A Microneutralization Test for Determination of Antibodies to Rubeola Virus (33499)

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(Introduced by H. A. Wenner)

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The determination of neutralizing antibodies for rubeola gives the most reliable correlation with immune status (1). Nevertheless, the available techniques (2) are laborious and not well suited for testing a large number of sera in epidemiological studies and other investigative work. Although the incidence of natural measles infections is decreasing in North America as a result of widespread immunization practices, rubeola continues to pose a public health problem in many developing countries. In addition, vaccine modified measles infections in this country may become increasingly difficult to diagnose on purely clinical grounds. The present report presents a method for determination of neutralizing antibodies to rubeola in microtiter plates which can be read macroscopically. This is an application of a method previously described (3) and now extensively used in this laboratory in epidemiological investigations.

Materials and Methods. Equipment and technique. Disposable Linbro[4] "U" microtiter plates[5] were immersed in 70% alcohol for 1 hr, inverted, and dried under ultraviolet light. Calibrated dropping pipettes and transferring loops were used as described by Lennette (4).

Monkey kidney cells. A suspension of secondary rhesus monkey kidney cells containing 200,000 cells/ml was prepared in medium 199 containing 0.5% lactalbumin hydrolysate, 2% inactivated chicken serum (30 min at 56°), and 1.68 g/liter of $NaHCO_3$.

Serum dilution. Initial 1:8 serum dilutions were made in medium 199 to which 1.68 g/liter of $NaHCO_3$ were added. These dilutions were inactivated at 56° for 30 min.

Virus strains. The strain of measles used initially in our neutralization tests was recovered in Kansas City in 1967 (PABS 25S). More recently, we have used Philadelphia strain 26[6]. The virus pools were prepared in primary rhesus monkey kidney cells and stored at −70° until used.

For the microtiter tests, 300 $TCID_{50}$ of virus was used. A virus titration was included in each test as described previously (3). All virus dilutions were made in medium 199 containing 2% inactivated chicken serum (56° for 30 min) and 1.68 g/liter of $NaHCO_3$.

Antibody assay. The procedure for antibody assay was described in detail (3). The only modification was the addition of 300 $TCID_{50}$ of virus to each well.

On the fifth day of incubation, a virus titration plate was tested by adding to each well 0.025 ml of a 0.9% African green monkey erythrocyte suspension in phosphate buffered saline. Erythrocytes can be stored for a period of 3 weeks at 4° in dextrose–gelatin–Veronal (5). After addition of erythrocyte suspension, the plates were incubated

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[4] Use of trade name is for identification purposes only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

[5] Linbro No. IS-MRC-96.

[6] Kindly supplied by Dr. Matthew A. Bucca, National Communicable Disease Center, Atlanta, Georgia.

TABLE I. Comparison of Serum Neutralizing Antibody Titers for Measles Using the New Microtechnique and Tube Tests.

	Serum neutralizing antibody titers		
	Microtest	Tube-test	
Serum no.	PABS 25S strain	PABS 25S strain	Edmonston strain
1	1:16	1:16	1:16
2	1:16	1:8	1:16
3	1:16	1:16	1:16
4	1:16	1:16	1:32
5	1:16	1:8	1:32
6	1:32	1:32	1:16
7	1:16	1:16	1:16
8	1:32	1:16	NT[a]
9	1:16	1:8	NT
10–17	<1:8	<1:8	<1:8

[a] NT = not tested.

first at 4° for 45 min, and then kept at room temperature an additional 10–20 min. Reading was done macroscopically by hemagglutination pattern. When Philadelphia strain 26 was used as antigen, the microtiter plates were incubated for 1 hr at 4° and kept at room temperature for an additional hour after the addition of erythrocytes.

Tube neutralization tests. Sera evaluated by the new method were also tested by standard tube neutralization procedures (2). For the tube tests both the PABS 25S and Edmonston strains[7] were used. The tube tests with PABS 25S antigen were read by inhibition of hemagglutination of African green monkey erythrocytes, those with Edmonston strain, by inhibition of cytopathic effect.

Results. Table I compares the serum neutralizing antibody titers determined by the new microtiter method and by the two tube neutralization tests. There was very good correlation between values obtained in the three tests. In no instance was there a greater than 2-fold deviation of titers comparing the determinations made in microtiter plates with those of each of the tube tests. Sera which were negative (<1:8) in microtiter plates were also negative in tubes.

[7] Kindly supplied by Dr. Chien Liu, Professor of Medicine and of Pediatrics, University of Kansas School of Medicine, Kansas City, Kansas.

Preliminary experiments using the Philadelphia 26 strain as antigen demonstrated that more distinct end points could be obtained than by using the PABS 25S strain. It was also possible to perform tests using only 30 $TCID_{50}$ of virus per well. Preliminary experiments showed that tests using this lower virus challenge gave slightly higher antibody titers (1 or 2 dilutions).

Discussion. Neutralization tests for determining antibodies for measles have been found to provide the most reliable correlation with immunity. Both complement-fixation and hemagglutination-inhibition tests are relatively insensitive (1). Our data indicate that the results of microneutralization tests correlate well with those of standard techniques.

The new microtechnique offers several advantages in comparison with a tube neutralization test. In microtiter plates, more sera can be tested in less time, using less volume of reagents and less laboratory space. The reading of the test is also greatly facilitated. A 500 sera test can be read by one technician in approximately 1 hr without using a microscope. Furthermore, it was our experience that neutralization tests which can be read by hemagglutination pattern have clearer end points than those read by cytopathic effect.

We were confronted with two minor difficulties in the development of the technique. First, spontaneous agglutination of the African green monkey erythrocytes by human sera occasionally occurred in the 1:4 dilutions of serum controls. But since this rarely happened in the 1:8 serum dilutions, we used this serum concentration as the initial dilution in our neutralization tests. Second, in tests using the PABS 25S strain, the agglutination pattern was stable for only approximately 30–40 min at 20°; a longer delay in reading resulted in elution of erythrocytes. The hemagglutination patterns obtained with the Philadelphia 26 strain were stable at 20° for at least 24 hr. Therefore, this antigen appears to be best suited for testing larger numbers of sera. The Edmonston strain proved unsatisfactory for use in microneutralization tests because of its weak ability to absorb and agglutinate green monkey ery-

throcytes.

Summary. A new microtiter method for determining serum neutralizing antibodies for measles is described. The test can be read macroscopically by the pattern of hemagglutination. The results obtained by the microtest compare well with those obtained in tube neutralization tests. Philadelphia strain 26 was technically the most satisfactory of the three measles strains evaluated for use in microneutralization tests.

1. Fulginiti, V. A. and Kempe, C. H., Am. J. Epidemiol. **82**, 135 (1965).

2. Enders, J. F. *in* "Diagnostic Procedures for Viral and Rickettsial Diseases" (E. H. Lennette and N. J. Schmidt, eds.), 3rd ed. Am. Public Health, New York (1964).

3. Wulff, H., Soeken, J., Poland, J. D., and Chin, T. D. Y., Proc. Soc. Exptl. Biol. Med. **125**, 1045 (1967).

4. Lennette, E. H., *in* "Diagnostic Procedures for Viral and Rickettsial Diseases" (E. H. Lennette and N. J. Schmidt, eds.), 3rd ed. Am. Public Health, New York (1964).

5. Work, T. H., *in* "Diagnostic Procedures for Viral and Rickettsial Diseases" (E. H. Lennette and N. J. Schmidt, eds.), 3rd ed. Am. Public Health, New York (1964).

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