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Enzyme Immunoassays for Measurement of Cytomegalovirus Immunoglobulin M Antibody

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The diagnosis of congenital cytomegalovirus (CMV) infection is often accomplished by the detection of circulating antibody directed against CMV. We devised a method for measuring CMV-specific immunoglobulin M (IgM) based on the isolation of IgM antibody by reaction with a solid phase coated with antihuman IgM. The determination of IgM antibody specific for CMV was accomplished by the subsequent addition of CMV or control antigen and enzyme-labeled CMV antibody (solid phase-IgM method). We compared the sensitivity and specificity of this method with those of a conventional form of solid-phase enzyme immunoassay in which CMV antigen is bound to the solid phase (solid phase-antigen method). Both assay systems were capable of detecting CMV-specific IgM antibody in the sera of 10 babies with documented CMV infection and in those of the mothers of 4 of these babies. The solid phase-IgM method yielded negative results in all 66 sera available from babies who did not have congenital CMV infection. On the other hand, the solid phase-antigen system yielded false-positive results in 12 (18%) of these sera. In addition, the solid phase-antigen system yielded false-positive results in 8 of 12 sera obtained from patients with demonstrable rheumatoid factor. However, the solid phase-IgM system yielded negative results for the rheumatoid sera, provided that appropriate control reactions were performed. The solid phase-IgM system is thus a specific and sensitive method for the determination of CMV IgM antibody.

Cytomegalovirus (CMV) is an important cause of congenital infections (9, 28). Accurate diagnosis of congenital CMV infection is desirable to insure the optimum management of affected children (13). Since viral isolation can require several days to perform and often cannot distinguish between cogenital and perinatal infection, the diagnosis of congenital CMV infection is often made by the demonstration of an immune response to the virus (9, 19, 25). However, since a baby can acquire CMV antibody of the immunoglobulin G (IgG) class by transplacental diffusion in the absence of active infection (18), it is necessary to detect specific CMV antibody of the IgM class to establish the diagnosis of congenital CMV infection in the immediate postnatal period (10, 24). For this reason, a number of solid-phase immunoassays for the measurement of CMV antibody of the IgM class have been described. These assays are based on the binding of CMV antigen or infected cells to a solid phase with the subsequent reaction of the test specimen and antihuman IgM labeled with an enzymatic, fluorescent, or radioactive marker (12, 23, 24).

Recently, a new form of solid-phase immunoassay has been described for the measurement of IgM viral antibody. This system consists of binding IgM to the solid phase by means of antibody to human IgM. Specific antiviral IgM is subsequently identified by the addition of viral antigen and enzyme-labeled viral antibody (5, 7, 27). This report describes such an enzyme immunoassay (EIA) for the measurement of anti-CMV IgM and compares the sensitivity and specificity of this assay system with those of an EIA which utilizes CMV antigen bound to the solid phase.

MATERIALS AND METHODS

Reagents. CMV antiserum was prepared by the immunization of goats with CMV (strain AD-169) purified by ultracentrifugation as previously described (22). The IgG fraction of this serum was isolated by chromatography with diethylaminoethyl-Sephadex and labeled with horseradish peroxidase by the method of Wilson and Nakane (29). (Peroxidase-labeled goat CMV antibody was provided by Bruce Ferguson, Dynatech Diagnostics, South Windham, Maine.) CMV complement fixation antigen and control antigen were obtained from Flow Laboratories,

Inc. (Alexandria, Va.). Antihuman IgM prepared in goats and purified by affinity chromatography was obtained from Antibodies, Inc. (Davis, Calif.). A portion of this antiglobulin was conjugated with alkaline phosphatase (type VII; Sigma Chemical Co., St. Louis, Mo.) by the method of Engvall and Perlmann (6).

Specimens. A total of 10 specimens were available from babies with congenital CMV infection. The sera were obtained during a prospective study of congenital CMV infection. (This study was approved by The Joint Committee for Clinical Investigation, The Johns Hopkins Medical School.) A baby was considered to be congenitally infected with CMV if the virus was isolated from the initial voided urine or if clinical signs of congenital CMV were present or both. These sera were obtained from 6 days to 1 month after birth. Four sera were available from the mothers of children with congenital CMV infection. In addition, a total of 66 sera were available from children without evidence of CMV infection as documented by the absence of cultivatable CMV in at least two urine specimens. The CMV-negative sera were obtained from normal babies, as well as from babies with medical problems such as prematurity, hyaline membrane disease, and bacterial infection. In addition, a total of four sera from babies with documented congenital rubella infection were available for testing. (Rubella sera were supplied by John Sever, National Institutes of Health, Bethesda, Md.) We also tested sera which were positive for rheumatoid factor by latex agglutination with IgG-coated microspheres (14). These sera were obtained from adults with suspected rheumatoid disease.

EIA: S-IgM method. For the solid-phase IgM (S-IgM) method, the optimal dilution of all reagents was determined by checkerboard titration. A volume of 0.1 ml was used for all incubations. Washing steps were performed by the addition of 0.2 ml of phosphate-buffered saline containing 0.05% Tween 20 (PBST) followed by the immediate removal of wash fluid with a vacuum manifold (32). This process was repeated five times in each washing step. Preliminary studies with known positive sera revealed that in both EIA systems, sera at dilutions more concentrated than 1:160 did not give specific activities greater than those found at a dilution of 1:160. In addition, the testing of sera at dilutions more concentrated than 1:160 led to a higher degree of nonspecific activity. Thus, subsequent sera specimens were tested at fourfold dilutions starting at 1:160. The sera were diluted in PBST containing 1% normal goat serum and 0.5% gelatin (PBSTS).

The wells of polyvinyl, round-bottomed microtiter plates (Dynatech, model 220-224) were coated with goat antihuman IgM immunoglobulin at a concentration of 1 μg/ml in 0.06 M carbonate buffer, pH 9.6. After incubation for 14 h at 4°C, the plates were either used immediately or stored until use. Before use, the plates were washed five times with PBST, and each dilution of specimen was added to four microtiter wells. After another incubation for 2 h at 37°C, the plates were washed, and samples of either CMV complement fixation antigen or control antigen, both diluted 1:160 in PBSTS, were added to the plate in such a way that each specimen dilution was tested in duplicate wells to which CMV antigen had been added and duplicate wells to which control antigen had been added. After incubation for 1 h at 37°C, the plates were washed, and peroxidase-labeled goat anti-CMV diluted 1:200 in PBSTS was added. After incubation for 1 h at 37°C, the plates were washed, and substrate solution was added. This substrate solution was prepared by adding 40 mg of *o*-phenylenediamine and 40 μl of 30% hydrogen peroxide to 100 ml of 0.01 M citrate buffer (pH 5.0) immediately before use. After incubation for 30 min at 37°C, 25 μl of 2 M H_2SO_4 was added, and the amount of color generated by the binding of peroxidase-labeled anti-CMV to the solid phase was measured in a microplate colorimeter at a wavelength of 492 nm (4). For each dilution of specimen a specific activity was calculated by subtracting the mean optical density in the wells to which control antigen had been added from the mean optical density of the specimen dilution tested in wells to which CMV had been added (30). At least three negative control specimens consisting of normal adult sera or buffer were included with each plate. A dilution was considered to be positive for CMV IgM antibody if it yielded a specific activity which was 2 standard deviations greater than the mean of the negative control specimens.

CMV IgM EIA: S-Ag method. The solid phase-antigen (S-Ag) method was modified slightly from that of Schmitz et al. and Voller et al. (23, 27). The wells of polyvinyl microtiter plates were coated alternately with CMV antigen or control antigen. After overnight incubation at 4°C, the plates were washed, and the specimen dilutions were added to duplicate CMV antigen wells and duplicate control antigen wells. After incubation for 2 h at 37°C, the plates were washed, and alkaline phosphatase-labeled goat antihuman IgM was added. After incubation for 1 h at 37°C, the plates were washed, and *p*-nitrophenyl phosphate substrate, diluted 1 mg/ml in 10% diethanolamine buffer, was added. The plates were incubated for another 30 min at 37°C and the amount of color was measured in the microplate colorimeter at a wavelength of 405 nm. A specific activity was calculated by subtracting the mean optical density of the sera in the wells coated with the control antigen from that in the wells coated with the specific antigen. Positivity was determined as described for the S-IgM assay.

EIA for rheumatoid factor. Sera were tested for rheumatoid factor by an EIA method modified from that of Ziola et al. (33). Human IgG was diluted to a concentration of 1 μg/ml in 0.06 M carbonate buffer (pH 9.6) and added to the wells of polyvinyl microtiter plates. After incubation for 14 h at 4°C, the plates were washed, and the test sera were diluted 1:160 in PBST gelatin before being added to the coated wells. After incubation for 1 h at 37°C, the plates were washed, and enzyme-labeled antihuman IgM was added. After incubation for 1 h at 37°C, the plates were washed, and substrate was added as described above. Three sera from healthy adults were added to each plate as negative controls. A specimen was considered to be positive for rheumatoid factor if it yielded an absorbance value which was 2 standard deviations greater than the mean of the negative control sera.

RESULTS

A total of 10 sera were available from children with congenital CMV infection and 4 sera from mothers of babies with congenital CMV infection acquired during the second trimester of pregnancy. All 14 gave positive results in both the S-IgM and S-Ag systems (Fig. 1). The geometric mean titer of the positive sera was 2,560 for the S-Ag assay and 2,334 for the S-IgM assay. The difference between these two values was not statistically significant. Although there were differences between individual titers, the correlation coefficient between the two systems was 0.61, and the Spearman rank coefficient (2) was 0.54. This Spearman rank coefficient indicates that the relationship between the titers obtained by the S-Ag and S-IgM systems was statistically significant ($P < 0.05$).

A total of 66 sera were available from babies known not to have CMV infection on the basis of repeated negative urine cultures for CMV. These included four sera from children known to have congenital rubella infection. All of these 66 control sera were negative for CMV IgM by the S-IgM assay method. However, 12 of the sera (18.2%), including 2 from children with congenital rubella infection, were positive for CMV IgM in the S-Ag system (Table 1). All 12 false-positive sera contained an IgM antibody reactive to solid-phase IgG as measured by the EIA for rheumatoid factor. In addition, 12 sera from patients with autoimmune diseases and positive for rheumatoid factor both by EIA and latex agglutination were available for testing. Of the 12, 8 yielded positive results for CMV IgM antibody as measured by the S-Ag system. In the case of the S-IgM system, 3 of the 12 sera reacted significantly in wells to which the antigen had been added. However, these sera reacted equally in the wells to which control antigen had been added, yielding a specific activity which was less than 2 standard deviations greater than the mean of the negative controls (Table 2). Thus, all 12 were negative by the criteria used to determine positivity. The S-IgM system was thus significantly more specific ($P < 0.01$) than the S-Ag system for the measurement of IgM CMV antibody.

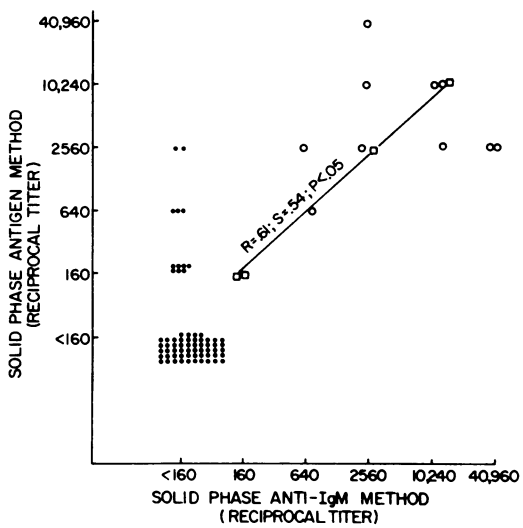

FIG. 1. *Determination of IgM anti-CMV by S-Ag and S-IgM methods. Titers were determined as described in the text. Symbols: ●, sera from babies without evidence of CMV infection; ○, sera from babies with congenital CMV infection as determined by viral cultivation from early voided urine; □, sera from mothers of babies with congenital CMV infection. R, Correlation coefficient of titers of sera from CMV-positive babies and mothers; S, Spearman rank coefficient of titers of sera from CMV-positive babies and mothers. P value was determined from the Spearman rank coefficient (2).*

DISCUSSION

A number of solid-phase immunoassays have been described for the measurement of specific IgM antibody directed against viral, bacterial, and parasitic antigens (16, 23, 27, 32). These assays are based on the binding of antigen to the solid phase and the subsequent addition of patient serum. If the serum contains antibody to the antigen, it will bind to the solid phase, whereas unreacted immunoglobulin and other irrelevant material will be removed in the subsequent washing step. The bound antibody is then identified as belonging to the IgM class by means of specific antihuman IgM labeled with an enzymatic, radioactive, or fluorescent marker. This system has the advantage of requiring only a single labeled reagent, the antihuman IgM, to identify IgM subclass antibodies to a number of

TABLE 1. *Comparison of EIA methods for IgG CMV antibody measurement*

Serum description	No. tested	No. positive[a] by:		P value
		S-IgM	S-Ag	
Proven CMV infection[b]	14	14	14	NS[c]
Babies, CMV negative	66	0	12	<0.01
Rheumatoid factor	11	0	8	<0.01

[a] At a minimum dilution of 1:160.

[b] Includes 10 sera from babies and 4 from mothers of babies with documented CMV infection.

[c] NS, Not significant.

TABLE 2. *Reactivity[a] rheumatoid factor sera for CMV IgM antibody*

Serum	S-Ag			S-IgM		
	CMV	CTR	Sp act	CMV	CTR	Sp act
A	0.641	0.281	0.360[b]	0.592	0.613	<0.01
B	0.294	0.163	0.131[b]	0.189	0.210	<0.01
C	0.228	0.187	0.041	0.242	0.259	<0.01
D	0.561	0.133	0.428[b]	0.260	0.268	<0.01
E	0.169	0.108	0.061	0.180	0.203	<0.01
F	0.382	0.227	0.155[b]	0.200	0.222	<0.01
G	1.031	0.153	0.878[b]	0.358	0.343	0.015
H	0.427	0.240	0.187[b]	0.191	0.175	0.016
I	0.352	0.271	0.081	0.172	0.166	0.006
J	0.309	0.203	0.106[b]	0.245	0.255	<0.01
K	0.253	0.086	0.167[b]	0.265	0.272	<0.01
L	0.150	0.113	0.037	0.239	0.221	0.014
Normal adult			0.035 ± 0.033			0.010 ± 0.014

[a] Optical density measurements of sera diluted 1:160. In the S-Ag assay, specimens were added to wells coated with CMV antigen or tissue culture control (CTR) antigen. In the S-IgM assay, specimens were run in wells to which CMV antigen or control antigen had been added. The specific activities were determined by subtracting the values in the control wells from that of the antigen wells. All serum specimens were obtained from patients with autoimmune diseases and were positive for rheumatoid factor by latex agglutination.

[b] Values are greater than 2 standard deviations above the mean of the normal adult sera (n = 9).

different antigens. In addition, this system does not require the pretreatment of the sera to isolate the immunoglobulin subclasses by means of physiochemical techniques, such as centrifugation or column chromatography to identify the antibody activity as belonging to the IgM subclass.

However, systems utilizing an antigen bound to a solid phase possess a number of theoretical disadvantages. These disadvantages are based on the fact that most sera which contain IgM antibodies to a particular antigen will also contain IgG antibodies to the same antigen (11, 32). Since the IgG antibodies can be present in higher concentrations than the IgM, it is possible that there will be competition for a limited number of antigenic sites and that small amounts of IgM might not be measured in the presence of an excess of IgG (16, 17, 26). In addition, the presence of rheumatoid factor, a class of IgM antibodies with a high affinity for IgG, will lead to a false-positive determination of IgM activity, since such rheumatoid factor antibodies will react with IgG bound to the solid-phase antigen and be detected by the labeled anti-IgM indicator antibody (12, 14, 21). Since the presence of serum rheumatoid factor is common in a number of disease processes, including acute infections in the neonatal period, the presence of rheumatoid factor could be an important confounding event in solid-phase antibody assays for the measurement of specific IgM antibodies (16, 21). These disadvantages are avoided by utilizing a different type of solid-phase assay as described by Duermeyer and Van Derveen and Flehmig et al. (5, 7). In this form of assay, antihuman IgM is bound to the solid phase. The serum specimen is then added. Antibodies of the IgM class bind to the solid phase, whereas IgG antibodies and other irrelevant reactants are removed in the subsequent washing step. The bound IgM antibody is identified as being directed against the antigen in question by the addition of a labeled antigen or, alternately, unlabeled antigen followed by labeled specific antibody directed against the antigen. Since IgG is removed in the first washing step, blocking of antigenic sites with IgG antibody should not be a problem (5, 7, 17). In addition, classical rheumatoid factor should not yield a false-positive result since there is little human IgG with which the IgM can react, provided that human IgG antibody is not used as the labeled indicator (15). To determine whether these theoretical advantages and disadvantages would result in practical differences in the measurement of IgM antibody directed against CMV, we developed a solid-phase anti-IgM EIA for the measurement of CMV anti-IgM and compared its sensitivity and specificity with an EIA based on the binding of CMV antigen to the solid phase. These experiments indicated that both assays had approximately equal sensitivity for the measurement of CMV IgM (Fig. 1). However, although the sensitivity of the assay systems was equivalent, the S-IgM system was more specific than the S-Ag system.

Thus, although no false-positive reactions were noted with the S-IgM system, 18% of the sera tested by the S-Ag system yielded positive results in spite of the fact that they were obtained from babies who had no evidence of CMV infection. All of the false-positive sera contained IgM antibody capable of reacting with IgG, as determined by a sensitive EIA for rheumatoid factor. In addition, sera from 8 of the 12 adults positive for rheumatoid factor showed positive reactivity in the S-Ag system for CMV IgM. With the S-IgM system, three of the rheumatoid factor sera did react with the reagents used to coat the solid phase in this system. However, the false-positive reactivity could be distinguished from true positive reactivity due to the fact that these sera reacted equally in wells with CMV antigen and in those with control antigen (30). Thus, the net specific activity of these sera was not greater than that of the negative control sera. The reactivity noted in the three sera was probably due to the presence of an IgM antibody directed against the goat immunoglobulin used as the enzyme-labeled reagent directed against CMV (30). Such nonspecific reactions do not appear to occur in S-IgM radioimmunoassays for the measurement of IgM antihepatitis antibody, provided that the specimens are diluted in a solution containing normal serum (5, 15). The higher rate of reactivity in EIA systems is probably due to the fact that antiglobulins have a higher affinity for polymerized enzyme-labeled antibody than for monomeric antibody labeled with radioactive iodine (22). It is thus necessary that control reactions be performed in the S-IgM EIA systems to recognize nonspecific reactivity due to antiglobulins.

One additional difference between the S-Ag and S-IgM systems described in this report is that alkaline phosphatase was used as the enzyme marker for the S-Ag system, whereas peroxidase was utilized as the marker for the S-IgM system. However, in previous studies we and others have shown that the two enzymes yield equivalent results in terms of sensitivity (1, 31). In addition, we conducted some experiments using alkaline phosphatase-labeled anti-CMV in the S-IgM system and peroxidase-labeled antihuman IgM in the S-Ag system. In all cases the results were equivalent to the ones presented above. It is therefore unlikely that the choice of enzymes could explain the increased specificity of the S-IgM system as compared to the S-Ag system.

Because of the confounding nature of rheumatoid factor in S-Ag antibody systems, a number of methods have been developed to reduce the influence of rheumatoid factor. These include the physical separation of the IgM fraction of the sera by means of gel exclusion chromatography, density sedimentation, or reaction with a reducing agent (3, 11, 20, 21, 30). In addition, the rheumatoid factor can be absorbed by the incubation of the sera with heat- or glutaraldehyde-aggregated IgG (12, 14, 23, 32). Alternately, some of the IgG can be removed from the sera by absorption with staphylococcal protein A (8). Although all of these methods can reduce the effect of rheumatoid factor to some extent, they do not always result in the complete removal of false-positive reactions due to IgM antiglobulins (7, 21, 26). In addition, the need for preparative reactions increases the time required for the performance of the IgM assay and limits large-scale testing. On the other hand, the S-IgM EIA system was not affected by the presence of rheumatoid factor in the sera as long as proper control reactions were performed. Our results are thus in keeping with those of Moller and Mathiesen, Naot et al., and Naot and Remington, who found the S-IgM method to be the preferred EIA system for measuring IgM antibody directed against hepatitis A (15) and *Toxoplasma gondii* (16, 17).

Schmitz et al. recently described an S-IgM EIA for the measurement of CMV IgM antibody that uses enzyme-labeled CMV antigen to react with the specific IgM bound to the solid phase (22). The results of their system were similar to ours in that excellent specificity and sensitivity were noted. However, their system requires the use of purified enzyme-labeled viral antigen, a reagent which is not widely available. On the other hand, our system, which requires unlabeled antigen but enzyme-labeled antibody, utilizes reagents which are commercially available. The widespread availability of assays capable of accurately measuring CMV antihuman IgM might markedly improve the rapid diagnosis of CMV infections in the prenatal and postnatal periods. In addition, the development of similar assays for the accurate measurement of IgM antibodies directed against other infectious antigens might prove to be useful for the rapid diagnosis of other infectious diseases.

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LITERATURE CITED

1. **Avrameas, S., T. Ternynck, and J. L. Guesdon.** 1978. Coupling of enzymes to antibodies and antigens. Scand. J. Immunol. 8(Suppl. 7):7-23.
2. **Brown, B., and M. Hollander.** 1977. Statistics—a

biomedical introduction, p. 293. John Wiley & Sons, Inc., New York.
3. **Capel, P. J., P. G. Gerlag, J. F. Hagemann, and R. A. Koene.** 1980. The effect of 2-mercaptoethanol on IgM and IgG antibody activity. J. Immunol. Methods **36**:77-80.
4. **Clem, T., and R. H. Yolken.** 1978. Development of a practical colorimeter for ELISA determination. J. Clin. Microbiol. **7**:55-58.
5. **Duermeyer, W., and J. VanDerveen.** 1978. Specific detection of IgM antibodies by ELISA, applied in hepatitis A. Lancet **ii**:684-685.
6. **Engvall, E., and P. Perlmann.** 1972. ELISA III—quantitation of specific antibodies by enzyme linked anti-immunoglobulin in antigen-coated tubes. J. Immunol. **109**:129-135.
7. **Flehmig, B., M. Ranke, J. H. Berthol, and H. J. Gerth.** 1979. A solid phase radioimmunoassay for detection of IgM antibodies against hepatitis A virus. J. Infect. Dis. **140**:169-175.
8. **Handsher, R., and A. Fogel.** 1977. Modified staphylococcal absorption method used for detecting rubella-specific immunoglobulin M antibodies during a rubella epidemic. J. Clin. Microbiol. **5**:588-592.
9. **Hanshaw, J. B.** 1976. Cytomegalovirus, p. 107-155. *In* J. Remington and J. Klein (ed.), Infectious diseases of the fetus and newborn infant. The W. B. Saunders Co., Philadelphia, Pa.
10. **Hanshaw, J. B., H. J. Steinfeld, and C. J. White.** 1968. Fluorescent antibody test for cytomegalovirus macroglobulin. N. Engl. J. Med. **279**:566-570.
11. **Kato, K., Y. Umeda, F. Suzuki, D. Hayashi, and A. Kosaka.** 1979. Use of antibody F(ab)' fragments to remove interference by rheumatoid factors with the enzyme-linked sandwich immunoassay. FEBS Lett. **102**:253-256.
12. **Knez, V., J. A. Stewart, and D. W. Ziegler.** 1976. Cytomegalovirus specific IgM and IgG response in humans studied by radioimmunoassay. J. Immunol. **117**: 2006-2013.
13. **Melish, M. E., and J. B. Hanshaw.** 1973. Congenital cytomegalovirus infection, developmental progress of infants detected by routine screening. Am. J. Dis. Child. **126**:190-194.
14. **Meurman, O. H., and B. R. Ziola.** 1978. IgM class rheumatoid factor interference in the solid phase radioimmunoassay of rubella-specific IgM antibodies. J. Clin. Pathol. **31**:483-487.
15. **Moller, A. M., and C. R. Mathiesen.** 1979. Detection of immunoglobulin M antibodies to hepatitis A by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **10**:628-632.
16. **Naot, Y., G. Desmonts, and J. S. Remington.** 1981. IgM enzyme-linked immunosorbent assay test for the diagnosis of congenital *Toxoplasma* infection. J. Pediatr. **98**:32-36.
17. **Naot, Y., and J. S. Remington.** 1980. An enzyme-linked immunosorbent assay for detection of IgM antibodies to *Toxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. J. Infect. Dis. **142**:757-761.
18. **Reynolds, D. W., S. Stagno, R. Reynolds, and C. A. Alford.** 1978. Perinatal cytomegalovirus infection: influence of placentally transferred maternal antibody. J. Infect. Dis. **137**:564-582.
19. **Reynolds, D. W., S. Stagno, K. G. Stubbs, A. J. Dahle, M. M. Livingston, S. S. Saxon, and C. A. Alford.** 1973. Maternal cytomegalovirus excretion and perinatal infection. N. Engl. J. Med. **289**:1-5.
20. **Robertson, P. W., V. Kertesz, and M. J. Cloonan.** 1977. Elimination of false-positive cytomegalovirus immunoglobulin M-fluorescent-antibody reactions with immunoglobulin M serum fractions. J. Clin. Microbiol. **6**:174-175.
21. **Salonen, E. M., A. Vaheri, J. Suni, and O. Wager.** 1980. Rheumatoid factor in acute viral infections—interference with determination of IgM, IgG and IgA antibodies in an enzyme immunoassay. J. Infect. Dis. **142**:250-255.
22. **Schmitz, H., Y. Deimling, and B. Flehmig.** 1980. Detection of IgM antibodies to cytomegalovirus using an enzyme-labelled antigen (ELA). J. Gen. Virol. **50**:59-68.
23. **Schmitz, H., H. W. Doerr, D. Kampa, and A. Vogt.** 1977. Solid phase enzyme immunoassay for IgM antibodies to cytomegalovirus. J. Clin. Microbiol. **5**:629-634.
24. **Schmitz, H., and R. Haas.** 1972. Determination of different cytomegalovirus immunoglobulins (IgG, IgA, IgM) by immunofluorescence. Arch. Gesamte Virusforsch. **37**:131-140.
25. **Spector, S., K. Schmidt, W. Ticknor, and M. Grossman.** 1979. Cytomegaloviruria in older infants in intensive care nurseries. J. Pediatr. **95**:444-446.
26. **Tuomanen, E., and K. R. Powell.** 1980. Staphylococcal protein A absorption of neonatal serum to facilitate early diagnosis of congenital infection. J. Pediatr. **97**: 238-243.
27. **Voller, A., D. Bidwell, and A. Bartlett.** 1980. Enzyme-linked immunosorbent assay, p. 359-371. *In* N. Rose and H. Friedman (ed.), Manual of clinical immunology. American Society for Microbiology, Washington, D.C.
28. **Weller, T. H., and J. B. Hanshaw.** 1962. Virological and clinical observations of cytomegalic inclusions disease. N. Engl. J. Med. **266**:1233-1344.
29. **Wilson, M. B., and P. K. Nakane.** 1978. Recent developments in the peroxidase method of conjugating horseradish peroxidase to antibodies, p. 215-224. *In* W. Knapp (ed.), Immunofluorescence and related techniques. Elsevier/North Holland Publishing Co., Amsterdam.
30. **Yolken, R. H., and P. J. Stopa.** 1979. Analysis of nonspecific reactions in enzyme-linked immunosorbent assay testing for human rotavirus. J. Clin. Microbiol. **10**: 703-710.
31. **Yolken, R. H., and P. J. Stopa.** 1980. Comparison of seven enzyme immunoassay systems for measurement of cytomegalovirus. J. Clin. Microbiol. **11**:546-551.
32. **Yolken, R. H., R. G. Wyatt, H. W. Kim, A. Z. Kapikian, and R. M. Chanock.** 1978. Immunological response to infection with human reovirus-like agent: measurement of anti-human reovirus-like agent immunoglobulin G and M levels by the method of enzyme-linked immunosorbent assay. Infect. Immun. **19**:540-546.
33. **Ziola, B., O. Meurman, M. T. Matikainen, A. Salmi, and J. Kalliomaki.** 1978. Determination of human immunoglobulin M rheumatoid factor by a solid-phase radioimmunoassay which uses human immunoglobulin G in antigen-antibody complexes. J. Clin. Microbiol. **8**: 134-141.