USE OF BLOOD COLLECTED ON FILTER PAPER DISKS IN NEUTRALIZATION TESTS FOR POLIOVIRUS ANTIBODY

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THE PREPARATION and preservation of serum samples in the field and the difficulty of obtaining specimens from infants are among the major problems encountered in serologic surveys. In an earlier study (*1*), we reported the usefulness of a method which surmounted these problems. The technique consisted of collecting a small amount of blood onto filter paper disks, which were then dried and stored until tested. A later report (*2*) described the successful use of the filter paper disk technique for standard complement fixation (CF) test for adenovirus and the hemagglutination (HAI) test for measles virus. Other laboratories have performed neutralization tests using filter paper disks, but their results were reported as erratic (*3*) or lacking quantitation (*4*). This report describes modifications of this technique which lead to the successful neutralization tests for three types of poliovirus.

Materials and Methods

The filter paper disks (*A*), described in previous reports (*1–3*), used in this study were similar to those used for antibiotic sensitivity tests. When saturated with whole blood, these disks contained about 0.07 ml. of serum. Whole blood was collected onto filter paper disks from 15 children at the Public Health Service Alaska Native Medical Center in Anchorage, by both routine venipuncture and from a fingerprick wound. For saturation of the filter paper disks, a finger was cleansed with alcohol and lanced, and the disks were held in a forceps directly on the puncture site. At least three disks could be saturated from a single fingerprick.

The disks were placed in 2-dram vials to dry overnight at room temperature and then stored at −20° C. until tested. Before testing, three disks were placed in 1 ml. of tissue culture maintenance media with antibiotics and eluted overnight at 4° C. This eluate was equivalent to a 1:5 serum dilution. The method of wringing fluid out of the saturated disks with a wooden applicator (*2*), was replaced by the more satisfactory method of dropping the wet disks into the chamber of a disposable 10-cc. syringe and forcibly squeezing the fluid back into the eluate. The disposable syringes could be cleaned with distilled water and used again. Approximately 0.75 ml. of eluate was recovered. This volume was at least 0.15 ml. greater than that recovered by previous methods and yielded enough fluid for performance of three neutralization tests at an initial dilution of 1:5. The eluate was then centrifuged for 10 minutes at 2,500 rpm to sediment any particles of paper. This step was essential to insure clear end points. After centrifuging, the supernatant eluate was poured off and used in the test.

Serums from the same children were separated from the blood in a routine manner and stored at −20° C. until tested. On the day of the test, 1 ml. of a 1:5 dilution was made from the serum and inactivated at 56° C. for 1 hour. The disk eluate was not inactivated because the fluids occasionally became turbid after heating.

Fourfold dilutions were made of the disk eluate and of the inactivated serums, and 0.15 ml. of each dilution was incubated for 1 hour

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at room temperature with an equal volume of virus suspension. The challenge dose was approximately 100 $TCID_{50}$ for each type of poliovirus, 0.1 ml. of each dilution was inoculated into each of two monolayer tissue culture tubes of rhesus monkey kidney or HEp–2 cells. The tissue culture tubes were observed daily for cytopathic effect. Disk and serum specimens from a given subject were examined in the same test.

Results

The poliovirus neutralization antibody titers obtained from the disk eluates and from the serums from 15 children are compared in the table. Titers are expressed as the reciprocal of the highest dilution at which neutralization occurred. In only 1 of 45 tests was there a fourfold difference observed between serum and disk (subject 7 for poliovirus I). In five other tests one tube of a dilution pair showed neutralization and the other did not. The extrapolated twofold difference between the disks and serum in these five tests was within the limits of laboratory variation.

Although the disks were not handled aseptically, there was no evidence of contamination in the tissue cultures.

Comparison of poliovirus neutralization antibody titers [1] in serum and disk eluates from the same subject

Subject number	Poliovirus I		Poliovirus II		Poliovirus III	
	Serum titer	Disk titer	Serum titer	Disk titer	Serum titer	Disk titer
1	80	80	<5	<5	5	5
2	<5	<5	<5	<5	80	80
3	<5	<5	<5	<5	<5	<5
4	5	5	20	20	20	20
5	5	5	5	2.5	<5	<5
6	20	20	5	5	80	80
7	20	80	20	20	20	10
8	<5	<5	5	5	20	20
9	5	5	5	5	5	5
10	<5	<5	80	80	20	20
11	<5	<5	<5	<5	<5	<5
12	<5	<5	5	5	5	5
13	<5	<5	80	40	<5	<5
14	<5	<5	<5	<5	20	20
15	80	160	20	20	2.5	5

[1] Presented as the reciprocal of the highest serum dilution showing neutralization.

Discussion

The successful adaptation of the filter paper disks method in poliovirus neutralization tests extends the growing list of serologic tests which can be done accurately by this simplified technique of blood specimen collection. Centrifuging the eluate probably removed particles of paper which, in previous studies, had interfered with the virus-antibody combination and resulted in inconsistent neutralizing titers. In our laboratory, the disk technique has been successfully used in HAI tests for measles virus, arboviruses, enteroviruses, influenza and parainfluenza types I and III, and CF tests for adenoviruses, respiratory syncytial virus, and influenza and parainfluenza III. Other investigators have used the disk technique successfully for mumps HAI tests (*5*), neutralization tests for Eastern equine encephalitis virus (*6*), and vesicular stomatitis virus (*7*). The disk technique has also been applied to fluorescent treponemal antibody surveys for syphilis, yaws, and pinta (*8*).

When the new method of squeezing the eluate out of the disks with a syringe is used, three different neutralization tests can be carried out on three disks at a 1:5 dilution. Three disks can easily be obtained from one finger puncture; however, more disks may require another puncture which would be time consuming. This limits the number of agents which can be tested by the neutralization test. This limitation does not apply if microtiter CF or HAI tests are used (*2*). The difficulties and expense of collecting serum samples, especially under field conditions, and the problems of venipuncture on infants and small children can be eliminated. Further, the disks can be preserved at room temperature for 6 months (*2*) and sent through the mail without apparent loss of titer (*9*).

Summary

Routine neutralization tests for the three types of poliovirus antibody were performed on children at the Public Health Service Alaska Native Medical Center in Anchorage. A simplified method of collecting a small amount of blood on filter paper disks was used. Results obtained were almost identical to those obtained with the use of serum.

A new method to remove eluate from the disks with a disposable syringe increased the amount of fluid recovered from the disks and permitted the performance of at least three neutralization tests on the usual three-disk specimen.

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SUPPLY REFERENCE

(A) Filter paper disks (740–E–½-inch): Carl Schleicher & Schuell Co., Keene, N.H.

Education Notes

Operational Aspects of Radiation Surveillance. This Robert A. Taft Sanitary Engineering Center course scheduled for April 25–May 6, 1966, is designed for professionals who plan and conduct environmental radiation surveillance programs.

Among course topics are sources of radionuclides in the environment, meteorological aspects of airborne radionuclides, design of sampling programs and techniques, statistical analysis of data, and application of radiation protection standards. Basic Radiological Health (211), or equivalent training, is prerequisite. Additional information is available from Director, Training Program, Robert A. Taft Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati, Ohio 45226.

Doctoral Study in Social Sciences. The University of Michigan School of Social Work offers an interdepartmental doctoral program in social work and social science which leads to the doctor of philosophy degree and combines social work and sociology, social psychology, psychology, or economics.

Traineeships of $1,800 to $3,600 plus tuition are available from the National Institute of Mental Health and from the Russell Sage Foundation. Application deadlines are February 15, 1966, for fellowships, and May 1, 1966, for admission. Additional information is available from Doctoral Program, School of Social Work, University of Michigan, Ann Arbor.

Medical Care Organization Degree. Fellowships for a program of study leading to the Ph.D. degree in medical care organization are available from the School of Public Health, University of Michigan, Department of Medical Care Organization.

Designed to be completed in 3 years, the program is intended to prepare students for careers in teaching, research, and policy formulation in the sociological, economic, and administrative aspects of medical care organization. Fellowship stipends begin at $2,400 for the first year with increments for succeeding years, full tuition, and supplements of $500 for each dependent.

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