Indirect Fluorescent Antibody Test for Malaria Antibody

WILLIAM A. SODEMAN, Jr., M.D., and GEOFFREY M. JEFFERY, Sc.D.

THE INDIRECT fluorescent antibody (FA) test for malaria antibody, which has been used extensively in a few research centers, has a demonstrated utility in clinical and research investigations of malarial infections in man and experimental hosts. Saliou (*1*) and Tobie (*2*) have previously reviewed the application of this method to studies on malaria. Specificity of the tests has been discussed in the literature, but so far as we know there is no published evaluation of the test for sensitivity, reproducibility, and ease of application. Our study seeks to point out and discuss sources of error and limitations in interpretation resulting from the way the technique is applied. The method we evolved and used will be presented in detail.

Methods

In a general sense, all investigators presently using the FA test for malarial antibody have adopted the method described by Kuvin and associates (*3*). Air-dried thin films of parasitized blood are used as antigen preparations, and these slides are treated as follows:

Steps of procedure	*Minutes*
0.1 N HCl	5
Distilled water (dip)	-----
PBS pH 7–7.2 (first wash)	5
PBS pH 7–7.2 (second wash)	5
Test serum	20–30
PBS pH 7–7.2 wash	10
Specific conjugated antiglobulin	20–30
PBS pH 7–7.2 wash	10
Mount in buffered glycerin pH 7–7.2	-----

NOTE: PBS—phosphate-buffered saline.

Smears either are used fresh or are preserved in a freezer until time for treatment and are then brought to room temperature in a dessicator. The slides are treated at room temperature, the films not being permitted to dry between steps of the procedure. After the smears have been mounted, they are examined for fluorescence on a suitable microscope.

For the fluorescence microscopy in the present study a Leitz SM fluorescence microscope (*A*) with an Osram HB–200 light source (*B*) was used with a UG–2 or BG–12 exciter filter and a UV absorbing secondary filter. The parasites were located by using the BG–12 filter; the degrees of fluorescence were determined with the UG–2 filter. Fluorescence is graded on an arbitrary scale of 0 (no fluorescence) to 4+ (maximum fluorescence). Titers are derived from the final dilution of test serum in which fluorescence can be demonstrated.

In adapting the method of Kuvin and associates to a specific test protocol, we have attempted to adhere to recognized principles in the performance of serologic tests. Specifically our concerns have been with reagent standardization, quantitation of antigen, and

Dr. Sodeman is chief, gastroenterology section, Veterans Administration Hospital, Ann Arbor, Mich., and instructor in medicine, University of Michigan Medical School, Ann Arbor. Dr. Jeffery is acting chief, Laboratory of Parasite Chemotherapy, National Institute of Allergy and Infectious Diseases, Public Health Service.

with total volume of reactants, all of which require particular consideration within the mechanics of the test as described. We have evolved the following protocol:

Air-dried thin films of parasitized blood are prepared for testing by delineating circular areas of uniform size, hereafter called wells. Our practice is to prepare each slide with three wells 8 mm. in diameter. The wells are formed by printing the pattern with a spongelike plastic foam templet saturated with a 4 percent water-soluble silicone compound, Siliclad ®(C). The only areas of the slide not covered by a silicone film are the wells. The slides are dried on their sides in racks at 37° C. for 30 minutes, the position of the wells being marked with a diamond scribe. After the drying period, the slides are passed through 0.1 N HCl for 5 minutes, through distilled water (a dip), and finally, through two phosphate-buffered saline pH 7.2 washes of 5 minutes each. After removal from the final wash, the slide is tapped on its side on an absorbent surface. Aqueous solutions will normally adhere only to the area of the wells; occasional droplets persisting elsewhere on the slide may be removed by touching them with absorbent paper. In this manner, wells of a uniform size containing uniform volumes of PBS may be quickly formed.

Serums to be tested are initially diluted 1 to 5 with PBS and then are prepared in serial threefold tube dilutions with PBS as a diluent. Dilutions are prepared by a loop microtitration technique commonly used in virus titrations (*4*). This method yields 0.05 ml. of each dilution of test serum with an error of ±1 percent. The test is quickly performed, speed being increased without sacrifice of accuracy. To maintain a uniform volume of reactant, the total volume of each dilution is aspirated from the diluting cup and transferred to the antigen well. A melting point capillary fitted with a perforated rubber bulb provides a convenient disposable transfer pipette. Slides are placed in a moist chamber at room temperature for 20 minutes. After the reaction period, slides are rinsed with PBS and washed in PBS on a slide rotator for 10 minutes. After the washing, slides are removed from the PBS and tapped on their sides to remove excess PBS as previously described. Fluorescein-conjugated globulin specific for the type of serum to be tested is added to each well; 0.05 ml. of a globulin solution is used for each well. Slides are again placed in a moist chamber for 20 minutes and rinsed, then washed in PBS for 10 minutes. After the slides are removed from PBS, a drop of 0.02 percent Evans blue is placed on each well as a counterstain. Reaction is permitted in a moist chamber for 15 minutes. A PBS rinse follows, then a 10-minute PBS wash on a slide rotator. Smears are mounted in buffered glycerin (pH 7.2) for viewing. Titers are derived from the final dilution of test serum in which fluorescence of 2+ or greater can be demonstrated. We found this endpoint, which was suggested by Voller and Bray (*5*), to be the most reproducible and easily observable one.

Results and Discussion

In the titration of test serums for the endpoint of fluorescence, most investigators have used serial twofold dilutions. A change of two or more tube dilutions has generally been considered to represent a significant change in antibody titer. In our hands, replicate titrations of a single serum diluted in this fashion did not, however, result in consistent endpoints within the range of a single ± tube dilution; about 1 in 20 titrations varied from the mode by 2 dilutions (table 1). We believe that this inconsistency reflects the cumulative error inherent both in the multiple manipulations and in the assumptions associated with the test as performed in our laboratory—an error which efforts at standardization of reagents and techniques failed to eliminate. Because the test apparently could not be depended upon to distinguish antibody concentrations differing by as much as three twofold tube dilutions, we adopted a protocol using threefold dilutions. When the test was performed as outlined previously, but using this protocol, antibody titers on replicate samples remained within a single ± threefold tube dilution of the modal titer. We tested this protocol by replicate observations on individual serums (table 1) and consider that by this method a difference of two or more threefold dilutions represents a significant change in antibody titer. This protocol decreases the sensitivity of the test but makes interpretation easier.

Table 1. Comparison of twofold and threefold dilution of serums for indirect fluorescent antibody determinations

Serum and antigen	Titer	Times observed
	Serial twofold dilutions [1]	
Atlanta A with Atlanta I, *Plasmodium cynomolgi* R	1:640	0
	1:320	4
	1:160	9
	1:80	9
	1:40	0
174/9/15 with Atlanta II, *P. cynomolgi* R	1:640	0
	1:320	4
	1:160	15
	1:80	9
	1:40	2
	1:20	0
173/9/15 with Atlanta II, *P. cynomolgi* R	1:2, 560	0
	1:1, 280	1
	1:640	3
	1:320	2
	1:160	1
	1:80	0
	Serial threefold dilutions [2]	
173/9/15 with Atlanta II, *P. cynomolgi* R	1:3, 645	0
	1:1, 215	6
	1:405	12
	1:135	9
	1:45	0

[1] Dilutions started at 1:10.
[2] Dilutions started at 1:5.

Evidence that the mechanics of the test may affect its sensitivity prompted us to examine carefully each of the materials and methods used in the test, including the antigen, the test serum, the specific antiglobulin conjugate, and the method of counterstaining.

Antigen. The use of thin films of parasitized blood as the antigen preparation raises several questions about uniformity of this reactant. We are not aware of an infallible method to prepare large numbers of thin blood films of a uniform cell density and cell distribution. For this purpose we prepare as many films as practicable from a single source of parasitized blood, attempting in each film to obtain approximately the same thickness (cell density) and an even distribution of cells in the central areas which are to be occupied by the wells. Slides which are obviously too thick or too thin or which lack uniformity are discarded. Within the recognized limitations, among a selected set of slides prepared from a single source of parasitized blood, the total antigen content of each well can be considered relatively uniform as to parasite size and number.

While this crude quantitation of antigen may assure reasonable consistency of the total amount of antigen in each test preparation and thus the number of antibody binding sites, there are other problems related to the antigen. The amount of fluorescence within the test well is seen as discrete fluorescent units or masses (parasites) rather than diffuse fluorescence of the total quantity of antigen in the well. Furthermore, in this case, fluorescence represents light emitted by fluorescein-tagged antibodies bound to antibodies which are, in turn, bound to antigen. This situation is further complicated because fluorescence is measured in subjective units of brightness rather than as a precise physical unit. The comparative size of the parasites on the antigen slide apparently materially affects the brightness of fluorescence; that is, large parasites are brighter than small parasites. In titrations of FA endpoints, fluorescence of the small parasites (rings) on a blood film will fall to less than 2+ two dilutions before that of the larger parasites (schizonts). Thus, test sensitivity will vary by two tube dilutions depending on the size of the parasites used as an indicator. When the staining titers of a serum are compared among a number of different antigen preparations, a slide containing many small parasites may not be positive, while one of the same species with a few large parasites may show strong fluorescence. The only solution seems to be to use a single lot of antigen slides for a single experiment and to recognize that significant changes in titer may occur with a change in lot of the antigen slides.

Another difficulty that we have encountered with antigen is in its in vivo binding of antibody. Such binding has been reported in *Plasmodium berghei* infection in mice (*6*), and we have demonstrated this binding phenomenon in *P. cynomolgi* infection in rhesus monkeys when slides were obtained late in the course of infection. To insure the desired reproducibility of the test, antigen should be obtained before significant specific antibody appears.

Test serum. We have already discussed the use of threefold dilutions rather than twofold dilutions. An equal volume of test serum should be placed on each well. This volume helps determine the sensitivity of the test, for if the volume varies from one titration to the next, replicate determinations cannot be made. The simplest illustration of the effect of serum volume that we have been able to perform was as follows: A slide was prepared with three wells. The first received 0.05 ml. of the titer (endpoint) dilution of a serum that had been previously run. The second well received 0.1 ml. of the next higher dilution, and the third well received 0.2 ml. of the next higher dilution. Reaction was carried out per protocol. All three wells stained with the brightness of 2+ as confirmed by three observers. This result suggests that to maintain a uniform sensitivity, constant quantities of each dilution must be applied.

Specific antiglobulin conjugate. Quantitative considerations with regard to antigen and test serum are less important with specific antiglobulin conjugate than with other reagents provided one rule is observed. Enough conjugated antiglobulin must be added to saturate the binding sites of all globulin in the antigen preparations. For economy, we use the maximum effective dilution of conjugate. We determine this for each lot of conjugated antiserum, using a known positive test serum to find the highest dilution of conjugate that will still give maximum fluorescence.

A detailed discussion of the techniques of conjugation is beyond the scope of this paper. We recommend the methods of Marshall and associates (*7*) for conjugation and those of Riggs and associates (*8*) for fractionation of the conjugate. Conjugated globulin obtained by diethylaminoethyl (DEAE) cellulose fractionation of tagged serum is our reagent of choice. This reagent proved superior to conjugates absorbed with tissue powder, for it resulted in less background staining. Suitable commercial products are available. By using single lots of serum for each experiment, we have avoided the necessity for more sophisticated reagent standardization. When indicated, determination of fluorescein-protein ratios and use of quantitative precipitin tests would permit better standardization.

Counterstain. Diggs and Sadun (*9*) suggest the use of Evans blue as a counterstain, and we have found this reagent of singular help in determining the endpoint of the titration. Without a counterstain, the fluorescence falls gradually from 4+ to the 0 level of background fluorescence. The color change is from bright apple-green to faint apple-green. With the counterstain, the color changes from bright apple-green to red. The change is distinct, sharp, and less questionable from the subjective point of view than without a counterstain. Properly applied, the counterstain appears to cause little, if any, artifact from the quenching of specific staining.

The question has been raised of comparability of results from the various centers currently performing the test. We sent questionnaires to most of the investigators using this test, and a summary of the methods they reported appears in table 2. There is wide variation in the specific method. For example, there are wide differences in the areas of the slide stained (from 0.2 to 9.35 sq. cm.), and in the volumes of the test serum applied to the slide (from 0.05 to 0.5

Table 2. Variability of methods applied by a number of investigators

Variables	Investigators					
	A	B	C	D	E	F
Quantity of test serum applied to slide	0.05 ml	2 drops	1 drop	2–3 drops	0.5 ml	2 drops.
Fixation of antigen slide	Nil	Nil	Acetone	Acetone	Nil	Acetone.
Area of well	0.58 sq. cm.	1 sq. cm.	0.2 sq. cm.	0.54 sq. cm.	9.35 sq. cm.	1 sq. cm.
Preparation of conjugate	DEAE	Tissue powder.	Tissue powder.	Tissue powder.	Tissue powder.	Tissue powder.
Counterstain	Yes	No	No	No	No	Yes.

ml.). In the absence of careful comparison of the results obtained by various laboratories on the same serums, there can be little meaningful comparison at this time between titers obtained by the various centers.

Summary

The indirect fluorescent antibody test for malaria antibody can provide a useful measurement of the immunological status during malarial infection. Variations in the method of performance of the test, however, may alter its sensitivity and reproducibility. In antigen preparation, special attention should be directed toward obtaining smears with a uniform distribution of parasites and toward maintaining uniformity of size in the area to be stained. The size or stage of the parasites to be stained will also affect the interpretation of results. Uniform quantities of the serum dilutions should be used for staining. The sensitivity of the test must be adjusted to the precision of performance. Use of Evans blue as a counterstain simplifies the reading of these tests for malarial antibodies.

REFERENCES

(*1*) Saliou, P.: Diagnostic sérologique du paludisme humain par l'immuno-fluorescence. Bosc Frères, Lyon, France, 1964.

(*2*) Tobie, J. E.: Detection of malaria antibodies—Immunodiagnosis. Amer J Trop Med & Hyg 13: 185–203 (1964).

(*3*) Kuvin, S., et al.: Antibody production in human malaria as determined by the fluorescent antibody technique. Science 135: 1130–1131 (1962).

(*4*) Sever, J. L.: Application of a microtechnique to viral serologic titrations. J Immun 88: 320–329 (1962).

(*5*) Voller, A., and Bray, R. S.: Fluorescent antibody staining as a measure of malarial antibody. Proc Soc Exp Biol Med 110: 907–910 (1962).

(*6*) Kreier, J. P., and Ristic, M.: Detection of a *Plasmodium berghei*-antibody complex formed in vivo. Amer J Trop Med & Hyg 13: 6–10 (1964).

(*7*) Marshall, J. D., Eveland, W. C., and Smith, C. W.: Superiority of fluorescein isothiocynate (Riggs) for fluorescent-antibody technic with a modification of its application. Proc Soc Exp Biol Med 98: 898–900 (1958).

(*8*) Riggs, J. S., Loh, P. C., and Eveland, W. C.: A simple fractionation method for preparation of fluorescein-labeled gamma globulin. Proc Soc Exp Biol Med 105: 655–658 (1960).

(*9*) Diggs, C. L., and Sadun, E. H.: Serological cross-reactivity between *Plasmodium vivax* and *Plasmodium falciparum* as determined by a modified fluorescent antibody test. Exp Parasit 16: 217–223 (1965).

EQUIPMENT REFERENCES

(*A*) Osram HBO–200 lamp, Unex Products Corp., New York, N.Y.

(*B*) Leitz model SM fluorescence microscope, E. Leitz, Inc., New York, N.Y.

(*C*) Siliclad®, Clay Adams Co., New York, N.Y.

Selective Typhoid Immunization

The Public Health Service Advisory Committee on Immunization Practices advises against routine typhoid immunization in the United States. Instead, it recommends selective immunization for people intimately exposed to a known typhoid carrier as would occur with continued household contact, living in communities or institutions with outbreaks of typhoid fever, or traveling to foreign areas where typhoid fever is endemic.

The committee also recommends discontinuing the practice of vaccinating people in summer camps and in flooded areas. It holds that paratyphoid A and B antigens when combined with typhoid vaccine may increase the occurrence of vaccine reactions and consequently discourages the use of paratyphoid A and B vaccines.

The committee states that less than 500 cases of typhoid fever are reported in the United States annually and a continuing downward trend can be expected.

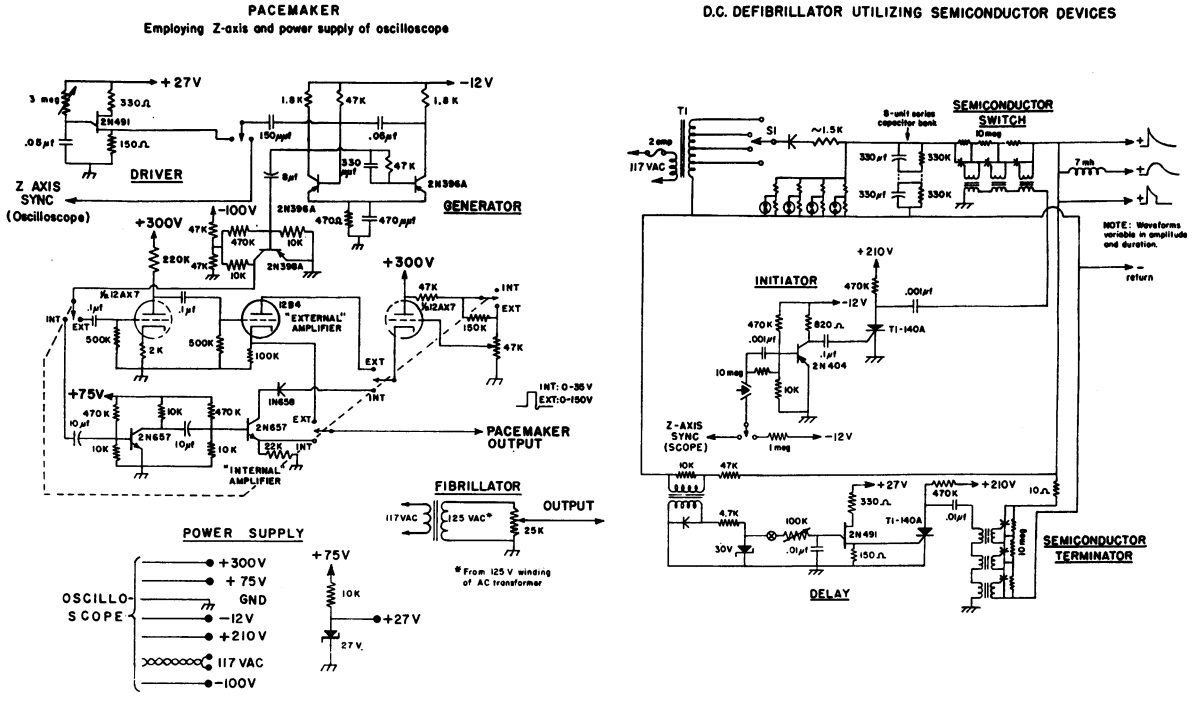
Single Unit Cardiac Monitor-Defibrillator-Pacemaker

Rapidly accumulating information concerning cardiac arrest points to the need for a single unit device for monitoring, d.c. defibrillation, and pacemaking that is readily available, simple to operate, reasonable in cost, and of maximum efficiency. Time, the crucial factor in such cases, must be minimized. The simpler the combined unit, the greater the chance of successful application in an extreme emergency; the cheaper the cost of such a unit, the more likely will one be available near a stricken person.

Recently developed, elaborate cardiac units have demonstrated convincingly the opportunities for reducing mortality by prompt observation, diagnosis, and treatment of cardiac arrest. The comprehensive units are, however, still too cumbersome, heavy, and complicated for portability. A compact, inexpensive, but highly efficient combined unit could be placed in every surgical operating room, emergency suite, cardiac patient's hospital room, and ambulance.

The unit illustrated combines the three effective modalities. It has one power cord. Combining the three units in a single small cabinet eliminates duplication of many parts, thus reducing weight to approximately 38 pounds—a nurse could carry the apparatus if necessary. In addition, it has only one cable

Circuit diagram of the single unit



NOTE: Six 1,600-volt silicon-controlled rectifiers make possible rapid charge and discharge of the condensers. A maximum 200-volt transistor makes possible a pacemaker without vacuum tubes.

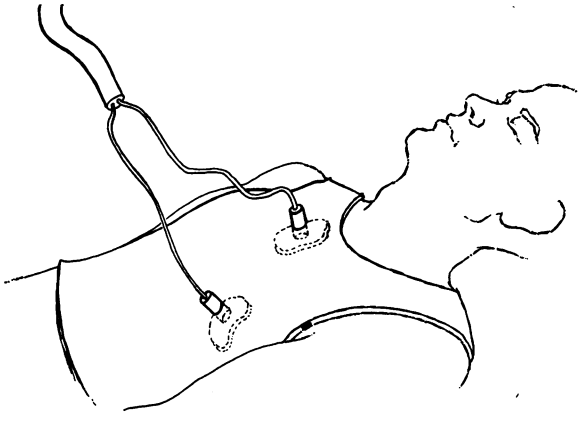
to the patient. The contour-fitted defibrillating-type monitor electrodes produce an electrocardiogram of the highest fidelity, although the electrodes are placed in the customary position for defibrillation. The electrodes used for monitoring can be instantly converted to defibrillating or pacemaking use. Thus, cardiac disaster can be treated the instant it is indicated by the monitor.

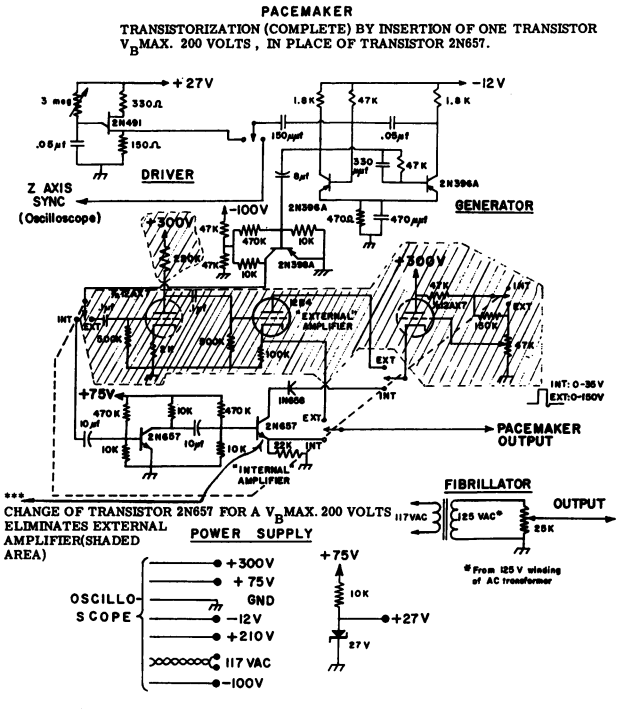
Although large, cumbersome, complicated units sell for approximately $4,000, the unit described can be produced to sell for about $1,200. The instrument panel is so simple that its operation can be learned instantly. The low cost, compactness, and simplicity of operation of the unit make mass production and therefore widespread distribution possible.—J. Robert Close, M.D., *Orthopaedic Hospital, Los Angeles, Calif.*

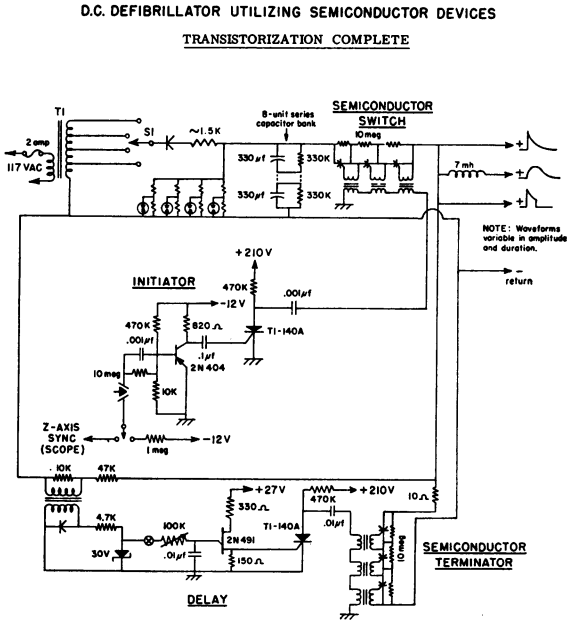
This invention was developed under Public Health Service grant No. NB–2300.

cardiac monitor-defibrillator-pacemaker

Electrodes incorporated in a "vest" are convenient and reliable for use with patients prepared for surgery

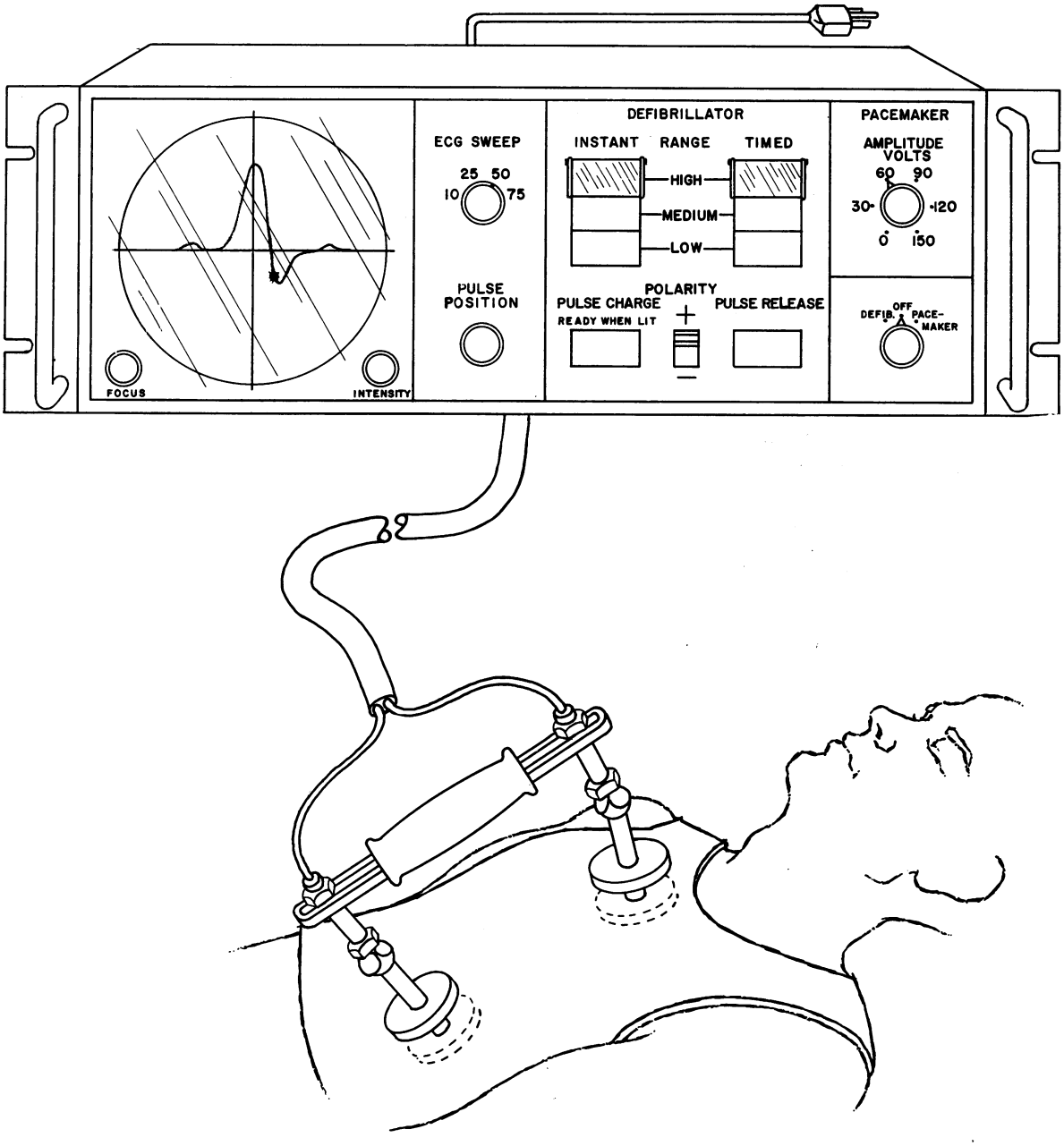






(over)

Electrodes (physically separated) can be immediately applied in an emergency



NOTE: The electrodes are bipolar and ground isolated. The chassis is grounded. No ground wire is connected to the patient lest stray voltages occur. The bar separating the electrodes is a safety device for the individual operating the unit.

Federal Publications

Hepatitis. *PHS Publication No. 446, Health Information Series No. 82; revised 1966; leaflet; 5 cents, $2 per 100.* Defines hepatitis and explains the difference between infectious hepatitis and serum hepatitis. Describes symptoms, recommends treatment by a physician, and advises how to reduce risk of infection.

Tapeworm. *PHS Publication No. 158, Health Information Series No. 48; revised 1966; leaflet; 5 cents, $2 per 100.* Describes beef, dwarf, and pork tapeworms that occur in humans. Warns against self-diagnosis and self-treatment. Explains preventive methods.

Ringworm. *PHS Publication No. 46, Health Information Series No. 6; revised 1966; leaflet; 5 cents, $2 per 100.* Describes symptoms, preventive practices, and warns against self-diagnosis and self-treatment for ringworm of the feet (athlete's foot), nails, body, and scalp.

Public Health Service Film Catalog, 1966. *PHS Publication No. 776; revised March 1966; 99 pages; $1.* Revised list of health and medical films available from the Public Health Service Audiovisual Facility, Communicable Disease Center, Atlanta, Ga.

Hospital Environment Makes the Difference. *PHS Publication No. 930–C–14; 1966; leaflet.* Outlines the significance of the hospital environment to patients, staff, and community in terms of infection control, personnel safety, and hospital utilization. Summarizes the Division of Hospital and Medical Facilities' programs in research, education, and consultation specifically directed to solving hospital environmental problems.

Film Reference Guide for Medicine and Allied Sciences. *PHS Publication No. 487; revised 1966; 397 pages; $2.50.* Includes entries for selected medical films and filmstrips that are useful in the medical training programs of one or more of the member agencies that comprise the Federal Advisory Council on Medical Training Aids. Members include Department of the Army, Department of the Navy, Department of the Air Force, Veterans Administration, and the Public Health Service. Provides film descriptions arranged by subject with a title index and a category listing. Lists materials currently available for borrowing or renting; no films listed are for sale only.

Reported Tuberculosis Data, 1964. *PHS Publication No. 638; 1966; 41 pages; 30 cents.* Presents data and statistics on tuberculosis morbidity and mortality and includes recent information about the status of the disease in the United States. Points to changes that have influenced recent trends and pinpoints the variety of components that require consideration in assessing the applicability and effectiveness of local, State, or national tuberculosis control activities.

Grade "A" Pasteurized Milk. *PHS Publication No. 1011; 1966; leaflet; 5 cents.* Gives the history of the Cooperative State-PHS Program for Certification of Interstate Milk Shippers. Describes the operation of the program, its growth, benefits, and accomplishments.

Elementary Rehabilitation Nursing Care. *PHS Publication No. 1436; 1966; 100 pages, illustrated; 55 cents.*

Presents a manual for teaching the fundamentals of rehabilitation nursing care to nursing and ancillary personnel in nursing homes, hospitals, convalescent facilities, and public health agencies. Employs practical and effective rehabilitation procedures based on the use of inexpensive equipment and a minimum of personnel. Provides the nursing personnel, through application of the principles and training techniques, with better understanding of the physical, mental, and social needs of the patient as a basis for improving nursing care, patient morale, and job satisfaction of nursing staff.

The manual was prepared by the public health nursing section of the Colorado State Department of Public Health as part of a demonstration project to determine the extent of rehabilitation care that can be maintained by nursing personnel and the rehabilitation nursing that can be taught on an inservice basis.

Depuration Plant Design. *PHS Publication No. 999–FP–7; 1966; 119 pages; 75 cents.* Outlines technical points in the design of shellfish depuration plants which safeguard oysters, clams, and mussels through the purification process known as shellfish depuration.

Radionuclide Analysis of Large Numbers of Food and Water Samples. *PHS Publication No. 999–RH–17; 1966; by Esther Ferri, Paul J. Magno, and Lloyd R. Setter; 28 pages.* Describes the processing of large numbers of food and water samples for gross and specific radioactivity as conducted by a regional laboratory of the Division of Radiological Health. Describes techniques for a series of radionuclides of interest and for certain stable elements in foods, gross alpha and beta determinations, gamma spectral analysis, and determination of radium 226 and strontium 90 in water. Briefly discusses manpower and equipment requirements for various measurements.

This section carries announcements of new publications prepared by the Public Health Service and of selected publications prepared with Federal support.

Unless otherwise indicated, publications for which prices are quoted are for sale by the Superintendent of Documents, U.S. Government Printing Office, Washington D.C., 20402. Orders should be accompanied by cash, check, or money order and should fully indentify the publication. Public Health Service publications which do not carry price quotations, as well as single sample copies of those for which prices are shown, can be obtained without charge from the Public Inquiries Branch, Public Health Service, Washington, D.C., 20201.

The Public Health Service does not supply publications other than its own.

PHR SYNOPSES

STOCKWELL, EDWARD G. (University of Connecticut): *Use of socioeconomic status as a demographic variable. Public Health Reports, Vol. 81, November 1966, pp. 961–966.*

The relations between the three processes of demographic change (fertility, mortality, and migration) and socioeconomic status have been analyzed. The data show that levels of fertility and migration tend to be positively associated with socioeconomic status, whereas levels of mortality are negatively correlated with all the various socioeconomic indexes: occupation, education, income. The data also show that the demographic variables are not necessarily related in any consistent fashion to all the socioeconomic variables. Mortality, for example, was more related (negatively) to income than to any other variable whereas education appeared (positively) to be the most significant factor in the migration socioeconomic differential.

Two conclusions have been drawn on the basis of these observations. First, social and economic factors are major determinants of demographic behavior, but there is a need for more research to discover more precisely the nature and extent of the relations between particular demographic variables and particular indexes of socioeconomic status. Second, research in this endeavor must clearly be cognizant of the multidimensional nature of socioeconomic status. What is needed is a research approach which recognizes that socioeconomic status is many things: that it is composed of a number of different variables, that each of these variables may act independently of one another in specific situations, and that each does not necessarily have to bear the same relation to any given behavioral phenomena.

EISENTHAL, SHERMAN (Veterans Administration), FARBEROW, NORMAN L., and SHNEIDMAN, EDWIN S.: *Followup of neuropsychiatric patients in suicide observation status. Public Health Reports, Vol. 81, November 1966, pp. 977–990.*

A followup study was conducted of 912 patients in a Veterans Administration neuropsychiatric hospital who had been placed in suicide observation status between 1954 and 1958. Complete followup data were obtained for 90 percent of the patients. Forty percent of these patients subsequently manifested further suicidal behavior; 6 percent committed suicide, 17 percent made nonlethal suicidal attempts, and 17 percent manifested suicidal ideation. Analysis of the patients' suicide histories, demographic information, and neuropsychiatric hospitalization data did not reveal any single pattern typifying the suicidal person.

The success of the best predictors in forecasting suicide was from 8 to as high as 13 percent, while the best predictors of attempted suicide forecast the event with success ranging from 23 to as high as 29 percent. In this particular population, suicide is much more probable than in the neuropsychiatric hospital population or the general population.

BOURDILLON, JAQUES (New York State Department of Health), and VANDERLINDE, RAYMOND E.: *An improved screening procedure for blood phenylalanine. Public Health Reports, Vol. 81, November 1966, pp. 991–995.*

An improved method is described for the automated screening of blood phenylalanine. When dry blood spots on filter paper are exposed to hot steam, the proteins are irreversibly bound to the paper, whereas the other solutes can be subsequently released by water elution. This step takes the place of dialysis and permits bypassing the dialyzer in the Technicon automated methods, resulting in a fivefold increase in sensitivity. Dry blood disks containing added phenylalanine and subjected to autoclaving for 3 minutes at 121° C. gave a phenylalanine recovery of about 75 percent. Under controlled conditions, this figure was constant and independent of the concentration. The fluorescence of nonphenylalanine substance was equivalent to only 0.8 mg. of phenylalanine per 100 ml.

With a smaller fluorometric cell, the method could be run at 60 specimens per hour without carryover. A modified pump allowed the speed to be increased to 120 specimens per hour. The method, which requires only a one-quarter-inch paper disk of dried blood, is considered accurate enough for screening purposes and sensitive enough to detect borderline cases.

PHR

SYNOPSES

COATS, G. I. (Public Health Service), and GOLDIN, A. S.: ***Energy alinement of gamma spectrometers. Public Health Reports, Vol. 81, November 1966, pp. 999–1007.***

One of the key requisites to proper operation of a gamma spectrometer is accurate energy alinement. Prominent among the characteristics of such systems which can affect energy alinement are the nonlinear response of scintillation crystals, the effects of the geometry of the source, and electronic performance—such as the variability in response to different counting rates and the shifts which occur in the zero and gain settings.

Regular checking of instruments, followed by any corrective adjustments indicated, is requisite to proper operation of a gamma spectrometer. In addition, control charts for documenting and monitoring instrument performance need to be set up. Regular application of such procedures will provide continual assurance that the data obtained from such a system are precise and accurate. Under normal conditions, information thus obtained will afford an accurate foundation on which to base public health evaluations of given radiological contaminations. In the event of elevated levels, the accuracy of such data will be indispensable to the application of sound procedures for corrective public health action.

The recommendations in the paper are directed particularly to public health personnel who have not had extensive experience in the detailed use and calibration of gamma spectrometers.

FEORINO, PAUL M. (Public Health Service), and HANNON, WILLIAM H.: ***Use of DEAE dextran in agar overlays to enhance size of ECHO virus plaques. Public Health Reports, Vol 81, November 1966, pp. 1015–1018.***

Many ECHO virus strains produce minute, faint plaques on monolayers of rhesus monkey kidney when the plaque inhibition test is performed by standard overlay technique. Plaques of some types are so small or so faint that work with them is difficult.

The authors tested the effect on plaque size of overlaying plates of ECHO virus types 1 through 27 with agar containing diethylaminoethyl dextran (DEAE-D). The presence of DEAE-D in the agar overlay enlarged the plaques of ECHO viruses types 1, 2, 3, 5, 6, 9, 13, 20, 25, and 26. Addition of DEAE-D also enhanced the size of contaminating simian viruses.

All types of ECHO viruses produced discernible plaques in the presence of DEAE-D, and its presence in the agar apparently did not harm the monkey kidney cell layer. Its only obvious effect was to change the plaque characteristics of some ECHO virus types.

KATZ, GERALD (Beth Israel Medical Center, New York City): ***Pilot study of medical practices in medical arts buildings. Public Health Reports, Vol. 81, November 1966, pp. 1025–1030.***

The results of a pilot study suggest that a range in the organization of medical practice exists, with solo practice at one extreme and group practice at the other. In addition, differences exist between physicians who establish arrangements with associates and those who do not. These arrangements, relating to coverage of practice and sharing of income, expenses, personnel, equipment, and some responsibility for patient care, suggest that practice in association has some characteristics of group practice.

The data indicate that medical arts buildings in the District of Columbia are chosen by a large proportion of practicing physicians as a site of office practice. Both the large number of physicians and broad range of specialties represented, despite the virtual absence of pediatricians and general practitioners, suggest that a survey of practices in medical arts buildings is valuable in studying the organization of private medical practice.

Perhaps this pilot study will help to bridge the gap in information about the organization of medical practice. It is hoped that it will provoke similar studies in other areas of the country, as well as studies examining the situation in greater depth.

PHR SYNOPSES

HULKA, BARBARA S. (University of Pittsburgh): *Motivation techniques in a cancer detection program. Public Health Reports, Vol. 81, November 1966, pp. 1009–1014.*

Mailing a notice with welfare checks was an effective method to stimulate medically indigent women to obtain an examination in a cervical cancer control program in Pittsburgh and Allegheny County, Pa. Of 23,000 women contacted 7,221 or 31.4 percent returned cards requesting a clinic appointment.

Of the 7,221 women requesting appointments, 3,719 or 51.5 percent came for examination. A prolonged delay in giving appointments because of limited personnel and examining facilities probably decreased clinic attendance. If the first clinic appointment was not kept, second and third appointment notices were sent. Among all women attending clinics, 28.7 percent came in response to the second notice and 7.9 percent in response to the third notice.

If three appointment notices were ignored, a telephone call was made, but even a skilled interviewer had limited success by telephone in convincing women to attend a clinic. About 10.2 percent of total calls resulted in clinic visits.

Home visiting of nonrespondents was superior to telephoning in one respect: The most financially deprived women did not have telephones. However, a response of 17.1 percent (49 women attended a clinic as the result of 287 home visits) hardly warrants the time and expense required for home visiting.

Nurse and caseworker referrals brought a greater percentage of the women contacted to examination than a notice with the welfare checks. Health department nurses were especially effective. Of 1,479 women contacted, 447 or 30.3 percent attended clinics, whereas the mailing technique effected a clinic attendance of 16.1 percent of the target population.

Unkept appointments were a major problem. Approximately one of every three women who requested appointments through nurses and caseworkers actually attended a clinic.

GREENBERG, JEROME H. (U.S. Army Medical Corps), SCHMIDT, EDWARD A., and BELL, FRED S., Jr.: *A common source epidemic of shigellosis. Public Health Reports, Vol 81, November 1966, pp. 1019–1024.*

An outbreak of illness following a bowling league banquet did not come to the attention of public health personnel until it had been underway for a week. Investigation was then undertaken by Fort Sam Houston and the San Antonio Metropolitan Health District.

Questionnaires were completed by 276 of the 320 persons who attended the banquet. One hundred ninety-six persons reported that they had been ill. Of these, 104 required medical attention. Fifty-six patients yielded stool specimens positive for *Shigella flexneri 4a*. All of those whose stool cultures were positive and many others whose stools were not cultured received tetracycline compounds. At the completion of the investigation, 217 persons, including the 56 whose stool specimens were initially positive, yielded at least one negative specimen.

On the basis of food-consumption histories, potato salad served at the banquet was the suspected vehicle of transmission. Subsequent investigation revealed that the potatoes had been handled under very insanitary conditions after cooking and that several periods existed during which the potatoes and later the potato salad were not refrigerated. No history of illness was elicited from the workers who prepared the food for the banquet, and rectal swabs taken from them 9 days later were negative for causative organisms.

The findings show the difficulties inherent in detecting the source of a food infection outbreak which has already been underway for a week. The necessity for maintaining high standards of sanitation during food preparation and the need for adequate refrigeration of prepared foods are clearly demonstrated.

PHR

SYNOPSES

RICHARDSON, JOHN H. (Georgia Department of Public Health), RAMSEY, RALPH L., and STARR, L. E.: *Bat rabies in Georgia, 1956–65. Public Health Reports, Vol. 81, November 1966, pp. 1031–1035.*

Rabies infection in an insectivorous bat from Georgia was first reported in 1956. For the period 1956–65 rabies was diagnosed in 36 (6.7 percent) of 531 bats examined. Rabid bats were found in four of the six physiographic regions of the State.

Infection rates were more than 10 times higher in noncolonial bats (14 percent) than in colonial bats (1.3 percent). Thirty-two (89 percent) of the 36 cases occurred in bats of the genus *Lasiurus*. Twenty-six (64 percent) of the 36 cases occurred in red bats, *Lasiurus borealis*. Infection occurred predominantly in mature bats with equal distribution among males and females. The peak incidence was in late summer. No cases were reported during the winter months of December, January, and February.

Negri bodies were demonstrated in 33 of 35 rabid bats, by microscopic examination of whole brain cross section impressions stained by Sellers' method. Rabies virus was demonstrated, by mouse inoculation tests, in the salivary glands of 12 of 21 rabid bats examined.

Bat rabies in Georgia has occurred predominantly in counties that were free of rabies in terrestrial mammals. No epidemiologic evidence exists to date which suggests the transmission of rabies by bats to other animals.

SODEMAN, WILLIAM A., Jr. (Veterans Administration), and JEFFREY, GEOFFREY M.: *Indirect fluorescent antibody test for malaria antibody. Public Health Reports, Vol. 81, November 1966, pp. 1037–1041.*

The indirect fluorescent antibody test for malaria antibody can provide a useful measurement of the immunological status during malarial infection. Variations in the method of performance of the test, however, may alter its sensitivity and reproducibility. In antigen preparation, special attention should be directed toward obtaining smears with a uniform distribution of parasites and toward maintaining uniformity of size in the area to be stained. The size or stage of the parasites to be stained will also affect the interpretation of results. Uniform quantities of the serum dilutions should be used for staining. The sensitivity of the test must be adjusted to the precision of performance. Use of Evans blue as a counterstain simplifies the reading of these tests for malarial antibodies.