.3	1.1	17.5	Vero	>100.0	>5.7	10/30/86
	SF	3.0	1.0	20.8	Vero	>100.0	>4.8	11/07/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

Shipment No. 5

AVS No.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
197	VV	0	0	--	Vero	›320.0	--	8/7/86
	VSV	0	0	--	L-929	100.0	--	8/1/86
	Ad	0	0	--	HEp-2	›320.0	--	9/25/86
	PT	1.7	0.6	73.0	Vero	›320.0	›4.4	10/30/86
	SF	3.4	1.1	49.7	Vero	›320.0	›6.4	10/29/86
206	VV	1.2	0.4	85.3	Vero	100.0	1.2	8/7/86
	VSV	0.6	0.2	295.0	L-929	100.0	0.3	7/18/86
	Ad	0.8	0.3	238.8	HEp-2	320.0	1.3	9/25/86
	PT	1.5	0.5	73.0	Vero	320.0	4.4	10/30/86
222	VV	0	0	--	Vero	100.0	--	8/7/86
	VSV	0	0	--	L-929	100.0	--	7/18/86
	Ad	0	0	--	HEp-2	100.0	--	9/25/86
	PT	0	0	--	Vero	›32.0	--	10/30/86
	SF	0	0	--	Vero	10.0	--	11/05/86
245	VV	0.3	0.1	--	Vero	320.0	--	8/7/86
	VSV	0	0	--	L-929	100.0	--	7/18/86
	Ad	0	0	--	HEp-2	320.0	--	9/25/86
	PT	0.3	0.1	--	Vero	›320.0	--	10/30/86
	SF	1.4	0.5	--	Vero	›100.0	--	10/29/86
257	VV	1.4	0.5	174.1	Vero	320.0	1.8	8/7/86
	VSV	1.6	0.5	60.9	L-929	3.2	0.1	8/1/86
	Ad	0.7	0.2	259.9	HEp-2	320.0	1.2	9/25/86
	PT	0.6	0.2	229.0	Vero	320.0	1.4	10/30/86
	SF	1.2	0.4	150.7	Vero	100.0	0.7	10/30/86
286	VV	≥1.9	0.6	1.0	Vero	‹0.32	0.3	8/7/86
	VV	1.5	0.5	--	Vero	0.1	--	10/16/86
	VSV	2.6	0.9	1.0	L-929	‹0.32	0.3	7/18/86
	Ad	0	0	--	HEp-2	0.03	--	9/25/86
	PT	0	0	--	Vero	0.32	--	11/13/86
	SF	0	0	-	Vero	0.1	--	10/30/86
332	VV	0.2	0.07	--	Vero	10.0	--	8/7/86
	VSV	0.3	0.1	--	L-929	10.0	--	7/18/86
	Ad	0.4	0.1	--	HEp-2	3.2	--	9/25/86
	PT	0	0	--	Vero	10.0	--	11/13/86
	SF	0.9	0.3	63.6	Vero	3.2	0.1	10/30/86
345	VSV	0	0	--	L-929	10.0	--	7/18/86
	Ad	0	0	--	HEp-2	10.0	--	9/25/86
	PT	0.3	0.1	--	Vero	0.32	--	11/13/86
	SF	1.0	0.3	88.0	Vero	100.0	1.1	10/30/86
701	VV	0	0	--	Vero	3.2	--	8/7/86
	VSV	0	0	--	L-929	32.0	--	7/18/86
	Ad	0	0	--	HEp-2	10.0	--	9/25/86
	PT	0	0	--	Vero	1.0	--	11/13/86
	SF	1.2	0.4	55.6	Vero	32.0	0.6	10/30/86
703	VV	0	0	--	Vero	10.0	--	8/7/86

	VSV	0	0	--	L-929	32.0	--	7/18/86
	Ad	0.2	0.07	--	HEp-2	100.0	--	9/25/86
	PT	0	0	--	Vero	32.0	--	11/13/86
	SF	1.6	0.5	52.7	Vero	32.0	0.6	11/03/86
866	VV	0	0	--	Vero	100.0	--	10/16/86
	VSV	0	0	--	L-929	10.0	--	7/18/86
	Ad	0	0	--	HEp-2	100.0	--	10/16/86
999	VV	0	0	--	Vero	320.0	--	8/7/86
	VSV	0	0	--	L-929	100.0	--	8/1/86
	Ad	0	0	--	HEp-2	100.0	--	9/25/86
	PT	0	0	--	Vero	320.0	--	11/14/86
	SF	0	0	--	Vero	3.2	--	11/03/86
1089	VV	1.7	0.6	81.4	Vero	320.0	3.9	8/7/86
	VSV	2.2	0.7	7.8	L-929	1.0	0.1	8/1/86
	Ad	1.0	0.3	3.2	HEp-2	10.0	3.1	9/25/86
	PT	3.4	1.1	0.9	Vero	3.2	3.6	11/14/86
	SF	5.2	1.7	1.0	Vero	>100.0	>100.0	11/03/86
1160	VV	≥5.6	1.9	0.6	Vero	100.0	166.7	8/7/86
	VSV	3.2	1.1	<0.32	L-929	1.0	3.1	7/18/86
	VSV	2.6	0.9	1.0	L-929	3.2	3.2	8/15/86
	Ad	0.7	0.2	3.2	HEp-2	3.2	1.0	9/25/86
	PT	1.0	0.3	51.4	Vero	0.7	0.01	11/14/86
	SF	1.0	0.3	70.4	Vero	100.0	1.4	11/03/86
1192	SF	0.3	0.1	--	Vero	0.01	--	11/03/86
1250	VV	0.8	0.3	21.0	Vero	10.0	0.5	8/7/86
	VSV	0	0	--	L-929	3.2	--	7/18/86
	Ad	≥2.0	0.7	1.3	HEp-2	3.2	2.5	9/25/86
	Ad	0	0	--	HEp-2	10.0	--	10/16/86
2135	VV	0	0	--	Vero	>320.0	--	8/7/86
	VSV	0	0	--	L-929	320.0	--	7/18/86
	Ad	0.3	0.1	--	HEp-2	>320.0	--	9/25/86
	PT	0.6	0.2	--	Vero	>320.0	--	11/14/86
	SF	0	0	--	Vero	>320.0	--	11/04/86
2136	VV	1.7	0.6	144.2	Vero	>320.0	>2.2	8/7/86
	VSV	0	0	--	L-929	10.0	--	7/18/86
	Ad	0	0	--	HEp-2	>320.0	--	9/25/86
	PT	0.3	0.1	--	Vero	32.0	--	11/07/86
	SF	1.4	0.5	88.0	Vero	320.0	3.6	11/04/86
2137	VV	0	0	--	Vero	100.0	--	8/7/86
	VSV	0	0	--	L-929	100.0	--	7/18/86
	Ad	0	0	--	HEp-2	32.0	--	9/25/86
	PT	1.2	0.4	313.4	Vero	100.0	0.3	11/07/86
	SF	0	0	--	Vero	320.0	--	11/04/86
2138	VV	0	0	--	Vero	100.0	--	8/7/86
	VSV	0	0	--	L-929	32.0	--	7/18/86
	Ad	0	0	--	HEp-2	320.0	--	9/25/86
	PT	1.4	0.5	236.3	Vero	>320.0	>1.4	11/07/86
	SF	0.9	0.3	91.8	Vero	320.0	3.5	11/04/86
2139	VV	0	0	--	Vero	100.0	--	8/7/86

	VSV	0	0	--	L-929	100.0	--	7/18/86
	Ad	0	0	--	HEp-2	32.0	--	9/25/86
	PT	0.8	0.3	--	Vero	)320.0	--	11/07/86
	SF	0.7	0.2	262.5	Vero	32.0	0.1	11/04/86
2140	VV	0	0	--	Vero	)320.0	--	8/7/86
	VSV	0	0	--	L-929	)320.0	--	7/18/86
	Ad	0	0	--	HEp-2	320.0	--	9/25/86
	PT	0.6	0.2	--	Vero	)320.0	--	11/07/86
Ara-A	VV	2.6	0.9	17.0	Vero	100.0	5.9	8/7/86
	VV	3.2	1.1	2.7	Vero	32.0	11.9	10/16/86
C-3-deaza-Ado	VSV	3.0	1.0	3.1	L-929	10.0	3.2	7/18/86
Selenazole	VV	2.2	0.7	18.8	Vero	10.0	0.5	10/16/86
	Ad	1.7	0.6	9.7	HEp-2	32.0	3.3	9/25/86
	Ad	2.0	0.7	8.1	HEp-2	32.0	4.0	10/16/86
Ribavirin	Ad	1.7	0.6	32.0	HEp-2	100.0	3.1	10/16/86
	PT	1.5	0.5	47.0	Vero	100.0	2.1	10/30/86
	PT	2.5	0.8	13.8	Vero	)100.0	)7.2	10/30/86
	PT	2.4	0.8	33.9	Vero	100.0	2.9	11/07/86
	PT	2.4	0.8	9.2	Vero	)100.0	)10.9	11/07/86
	PT	2.4	0.8	18.8	Vero	100.0	5.3	11/13/86
	PT	1.5	0.5	47.3	Vero	100.0	2.1	11/14/86
	SF	4.5	1.5	5.2	Vero	)100.0	)19.2	10/29/86
	SF	2.6	0.9	25.9	Vero	)100.0	3.9	10/29/86
	SF	2.8	0.9	14.9	Vero	)100.0	)6.7	10/30/86
	SF	3.3	1.1	17.5	Vero	)100.0	)5.7	10/30/86
	SF	2.3	0.8	20.0	Vero	100.0	5.0	11/03/86
	SF	2.6	0.9	8.9	Vero	)100.0	)11.2	11/04/86
	SF	3.0	1.0	9.8	Vero	)100.0	)10.2	11/04/86
	SF	2.9	1.0	14.4	Vero	)100.0	)6.9	11/05/86
	SF	2.5	0.8	24.1	Vero	)100.0	)30.1	11/05/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

Shipment No. 6

AVS NO.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
2087	VSV	0	0	--	L-929	100.0	--	8/1/86
	VV	0	0	--	Vero	>320.0	--	8/20/86
	Ad	0	0	--	HEp-2	320.0	--	10/2/86
2088	VSV	0	0	--	L-929	10.0	--	8/1/86
	VV	0	0	--	Vero	32.0	--	8/20/86
	Ad	0	0	--	HEp-2	32.0	--	10/2/86
2089	VSV	0	0	--	L-929	10.0	--	8/1/86
	VV	0.1	0.03	--	Vero	10.0	--	8/20/86
	Ad	0	0	--	HEp-2	1.0	--	10/2/86
2090	VSV	0	0	--	L-929	100.0	--	8/1/86
	VV	0.5	0.17	320.0	Vero	100.0	0.3	8/20/86
	Ad	0	0	--	HEp-2	320.0	--	10/2/86
2091	VSV	0	0	--	L-929	>320.0	--	8/1/86
	VV	0	0	--	Vero	>320.0	--	8/20/86
	Ad	0	0	--	HEp-2	>320.0	--	10/2/86
2092	VSV	0	0	--	L-929	32.0	--	8/8/86
	VV	0	0	--	Vero	320.0	--	8/20/86
	Ad	0.7	0.23	320.0	HEp-2	>320.0	>1.0	10/2/86
2093	VSV	0	0	--	L-929	320.0	--	8/8/86
	VV	0	0	--	Vero	320.0	--	8/20/86
	Ad	0	0	--	HEp-2	320.0	--	10/2/86
2094	VSV	0	0	--	L-929	>320.0	--	8/8/86
	VV	0	0	--	Vero	>100.0	--	8/20/86
	Ad	0.1	0.03	--	HEp2	100.0	--	10/2/86
2095	VSV	0	0	--	L-929	>320.0	--	8/8/86
	VV	0	0	--	Vero	320.0	--	8/20/86
	Ad	0.2	0.07	--	HEp-2	>320.0	--	10/2/86
2096	VSV	0	0	--	L-929	10.0	--	8/8/86
	VV	0.5	0.17	9.5	Vero	10.0	1.1	8/20/86
	Ad	0	0	--	HEp-2	32.0	--	10/2/86
2097	VSV	0	0	--	L-929	320.0	--	8/8/86
	VV	0	0	--	Vero	320.0	--	8/20/86
	Ad	0	0	--	HEp-2	>320.0	--	10/2/86
2098	VSV	0	0	--	L-929	320.0	--	8/8/86
	VV	0	0	--	Vero	320.0	--	8/20/86
	Ad	0.5	0.17	--	HEp-2	>320.0	--	10/2/86
2099	VSV	0	0	--	L-929	100.0	--	8/8/86
	VV	0	0	--	Vero	100.0	--	8/20/86
	Ad	0	0	--	HEp-2	100.0	--	10/2/86
2100	VSV	0	0	--	L-929	32.0	--	8/8/86
	VV	0.4	0.13	320.0	Vero	320.0	1.0	8/20/86

	Ad	0	0	--	HEp-2	320.0	--	10/2/86
2101	VSV	0	0	--	L-929	100.0	--	8/8/86
	VV	0	0	--	Vero	320.0	--	8/20/86
	Ad	0.3	0.10	--	HEp-2	320.0	--	10/2/86
2102	VSV	0	0	--	L-929	10.0	--	8/8/86
	VV	0.8	0.27	32.0	Vero	10.0	0.3	8/20/86
	Ad	0	0	--	HEp-2	320.0	--	10/2/86
2103	VSV	0	0	--	L-929	32.0	--	8/8/86
	VV	0	0	--	Vero	3.2	--	8/20/86
	Ad	0	0	--	HEp-2	>32.0	--	10/2/86
	Ad	0	0	--	HEp-2	320.0	--	10/16/86
2104	VSV	0	0	--	L-929	3.2	--	8/8/86
	VV	0.2	0.07	--	Vero	1.0	--	8/20/86
	Ad	0	0	--	HEp-2	<0.3	--	10/2/86
	Ad	0	0	--	HEp-2	0.032	--	10/16/86
2105	VSV	0	0	--	L-929	10.0	--	8/8/86
	VV	0	0	--	Vero	320.0	--	8/20/86
	Ad	0	0	--	HEp-2	>320.0	--	10/2/86
2106	VSV	0	0	--	L-929	100.0	--	8/8/86
	VV	0	0	--	Vero	>320.0	--	8/20/86
	Ad	0	0	--	HEp-2	>320.0	--	10/2/86
2107	VSV	0	0	--	L-929	320.0	--	8/8/86
	VV	0	0	--	Vero	>320.0	--	8/20/86
	Ad	0.3	0.10	--	HEp-2	>320.0	--	10/2/86
2108	VSV	0	0	--	L-929	100.0	--	8/8/86
	VV	0	0	--	Vero	100.0	--	8/20/86
	Ad	0	0	--	HEp-2	10.0	--	10/2/86
2109	VSV	0	0	--	L-929	100.0	--	8/8/86
	VV	0.4	0.13	320.0	Vero	100.0	0.3	8/20/86
	Ad	0.3	0.10	--	HEp-2	320.0	--	10/2/86
2110	VSV	0	0	--	L-929	100.0	--	8/8/86
	VV	0	0	--	Vero	>320.0	--	8/20/86
	Ad	0	0	--	HEp-2	100.0	--	10/2/86
2111	VSV	0	0	--	L-929	32.0	--	8/8/86
	VV	0	0	--	Vero	10.0	--	8/20/86
	Ad	0	0	--	HEp-2	10.0	--	10/2/86
2112	VSV	0	0	--	L-929	100.0	--	8/12/86
	VV	0	0	--	Vero	>320.0	--	8/20/86
	Ad	0	0	--	HEp-2	100.0	--	10/2/86
C-3-deaza-Ado	VSV	2.3	0.77	6.8	L-929	10.0	1.5	8/1/86
	VSV	2.1	0.70	8.2	L-929	10.0	1.2	8/8/86
Ara-A	VV	2.9	0.97	8.1	Vero	100.0	12.3	8/20/86
Selenazole	VV	2.5	0.83	13.8	Vero	10.0	0.7	8/20/86
	Ad	1.5	0.50	9.6	HEp-2	32.0	3.3	10/2/86

	Ad	2.0	0.67	8.1	HEp-2	32.0	4.0	10/16/86
Ribavirin	Ad	1.7	0.57	30.0	HEp-2	100.0	3.3	10/2/86
	Ad	1.7	0.57	32.0	HEp-2	100.0	3.1	10/16/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

Shipment No. 7

AVS No.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
2113	VSV	0	0	--	L-929	320.0	--	8/12/86
	VV	0	0	--	Vero	320.0	--	8/28/86
	Ad	0.3	0.1	--	HEp-2	>320.0	--	10/9/86
2114	VSV	0	0	--	L-929	32.0	--	8/12/86
	VV	0	0	--	Vero	32.0	--	8/28/86
	Ad	0	0	--	HEp-2	10.0	--	10/9/86
2115	VSV	0	0	--	L-929	32.0	--	8/12/86
	VV	0	0	--	Vero	≥320.0	--	8/28/86
	Ad	0	0	--	HEp-2	320.0	--	10/9/86
2116	VSV	0	0	--	L-929	32.0	--	8/12/86
	VV	0	0	--	Vero	320.0	--	9/28/86
	Ad	0.3	0.1	--	HEp-2	32.0	--	10/9/86
2117	VSV	0	0	--	L-929	320.0	--	8/12/86
	VV	0	0	--	Vero	>320.0	--	8/28/86
	Ad	0.6	0.2	--	HEp-2	>320.0	--	10/9/86
2118	VSV	0	0	--	L-929	100.0	--	8/12/86
	VV	0.2	0.07	--	Vero	100.0	--	8/28/86
	Ad	0	0	--	HEp-2	320.0	--	10/9/86
2119	VSV	0	0	--	L-929	>320.0	--	8/12/86
	VV	0	0	--	Vero	320.0	--	8/28/86
	Ad	0	0	--	HEp-2	>320.0	--	10/9/86
2120	VSV	0	0	--	L-929	10.0	--	8/12/86
	VV	0	0	--	Vero	100.0	--	8/28/86
	Ad	0	0	--	HEp-2	32.0	--	10/9/86
2121	VSV	0	0	--	L-929	100.0	--	8/12/86
	VV	0	0	--	Vero	100.0	--	8/28/86
	Ad	0.2	0.07	--	HEp-2	100.0	--	10/9/86
2122	VSV	0	0	--	L-929	32.0	--	8/12/86
	VV	0.4	0.1	320.0	Vero	100.0	0.3	8/28/86
	Ad	0	0	--	HEp-2	320.0	--	10/9/86
2123	VSV	0	0	--	L-929	320.0	--	8/12/86
	VV	0.3	0.1	--	Vero	320.0	--	8/28/86
	Ad	0.3	0.1	--	HEp-2	>320.0	--	10/9/86
2124	VSV	0	0	--	L-929	32.0	--	8/12/86
	VV	0	0	--	Vero	320.0	--	8/28/86
	Ad	0	0	--	HEp-2	>320.0	--	10/9/86
2125	VSV	0	0	--	L-929	10.0	--	8/12/86
	VV	0	0	--	Vero	100.0	--	8/28/86
	Ad	0	0	--	HEp-2	10.0	--	10/9/86
2126	VSV	0	0	--	L-929	32.0	--	8/12/86
	VV	0	0	--	Vero	>320.0	--	8/28/86

	Ad	0	0	--	HEp-2	320.0	--	10/9/86
2127	VSV	1.0	0.3	23.4	L-929	100.0	4.3	8/15/86
	VV	0.4	0.1	100.0	Vero	100.0	1.0	8/28/86
	Ad	0.2	0.07	--	HEp-2	32.0	--	10/9/86
2128	VSV	0.4	0.1	31.0	L-929	32.0	1.0	8/15/86
	VV	0.6	0.2	--	Vero	10.0	--	8/28/86
	Ad	0.8	0.3	10.0	HEp-2	32.0	3.2	10/9/86
2129	VSV	0	0	--	L-929	320.0	--	8/15/86
	VV	0	0	--	Vero	>320.0	--	8/28/86
	Ad	0	0	--	HEp-2	100.0	--	10/9/86
2130	VSV	0	0	--	L-929	>320.0	--	8/15/86
	VV	0	0	--	Vero	>320.0	--	8/28/86
	Ad	0	0	--	HEp-2	>320.0	--	10/9/86
2131	VSV	0	0	--	L-929	>320.0	--	8/15/86
	VV	0	0	--	Vero	>320.0	--	8/28/86
	Ad	0.3	0.1	--	HEp-2	>320.0	--	10/9/86
2132	VSV	0	0	--	L-929	320.0	--	8/15/86
	VV	0.5	0.2	100.0	Vero	100.0	1.0	8/28/86
	Ad	0	0	--	HEp-2	320.0	--	10/9/86
2133	VSV	0	0	--	L-929	100.0	--	8/15/86
	VV	0	0	--	Vero	>320.0	--	8/28/86
	VV	0.1	0.03	--	Vero	100.0	--	10/23/86
	Ad	0	0	--	HEp-2	100.0	--	10/23/86
2134	VSV	0	0	--	L-929	320.0	--	8/15/86
	VV	0	0	--	Vero	>320.0	--	8/28/86
	Ad	0	0	--	HEp-2	>320.0	--	10/9/86
2147	VSV	0	0	--	L-929	100.0	--	8/15/86
	VV	0	0	--	Vero	>320.0	--	8/28/86
	Ad	0	0	--	HEp-2	320.0	--	10/9/86
2148	VSV	0	0	--	L-929	100.0	--	8/15/86
	VV	0	0	--	Vero	100.0	--	8/28/86
	Ad	0	0	--	HEp-2	32.0	--	10/9/86
2150	VSV	0	0	--	L-929	100.0	--	8/15/86
	VV	0.3	0.1	--	Vero	100.0	--	8/28/86
	Ad	0	0	--	HEp-2	100.0	--	10/9/86
2152	VSV	0	0	--	L-929	100.0	--	8/15/86
	VV	0	0	--	Vero	100.0	--	8/28/86
	Ad	0	0	--	HEp-2	>320.0	--	10/9/86
C-3-deaza-Ado	VSV	2.1	0.7	3.1	L-929	10.0	3.2	8/12/86
	VSV	3.3	1.1	3.1	L-929	32.0	10.3	8/15/86
Ara-A	VV	2.1	0.7	9.1	Vero	32.0	3.5	8/28/86
	VV	2.5	0.8	5.7	Vero	10.0	1.7	10/23/86
Selenazole	VV	2.7	0.9	6.3	Vero	10.0	1.6	8/28/86
	VV	2.5	0.8	7.5	Vero	10.0	1.3	10/23/86

	Ad	1.4	0.5	9.6	HEp-2	32.0	3.3	10/9/86
	Ad	1.3	0.4	9.7	HEp-2	32.0	3.3	10/23/86
Ribavirin	Ad	1.9	0.6	30.7	HEp-2	100.0	3.3	10/9/86
	Ad	1.9	0.6	30.7	HEp-2	100.0	3.2	10/23/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

SHIPMENT NO. 8

AVS NO.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
2145	VSV	0	0	--	L929	>320.0	--	8/29/86
	VV	0	0	--	Vero	>320.0	--	10/16/86
	Ad	0	0	--	HEp-2	>320.0	--	10/16/86
2146	VSV	0	0	--	L929	>320.0	--	8/29/86
	VV	0	0	--	Vero	>320.0	--	10/16/86
	Ad	0	0	--	HEp-2	>320.0	--	10/16/86
2163	VSV	0	0	--	L929	32.0	--	8/29/86
	VV	0.2	0.07	--	Vero	>320.0	--	10/23/86
	Ad	0	0	--	HEp-2	>320.0	--	10/23/86
2164	VSV	0	0	--	L929	32.0	--	8/29/86
	VV	0	0	--	Vero	>320.0	--	10/23/86
	Ad	0	0	-	HEp-2	1.0	--	10/23/86
2165	VSV	0	0	--	L929	100.0	--	8/29/86
	VV	0	0	--	Vero	100.0	--	10/16/86
	Ad	0	0	--	HEp-2	320.0	--	10/16/86
2166	VSV	0	0	--	L929	1.0	--	8/29/86
	VV	0.5	0.2	--	Vero	10.0	--	10/16/86
	Ad	0	0	--	HEp-2	10.0	--	10/16/86
2167	VSV	0	0	--	L929	32.0	--	8/29/86
	VV	0.6	0.2	87.7	Vero	100.0	1.1	10/23/86
	Ad	0.3	0.1	--	HEp-2	320.0	--	10/23/86
2168	VSV	0	0	--	L929	100.0	--	8/29/86
	VV	0	0	--	Vero	100.0	--	10/23/86
	Ad	0	0	--	HEp-2	100.0	--	10/23/86
2169	VSV	0	0	--	L929	100.0	--	9/5/86
	VV	0.4	0.1	--	Vero	>320.0	--	10/16/86
	Ad	0	0	--	HEp-2	>320.0	--	10/16/86
2170	VSV	0	0	--	L929	3.2	--	9/5/86
	VV	4.7	1.6	1.3	Vero	10.0	7.7	10/16/86
	Ad	0.5	0.2	--	HEp-2	32.0	--	10/16/86
2171	VSV	0	0	--	L929	320.0	--	9/5/86
	VV	0	0	--	Vero	>320.0	--	10/16/86
	Ad	0	0	--	HEp-2	>320.0	--	10/16/86
2172	VSV	0	0	--	L929	10.0	--	9/5/86
	VV	0	0	--	Vero	32.0	--	10/23/86
	Ad	0	0	-	HEp-2	32.0	--	10/23/86
2173	VSV	0	0	--	L929	32.0	--	9/5/86
	VV	0.5	0.2	100	Vero	32.0	0.3	10/23/86
	Ad	1.1	0.4	27.3	HEp-2	100.0	3.6	10/23/86
2174	VSV	0	0	--	L929	100.0	--	9/5/86
	VV	0.6	0.2	100	Vero	100.0	1.0	10/16/86

	Ad	0	0	--	HEp-2	320.0	--	10/16/86
2175	VSV	0	0	--	L929	32.0	--	9/5/86
	VV	0.2	0.07	--	Vero	100.0	--	10/23/86
	Ad	0.1	0.03	--	HEp-2	32.0	--	10/23/86
2176	VSV	0	0	--	L929	320.0	--	9/5/86
	VV	0.2	0.07	--	Vero	100.0	--	10/16/86
	Ad	0	0	--	HEp-2	320.0	--	10/16/86
2177	VSV	0	0	--	L929	320.0	--	9/5/86
	VV	0	0	--	Vero	>320.0	--	10/16/86
	Ad	0.5	0.2	--	HEp-2	>320.0	--	10/16/86
2178	VSV	0	0	--	L929	10.0	--	9/5/86
	VV	0.6	0.2	93.9	Vero	32.0	0.3	10/23/86
	Ad	0	0	--	HEp-2	100.0	--	10/23/86
2179	VSV	0	0	--	L929	32.0	--	9/5/86
	VV	0.3	0.1	--	Vero	>320.0	--	10/16/86
	Ad	0.3	0.1	--	HEp-2	>320.0	--	10/16/86
2180	VSV	0	0	--	L929	1.0	--	9/5/86
	VV	0.1	0.03	--	Vero	10.0	--	10/16/86
	Ad	0	0	--	HEp-2	0.32	--	10/16/86
2181	VSV	0	0	--	L929	≤0.32	--	9/5/86
	VV	0	0	--	Vero	100.0	--	10/23/86
	Ad	0	0	--	HEp-2	100.0	--	10/23/86
2182	VSV	0	0	--	L929	100.0	--	9/5/86
	VV	0	0	--	Vero	>320.0	--	10/23/86
	Ad	0.2	0.07	--	HEp-2	>320.0	--	10/23/86
2184	VSV	0	0	--	L929	100.0	--	9/5/86
	VV	0.9	0.3	210	Vero	320.0	1.5	10/16/86
	Ad	0.3	0.1	--	HEp-2	320.0	--	10/16/86
2185	VSV	0	0	--	L929	32.0	--	9/5/86
	VV	0	0	--	Vero	320.0	--	10/16/86
	Ad	0.5	0.2	--	HEp-2	0.32	--	11/06/86
2186	VSV	0	0	--	L929	>320.0	--	9/5/86
	VV	0	0	--	Vero	>320.0	--	10/23/86
	Ad	0.3	0.1	--	HEp-2	>320.0	--	10/23/86
2187	VSV	0	0	--	L929	10.0	--	9/5/86
	VV	0.4	0.1	--	Vero	10.0	--	10/16/86
	Ad	0.4	0.1	--	HEp-2	10.0	--	10/16/86
C-3-Deaza-Ado	VSV	1.8	0.6	8.6	L929	3.2	0.4	8/29/86
	VSV	2.0	0.7	3.2	L929	3.2	1.0	9/5/86
Ribavirin	Ad	1.7	0.6	32	HEp-2	100.0	3.1	10/16/86
	Ad	1.9	0.7	30.7	HEp-2	100.0	3.2	10/23/86
	Ad	1.9	0.7	19.7	HEp-2	100.0	5.1	11/06/86
Selenazole	VV	2.5	0.8	7.5	Vero	10.0	1.3	10/23/86

	Ad	2.0	0.7	8.1	HEp-2	32.0	4.0	10/16/86
	Ad	1.3	0.4	9.7	HEp-2	32.0	3.3	10/23/86
	Ad	1.6	0.5	7.2	HEp-2	32.0	4.4	11/06/86
Ara-A	VV	2.5	0.8	5.7	Vero	10.0	1.7	10/23/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797 - 801, 1971).

Shipment No. 9

AVS NO.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
890	PT	0.5	0.2	--	Vero	>320.0	--	11/07/86
1052	PT	0.2	0.1	--	Vero	320.0	--	11/07/86
2024								
2025								
2026	PT	1.8	0.6	17.3	Vero	100.0	5.8	11/07/86
2027	PT	0.4	0.1	--	Vero	32.0	--	11/10/86
2028	PT	1.2	0.4	--	Vero	100.0	--	11/10/86
2029	PT	0.3	0.1	--	Vero	3.2	--	11/10/86
2030	PT	1.0	0.3	--	Vero	>100.0	--	11/10/86
2031	PT	0.1	0	--	Vero	0.32	--	11/10/86
2032	PT	0	0	--	Vero	1.0	--	11/05/86
2033	PT	0.3	0.1	--	Vero	1.0	--	11/05/86
2034	PT	2.1	0.7	6.8	Vero	10.0	1.5	11/05/86
2035	PT	0.2	0.1	--	Vero	0.1	--	11/05/86
2036	PT	0.4	0.1	--	Vero	10.0	--	11/05/86
2037	PT	0.3	0.1	--	Vero	1.0	--	11/12/86
2038	PT	0.6	0.2	--	Vero	10.0	--	11/12/86
2159	VV	0	0	--	Vero	>320.0	--	10/23/86
	Ad	0.5	0.2	--	HEp-2	>320.0	--	10/23/86
	VSV	0.4	0.1	--	L929	100.0	--	9/19/86
	PT	0.4	0.1	--	Vero	10.0	--	11/12/86
	SF	3.8	1.3	<0.32	Vero	>320.0	>1000.0	10/31/86
2160	VV	0.2	0.07	--	Vero	32.0	--	10/23/86
	Ad	0.3	0.1	--	HEp-2	32.0	--	10/23/86
	VSV	0	0	--	L929	100.0	--	9/19/86
	PT	0.5	0.2	--	Vero	10.0	--	11/12/86
	SF	3.1	1.0	<0.32	Vero	32.0	>100.0	10/31/86
2161	VV	1.2	0.4	47.3	Vero	32.0	0.7	10/23/86
	Ad	0.4	0.1	--	HEp-2	100.0	--	10/23/86
	VSV	0	0	--	L929	32.0	--	9/19/86
	PT	0.4	0.1	--	Vero	>100.0	--	11/12/86
	SF	1.8	0.6	--	Vero	100.0	--	10/31/86
2162	VV	0.6	0.2	100.0	Vero	320.0	3.2	10/23/86
	Ad	0	0	--	HEp-2	320.0	--	10/23/86
	VSV	0	0	--	L929	100.0	--	9/19/86
	PT	1.3	0.4	--	Vero	>100.0	--	11/12/86

	SF	2.6	0.9	2.1	Vero	320.0	--	10/31/86
2188	VV	0.4	0.1	--	Vero	3.2	--	10/23/86
	Ad	0	0	--	HEp-2	3.2	--	10/23/86
	VSV	0.5	0.2	--	L929	100.0	--	9/19/86
	PT	0.2	0.1	--	Vero	10.0	--	11/12/86
	SF	2.6	0.9	<0.32	Vero	32.0	100.0	10/31/86
2189	VV	0	0	--	Vero	320.0	--	10/23/86
	Ad	0	0	--	HEp-2	3.2	--	10/23/86
	VSV	0	0	--	L929	32.0	--	9/19/86
	PT	0.2	0.1	--	Vero	>100.0	--	11/12/86
	SF	0.4	0.1	--	Vero	100.0	--	11/04/86
2190	VV	0.1	0.03	--	Vero	100.0	--	10/23/86
	Ad	0	0	--	HEp-2	32.0	--	10/23/86
	VSV	0	0	--	L929	100.0	--	9/19/86
	PT	0.3	0.1	--	Vero	32.0	--	11/12/86
	SF	0.2	0.1	--	Vero	320.0	--	11/04/86
2191	VV	0.5	0.2	--	Vero	10.0	--	10/23/86
	Ad	0	0	--	HEp-2	1.0	--	10/23/86
	VSV	0	0	--	L929	3.2	--	9/19/86
	PT	0.4	0.1	--	Vero	32.0	--	11/12/86
	SF	0.4	0.1	--	Vero	32.0	--	11/04/86
2192	VV	0	0	--	Vero	100.0	--	10/30/86
	Ad	0	0	--	HEp-2	100.0	--	10/30/86
	VSV	0	0	--	L929	10.0	--	9/19/86
	SF	0	0	--	Vero	100.0	--	11/04/86
2193	VV	0.2	0.07	--	Vero	100.0	--	10/30/86
	Ad	0	0	--	HEp-2	32.0	--	10/30/86
	VSV	0	0	--	L929	3.2	--	9/19/86
	SF	0	0	--	Vero	32.0	--	11/04/86
2194	VV	0	0	--	Vero	>320.0	--	10/23/86
	Ad	0	0	--	HEp-2	>320.0	--	10/23/86
	VSV	0	0	--	L929	>320.0	--	9/19/86
2195	VV	0	0	--	Vero	320.0	--	10/30/86
	Ad	0	0	--	HEp-2	320.0	--	10/30/86
	VSV	0	0	--	L929	32.0	--	9/19/86
2196	VV	0	0	--	Vero	320.0	--	10/30/86
	Ad	0	0	--	HEp-2	32.0	--	10/30/86
	VSV	0	0	--	L929	320.0	--	9/19/86
2197	VV	0	0	--	Vero	>320.0	--	10/30/86
	Ad	0	0	--	HEp-2	32.0	--	10/30/86
	VSV	0	0	--	L929	100.0	--	9/19/86
2198	VV	0.2	0.07	--	Vero	320.0	--	10/30/86
	Ad	0.4	0.1	--	HEp-2	320.0	--	10/30/86
	VSV	0	0	--	L929	32.0	--	9/19/86
2199	VV	0.4	0.1	--	Vero	100.0	--	10/30/86
	Ad	0	0	--	HEp-2	320.0	--	10/30/86
	VSV	0	0	--	L929	320.0	--	9/19/86

2200	VV	0	0	--	Vero	>320.0	--	10/30/86
	Ad	0	0	--	HEp-2	>320.0	--	10/30/86
	VSV	0	0	--	L929	320.0	--	9/19/86
2201	VV	0	0	--	Vero	>320.0	--	10/30/86
	Ad	0.3	0.1	--	HEp-2	320.0	--	10/30/86
	VSV	0	0	--	L929	10.0	--	9/19/86
2202	VV	0.1	0.03	--	Vero	320.0	--	10/30/86
	Ad	0	0	--	HEp-2	320.0	--	10/30/86
	VSV	0	0	--	L929	320.0	--	9/19/86
2203	VV	0	0	--	Vero	100.0	--	10/30/86
	Ad	0	0	--	HEp-2	320.0	--	10/30/86
	VSV	0	0	--	L929	100.0	--	9/19/86
	SF	0.3	0.1	--	Vero	32.0	--	11/05/86
2204	VV	0	0	--	Vero	100.0	--	10/30/86
	Ad	0.2	0.07	--	Vero	100.0	--	10/30/86
	VSV	0	0	--	L929	320.0	--	9/19/86
	SF	0.3	0.1	--	Vero	320.0	--	11/05/86
2205	VV	0	0	--	Vero	320.0	--	10/30/86
	Ad	0	0	--	HEp-2	320.0	--	10/30/86
	VSV	0	0	--	L929	32.0	--	9/19/86
	SF	0	0	--	Vero	320.0	--	11/05/86
2206	VV	0	0	--	Vero	320.0	--	10/30/86
	Ad	0	0	--	HEp-2	1.0	--	10/30/86
	VSV	0	0	--	L929	32.0	--	9/19/86
	SF	0	0	--	Vero	32.0	--	11/05/86
2208	VV	0.3	0.1	--	Vero	100.0	--	10/30/86
	Ad	0	0	--	HEp-2	100.0	--	10/30/86
	VSV	0	0	--	L929	100.0	--	9/19/86
	SF	0	0	--	Vero	100.0	--	11/05/86
Ara-A	VV	2.5	0.8	5.7	Vero	10.0	1.7	10/23/86
	VV	2.4	0.8	3.2	Vero	10.0	3.1	10/30/86
Ribavirin	Ad	1.9	0.6	30.7	HEp-2	100.0	3.2	10/23/86
	Ad	1.2	0.4	26.2	HEp-2	32.0	1.2	10/30/86
	PT	2.4	0.8	33.9	Vero	100.0	2.9	11/07/86
	PT	2.4	0.8	9.2	Vero	>100.0	>10.9	11/07/86
	PT	2.6	0.7	18.5	Vero	>100.0	>5.4	11/05/86
	PT	2.8	0.9	32.9	Vero	>100.0	>3.0	11/10/86
	PT	2.7	0.9	22.2	Vero	>100.0	>4.5	11/12/86
	PT	1.8	0.6	23.9	Vero	>100.0	>4.2	11/12/86
	SF	3.4	1.1	3.2	Vero	>100.0	>31.3	10/31/86
	SF	2.6	0.9	8.9	Vero	>100.0	>11.2	11/04/86
	SF	3.0	1.0	9.8	Vero	>100.0	>10.2	11/04/86
	SF	2.9	1.0	14.4	Vero	>100.0	>6.9	11/05/86
	SF	2.5	0.8	24.1	Vero	>100.0	>30.1	11/05/86
Selenazole	VV	2.5	0.8	7.5	Vero	10.0	1.3	10/23/86
	VV	2.2	0.7	17.4	Vero	10.0	0.6	10/30/86
	Ad	1.3	0.4	9.7	HEp-2	32.0	3.3	10/23/86
	Ad	1.4	0.5	50.7	HEp-2	32.0	0.6	10/30/86

C-3-Deaza-Ado	VSV	2.0	0.7	6.9	L929	3.2	0.5	9/19/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

SUMMARY OF ANTIVIRAL TEST RESULTS--CONTRACT DAMD17-86-C-6013 (SoRI)

SHIPMENT NO. 9

AVS NO.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
1	FeLV	2.3	0.8	3.9	81C	3.2	0.8	11/07/86
70	FeLV	1.3	0.4	0.7	81C	<0.1	0.1	11/07/86
79	FeLV	1.5	0.5	0.47	81C	1.0	2.1	11/07/86
111	FeLV	1.6	0.5	5.4	81C	3.2	0.6	11/07/86
122	FeLV	2.5	0.8	1.0	81C	0.32	0.3	11/07/86
136	FeLV	1.2	0.4	--	81C	32.0	--	11/14/86
148	FeLV	0	0	--	81C	<0.32	--	11/14/86
200	FeLV	1.3	0.4	27.5	81C	32.0	1.2	11/14/86
202	FeLV	1.5	0.5	--	81C	32.0	--	11/14/86
206	FeLV	1.9	0.6	15.8	81C	32.0	2.0	11/14/86
215	FeLV	2.4	0.8	11.1	81C	32.0	2.9	11/14/86
222	FeLV	1.5	0.5	67.7	81C	32.0	0.5	11/14/86
245	FeLV	2.1	0.7	80.8	81C	100.0	1.2	11/14/86
345	FeLV	1.3	0.4	17.8	81C	32.0	1.8	11/14/86
646	FeLV	1.0	0.3	2.58	81C	3.2	1.2	11/14/86
999	FeLV	2.7	0.9	14.8	81C	100.0	6.8	11/14/86
Azidothymidine	FeLV	3.7	1.2	5.8	81C	>78.4	>13.5	8/15/86
Dideoxycytidine	FeLV	3.0	1.0	2.6	81C	7.3	2.8	9/17/86
	FeLV	2.0	0.7	0.76	81C	10.0	13.2	10/10/86
	FeLV	4.0	1.3	0.4	81C	10.0	25.0	11/07/86
	FeLV	3.2	1.1	0.98	81C	10.0	10.2	11/14/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801. 1971).

Updated 12/05/86:ADB

SHIPMENT NO. 10

AVS NO.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
2209	SF	0.1	0.03	--	Vero	≤0.32	--	11/06/86
	VV	0	0	--	Vero	320.0	--	10/30/86
	Ad	0	0	--	HEp-2	100.0	--	10/30/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2210	SF	1.0	0.3	--	Vero	32.0	--	11/06/86
	VV	0	0	--	Vero	100.0	--	10/30/86
	Ad	0	0	--	HEp-2	0.1	--	11/13/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2211	VSV	0	0	--	L929	0.32	--	10/03/86
2212	VV	0	0	--	Vero	10.0	--	11/06/86
	Ad	0	0	--	HEp-2	10.0	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2213	VV	0.2	0.07	--	Vero	100.0	--	11/06/86
	Ad	0.8	0.3	--	HEp-2	≥320.0	--	11/06/86
	VSV	0	0	--	L929	100.0	--	10/03/86
2214	SF	1.4	0.5	--	Vero	10.0	--	11/10/86
	VV	0.2	0.07	--	Vero	10.0	--	11/06/86
	Ad	0.2	0.07	--	HEp-2	32.0	--	11/06/86
	VSV	0	0	--	L929	10.0	--	10/03/86
2215	SF	1.3	0.4	--	Vero	320.0	--	11/10/86
	VV	0	0	--	Vero	≥320.0	--	11/06/86
	Ad	0	0	--	HEp-2	≥320.0	--	11/06/86
	VSV	0	0	--	L929	320.0	--	10/03/86
2216	SF	1.2	0.4	173.0	Vero	100.0	0.6	11/10/86
	VV	0.4	0.1	--	Vero	320.0	--	11/06/86
	Ad	0	0	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	100.0	--	10/03/86
2217	SF	1.3	0.4	1.9	Vero	3.2	1.7	11/12/86
	VV	0.4	0.1	--	Vero	32.0	--	11/06/86
	Ad	0	0	--	HEp-2	32.0	--	11/06/86
	VSV	0	0	--	L929	10.0	--	10/03/86
2218	SF	1.0	0.3	7.2	Vero	3.2	0.4	11/12/86
	VV	0.9	0.3	21.1	Vero	32.0	1.5	11/06/86
	Ad	0.2	0.07	--	HEp-2	10.0	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2219	SF	2.5	0.8	2.5	Vero	32.0	12.8	11/12/86
	VV	0.8	0.3	78.4	Vero	100.0	1.3	11/06/86
	Ad	0.2	0.07	--	HEp-2	32.0	--	11/06/86
	VSV	0	0	--	L929	100.0	--	10/03/86
2220	SF	3.2	1.1	<0.32	Vero	32.0	≥100.0	11/12/86
	VV	0.2	0.07	--	Vero	100.0	--	11/06/86
	Ad	0	0	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	100.0	--	10/03/86

2221	SF	1.2	0.4	1.8	Vero	3.2	1.8	11/12/86
	VV	0.6	0.2	29.8	Vero	32.0	1.1	11/06/86
	Ad	0	0	--	HEp-2	10.0	--	11/06/86
	VSV	0	0	--	L929	10.0	--	10/03/86
2222	SF	0.6	0.2	10.4	Vero	10.0	1.0	11/13/86
	VV	1.0	0.3	96.9	Vero	100.0	1.0	11/06/86
	Ad	0	0	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2223	SF	1.1	0.4	79.8	Vero	100.0	1.3	11/13/86
	VV	0	0	--	Vero	100.0	--	11/06/86
	Ad	0	0	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	100.0	--	10/03/86
2224	SF	0.1	0.03	--	Vero	32.0	--	11/13/86
	VV	0	0	--	Vero	32.0	--	11/06/86
	Ad	0	0	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	1.0	--	10/03/86
2225	SF	1.0	0.3	--	Vero	3.2	--	11/13/86
	VV	0.6	0.2	10.0	Vero	10.0	1.0	11/06/86
	Ad	0	0	--	HEP-2	3.2	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2226	SF	1.4	0.5	8.8	Vero	32.0	3.6	11/13/86
	VV	0.3	0.1	--	Vero	32.0	--	11/06/86
	Ad	0.2	0.07	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2227	SF	0	0	--	Vero	10.0	--	11/13/86
	VV	0.2	0.07	--	Vero	3.2	--	11/06/86
	Ad	0	0	--	HEp-2	10.0	--	11/06/86
	VSV	0	0	--	L929	10.0	--	10/03/86
2228	SF	1.3	0.4	17.4	Vero	10.0	0.6	11/13/86
	VV	0.3	0.1	32.0	Vero	10.0	0.3	11/06/86
	Ad	0	0	--	HEp-2	3.2	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2229	SF	0	0	--	Vero	100.0	--	11/13/86
	VV	0.2	0.07	--	Vero	32.0	--	11/06/86
	Ad	1.0	0.3	28.0	HEp-2	100.0	3.6	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2230	SF	0	0	--	Vero	320.0	--	11/13/86
	VV	0	0	--	Vero	320.0	--	11/06/86
	Ad	0	0	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	100.0	--	10/03/86
2231	SF	0.7	0.2	--	Vero	32.0	--	11/13/86
	VV	0.9	0.3	210.0	Vero	320.0	1.5	11/06/86
	Ad	0.3	0.1	--	HEp-2	320.0	--	11/06/86
	VSV	0	0	--	L929	100.0	--	10/03/86
2232	VV	0.5	0.2	--	Vero	320.0	--	11/06/86
	Ad	0.3	0.1	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86

2233	VV	0.3	0.1	--	Vero	320.0	--	11/06/86
	Ad	0	0	--	HEp-2	100.0	--	11/06/86
	VSV	0	0	--	L929	100.0	--	10/03/86
2234	VV	0	0	--	Vero	32.0	--	11/06/86
	Ad	0.5	0.2	--	HEp-2	32.0	--	11/06/86
	VSV	0	0	--	L929	10.0	--	10/03/86
2235	VV	1.4	0.5	37.3	Vero	32.0	0.9	11/06/86
	Ad	0	0	--	HEp-2	10.0	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
2236	VV	0	0	--	Vero	>320.0	--	11/06/86
	Ad	0	0	--	HEp-2	>320.0	--	11/06/86
	VSV	0	0	--	L929	32.0	--	10/03/86
C-3-deaza-Ado	VSV	2.8	0.9	3.1	L929	32.0	10.3	10/03/86
Ara-A	VV	2.4	0.8	3.2	Vero	10.0	3.1	10/30/86
	VV	3.3	1.1	2.7	Vero	32.0	11.9	11/06/86
Ribavirin	SF	3.1	1.0	14.4	Vero	>100.0	>6.9	11/06/86
	SF	4.4	1.5	2.3	Vero	>100.0	>43.5	11/10/86
	SF	3.4	1.1	14.1	Vero	>100.0	>7.1	11/12/86
	SF	4.0	1.3	0.92	Vero	>100.0	>109.0	11/12/86
	SF	4.0	1.3	6.1	Vero	>100.0	>16.4	11/13/86
	SF	4.4	1.5	2.9	Vero	>100.0	>34.5	11/13/86
	SF	3.0	1.0	10.0	Vero	>100.0	>10.0	11/13/86
	Ad	1.2	0.4	26.2	HEp-2	32.0	1.2	10/30/86
	Ad	1.9	0.6	19.7	HEp-2	100.0	5.1	11/06/86
	Ad	1.9	0.6	25.5	HEp-2	100.0	3.9	11/13/86
Selenazole	VV	2.2	0.7	17.4	Vero	10.0	0.6	10/30/86
	VV	2.7	0.9	7.6	Vero	10.0	1.3	11/06/86
	Ad	1.4	0.5	50.7	HEp-2	32.0	0.8	10/30/86
	Ad	1.6	0.5	7.2	HEp-2	32.0	4.4	11/06/86
	Ad	0.9	0.3	32.0	HEp-2	10.0	0.3	11/13/86

VR* = Virus Rating calculated according to the method of Sidwell and Huffman (Appl. Microbiol. 22: 797-801, 1971).

SHIPMENT NO. 11

AVS NO.	VIR	VR	VR*	ID50	CELL	MTC	TI	DATE
2276	VV	0.3	0.1	--	Vero	320.0	--	11/13/86
	Ad	0.2	0.07	--	HEp-2	320.0	--	11/13/86
	VSV	0	0	--	L929	100.0	--	10/24/86
2277	VV	0.9	0.3	217.0	Vero	320.0	1.5	11/13/86
	Ad	1.1	0.4	100.0	HEp-2	320.0	3.2	11/13/86
	VSV	0	0	--	L929	320.0	--	10/24/86
2278	VV	0.4	0.1	--	Vero	>320.0	--	11/13/86
	Ad	0.1	0.03	--	HEp-2	320.0	--	11/13/86
	VSV	0	0	--	L929	320.0	--	10/24/86
2279	VV	0.2	0.07	--	Vero	32.0	--	11/13/86
	Ad	0.7	0.2	10.0	HEp-2	32.0	3.2	11/13/86
	VSV	0	0	--	L929	32.0	--	10/24/86
2280	VV	0	0	--	Vero	>320.0	--	11/13/86
	Ad	0.6	0.2	--	HEP-2	>320.0	--	11/13/86
	VSV	0	0	--	L929	320.0	--	10/24/86
2281	VV	0	0	--	Vero	>320.0	--	11/13/86
	Ad	0.6	0.2	--	HEp-2	>320.0	--	11/13/86
	VSV	0	0	--	L929	320.0	--	10/24/86
2282	VV	0.2	0.07	--	Vero	320.0	--	11/13/86
	Ad	0.2	0.07	--	HEp-2	320.0	--	11/13/86
	VSV	0.5	0.2	307.0	L929	320.0	1.0	10/24/86
2283	VV	0	0	--	Vero	320.0	--	11/13/86
	Ad	0.6	0.2	--	HEp-2	320.0	--	11/13/86
	VSV	0	0	--	L929	320.0	--	10/24/86
2284	VV	0.2	0.07	--	Vero	>320.0	--	11/13/86
	Ad	0	0	--	HEp-2	320.0	--	11/13/86
	VSV	0	0	--	L929	320.0	--	10/24/86
2285	VV	0.5	0.2	--	Vero	>320.0	--	11/13/86
	Ad	0.6	0.2	--	HEp-2	>320.0	--	11/13/86
	VSV	0	0	--	L929	100.0	--	10/24/86
2286	VV	0	0	--	Vero	320.0	--	11/13/86
	Ad	0	0	--	HEp-2	100.0	--	11/13/86
	VSV	0	0	--	L929	320.0	--	10/24/86
2287	VV	0.1	0.03	--	Vero	320.0	--	11/13/86
	Ad	0	0	--	HEp-2	100.0	--	11/13/86
	VSV	0	0	--	L929	32.0	--	10/24/86
2288	VV	0	0	--	Vero	320.0	--	11/13/86
	Ad	0.4	0.1	--	HEp-2	100.0	--	11/13/86
	VSV	0	0	--	L929	100.0	--	10/24/86

2289	VV	0.7	0.2	79.8	Vero	100.0	1.2	11/13/86
	Ad	0.8	0.3	262.0	HEp-2	320.0	1.2	11/13/86
	VSV	0	0	--	L929	320.0	--	10/24/86
2290	VV	3.2	1.1	5.7	Vero	32.0	5.6	11/13/86
	Ad	1.0	0.3	25.4	HEp-2	32.0	1.3	11/13/86
	VSV	0	0	--	L929	100.0	--	10/24/86
2291	VV	1.9	0.6	4.7	Vero	10.0	2.1	11/13/86
	Ad	0	0	--	HEp-2	10.0	--	11/13/86
	VSV	0	0	--	L929	10.0	--	10/24/86
2292	VV	0	0	--	Vero	10.0	--	11/13/86
	Ad	0	0	--	HEp-2	32.0	--	11/13/86
	VSV	0	0	--	L929	32.0	--	10/24/86
2293	VV	0	0	--	Vero	>320.0	--	11/13/86
	Ad	0	0	--	HEp-2	>320.0	--	11/13/86
	VSV	0	0	--	L929	100.0	--	11/04/86
2295	VV	0	0	--	Vero	3.2	--	11/13/86
	Ad	0.3	0.1	--	HEp-2	10.0	--	11/13/86
	VSV	0	0	--	L929	10.0	--	11/04/86
2296	VV	(On Retest)						
	Ad	(On Retest)						
	VSV	0	0	--	L929	10.0	--	11/04/86
2297	VV	(On Retest)						
	Ad	(On Retest)						
	VSV	0	0	--	L929	100.0	--	11/04/86
2298	VV	0.3	0.1	--	Vero	1.0	--	11/13/86
	Ad	0	0	--	HEp-2	1.0	--	11/13/86
	VSV	0	0	--	L929	1.0	--	11/04/86
2299	VV	0.2	0.07	--	Vero	32.0	--	11/13/86
	Ad	0.1	0.03	--	HEp-2	10.0	--	11/13/86
	VSV	0	0	--	L929	3.2	--	11/04/86
2301	VSV	0	0	--	L929	100.0	--	11/04/86
2302	VSV	0	0	--	L929	3.2	--	11/04/86
2303	VSV	0	0	--	L929	320.0	--	11/04/86
2304	VSV	0	0	--	L929	320.0	--	11/04/86
2305	VSV	0	0	--	L929	320.0	--	11/04/86
2307	VSV	0	0	--	L929	32.0	--	11/04/86
2308	VSV	0	0	--	L929	1.0	--	11/04/86
2309	VSV	0	0	--	L929	3.2	--	11/04/86
2310	VSV	0	0	--	L929	10.0	--	11/04/86
C-3-Deaza-Ado	VSV	2.5	0.8	3.2	L929	10.0	3.1	10/24/86

	VSV	2.5	0.8	3.2	L929	10.0	3.1	11/04/86
Ara-A	VV	3.3	1.1	2.7	Vero	32.0	11.8	11/13/86
Selen-azole	VV	2.8	0.9	5.9	Vero	3.2	0.5	11/13/86
	Ad	0.9	0.3	32.0	HEp-2	10.0	0.3	11/13/86
Ribavirin	Ad	1.9	0.6	25.5	HEp-2	100.0	3.9	11/13/86

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Updated 12/12/86:ADB

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