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Microneutralization Test for the Determination of Mumps Antibody in Vero Cells

M. T. KENNY, K. L. ALBRIGHT, AND R. P. SANDERSON

Department of Infectious Diseases, Dow Human Health Research and Development Laboratories, The Dow Chemical Company, Zionsville, Indiana 46077

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A sensitive and reproducible test for the determination of mumps serum-neutralizing antibody with Vero cells grown in disposable plastic microtiter plates is described. Serum end point titers obtained by this method showed excellent correlation with those obtained by the primary chick embryo tissue culture-hemadsorption tube test.

Since its development by Takatsy et al. (11) and modification by Sever (10), an increasing number of routine serological tests and tissue culture assays have been adapted to the microtiter system. The advantages of this technique lie in the conservation of reagents, valuable samples, and time. This report describes a simple, accurate and reproducible mumps micro serum neutralization (SN) test with Vero cells. The results were compared with SN titers obtained by the primary chick embryo tissue culture-hemadsorption mumps SN test performed in stationary culture tubes.

MATERIALS AND METHODS

Tissue culture. Stock Vero cells (7, 12) were grown on Eagle's minimal essential medium in Earle's balanced salt solution (MEM-E) containing 0.1% bicarbonate, 10% fetal calf serum (FCS), 100 μg of streptomycin per ml, and 100 μg of neomycin per ml. The bicarbonate concentration was increased to 0.2% and the FCS concentration was reduced to 2% for cell maintenance. Vero cells between passage levels 135 and 160 were used for SN tests.

Primary chick embryo tissue cultures (CETC) were prepared from 9- to 10-day-old embryos and grown in Earle's balanced salt solution containing 5% FCS, 0.5% lactalbumin hydrolysate (LAH), 400 μg of streptomycin per ml, and 400 units of penicillin per ml. Medium 199 containing 0.22% bicarbonate, 1% FCS, 100 μg of streptomycin per ml, and 100 μg of neomycin per ml was used for cell maintenance.

Challenge virus. The CETC-propagated Jeryl Lynn strain of mumps virus was obtained from Merck Sharp and Dohme Laboratories, West Point, Pa. The virus was passed two times in Vero cells for use in the microtiter test.

Test sera. Serum samples from our files were divided into three groups: (i) children convalescing from natural mumps; (ii) children vaccinated with live, attenuated mumps virus vaccine (Mumpsvax, Merck Sharp and Dohme); and (iii) randomly selected sera from adults and children who were not convalescing from natural mumps and who had not received mumps vaccine. To obviate prejudicing of results, the sera were coded and tested by the microtiter and tube SN tests separately in two different laboratories.

Tube SN test. Tube SN tests were performed in CETC by the methods described by Buynak and Hilleman (1), except that tests were read for hemadsorption after 6 rather than 5 days.

Microtiter SN test. Presterilized, flat-bottom plastic plates and lids (Microtest II, Falcon Plastic, Inc.) were used without further treatment. Disposable 1.5- and 6.0-ml droppers and 25- and 50-μliter dropping tips were purchased from the Linbro Chemical Co., New Haven, Conn., and sterilized by autoclaving (121 C, 15 min). Fifty-microliter tulip diluters and handles were obtained from Microbiological Associates, Bethesda, Md.

Test medium (TM) for the microtest consisted of MEM-E containing 0.1% bicarbonate, 2% FCS, 0.5% LAH, 100 μg of streptomycin per ml, and 100 μg of neomycin per ml.

For each sample to be tested, 50 μliters of heat-inactivated (56 C, 30 min) serum was added to each of four wells containing 50 μliters of TM. Using the 50-μliter diluters, the serum was diluted in twofold steps in TM from 1:2 through 1:64. Each well of the first three serum dilution series then received an equal volume (50 μliters) of challenge virus diluted in TM to give 30 to 100 $TCID_{50}$/50 μliters at 6 days. Each well of the remaining serum dilution series received 50 μliters of TM and served as the serum control. After an incubation period of 1 hr at 35 C (humidified CO_2 incubator; 5% CO_2, 95% air), each well of the test received 100 μliters of Vero cell suspension in TM adjusted to contain 10^5 viable cells per ml, and the test was incubated at 35 C in a humidified CO_2 incubator. A known positive and negative serum control and Vero cell control (100 μliters of TM plus 100 μliters of cells) were included in each test. To determine the amount of challenge virus used, a virus back titration was also performed with each test. Fifty-microliter amounts of undiluted and 10-fold dilutions of the challenge virus suspension used in the test were added

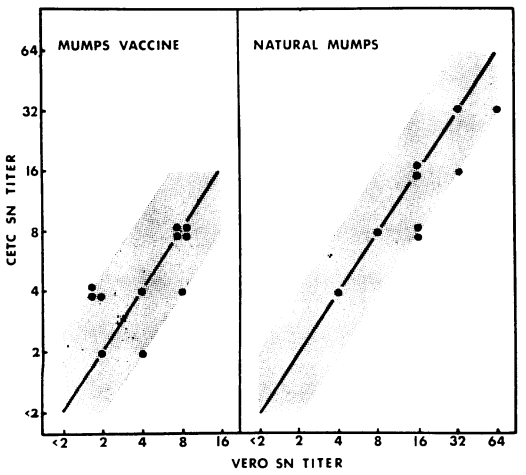

FIG. 1. *Comparison of mumps SN titer obtained by the CETC macrotiter and Vero microtiter systems. Sera were obtained from children convalescing from natural mumps and from children who had received live, attenuated mumps virus vaccine. The diagonal line represents equivalence in titers, and the shaded area represents twofold variation from equivalence.*

to each of 10 wells containing 50 μliters of TM. A 100-μliter amount of Vero cell suspension was then added to each well, and the back titration was incubated with the test. Microtiter tests were routinely read at 6 days by using an inverted microscope. The mumps SN titer was taken as the highest initial serum dilution negative for mumps cytopathology in two to three of the test wells at that dilution.

TABLE 1. *Reproducibility of mumps SN titers obtained by the Vero microtiter system*

Serum	Reciprocal mumps SN titer		
	Test 1	Test 2	Test 3
R.B.	2	2	2
W.M.	4	4	4
G.A.	8	8	8
B.B.	4	4	4
Human pool[a]	4	4	8
Positive horse	128	128	256
Negative horse	<2	<2	<2
Challenge virus titer ($TCID_{50}$/50 μliters)	100	50	100

[a] Hyland Laboratories, Inc., Los Angeles, Calif.

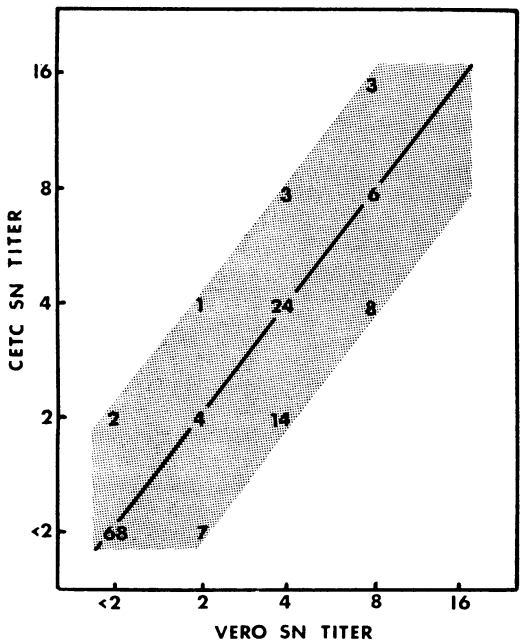

FIG. 2. *Comparison of mumps SN titers obtained by the CETC macrotiter and Vero microtiter systems. Sera were obtained from adults and children who had not received mumps vaccine and were not convalescing from natural mumps. The diagonal line represents equivalence in titers, and the shaded area represents twofold variation from equivalence. Numbers indicate number of sera tested.*

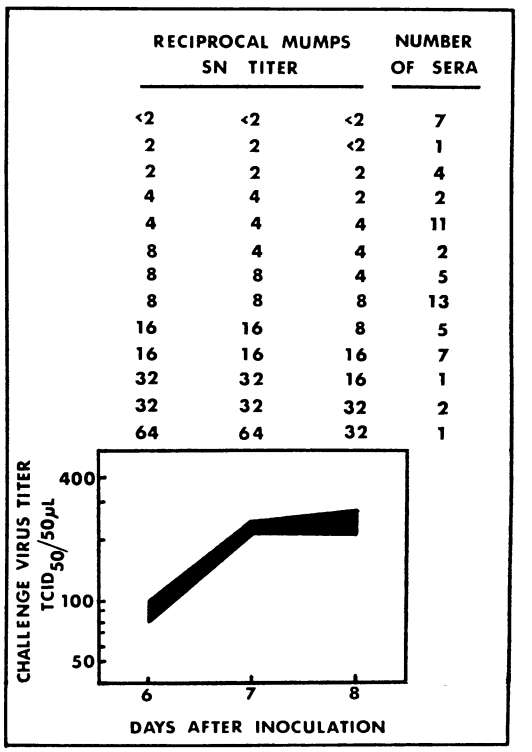

FIG. 3. *Effect of challenge virus titer and reading time on mumps SN titers obtained on 61 randomly selected human sera 6, 7, and 8 days after inoculation.*

RESULTS AND DISCUSSION

Tissue culture SN tests have been successfully adapted to the microtiter system by a number of investigators. A high degree of correlation between serum antibody end points determined by conventional and microtiter tests was found with the polioviruses (3, 8), the reoviruses (9), rubeola virus (5, 6), a rhinovirus and coxsackievirus A-4 (4), and rubella virus (2).

Figures 1 and 2 show that there is good correlation between mumps SN titers obtained by the microtiter and CETC tube tests. Of the 160 human sera tested, 113 or 70.5% showed equilvalent titers in both systems, 35 or 22% showed twofold higher titers in the Vero microtiter system, and 12 or 7.5% showed twofold higher titers in the CETC tube test.

SN titers obtained by the Vero microtiter system were found to be reproducible from test to test. Table 1 summarizes mumps SN titers obtained on seven serum samples tested at 3 weekly intervals. The titers were based on 6-day readings, and it was found that there was little variation from test to test.

Figure 3 summarizes two experiments showing the effect of challenge virus titers and reading time on mumps SN titers of 61 randomly selected human sera. Cultures were read for SN titers 6, 7, and 8 days after inoculation. Challenge virus titers increased from 80 to 100 $TCID_{50}$/50 μliters at 6 days to 200 to 250 $TCID_{50}$/50 μliters at 7 days. There was little change in challenge virus titer at 8 days (200 to 300 $TCID_{50}$/50 μliters). Two serum samples (3%) showed a twofold reduction in SN titer at 7 days, whereas a total of 17 or 36% showed a twofold reduction in SN titer by 8 days. In one case, this reduction in titer would have resulted in a false-negative reading (2 to <2) had the test been initially read at 8 days. A greater than fourfold reduction in SN titer was not seen in SN tests read between 6 and 8 days. Based on these findings, SN tests were routinely read 6 days after inoculation.

Cell survival and serum toxicity at low dilutions have not been problems in the microtiter mumps SN test. Cell sheets remain intact for at least 8 days and less than 0.5% of approximately 4,000 human sera tested by the microtest have demonstrated cell toxicity at 1:2 serum dilutions. Care must be taken, however, to insure that the bottoms of the wells are not excessively scratched by the microdiluters during the serum dilution step of the test. Such abrasions may interfere with proper cell attachment and formation of intact cell sheets which are necessary for proper interpretation of test results.

The microtiter mumps SN test described here is sensitive and highly reproducible and is especially suitable for epidemiological and vaccine studies in which large numbers of sera must be accurately tested.

ACKNOWLEDGMENTS

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