THE TELEMETRIC AUTOMATED MICROBIAL IDENTIFICATION SYSTEM (TAMIS) AND SUBSYSTEMS

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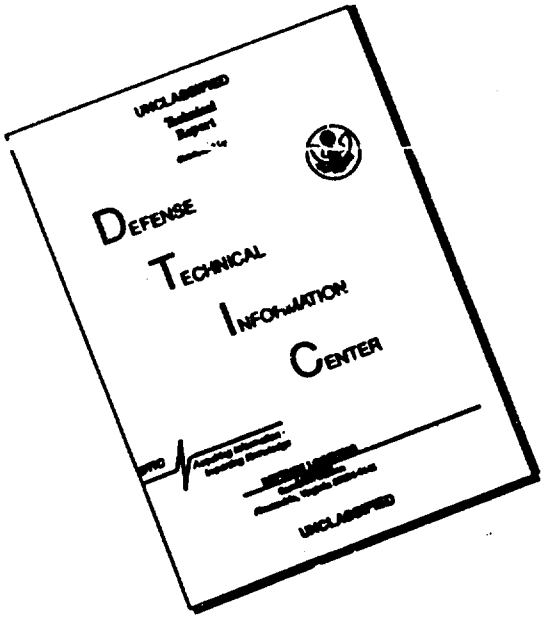

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FOREWORD

The research detailed in this review evolved from instrumented approaches to the quantitation of a certain type of immunochemical analysis initiated at the USAF School of Aviation Medicine in 1955. Actual telemetry started in 1961 and interhemispheric transmission of analyses of human serum was accomplished in 1962. The purposes of the study required the integration of immunology and electronics in a manner not previously demonstrated in science. Currently, the completion of this research provides the USAF and the Department of Defense with an interdisciplinary tool.

This comprehensive report brings the potential, feasibility, and demonstrated capabilities of the telemetry of microbiologic analyses into clear focus for mission-oriented aerospace and terrestrial applications.

In an undertaking of the magnitude of TAMIS, faith, vision, encouragement, and administrative support were essential to success. For these contributions the author is grateful to Lt. Colonel Irving Davis, Lt. Colonel Laurence A. Irvine, and Dr. E. Staten Wynne, of the Biosciences Branch, USAF School of Aerospace Medicine.

During the development of the system, the interdisciplinary approach required the knowledge and skills of several people in electronics, instrumentation, and immunology. Specific contributions were made by Wesley E. Prather, Electronics Engineer, and Heinz A. Jaeger, Instrument Designer. Support in microbiology was capably given by Dr. Warren J. Russell, James R. Ralston, Mrs. Iowa W. Marable, and Sergeant Michael De Buysere.

The author is also indebted to Colonel Nicholas H. Cox and his staff at the Air Force Armament Laboratory, Elgin Air Force Base, Fla., for their support of the project during the later phases of the study.

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THE TELEMETRIC AUTOMATED MICROBIAL IDENTIFICATION SYSTEM (TAMIS) AND SUBSYSTEMS

INTRODUCTION

TAMIS is a research technic utilizing proved immunochemical and electronic methods. These methods were accomplished by system and subsystem components combined into a complete complex of instruments and designed and integrated for microbiologic analyses. TAMIS exemplifies concepts of analyses and data handling adaptable to current and future requirements of terrestrial, marine, and aerospace exploration.

The application and versatility of the immunoelectronic instruments and procedures to be described in this review have been explained in earlier reports (3, 6-20). The existence and functions of the computer, several associated subsystem components, the complete semiautomatic method of processing microorganisms (19), preparation of reference reactions (20), the most recent results of the comparison between known and unknown bacterial extracts, and the field tests of a subsystem for aerosol identification have not been previously documented and are detailed in this report from both an electronic and a microbiologic viewpoint.

A subsystem module of TAMIS called BAIT (Bacterial Automated Identification Technic) specifically designed for aerosol sampling was field-tested in May 1967. The pertinent results of these tests serve as the basis in this report for criteria appropriate for evaluation of detection and identification systems.

BACKGROUND

A combination of known ideas and concepts in immunology and electronics was integrated and first presented by the author in September 1962 under the title "The Bio-Courier Project" (bio - life, courier - special messages) (13). This project proposed to free scientists from the tedium of field collecting, tiring travel, and the disadvantages of transporting certain biologic specimens. The approach was to make it possible for semiskilled assistants to

collect and process samples and to initiate analyses at field sites. The quantitative information from special instruments would be relayed by radio or telephone to the laboratory. One scientist could then monitor analyses from several remote sites and, in addition, relay, compare, and store instrumented assays being performed by colleagues over the entire world.

The purpose of the Bio-courier project, as applied to biologic analyses of the body fluids of astronauts in orbit and the biologic exploration of satellites and planets was expressed in 1963 as follows:

> 1. There is an immediate Air Force and civilian need for a suitable procedure whereby . . . scientific or non-scientific personnel on Earth who are located in remote laboratories or on field explorations can initiate meaningful "on the spot" analyses of biological solutions and extracts. These analyses will help to:
>
> a. Detect the causative agents of infection and changes in such agents (bacteria, viruses).
>
> b. Identify vectors and host of disease.
>
> c. Screen groups of people for immunity to disease (antibodies).
>
> d. Explore both endemic and exotic diseases.
>
> On this planet, Earth, we usually term such efforts preventive epidemiology, and the Bio-Courier can be applied to this aspect. (7)

TAMIS, the acronym coined for the immunoelectronic system used to demonstrate the feasibility of microbiologic analyses by telemetry on earth, has used this original purpose of the Bio-courier project as a guide.

It was soon obvious that the modified instruments (figs. 1 and 2) originally used to demonstrate the principle of immunobiologic analysis by telemetry (9, 13) were not adequate for field studies. Consequently, an intensive period of instrumentation development and evaluation started. This was interrupted during the five years by brief explorations in performing biologic exercises to anticipate problem areas and reorient direction.

As the instrumentation became more sophisticated (8, 10), demonstrations of both short- and long-range telemetry of biologic reactions were successfully completed. In 1962, the Military Affiliated Radio Service cooperated in achieving combinations of radio

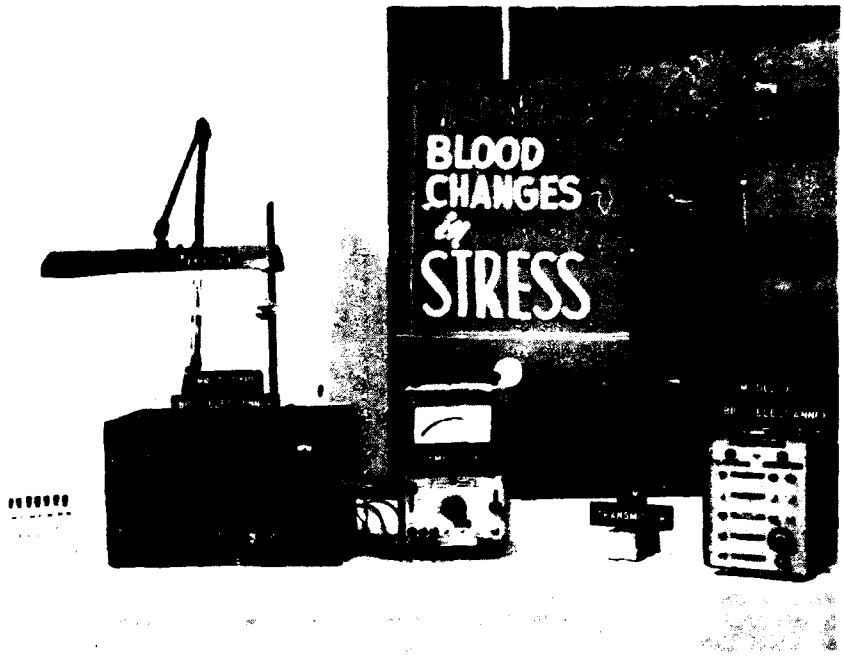


FIGURE 1

Working prototype instrumentation for microbiologic analyses by telemetry used in 1961 (for details see ref. 9).

and telephone links for the transmission of analyses of human serum over several distances up to 8,000 miles in both intra- and intercontinental experiments (fig. 3) (8). Several other interconnections between the laboratory and remote sites on earth were also explored during the same period (figs. 4 and 5) (17). In April 1963, one of the instrumentation modules of the future TAMIS was the basis for an exhibit at the annual meeting of the Aerospace Medical Association, Los Angeles, Calif. (fig. 6). At that time, five radio-telephone telemetry analyses were conducted from Randolph Air Force Base, Tex., to March Air Force Base, Calif., then by telephone directly into the exhibit booth of the hotel.

The biologic analyses used in the TAMIS evolved from the comparison of unknown with known reactions by immunochemical

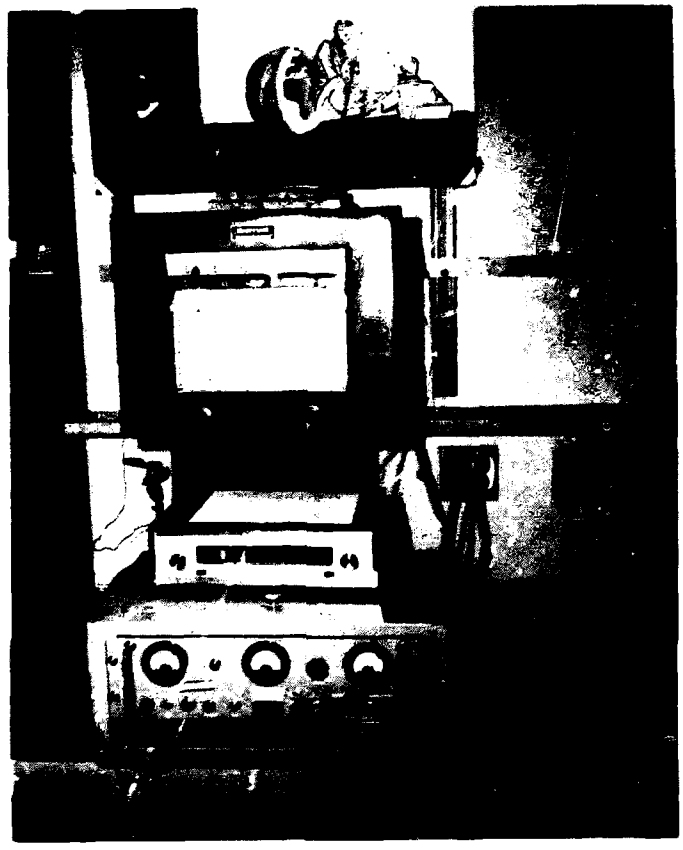


FIGURE 2

Prototype receiver-recorder combination for the Bio-courier project. Telemetered data are received via the "rabbit ear" antenna, relayed to the FM receiver, thence to the discriminator, and from there to the recorder. Note the contrast between this 1961 instrumentation, the 1963 version (fig. 6), and the complete 1966 console (fig. 8).

analyses in a manner not too dissimilar from that used since 1897. Biologic solutions will, under appropriate conditions, react together to form a precipitate. This antigen-antibody precipitin reaction will gather or stratify into zones rather than settle to the bottom when performed in a narrow tube or column containing a semisolid

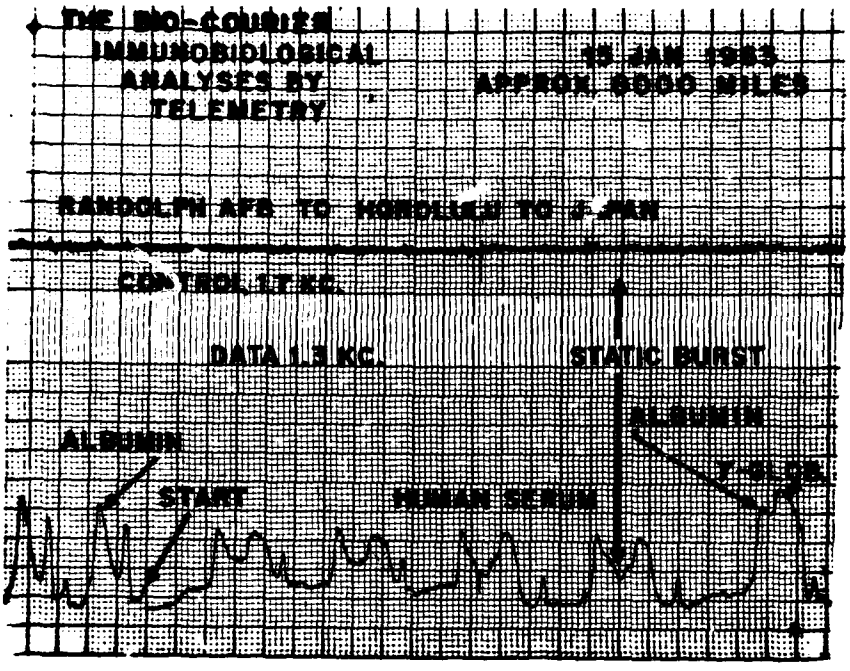


FIGURE 8

Graphic records of telemetered immunochemical analyses of human serum after transmission 8,000 miles in an interhemispheric experiment.

medium (agar gel). The zones have various shapes, densities, and pattern configurations (fig. 7). They reflect the ability of molecules to diffuse and combine with other molecules. From such intermingling in this type of precipitin reaction, the biologic relationships of unknown solutions—their concentrations, identification, and phylogenetic and ontogenetic backgrounds—can be determined. To draw such inferences correctly, the characteristics of both of the two reacting solutions (reference solutions) are determined beforehand. When unknown solutions are tested against either of these previously calibrated reactants, deductions concerning the relationship of the unknown materials can be made.

The physical configuration of the glass columns in which the precipitin reaction can be performed is very suitable for densitometry. This characteristic has helped the development of scanning

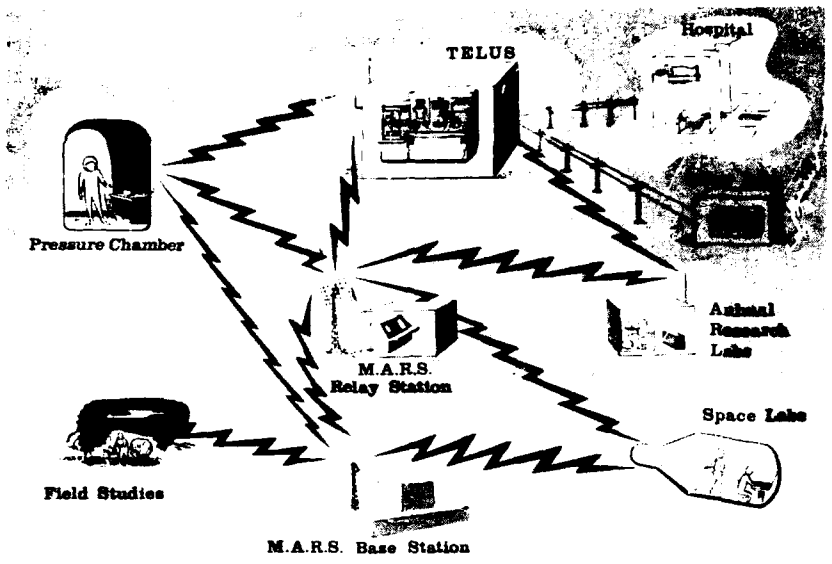


FIGURE 4

Applicable sites for field studies via communication links as envisioned in 1962.

instruments. The current scanning instruments that have been fabricated (10) for the various applications of the TAMIS emit an electrical signal that can be directly quantitated on a strip-chart recorder for on-the-site densitometry. As an alternative, the same signals can be transformed into audio or electromagnetic form for transmission by telephone or radio, respectively.

INSTRUMENTATION

The immunoelectronic system and subsystems that constitute the electronic components of TAMIS are sufficiently versatile for physiologic as well as microbiologic analyses. Mission requirements and anticipated future utilizations of the analytic data determine what instruments and interconnections are ultimately used in the task.

TELUS

The Telemetric Universal Sensor (TELUS) (fig. 8) (14-16) is a laboratory console consisting of three modules together measuring 16 ft. long, 3 ft. deep, and 6 ft. high. This combination of instruments was designed to:

1. Receive telemetered signals directly or indirectly from air or ground by radio, telephone, or magnetic tape.

2. Initiate or relay data to space or ground stations.

3. Decode frequency modulated signals.

4. Place five separate binary codes (000-999) on both incoming and outgoing data and utilize these codes through an automatic searching system to retrieve information from magnetic tape.

5. Present channels of data for viewing on a dual channel oscilloscope and a 2-channel strip-chart recorder.

6. Upon telemetered signal, integrate analog data and convert this integration into a binary code, automatically printing these and other data on preselected recorder tracks.

7. Provide the capability of screening and retaping appropriate data.

8. Simultaneously receive 4 channels of the same or different information (either frequency modulated or direct voice). This information can be simultaneously recorded on any of 28 tracks on magnetic tape.

The TELUS main console has the following components interconnected for functional modes of operation:

1. Receiver.

2. Discriminators.

3. Amplifiers.

4. Dual channel oscilloscope.

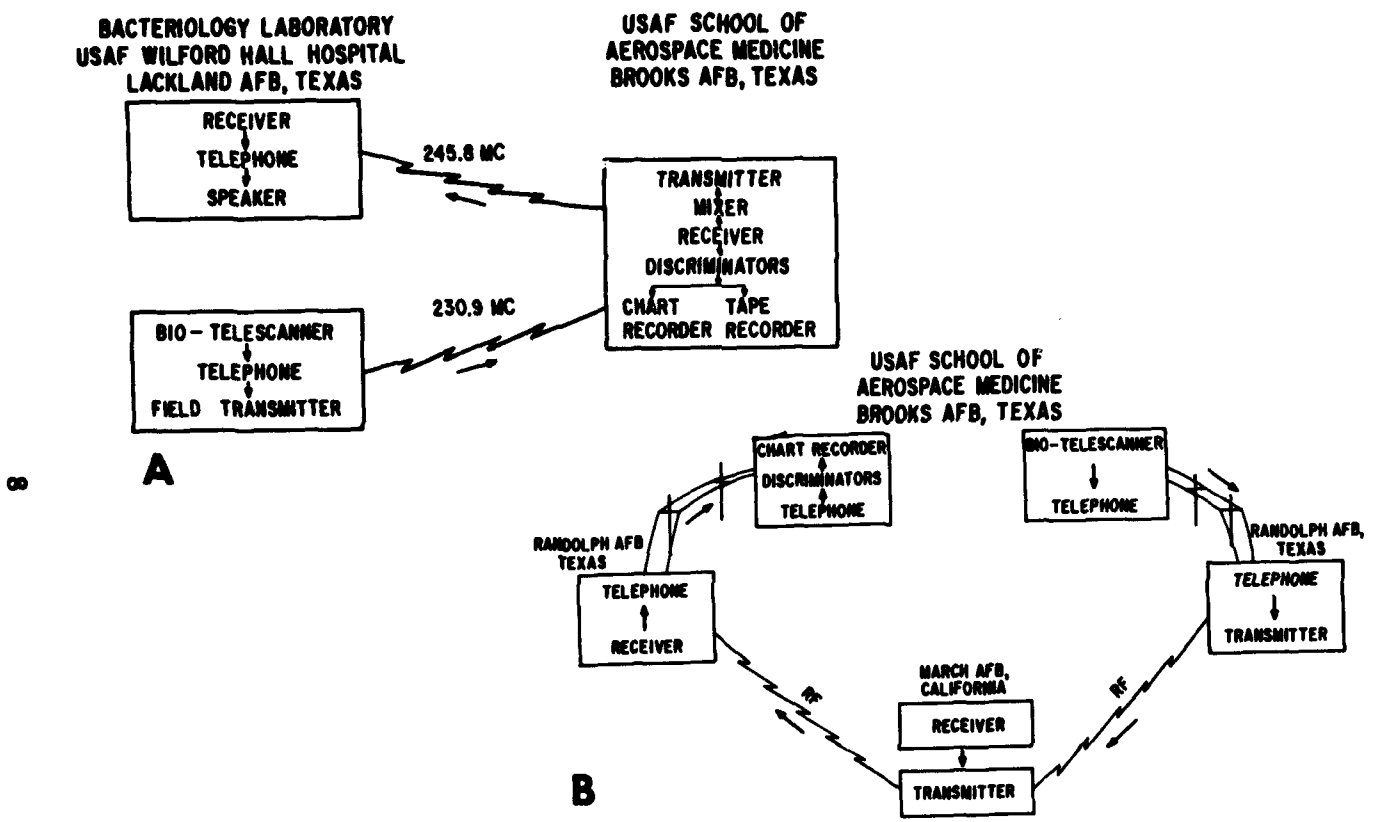
5. Voltage-frequency converter.

6. Counter.

7. Biologic identification coder, 48-bit.

8. Frequency counter.

9. Dual-channel strip-chart recorder.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USAF WILFORD HALL HOSPITAL
LACKLAND AFB, TEXAS
RECEIVER
TELEPHONE
SPEAKER
245.8 MC
USAF SCHOOL OF
AEROSPACE MEDICINE
BROOKS AFB, TEXAS
TRANSMITTER
MIXER
RECEIVER
DISCRIMINATORS
CHART RECORDER
TAPE RECORDER
230.9 MC
BIO-TELESCANNER
TELEPHONE
FIELD TRANSMITTER
A
USAF SCHOOL OF
AEROSPACE MEDICINE
BROOKS AFB, TEXAS
CHART RECORDER
DISCRIMINATORS
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BIO-TELESCANNER
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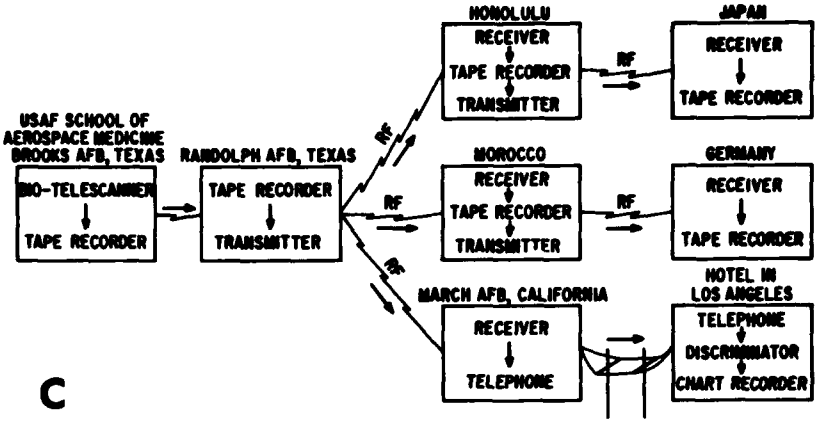

FIGURE 5

Radio and communication links tested in (A) local, (B) intercontinental, and (C) interhemispheric telemetry of microbiologic analyses in the TAMIS.

10. Interval timer.
11. Dual telephones.
12. Two headsets.
13. Pegboard and patch board interconnections.

The module on the right of figure 8 houses 4 magnetic tape coders and 4 magnetic tape searchers. On the left side of the main console are 2 tape recorder–reproducers. Switching and viewing any combination of the 28 record and 28 reproduce tracks into the 14 channels of the main console is made possible by 2 multiplex rotary switching units located below the recorders.

TAMIS subsystem components

TAMIS subsystem components specifically designed for portability and transporting to field sites are as follows:

1. Biotelescanner, model X 66-A (fig. 9).
2. Biotelescanner, model X 65-D (fig. 10).
3. Biotransceiver, model X 63-BR (fig. 10).

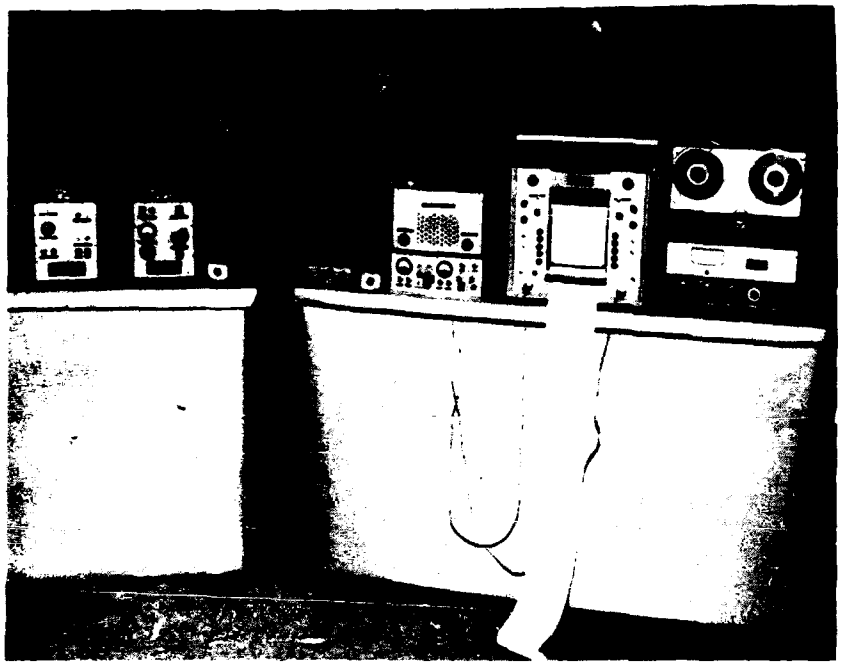

FIGURE 6

Instrumentation used in 1968 for demonstrating telemetry of biologic reactions. The Biotelescanner and Biotransceiver on the left represent field locations. On the right are receiving instruments and a strip-chart recorder. The magnetic tape recorder was used to program the scanner to initiate the scan cycle, magazine-turn mechanism, etc. This was another step in the evolution of the complete TAMIS instrumentation. The reader can contrast the receiving section shown here with that shown in figure 8 taken in 1966.

4. Interconnect for model 65-A (fig. 11).
5. Field transmitter model 65-A (fig. 11).
6. Microfil, model X 66-A (fig. 9).
7. Sonicator, model S-75 (fig. 12).
8. Filtration reservoir (fig. 12).
9. Receiver-discriminator combination, model 70-258 (fig. 11).
10. One-channel strip chart recorder, 6 in. (fig. 12).

FIGURE 7

Orientation of biologic reactants in single diffusion columns (for details see ref. 14).

11. **Instrumentation recorder-reproducer, 4-channel, portable (fig. 11).**

Supporting subsystem components are:

1. **Battery pack, model 44-257.**
2. **Recharger, model 1031.**
3. **Industrial abrasive unit, model F (fig. 16).**
4. **Direct current power supply, model TW 4005.**
5. **Liquid nitrogen storage tank, model LNR – 185 (fig. 15).**

The electronic subsystem instruments, in general, derive power from nickel-cadmium batteries that can be recharged as required from a 110 v. electrical source. Some instruments, such as field transmitter and sonicator, require direct access to a 110 v., 60 cps circuit.

While the type of instruments that are used to initiate field analyses depends upon the objectives of the task, certain sequential connections are common to most requirements. One such system is shown in figure 12.

Two Biotelescanners (model X 66-A) (fig. 9) and (model X 65-D) (fig. 10) are listed under subsystem components. Model X 66-A is a single-purpose instrument whose output is usually directly connected to a strip-chart recorder with or without an interconnection to a battery-powered recorder-reproducer. In essence, it is a densitometer with a 30-position magazine that is

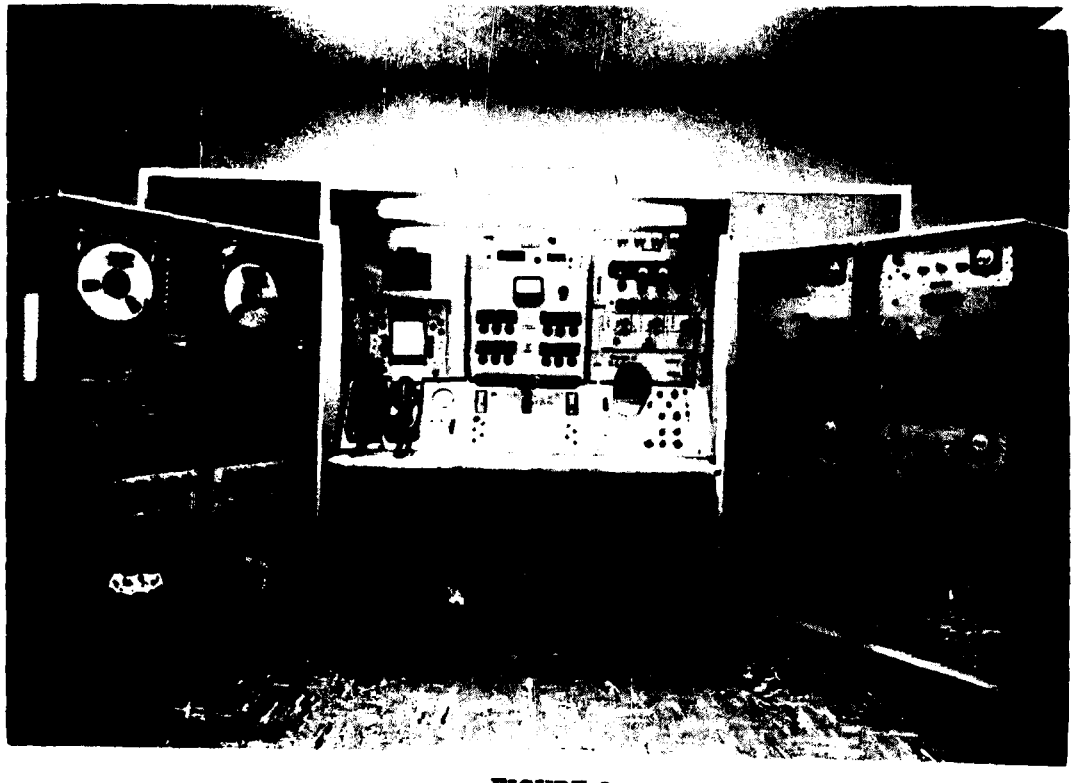

FIGURE 8

The complete TELUS (Telemetric Universal Sensor) used as a research tool for microbiologic analyses in the central laboratory.

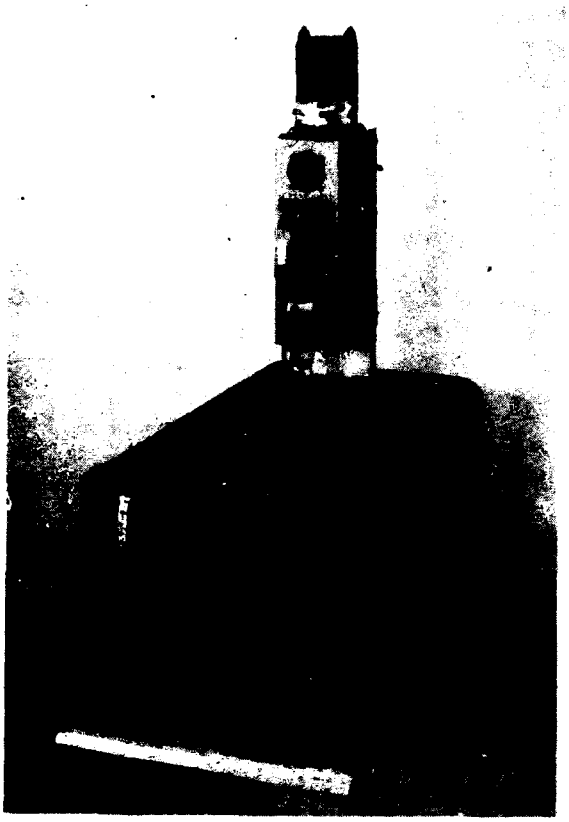

FIGURE 9

Biotelescanner, model X 66-A, with Microfil, model X 66-A attached on top. This scanner was made for a direct readout into a strip-chart or tape recorder. The Microfil can be adjusted to overlay as little as 0.05 ml. of a bacterial extract on top of a semisolid agar-antiserum mixture previously placed in glass columns. The 30-position magazine accommodates 5 calibration positions and an additional 25 reaction columns.

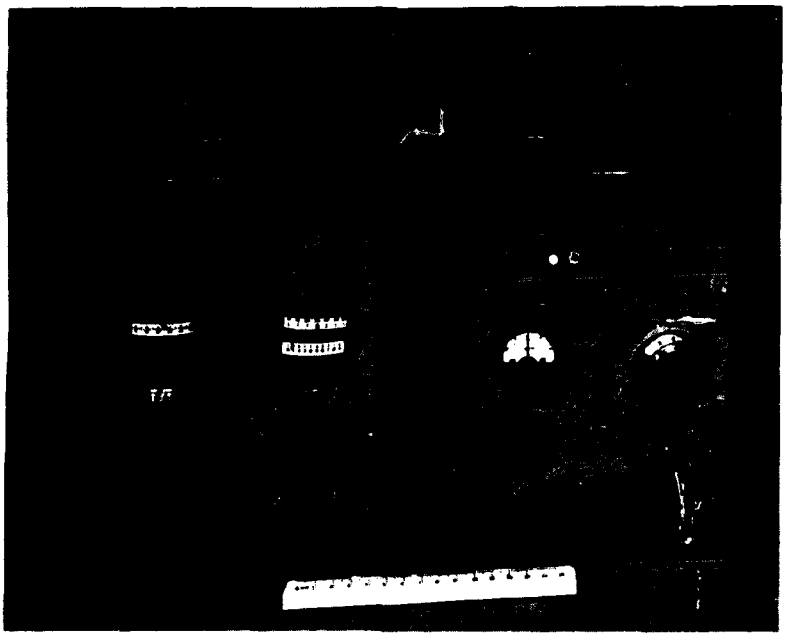

FIGURE 10

Biotelescanner, model X 66-D, and Biotransceiver, model X 63-BR. The scanner has 5 calibration slots and an additional 55 spaces for diffusion reaction columns. This is a complete telemetering instrument connected to a combination transmitter and receiver that permits 2-way telemetry between the field site and the laboratory. Both instruments are transistorized and battery-powered. The meter readings on the face of the scanner are also monitored by the scientist in the laboratory via radio or telephone signals (see fig. 14). Condition of the batteries in each vital circuit can be observed by a green-red indicator on the back of the scanner.

rotated manually. It is operated on 28 v. direct current from a power supply. Primarily the X 66-A is used as part of a compact, self-contained system for performing "on-the-bench studies" of a clinical laboratory nature (fig. 12). In the past, the same Biotelescanner and associated instruments were coupled to a high-volume air sampler for identification of bacteria in aerosols (see below).

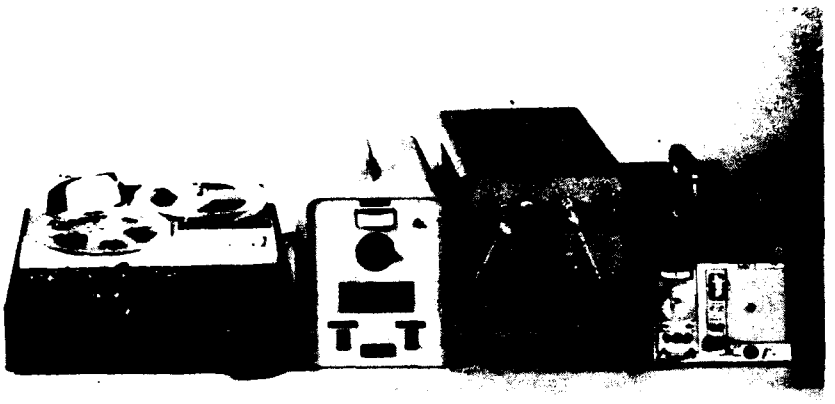

FIGURE 11

Supporting recording, transmitting, and receiving equipment for field site applications. The battery-operated instrumentation tape recorder-reproducer has 3 FM and 1 AM channel; Interconnect (model 65-A) makes it possible to radiotape data; and the Field Transmitter (model 65-A) (230.9 mc.) matched with a special receiver-discriminator combination (model A 180-3) completes the 2-way telemetry field instrumentation for approximately a 20-mile range.

In contrast, the Biotelescanner, model X 65-D (fig. 10) is a highly sophisticated instrument, approximately 28 by 16 by 21 cm. (weighing 14 lb.), and is powered by rechargeable batteries. It is the fourth model of its type. In this instrument when a light beam and photoresistor combination vertically scans biologic reactions (fig. 13), the analog changes in the photoresistor are transmitted by radio in subcarrier form (1.3 kc.) to TELUS. The calibration of density changes is made from the transmission of scans of 0.2 and 0.6 neutral density filters permanently fixed in 5 positions of the Biotelescanner. In this manner, the densities of the precipitin system areas in the glass columns subsequently scanned can be transposed into neutral density units.

The areas of the densities are automatically integrated by digitizing the curves for preset time intervals during the scanning cycles. This digitizing is automatically initiated by a pulse. A 3.0 kc. subcarrier transmits a synchronization pulse for either of two preset periods while the column is being scanned. For example,

FIGURE 12

The BAIT (Bacterial Automated Identification Technic) set up for "bench top" analyses without telemetry. The bacterial cultures are injected into the sonic chamber and the reacting diffusion columns are incubated in the scanner, model X 66-A. For additional "back up" record or telemetry later, the analog data in the strip-chart recorder can be simultaneously taped, then reproduced, on the precision magnetic tape recorder.

when the scanning beam is at the interface of the reactants on the "down" scan, a microswitch is energized and triggers the pulse (3.0 kc.) that is used to start an analog to digital converter and counter in TELUS. This pulse remains high for a preset 5 or 10 mm. vertical movement of the scanning head. When the light beam moves farther down, the microswitch opens, the pulse ceases, and the analog to digital conversion automatically stops (15). (Direct digital transmission was considered; however, the definition obtainable by such a system was believed to be incompatible with the requirements.)

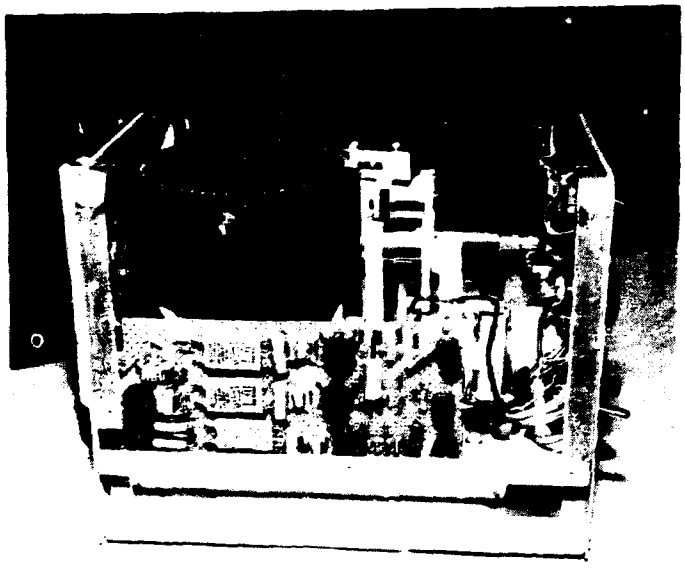

FIGURE 13

Internal view of the Biotelescanner, model X 63-B. In subsequent modifications the electronic deck was divided into two and reworked into plug-in units in a false bottom under the scanner. Easier access and replaceability was achieved by this change.

For biologic analyses by telemetry, it is essential that other information be available to the TELUS operator in the laboratory. This is achieved by means of a twelve subcarrier automatically transmitted from the Biotelescanner and used to provide four other parameters of information. During one-fourth of a revolution of a commutator attached to the scanning head cam, a thermistor in the Biotelescanner is sensed and the temperature in the field instrument is transmitted as a square wave that can be viewed or graphed on a previously calibrated range. The next one-fourth of a turn of the commutator allows the transmission of the "tens" position of a position-indicating rotostepper upon which the magazine turns. Following this, another one-fourth turn permits the "units" signal to be radioed. Both the "tens" and the "units" are

required to determine the complete position indication of the magazine since there are 60 positions in the Biotelescanner. Lastly, the remaining one-quarter of a revolution of the commutator permits a frequency to be transmitted that can be used as a "homing" signal when other instruments are also on the air. All of these commutated signals are visualized by the TELUS operator as square waves (fig. 14). Any spike or break in the configuration is a sign of interference in the transmission of data and is one of the methods of detecting errors by telemetry.

An instruction and maintenance manual for the TAMIS and all subsystem components has been completed. With the manual, assistants should be able to correctly operate both the laboratory and field instruments with a minimum of training.

BASIC ELECTRONIC IDENTIFICATION PROCEDURE

Reference data

Known extracts (antigens) reacted with quantitatively discriminating known solutions (antisera) are densitometrically measured at fixed time and temperature levels. Each scan for each reaction produces a curve in which the area under the curve is the important reactivity parameter. (For the reader unfamiliar with immunologic technics, the area under the densitometric curve represents the sum of all the antigen-antibody reactions and is an indirect measure of the amount of antibody, as well as of the relative strengths of the reactive biologic fluids. For this and other immunochemical reasons, this area is of considerable importance.) The telemetered analog signal for the curve is sensed by a voltage to frequency (V-F) converter, thence passed to a 12-bit binary coded decimal (BCD) counter that displays the integrated area in a 3-digit nixie-light readout. V-F quantitation is set for 0.500 second of the scanning time and the count start is initiated by a pulse emitted by telemetry from the scanner when the scanning beam is at a preset place in its vertical travel along the reaction column. At the end of 0.500 second, the BCD representing the curve area, in parts per 500 counts, and 4 other independent BCD series (which were manually set into 4 separate TELUS channels) are placed on 5 recorder tracks by an automatic print signal triggered by the

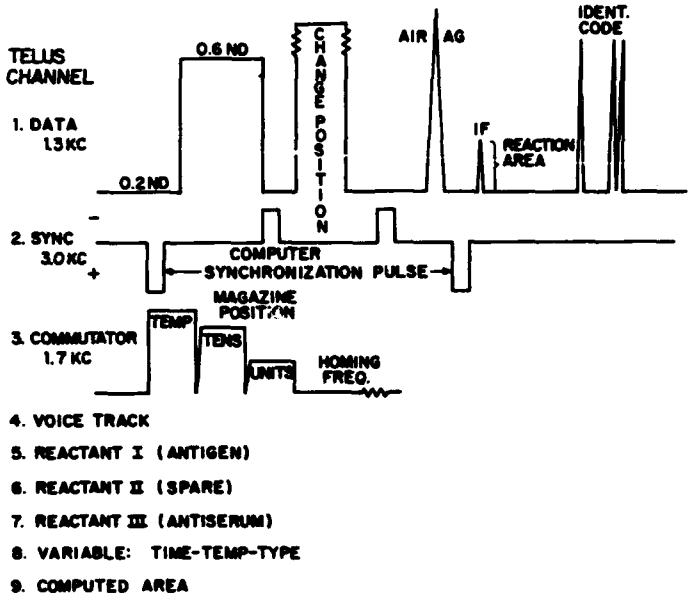

FIGURE 14

Composite of all signals sent via radio from Biotelescanner, model X 65-D, to TELUS as they appear on a strip-chart recorder. The calibration (0.2 to 0.6, neutral density) is shown on the 1.3 kc. data channel. If maximum neutral density of the reaction is to be recorded in binary coded decimals (BCD), it is generally placed on TELUS channel 8 and antiserum BCD and time-temperature-type of reaction moved up to channels 6 and 7, respectively. Significances of the other signals are explained in the text.

termination of the synchronization pulse. Simultaneously, a sequential BCD, which functions later as an area locator, is placed on a separate track every 20 seconds by a time-tape coder combination. (Experience showed that tape footage counters were unreliable when used for repeated tape searching.) The integrated area in BCD (3 BCDs), the extract identification number (antigen) (3 BCDs), antiserum number (3 BCDs), incubation time (1 BCD), incubation temperature (1 BCD), type of column (1 BCD) together with the BCD area locator (3 BCDs)—all comprise the reference data for known solutions reacted with known solutions.

Comparing known and unknown reactions

Data received from the field are taped at TELUS. In the analyses initiated in the field only one of two reactants (either antigen or antibody) is of concern and the problem at the receiving laboratory is to identify and quantitate the unknown solution. From telemetered information, the TELUS operator knows the incubation time (1 BCD), incubation temperature (1 BCD), type of reaction (1 BCD), and identity of one of the two reactants (3 BCDs). The area density is automatically measured (by voltage to frequency conversion) or the maximum density is manually computed as the scan is received (3 BCDs). This totals 9 BCDs in all. In an extensive file system, the operator can find a BCD sequence that is closest to the 9 BCDs accumulated above. There is also a tape reel number and a BCD tape area locator noted for each BCD sequence. By playback of the correct reel in conjunction with a magnetic tape searcher, the tape area of the sought BCD sequence can be located. Then, 3 tape searchers connected in parallel are armed to stop the tape recorder playback at the specific place where the 9-BCD sequence is located. The TELUS operator may then graphically display the reference scan most closely matching the one received by field telemetry. (This display, however, is not necessary to the identification. Upon finding the 9-BCD sequence in the file, another BCD of 3 numbers is shown on the file card. This BCD is the identification number of the reference material that best matches the unknown solution.) Since both reactants of the reference scan are known, within the limitations of the technic, the identity of both of the reactants initiated in the field is therefore also known—and the problem is solved.

SUPPORTING SUBSYSTEMS

Of the 5 supporting subsystems, 2 require further explanation as to the manner in which they contribute to the objectives of utilizing the TAMIS in the research and clinical laboratory.

Liquid nitrogen storage

Two complex biologic solutions (reactants) are primarily involved in microbiologic analyses of the types used in the TAMIS; namely, antigens (sonicated extracts of microbiologicals) and precipitating

antibodies (molecules produced in the sera of animals which have previously been injected with antigens). When the laboratory and animal manipulations are successful and good differentiating reactants are obtained, storage of them in a manner that may preserve their reactive configurations becomes paramount. The selection of extreme cold (—196° C.) produced by liquid nitrogen seemed to be the most promising condition of storage. A compromise between equipment capability and the strength of small glass storage ampuls determined the rate of cooling from 24° to —77° C. in 15 minutes in a controlled-rate freezer. Following this, the 1-ml. ampuls are stored directly in the liquid nitrogen holding tank (fig. 15). The tank has a capacity of 185 liters and is 89 cm. in diameter and 93 cm. high. When purchased, the tank did not contain any equipment for storing biologicals in an orderly fashion. By use of stainless steel throughout, partitions were made that divided the inside of the tank into 5 sections for each of which 6 covered drawers or trays (18.5 cm. square x 6.3 cm. deep) were fabricated. Each of these 30 drawers is divided into 225 sections suitable for storing glass ampuls (1 ml. capacity). A total of 6,750 ampuls can be placed in the tank. Since the condensation of moisture by the extreme cold does not permit visual observations of the inside of the tank, a mechanically guided probe was designed for retrieval of the trays. This retrieving mechanism is shown in position above the opening to the tank. The depth of the grasping clamp is preset by the operator; then a keyed rim mounted above the tank guides the probe into any 1 of the 5 sections. Once the clamp has been remotely closed upon a tray handle, the tray is removed safely without the operator coming in contact with the liquid nitrogen. To reduce errors in removing the desired vials from a tray, a correctly labeled Plexiglas template with precut access holes is chosen and placed over the open tray. Only certain vials are then accessible.

We have not been successful in labeling the ampuls in any manner that would assure permanent identifiaction. The locations of the 5,350 ampuls now stored are kept in an extensive inventory system.

Efforts to completely flame-seal all of the storage vials before placing them in liquid nitrogen have not been successful and, during the first year of operation, numerous vials exploded violently upon removal from the liquid. Rather than digress and attempt to

FIGURE 15

Liquid nitrogen storage tank.

device a faultless testing system for determining adequate sealing, it was reasoned that the inert character of the nitrogen would not affect the biologicals. The reactants have therefore been stored unsealed in narrow-neck ampuls.

Monitoring the adequacy of the supercold storage compared to the usual —20° C. deep-freeze temperature has been done periodically since October 1965. Bacterial antigens and antibodies are used for this purpose. To date there have not been any statistically significant differences among the same reactants stored separately under the two different temperatures. Quantitative gel diffusion analysis is used in these assays of storage effects.

Preparation of calibrated solutions often takes up several months and the induction of adequate antibodies may use 20 animals. This is an expensive and time-consuming undertaking; therefore, preservation of these biologicals for the longest possible period would seem to be warranted.

Numerical marking abrasive unit

Since all sources of error can not be completely eliminated in a remote analytic system, it is essential that they be recognized during the conduct of the work. With the TAMIS, it was evident that small glass columns preloaded with calibrated reactants could be mixed or placed in the magazine of the Biotelescanner in a position other than the one indicated by the voice telemetry sent from the field site. An adaptation of a commercially available abrasive unit (fig. 16) was therefore made (24) to score various sequences of bands completely around the bottom, top, or both ends of the columns sent to the field sites (fig. 17). When measured by the light and photoresistor combination of the field scanner, these band sequences can be visualized and converted to numers on an oscilloscope or strip-chart recorder and will provide the laboratory operator of the TELUS assurance that the correct columns were used. The sequences are actually modifications of the binary coded decimal system and permit 9,998 different combinations (fig. 18).

IMMUNOLOGIC DIFFERENTIATION

The utilization of the precipitin reaction for differentiation depends, in part, upon prior knowledge of the kinds of antigens with

which the antibodies will precipitate. Extracts of bacteria that are only partially like the antigens used to evoke the antibodies may also combine with them in an in vitro assay (heterologous reaction). Such heterologous reactions involving partially similar molecules and antibodies will generally not produce as much precipitate as antigen-antibody aggregations consisting of the antigens originally used to evoke the antibodies (homologous reaction). Thus, the antibodies from one animal may react with 2 or more bacterial antigens but not to the same extent. The word "extent" used in a quantitative sense is the basis for the differentiation of bacterial extracts described in this report. For example, figure 19 shows 3 different bacterial extracts, one in the upper part of each glass column. Note that only the two columns on the left show white precipitates in a curved arc below the reference scratch circling the tube (approximately two-thirds up from the

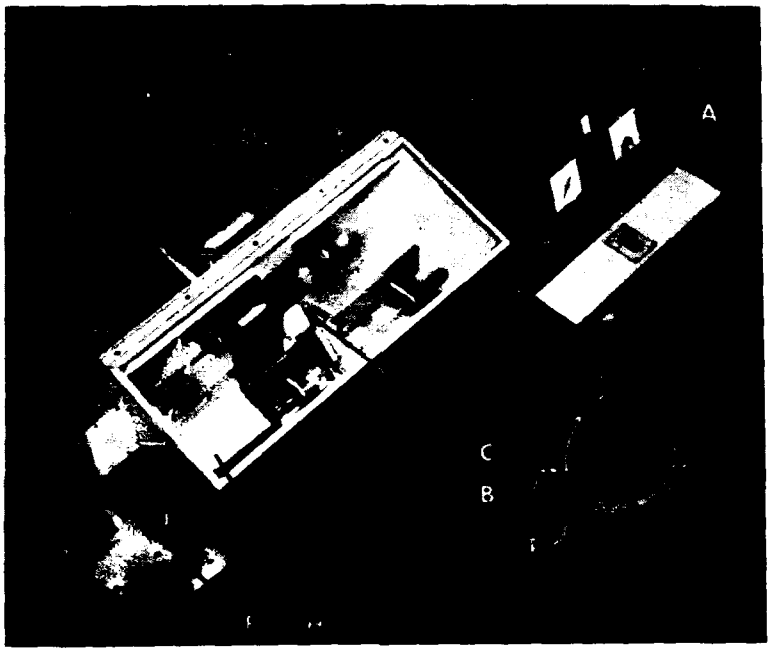

FIGURE 16

Numerical marking adhesive unit (see ref. 24 for details).

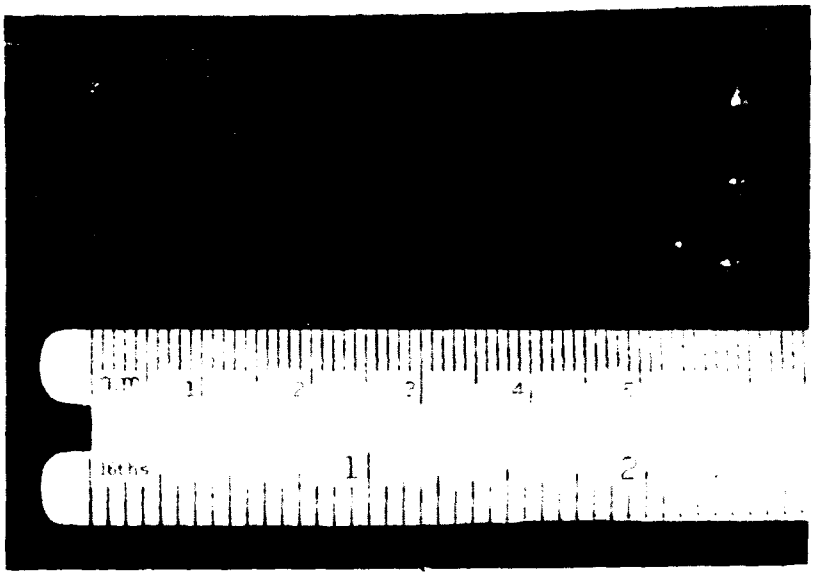

FIGURE 17

Column identification, abraded by the Numerical Marking Abrasive Unit. The upper column (nonabraded) can be compared with the 2 lower (abraded) columns (see also fig. 18).

bottom of the tube). The column on the extreme right is negative (no precipitate). As indicated in the legend of figure 19, the antigens in the upper part of the columns are different, but the antibodies incorporated in the lower two-thirds of each column are the same. One may note that the homologous reaction (on the far left) has more precipitate than the heterologous one (middle column).

If a small beam of white light is passed through the columns at the appropriate levels, the precipitates partially occlude the light passage and the amounts of light before and after the precipitates can be indirectly measured by a photoresistor (10). A comparison of figure 20 (30-minute reaction) with figure 21 (120-minute reaction) shows that the precipitates become more dense with increasing incubation time.

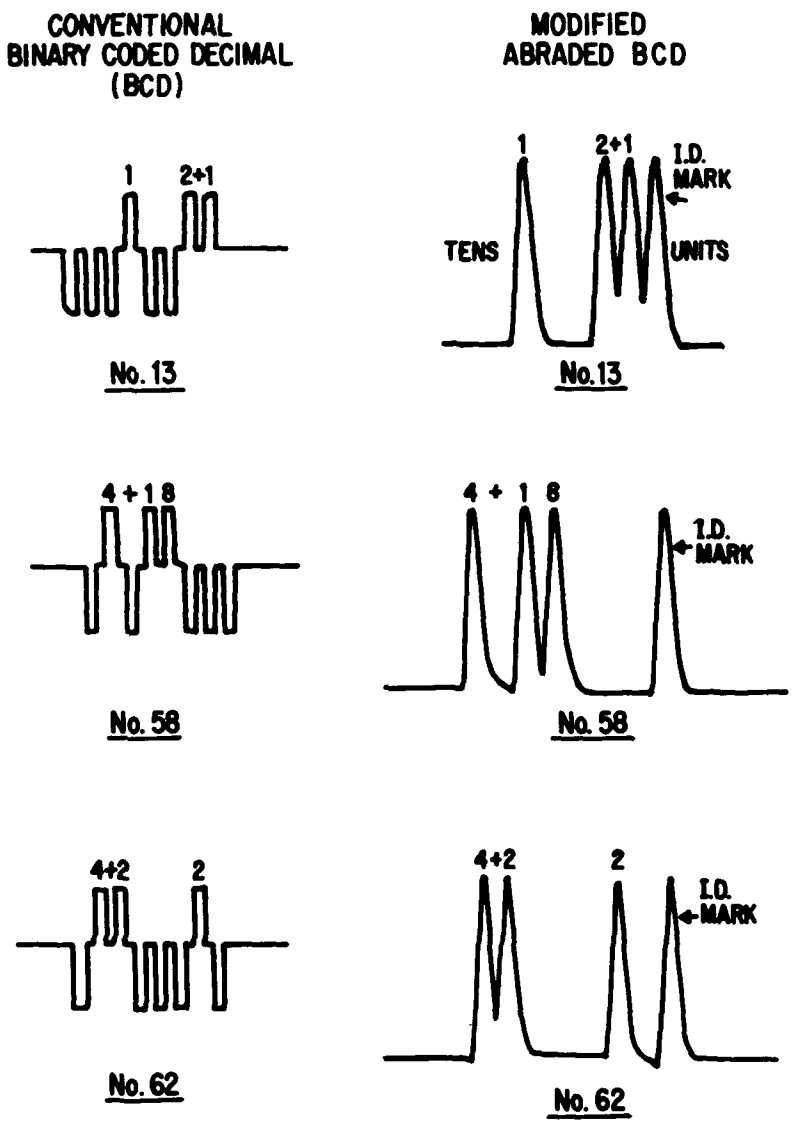

FIGURE 18

Strip-chart comparison of conventional binary coded decimal (BCD) and modified abraded BCD (see ref. 24 for details).

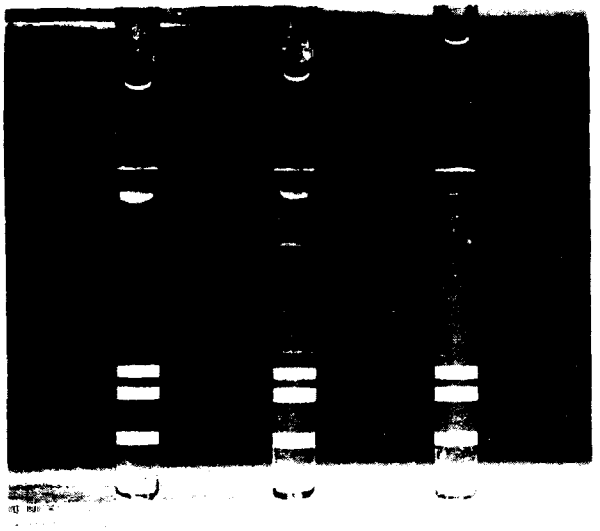

FIGURE 19

Single diffusion columns (for details see ref. 18).

The description above and figures 20 and 21 are classic examples in that differentiation conforms to tested immunologic axioms. Our extensive research with the TAMIS has indicated, however, that for bacterial-antibacterial reactions to conform to the axiom of "homologous reacting stronger than heterologous," determinations must be made before sonication of the approximate numbers of organisms to be disrupted. There were numerous instances in the standardization of reactants (19) where, if the number of organisms in the starting heterologous population exceeded the homologous, the heterologous reacted more strongly than the homologous reference system (20). These ambiguities do not, in themselves, indicate that identification of bacterial extracts by precipitin reactions is unsuitable. They emphasize the requirement of determining beforehand the concentration of the starting population by nephelometry (e.g., McFarland turbidity scale (25)) or by some other method. This estimate must be considered in the ultimate determination and comparison of the unknown reaction with the reference data for the known extracts. In this

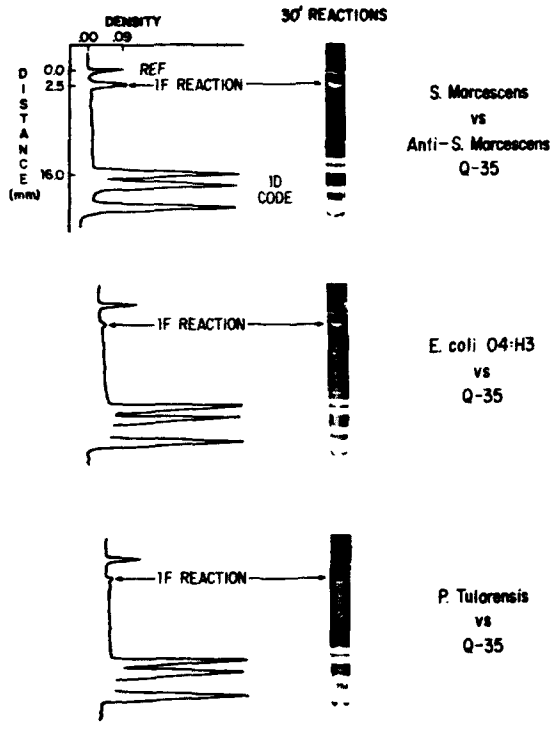

FIGURE 20

TAMIS densitometric and photographic comparison of homologous and heterologous bacterial-antibacterial reactions in single diffusion columns at 30-min. incubation time (for details see ref. 18).

manner, errors in identification of the unknown extracts can be minimized. To date, the TAMIS has been applied to 2,963 immunologic reactions to ferret out sources of error such as the one explained above.

Temperature effects

Compactness and portability of the Biotelescanner model X 65-D permitted studies of reactions performed with the scanner in a

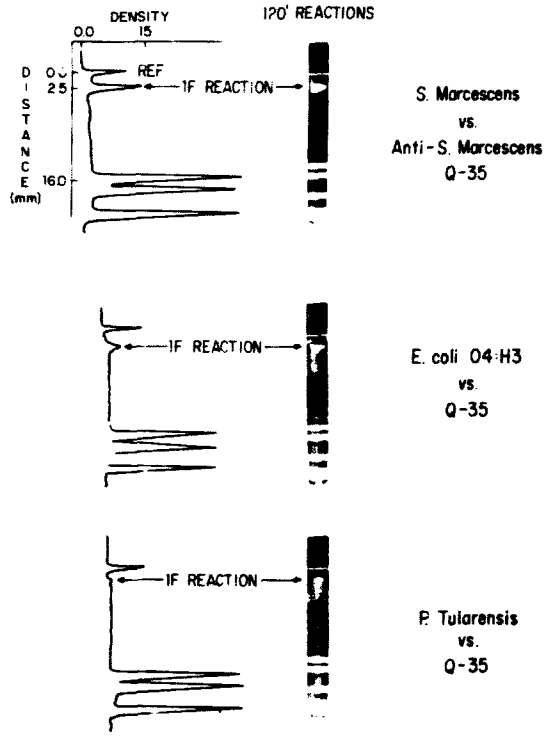

FIGURE 21

Densitometric and photographic comparison of the single gel diffusion reactions shown in figure 20. after 120-min. incubation (for details see ref. 18).

refrigerator (4° C.) and in an incubator (25° and 37° C.). Obviously, rates of reactivity of the solutions varied with the 5 concentrations of extracts. Using *Shigella dysenteriae* – anti-*Shigella dysenteriae* as a model system, the sensitivity and reproducibility of the measuring system precluded linearizing the slight changes at various incubation times up to and including 60 minutes. A difference of ± 0.022 neutral density units was therefore calculated as the standard deviation that would include temperature differences from 4° to 37° C.

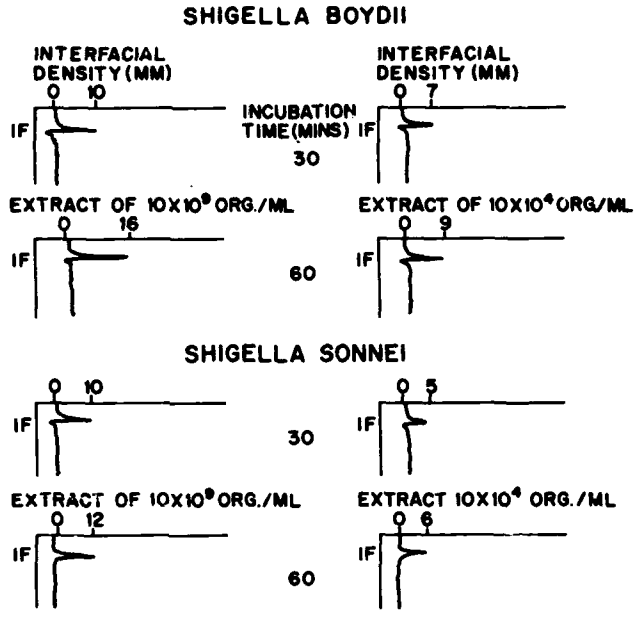

FIGURE 22

Densitometric graphs of homologous and heterologous precipitin reactions in single diffusion columns. The increased densities at the interface (IF) result from sonicated extracts of two populations of bacteria reacting separately with antiserum to Shigella boydii *at approximately 25° C. for 30 minutes and 60 minutes. Note the lower interfacial densities for extracts from 10×10^4 organisms per milliliter compared to 10×10^9 organisms per milliliter. Smaller densities with decreasing extract concentrations are reactions in antibody excess.*

Bacterial extracts

Clear extracts of bacteria obtained by disruption of the organisms and solubilization of the molecules on and within the organisms are essential to the TAMIS procedure. While sufficient organisms to produce an extract of adequate concentration may be obtained in a variety of ways, a biphasic culture technic, modified from a published method (31) is currently used. A sterile flask (250 ml.)

invaginated in four places is prepared with 50 ml. of 2.5% sterile heart infusion agar. After the agar has gelled, 10 ml. of sterile heart infusion broth is overlaid. The bacterial inoculum placed in the flask is either 1 ml. of a suspension of organisms washed from an agar slant after overnight incubation or 1 ml. of broth culture. After inoculation, the flask is incubated for 16 hours at 37° C. Faster growth is achieved by the use of a reciprocating shaker (200 strokes per minute, 4.2 in. per stroke).

Following growth, the broth culture from the flask is standardized to contain 10×10^9 organisms per milliliter when compared with a McFarland turbidity scale (25) measured at 660 $m\mu$. on a spectrophotometer. The nutrient medium is not removed for this measurement or for subsequent sonication.

Several methods were tested for their efficiency in preparing extracts of the organisms. These were concerned with various time combinations of boiling, grinding, extraction with benzene, and sonication. The best treatment applicable to most gram-negative organisms was sonication for 10 minutes of an 8 ml. bacterial suspension (10×10^9 organisms per milliliter) in a sealed chamber cooled by tap water. When required, the suspension may be only 4 ml. With either volume, consistent quantitative extractions were obtained in five minutes for certain gram-negative organisms; namely, *Escherichia coli* and *Serratia marcescens*. With both volumes a sonicator probe was used with a ½-inch tip operating at maximum power (Sonicator, model S-75, Heat Systems, Inc.) (19).

If the sonicate was for the induction of antisera, our studies showed that better antisera were produced when solubilized substances together with cellular debris were used for injection. If the sonicate is to be used as a reference or is from bacteria to be identified, the sonicate is processed by filtration (or alternatively, centrifugation, $12{,}000 \times g/15$ minutes) through the filtration reservoir shown in figure 11. To date, these explorations have resulted in the preparation of approximately 90 bacterial extracts.

Biotelescanner model X 66-A (fig. 8) has a Microfil, model X 66-A, attached to the top. This is used to automatically overlay a bacterial extract onto the menisci of preloaded columns containing calibrated antisera incorporated in agar. The telemetering

Biotelescanner, model X 65-D, does not have such a device and the columns (2 mm. x 60 mm.) are one-half the inside diameter of those used in model X 66-A. For this reason, the bacterial extracts overlaid onto columns in the telemetry scanner are filled by manual manipulation of an 18-gage, 2½-inch needle attached to a 2-ml. syringe.

Reference antisera

Since concentrating on the biologic aspects of the TAMIS during the past two years, approximately 200 rabbit and 2 goat antisera have been evaluated using single diffusion agar columns. For the quantitative specificity, reactivity, and sensitivity required in the TAMIS, the number of suitable sera obtained has been approximately 10%. The inherent limitations of the precipitin technic in the TAMIS approach indicate that these antisera rarely form precipitin aggregates with bacterial extracts of less than 0.15 mg. N per milliliter resulting from presonicated starting populations of not less than 5×10^9 organisms per milliliter. While most antisera will cross-react with at least 4 organisms, on occasion antisera have been produced that react with 14 extracts from different gram-negative organisms. Such broad reactivity is usually sufficient to provide clues for determining the *genus* of unknown preparations while *species* determinations are made with antisera that have a narrower reactivity range.

The results of these reactions from screening antisera, as well as final calibrations of selected reference antisera (figs. 23 and 24) to be used in subsequent identification of unknown bacteria, are stored on magnetic tape in the TELUS (fig. 9) or in strip-chart form (fig. 11).

Ultimately, extracts and antisera that are suitable for "blind" determinations are stored in liquid nitrogen as indicated previously.

Lastly, details of the most successful injection schedules should be mentioned. For producing rabbit antisera to whole bacterial sonicates, the suspension is adjusted to contain 0.6 mg. N per milliliter (equivalent to approximately 1.4 to 1.5×10^{10} organisms per milliliter). Table I shows a typical injection schedule used with adequate results for rabbits.

Reactions with viruses

Using the isolated experiments scattered throughout the literature pertinent to discrimination of viral extracts (1, 2, 4, 5, 21, 23, 26-30) as a starting point, the TAMIS was applied to the discrimination of 8 extracts. For this purpose homologous antisera were induced in rabbits using immunization schedules shown in table II.

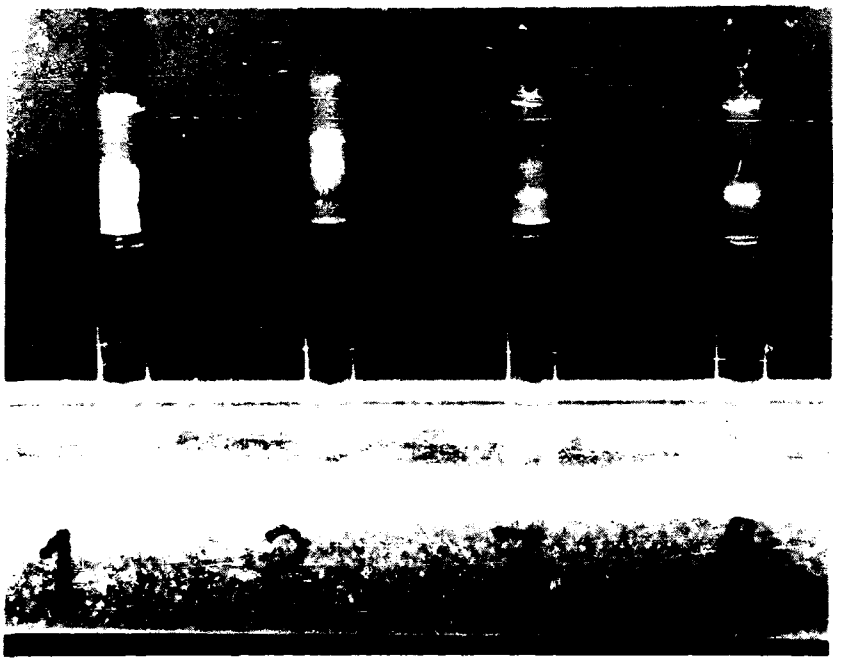

FIGURE 23

Double diffusion reactions in agar gel differentiating among several extracts of streptococci bacteria each reacted with the same antiserum to Streptococcus *group C. In contrast to single diffusion where the antigen is layered directly upon the antiserum-agar, in double diffusion the antigen is separated from the antiserum by an arena of agar. The reactions form in this arena. Tube 1, extract from group C; tube 2, group B; tube 3, group D; and tube 4, group G. The two white lines in the center of each column are reference marks 10 mm. apart to anticipate by the TAMIS where the reaction should occur. Incubation time, 92 hours at 22° to 24° C. Note the dissimilarities in the reactions that permit densitometric characterization shown in figure 24.*

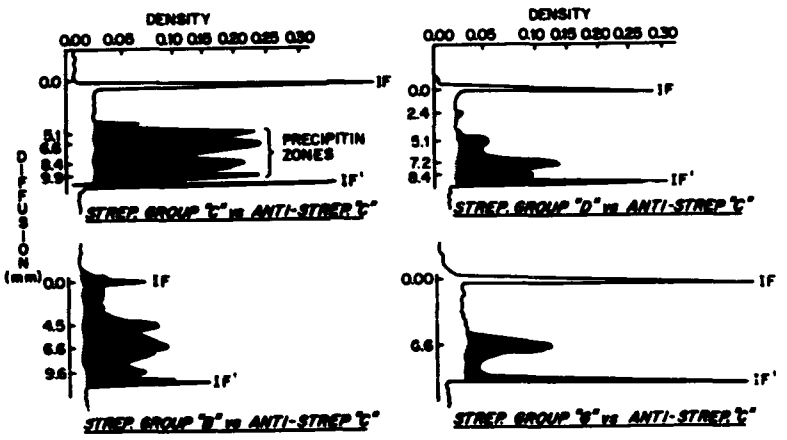

FIGURE 24

***TAMIS** tracings of the same reactions shown in figure 23. IF and IF' are the interfaces of the bacterial extract–agar reaction arena and the agar reaction arena and the antiserum, respectively. These are double diffusion reactions and establish the feasibility of differentiating among strains of* Streptococcus *spp. reacting with the same antiserum.*

To obtain the best differentiation, the gamma globulin fractions of these antisera were prepared by separation with diethylaminoethyl cellulose (DEAE). Following this, gel diffusion reactions were performed in a checkerboard-designed experiment including as controls appropriate normal sera and growth media. From these analyses, an antiserum was selected that had the broadest antibody spectrum as well as differentiating capabilities. Figure 25 shows the results of the analyses. The photographs provide clear evidence that all the different viral extracts listed in the legend gave dissimilar precipitin results when reacted with the gamma globulin fraction of antiserum prepared against poliomyelitis antigens I and III. It is also interesting to note the marked similarities among the 4 strains of influenza virus. Quantitation of the various reactions by densitometry resulted in the graphs also shown in figure 25. These reactions were reference ones to further establish the feasibility of the TAMIS approach.

TABLE I

General injection schedule for inducing rabbit antibacterial sera

Day	Vaccine
1	1.0 ml. s.c. (0.5 ml. sonicate with 0.5 ml. incomplete Freund adjuvant)
3	0.1 ml. I.V.
5	0.3 ml. I.V.
7	2.0 ml. s.c. (1.0 ml. sonicate with 1.0 incomplete Freund adjuvant)
9	0.6 ml. I.V.
11	1.0 ml. I.V.
18	1.5 ml. I.V.
24	Trial bleeding
25	Exsanguination

The vaccine was the whole sonicated suspension of approximately 1.4 to 1.5 × 10^{10} organisms per milliliter adjusted to contain 0.5 mg. N per milliliter.

FIELD TEST RESULTS

Clinical laboratory application

It was repeatedly shown that the analyses of immunologic reactions initiated in the clinical laboratory of a local military hospital could be successfully telemetered by a combination of telephone and radio from the laboratory to the TELUS. Logistic difficulties required that additional identifications be made from samples delivered to the TAMIS laboratory. These analyses were done in the BAIT system (fig. 11) without a radio or telephone link. Table III lists the test organisms, antisera now used, and results. In brief, 7 reference antisera have been used to distinguish among 25 organisms. Of 25 cultures submitted, 100% were correctly identified to the genus level and 65% to the species level. *Pseudomonas* spp. and *Proteus* spp. have caused the greatest difficulty in species identification because adequate differentiating reference sera were not on hand.

TABLE II

General injection schedule for inducing rabbit antiviral sera

Day	Vaccine	
1	1.0 ml. s.c.	0.5 ml. washed virus with 0.5 ml. complete Freund adjuvant for each injection
8	1.0 ml. s.c.	
17	1.0 ml. s.c.	
24	0.5 ml. I.V.	
31	Trial bleeding	
32	Exsanguination	
54	0.5 ml. I.V.	Additional injection for influenza and adenovirus antisera
61	Trial bleeding	
62	Exsanguination	

The vaccines were made from live viruses washed free of appropriate growth media.

Aerosol identification

The BAIT augmented with an electrostatic-type air sampler (10,000 liters per minute) (Applied Science Division, Litton Systems, Inc.) coupled to a continuous flow centrifuge (fig. 26) was housed in a mobile van and field-tested in May 1967. Two bacterial populations, live *Serratia marcescens* and killed *Escherichia coli* (strain 0119:B14:H6) were aerosolized in 9 test runs. Table IV details the results of these trials. (The analog output of the densitometer was also sent by telephone to a remote chart recorder several miles distant.) In summary:

1. In 4 out of 4 completed tests (table IV, mission 2, passes 3 and 4; mission 3, passes 3 and 4) involving *S. marcescens*, the BAIT correctly identified the organisms.

2. In mission 2, pass 2, the BAIT gave a misleading reaction indicating possibility of *S. marcescens* when the organisms actually aerosolized in 2 successive passes were *E. coli* (strain 0119:B14:H6).

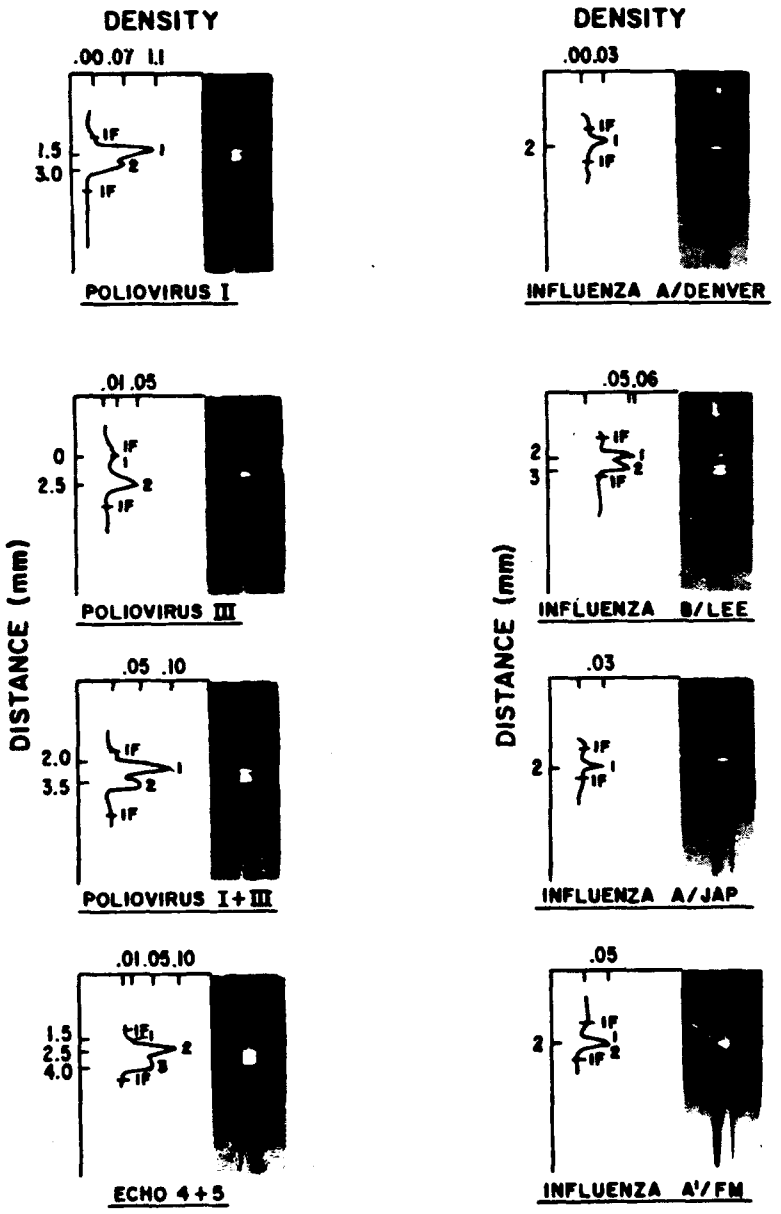

FIGURE 25

Densitometric graphs with accompanying photographs of viral-antiviral reactions in gel double-diffusion columns.

TABLE III

Results of "blind" analyses of sonicated extracts of bacteria of clinical importance completed in the TAMIS procedure

Actual organism	TAMIS identification
Shigella sonnei	*Shigella sonnei*
S. dysenteriae	*S. dysenteriae*
S. boydii	*S. boydii*
Salmonella enteritidis	*Salmonella enteritidis*
S. bredeney	*S. bredeney*
S. paratyphi B	*S. paratyphi B*
S. javiana	*S. javiana*
S. paratyphi B	*S. paratyphi B*
S. bredeney	*Shigella boydii*
S. javiana	*S. javiana*
Escherichia coli (0128)	*Escherichia coli* (0128 or 0127)
E. coli (0128)	*E. coli*
E. coli (0111)	*E. coli* (0111)
Proteus morganii	*Proteus rettgeri* or *mirabilis*
P. mirabilis	*P. vulgaris*
P. rettgeri	*P. vulgaris*
P. vulgaris	*P. mirabilis* or *rettgeri*
P. mirabilis	*P. rettgeri*
Pseudomonas pseudomallei	*Pseudomonas pseudomallei*
Pseudomonas spp.	*P. pseudomallei*
P. pseudomallei	*Pseudomonas spp.*
Bacillus anitratum	Other than those above or below
Salmonella taksony	*Salmonella taksony*
Proteus mirabilis	Other than those above or below
Citrobacter spp.	Other than those above

Extracts of bacteria were prepared by sonication of 10×10^9 bacteria per milliliter for 10 minutes and reacted for 4 hours at 22° to 25° C. To differentiate among these organisms the following antisera were used separately and simultaneously: *Salmonella enteritidis, S. typhi J, S. typhimurium, paratyphi B, Shigella dysenteriae, S. sonnei* (T-04), and *S. sonnei* (S-90).

3. Gelatine phosphate diluent only was aerosolized in mission 3, pass 1, and the BAIT identified *S. marcescens* in the aerosol. An inspection of the background count (live organisms) between passes 3 and 4 of mission 2 shows 2.94×10^3 organisms per liter. Superimposed upon this was the next aerosolization

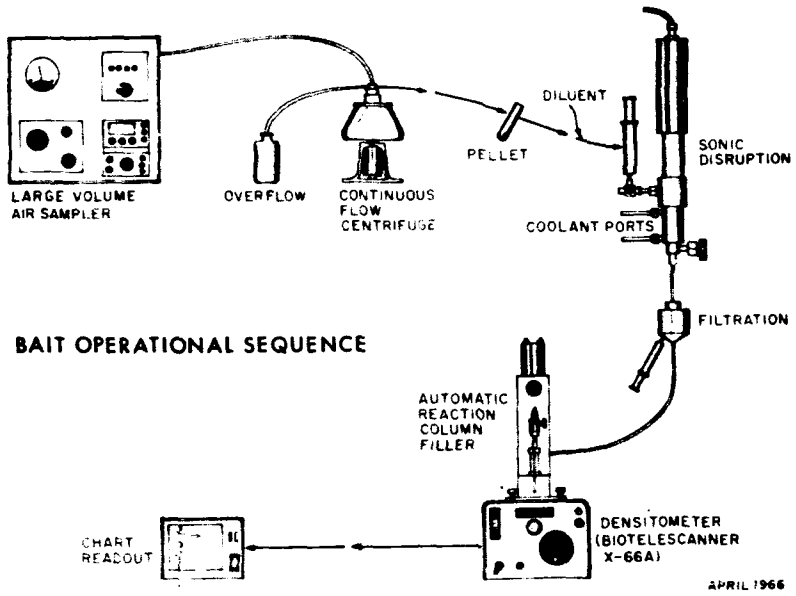

FIGURE 26

Flow diagram of BAIT (Bacterial Automated Identification Technic) for aerosol applications.

(mission 2, pass 4) of 1.07×10^7 *S. marcescens* organisms per liter. The following day, only gelatine phosphate was aerosolized (mission 3, pass 1), but no background count was made before that aerosolization. (When background counts were made, they were on live *S. marcescens* organisms only; the BAIT analyzes dead as well as live bacteria.) It is possible, therefore, that the collecting ducts and impaction filter of the BAIT air sampler and the area around the intake actually had sufficient killed or live *S. marcescens* to produce a reaction. Consequently, these data are inconclusive.

There are additional observations and conclusions from these trials that should be considered. The first 1900 hours test was aborted because the relative humidity was 87%. This ambient

TABLE IV

Details of aerosolization and results of identification of bacteria in field tests using the BAIT. Only two organisms—killed Escherichia coli, *0119:B14:H6*, and Serratia marcescens *(SM)—were used.*

Date (May 1967)	Mission number	Pass number	Time (CST)	Organism	Desired concentration (organisms per liter)	Actual concentration (organisms per liter)*	Background organisms per liter	BAIT		
								Concentration per liter air collected†	Total organisms collected	Identification results
8	1	1	1854-1907	SM	1.5×10^6	2.54×10^7	0	Shorted out—test aborted.		
9	2	1	1420-1429	*E. coli*	1.5×10^6	1.05×10^6	1.43×10^5	1.8×10^6	180×10^9	Neither SM nor *E. coli*
		2	1446-1456	*E. coli*	1.5×10^6	1.02×10^6		1.85×10^6	185×10^9	Slight reaction anti SM
		3	1828-1838	SM	1.5×10^6	3.12×10^7	2.94×10^3	4.9×10^6	490×10^9	SM
		4	1858-1908	SM	1.5×10^6	1.07×10^7		1.95×10^6	195×10^9	SM
10	3	1	1435-1445	Gelatin phosphate				1.8×10^5	18×10^9	SM
		2	1500-1510	*E. coli*	1.5×10^6	1.07×10^6	4.53×10^5	1.3×10^6	130×10^9	Neither SM nor *E. coli*
		3	1830-1840	SM	1.5×10^5	2.90×10^6	2.40×10^5	2.63×10^5	26.3×10^9	SM
		4	1855-1905	SM	1.5×10^3	1.65×10^6		5×10^6	50×10^9	SM

*Concentration for viable SM determined by plate count; concentration for killed *E. coli* determined by slide count.
†Determined by nephelometry before sonication.

relative humidity combined with the additional moisture of the aerosolized organisms caused repeated electrical shorting of the air sampler. It was not possible to maintain a potential of 25,000 v. or 20,000 v. between the collecting orifice and the positively charged rotating impaction table. For adequate collection, 20,000 v. would appear to be the minimum. A part of this test was conducted at 5,000 v. potential with resultant inability to collect sufficient organisms. Subsequently, other tests were conducted at relative humidities varying from 23% to 67%. At these humidities, only occasional difficulty was encountered in maintaining the 25,000 v. potential and this occurred at relative humidity conditions above 65%. To use a sampler of this type under conditions of high relative humidity, some engineering modifications would have to be made to reduce the relative humidity as the air passes through the intake.

Subsequent to the field test, it was learned by further laboratory investigations that the *E. coli* used for field trials was strain 0119:B14:H6. This did not react in the BAIT as strongly as strain 0111ab:B4:H12 upon which the BAIT had been precalibrated. In addition, the organisms that were aerosolized had been killed with beta-propiolactone. Laboratory tests later showed that beta-propiolactone appears to partially inhibit disruption of *E. coli* by sonication. It may also directly or indirectly affect the preparation of a satisfactory antigen extract. These observations together with the low concentrations of *E. coli* that were aerosolized could account for the inability of the BAIT to identify *E. coli* 0119:B14:H6.

Further studies showed that incorporating the organisms in 0.1 M gelatine phosphate makes only a negligible difference in disruption by sonication. Extracts of organisms with and without gelatine phosphate reacted similarly in the BAIT. This fact again points to beta-propiolactone as one of the factors adversely influencing the identification or preparation of a satisfactory sonicate of *E. coli* in the field tests.

From observations with TAMIS and the aerosol identification with BAIT, precepts can be formulated. These could be used to evaluate a microbiologic identification system applied to aerosols.

1. The system air sampler must provide efficient collection in marine, coastal, and inland environments at relative humidities and air compositions anticipated in both day and night operations.

2. The system must have constant or intermittent measurement of the organism population during recovery in order that subsequent processes will be programmed only when sufficient organisms have been collected.

3. The system must give unambiguous identification at a remote site for whole bacterial suspensions as well as for solutions and fractions thereof, including strains known or anticipated in biologic operations.

4. The system must have a processing and identification method that can not be counteracted by known or anticipated aerosol diluents, chemical potentiators, partial detoxification, carrier proteins, or fragments of protein or protein conjugates.

5. The system must be resistant to "jamming" by the addition into the aerosol of innocuous bacteria or substances similar to but not identical to the primary agent.

6. The system must provide unambiguous identification for both low and high concentrations of organisms. The signal-to-noise ratio in an instrumented readout must be adequate for low concentrations and distortion-free for high concentrations of organisms.

CONTRIBUTIONS

The purpose of the TAMIS within the framework of the original concepts of the Bio-courier project was to conduct research that would examine the feasibility of a telemetric immunoelectronic approach for identification and differentiation of certain biologic substances. In this regard, feasibility has been demonstrated and specific contributions to and extensions of existing knowledge have been made in both electronics and immunobiology. These are:

1. *Electronics and instrumentation*

a. Fabrication of portable field instruments for initiating, taping, and transmitting by radio or telephone six kinds of information concerned with densitometry.

b. Considerations in field instrumentation for recognizing and minimizing errors.

c. Automatic instrumentation for layering one reactant on top of another in gel diffusion columns.

d. Semiautomatic technics for the preparation of bacterial extracts by sonication and filtration.

e. A method for detecting mistakes in the selection of preloaded reactant columns.

f. An instrument modification for marking glass tubes.

g. A binary coded decimal system of 60 bits (15 numbers) that can be used to characterize each precipitin reaction for storage of data on magnetic tape and subsequent retrieval by automatic tape searchers.

h. Development and establishment of the versatility and capability of a laboratory console, TELUS, for receiving, storing, comparing, retrieving, integrating, and relaying immunobiologic, biochemical, and physiologic data from remote sites using both short- and long-range (8,000 miles) telemetry.

2. *Immunobiology*

a. Procedures for standardization of bacterial extracts prepared by sonication.

b. Technics for the utilization of differentiating antisera of the precipitin type.

c. Parameters of concentration, time, and temperature pertinent to the use of precipitin reaction for the identification of bacteria.

d. Differentiation and identification of enteric bacteria by quantitation of precipitin reactions.

e. Validation of the adequacy of the precipitin system for the identification of certain bacteria in aerosols.

f. Establishment of certain criteria to be considered in the problems associated with bacterial identification in aerosols.

g. A method of preparing, storing, and retrieving vials of biologicals stored in liquid nitrogen.

h. Evaluation of the potential of the precipitin system for identification of viruses.

There are numerous applications of these immunobiologic and electronic contributions for terrestrial and aerospace problems. The differentiation of numerous biologic solutions not demonstrated by these studies has, for the most part, either already been done by less sophisticated methods or can logically be assumed to be possible from present knowledge. The future use of the TAMIS and associated subsystems in the field, clinical, or aerospace laboratory depends upon the administrative and scientific recognition of problems consistent with the capability.

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