A Plaque Neutralization Test for Determining Mumps Antibodies (33452)

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The conventional procedures for measuring mumps antibodies are the complement fixation (CF) and hemagglutination-inhibition (HAI) tests. Both tests can be performed rapidly and both are suitable for measuring high levels of antibody. However, each of these assay methods has certain drawbacks which have been described in earlier studies. With the complement fixation test there is only an 80% correlation between previously diagnosed mumps infection and the presence of CF antibody; levels of both the early-appearing S and even the more stable V antibodies may fall below detectable levels (1, 2). The HAI test is inaccurate when low dilutions of serum are tested; many human sera contain nonspecific inhibitors of agglutination which cannot always be removed by chemical treatment (3, 4). The need for a more sensitive antibody assay method became evident with the development of a live attenuated mumps vaccine (5, 6). While the vaccine induces antibody in 90% of vaccinees, the levels observed are low in comparison to levels seen after natural mumps infection. The purpose of this report is to describe a plaque neutralization (PN) test which is both sensitive and reliable for measuring low levels of mumps antibody; a preliminary description of the method was presented earlier (7).

Materials and Methods. Virus. The Sporn strain of mumps virus was isolated in BS-C-1 cells from throat washings of a child with typical clinical mumps. The virus was then plaque-passaged 17 times in MA-104[1] (embryonic rhesus kidney) cell cultures. Seed pools containing 10^4 tissue culture infectious $doses_{50}$ ($TCID_{50}$) were prepared in MA-104 kidney cells. For use in the plaque assay, the virus pool was diluted in M199 with 10% skim milk to contain 50–60 plaque forming units/0.25 ml. Although other strains of $doses_{50}$ ($TCID_{50}$) were prepared in MA-104 cell monolayers, those produced by the MA-104 cell-adapted Sporn strain are larger (0.75–1.0 mm) than those produced by unadapted strains (0.5 mm).

Cell cultures. The MA-104 cell cultures (in 2-oz glass prescription bottles) were obtained from the Division of Biologics Standards' Tissue Culture Section. The cultures were grown in Eagle's minimum essential medium (MEM) with Hank's base, 200 m*M* glutamine, 10% calf serum, penicillin 100 units/ml and streptomycin 20 μg/ml. After healthy, confluent monolayers formed, maintenance medium consisting of Eagle's MEM with 200 m*M* glutamine, 2% fetal bovine serum and antibiotics [penicillin (100 units/ml) and streptomycin (20 μg/ml)] was substituted. The cultures were used usually within 5–6 days after preparation.

Sera. All test sera were inactivated at 56° for 30 min; twofold dilutions were made in Hanks balanced salt solution.

Test procedure. Equal volumes (1.0 ml) of diluted serum and virus were mixed. Virus controls consisting of diluent and virus (3.0 ml each) were mixed in the same fashion. All tubes were then incubated for 1 hr in a 37° waterbath. The serum–virus mixture was then dispensed in 0.5-ml amounts to 3 MA-104 cultures from which the medium had been removed. Ten cultures were inoculated with the virus–diluent controls; 5 at the beginning of the inoculation procedure and 5 at the end. Each bottle was rotated gently to insure maximum contact of cells and inoculum. The cultures were then incubated at 36° for 3 hr and the bottles were rotated at hourly intervals. After virus adsorption the

[1]These cells can be obtained from Microbiological Associates, Inc.

TABLE I. Geometric Mean Antibody Titers Following Natural Mumps.

Assay	Acute	Conv.	1 year	% Conversion[a]	Conversions[b]
PN	8.7	388	155	94	34/36
HAI	44	355	63	91	33/36
CF (viral)	4	102	15	91	33/36

[a] Value indicates percentage of children with clinical mumps who had at least a 4-fold rise in mumps antibody between their acute and convalescent sera.

[b] Numerator indicates children with at least 4-fold antibody rises, and denominator indicates number of children with clinical mumps tested.

cell monolayers were overlayed with 6.0 ml of twice concentrated M199 plaque base, without phenol red, containing 10% twice concentrated skimmed milk and 3.3% of 1:1000 neutral red. Nobel's agar (3% in distilled water) was melted and cooled to 43–45°. Equal volumes of agar and plaque medium (43–45°) were mixed thoroughly and this mixture was dispensed in 6-ml amounts using a foot pedal-operated soap dispenser.[2] This procedure was far more rapid than pipetting the agar by hand; with practice one can deliver the required 6 ml consistently. Since neutral red was present in the medium, the overlay procedure was carried out in a darkened room. After the agar solidified completely, the bottles were inverted and incubated at 36° for 7–10 days; usually, on day 8, the number of plaques can be counted and recorded. The plaque neutralizing antibody titer of a serum specimen is defined as the highest serum dilution resulting in an average plaque count which is 50% that of the average of the 6 virus controls.

Mumps vaccine. Live attenuated mumps vaccine prepared from the Jeryl Lynn strain of mumps virus was supplied by Dr. Maurice Hilleman; the history of this strain and the development of the vaccine are described elsewhere (5).

Results. A comparison of antibody levels measured with the plaque neutralization, CF and HAI tests is presented in Table I. The sera tested were obtained from 36 children involved in an outbreak of natural mumps at the Arkansas Children's Colony. Specimens were taken during the acute phase of illness, approximately 1 month after illness and 1 year later. There was a high percentage of sera showing 4-fold antibody rises, 94% by plaque neutralization test and 91% by either the CF or HAI procedure. It is of interest that after 1 year, the levels of PN antibody had dropped only 2-fold while HAI and CF antibody levels decreased about 10-fold.

There have been conflicting reports in the literature (8, 9) concerning the question of cross-reactions between mumps antibodies and parainfluenza antibodies in mumps hemagglutination inhibition tests. To evaluate the specificity of the plaque neutralization assay, we studied 3 specific hyperimmune antisera to Parainfluenza strains I, II, and III,[3] using the PN assay. There was no inhibition of mumps plaques by these antisera. This finding confirms the specficity of this test. It also indicates that some human sera which inhibit mumps hemagglutination, but have no mumps plaque neutralizing antibody, have nonspecific inhibitors and not cross-reacting parainfluenza antibodies.

A comparison of the geometric mean antibody titers (GMT) following natural and attenuated mumps infections is shown in Table II. The antibody levels produced by vaccination are about 10-fold lower than those present after natural mumps infection. Such low titers in the vaccinees' sera make it evident that certain sera will not show significant rises in antibody if assayed with a procedure which is not sensitive at low serum dilutions. Examples of this problem are shown in Table III, which illustrates the mumps antibody levels of 11 vaccinees prior to and 4 weeks after vaccination. There were

[2] The soap dispenser was purchased from Peck's Products Co., 610 E. Clarence Avenue, St. Louis 15, Missouri.

[3] These sera were purchased from Microbiological Associates, Inc., Bethesda, Maryland.

TABLE II. Comparison of Geometric Mean Antibody Titers (GMT) Following Natural and Attenuated Mumps Infections.

Assay system	Natural mumps (36 patients)	Attenuated mumps (36 vaccinees)
PN	388[a]	20[b]
HAI	355	17
CF (viral)	95	15

[a] Sera obtained approximately 1 month after diagnosis.

[b] Sera obtained 28 days postvaccination; only sera showing 4-fold increase in antibody titer are included.

25 other vaccinees who had definite fourfold rises in mumps antibodies by all three assay procedures. Ten of the 11 vaccinees all definitely converted by PN, but only one (M.W.) showed even a questionable rise in mumps antibody by HAI. These vaccinees did not show significant rises by HAI because of nonspecific inhibitors of agglutination present in their serum, which were not removed by treatment with either kaolin or potassium periodate. Four vaccinees (W.C., C.H., S.R., and M.W.) failed to show a significant rise in CF antibodies after vaccination.

Discussion. The results presented indicate that the mumps plaque neutralizing antibody assay (PN) is an improvement over the more conventional HAI and CF methods in titrating low serum levels of mumps antibody. The PN test is no better than the HAI or CF tests in diagnostic serology of natural mumps infection when antibody levels are high as occurs in convalescent sera, but the test has a decided advantage in determining mumps immunity prior to and after vaccination because of its greater sensitivity. Low levels of antibody have been thought to be protective against mumps but Henle (2) reported that 1–2% of individuals having CF antibodies subsequently contracted mumps. In addition, Hilleman has reported natural mumps infection in two vaccinees who had very low levels of vaccine-induced mumps antibodies prior to natural challenge (6). The PN procedure described here should help to clarify the problem of whether such low antibody levels are protective.

Summary. The mumps plaque neutralization assay procedure was found to be a sensi-

TABLE III. Comparison of Antibody Levels in Mumps Vaccinees Using Three Serologic Procedures.

	Plaque neutralization		HAI		CF (viral)	
Vaccinees	Pre[a]	Post[b]	Pre	Post	Pre	Post
1. P.A.	<2	8	8	16	<4	8
2. A.B.	4	16	8	16	4	32
3. S.B.	<2	32	8	16	<4	32
4. W.C.	<2	16	8	8	<4	<4
5. K.D.	<8	32	16	16	4	32
6. C.H.	<2	2[c]	8	8	4	8
7. G.L.	<2	16	8	16	<4	16
8. S.R.	<2	8	8	16	<4	4
9. H.T.	<2	32	8	16	<4	16
10. M.W.	<2	4	<8	8[c]	<4	<4
11. J.W.	2	16	8	16	<4	32
Conversions[d]/No. of vaccinees tested		10/11		0/11		7/11

[a] Serum sample obtained immediately prior to vaccination.

[b] Peak antibody titer obtained on either 30 or 90 day postvaccination sera by HAI and CF testing; PN antibodies were studied only on day 30.

[c] Not definite sero-converters. Lack of sera prevented testing at lower dilutions.

[d] Conversions—mumps serum antibodies rose 4-fold following vaccination.

tive indicator of low levels of mumps antibodies. Undiluted serum can be studied for the presence of mumps antibodies and there is no problem of nonspecific inhibition. The levels of antibody 1 year after natural mumps are more stable by the PN method than by HAI or CF tests. This assay procedure is valuable in studying sera where low levels of antibody may be present, as after remote natural disease, or vaccination with attenuated mumps strains.

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Size Distribution and Counting of Liver Nuclei by an Electronic Particle Counter* (33453)

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In order to correlate cellular and subcellular changes in a tissue with the physiological state of the animal, it is necessary to relate the contents of the cell to a so-called "fixed tissue constituent." Recently, DNA has been suggested as a cellular constant (1), but in certain tissues such as liver, heart, and brain, polyploidy occurs (2–4). Polyploidy is the result of an increase in nuclear DNA without cell division, which occurs in liver cells as tetraploidy, octoploidy, 16-ploidy and 32-ploidy (5). Because of this nonconstancy of DNA, it would be advantageous to express the data, on a per cell basis, but the homogenization procedures developed so far to disrupt liver tissue do not leave many of the cells intact. The next best choice appears to be an isolation and determination of the number of nuclei which could then be related to the number of liver cells. Such a calculation necessitates the assumption that binucleated liver cells have twice the cytoplasmic mass of mononucleated cells. Recently, Blobel and Potter (6) suggested a procedure which allows the recovery of more than 90% of the liver nuclei, with very little cytoplasmic contamination. By these procedures, we are able to obtain nuclear preparations that can be readily counted and sized in an electronic particle counter.

Materials and Methods. Male rats (Wistar strain) of various ages were housed in a room with controlled light from 6:00 a.m. to 6:00 p.m. and were fed an 18% casein agar-gel diet (7) between 8:00 a.m. and 12:00 noon. All rats were killed between 12:00 noon and 10:00 p.m. and were perfused with ice-cold 0.9% saline until all traces of blood had been removed from the tissues. The livers were removed and quickly immersed in several volumes of ice-cold 0.25 *M* sucrose in

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