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Immunoglobulin M Antibodies Detected by Enzyme-Linked Immunosorbent Assay and Radioimmunoassay in the Diagnosis of Cytomegalovirus Infections in Pregnant Women and Newborn Infants

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Immunoglobulin M (IgM) antibodies were detected by a commercially available enzyme-Linked Immunosorbent Assay (ELISA) in 36 of 49 (73%) pregnant women with primary cytomegalovirus (CMV) infection. A positive ELISA-IgM result occurred in 10 of 13 patients (77%) assessed within 8 weeks of seroconversion. The sensitivity of the radioimmunoassay (RIA) to identify primary infection in pregnant women was comparable, 78% in general and 86% for women tested within 16 weeks of seroconversion. Of the 36 women with primary infection who had detectable IgM antibodies by ELISA, 25 (69%) were delivered of congenitally infected infants, whereas of the 13 with undetectable IgM antibodies, 7 (54%) transmitted the infection in utero. IgM antibodies were detected by ELISA in only 5 of 43 (11%) women who experienced a recurrence of CMV which either did or did not result in congenital infection. RIA was less likely to measure CMV-specific IgM in recurrent infection, inasmuch as 1 of 19 (5.2%) women with proven recurrent infection had detectable IgM antibody, giving RIA a better specificity for primary infection. Specific IgM antibodies were detected by ELISA in 42 of 61 (69%) babies congenitally infected with CMV and in 4 of 70 (5.7%) uninfected control newborn infants. The RIA was superior for diagnosis of congenital CMV infection, with a sensitivity of 89% and a specificity of 100%. The lower sensitivity of the ELISA-IgM occurred in the category of congenitally infected infants born to mothers with recurrent infection (43%), a group that is at the lowest risk of disease or to develop sequalae. This commercially available ELISA-IgM could be used in combination with a CMV-specific IgG test for monitoring women during pregnancy for primary infection.

Cytomegalovirus (CMV) is the most common cause of congenital viral infections in humans, with an incidence ranging from 0.2 to 2.2% of all live births in different populations (19). In the United States only a small proportion (5%) of infected newborns have typical cytomegalic inclusion disease, and their prognosis is universally poor. Among those with inapparent infection 10% or perhaps 15% will subsequently develop abnormalities of hearing, vision, and intellectual function and dental defects (19). The factors that control these important complications of congenital CMV infection have been difficult to delineate, in part because primary as well as recurrent maternal infections can be transmitted in utero and because in pregnant women, as with most other groups, primary CMV infections are generally (95%) asymptomatic and thus go undetected (21, 23). Except for the period between conception and the first prenatal visit, gestational CMV infection may be diagnosed by demonstrating seroconversion with serial antibody determinations with an immunoglobulin G (IgG) antibody test such as immunofluorescence or an enzyme-linked immunosorbent assay (ELISA), an approach whose cost efffectiveness remains uncertain (4, 6, 9, 14, 17, 21, 24; G. A. Nankervis, M. L. Kumar, and E. Gold, Pediatr. Res. 8:427, 1974). To rule out the infection during the first trimester among those who are seropositive in the first postconceptional specimen, it is necessary to test that specimen for CMV-specific IgM antibodies (7). The presence of IgM antibodies helps discriminate between primary and recurrent infections, inasmuch as IgM antibodies are not or are only rarely found in recurrent CMV infections. Of the several assays available to measure CMV-specific IgM in pregnant women, the radioimmunoassay (RIA) has the highest specificity and sensitivity (1–3, 5, 7, 10, 11, 18, 25). This assay is also the most specific serological technique to identify cases of congenital CMV infection, with 90% concordance when compared with viral isolation (8, 11). However, the high cost of equipment and reagents, concern about radioactivity, the short shelf life of radiolabeled probes, and the lack of commercially available kits are well-recognized limitations of the RIA. The purpose of this study was to evaluate the ability of a commercially available ELISA-IgM kit to diagnose congenital CMV infections in infants and to distinguish primary from recurrent infections in pregnant women, irrespective of whether intrauterine transmission of CMV had occurred, and to compare results with an in-house prepared RIA-IgM.

MATERIALS AND METHODS

Study population. Study A was restricted to the examination of cord sera. Serum specimens were collected from 61 infants proven to be congenitally infected with CMV by means of viral isolation from urine in the first week of life. These serum specimens had been stored at −20°C from 1 month to 14 years and had been thawed on several occasions. Eleven of the babies had typical signs and symptoms

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of cytomegalic inclusion disease. Fifty infants were asymptomatic at birth. In 15 patients congenital infections resulted from primary maternal infections, inasmuch as seroconversion was documented during pregnancy. Fourteen patients were born to mothers who had recurrent CMV infection, inasmuch as IgG class-specific antibodies were shown to be present before conception. In the remaining 32 patients the type of maternal CMV infection could not be defined.

Control cord sera were collected from 70 babies proven not to be congenitally infected with CMV by failure to isolate virus from urine during the first week of life. A total of 8 control babies were born to CMV-seronegative women, and 62 were born to seropositive women. Among the latter, eight infants were born to mothers who had a primary CMV infection during pregnancy which did not result in intrauterine infection; eight infants had increased cord levels of total IgM; 10 babies were included because their cord sera contained rheumatoid factors (RF).

Study B included serum samples collected from pregnant women during a series of prospective studies. These patients are divided into four groups: group 1, patients with proven primary CMV infection (seroconversion) during pregnancy; group 2, women shown to possess IgG antibodies to CMV before conception who were delivered of congenitally infected infants (recurrent infection with transmission in utero; group 3, women shown to possess IgG antibodies to CMV before conception who excreted CMV from the cervix or urine, or both, during pregnancy but whose infants were not infected (recurrent infection without transmission in utero; and group 4, random seropositive women in the early stages of pregnancy (mean, 9.3 weeks of gestation). Overall, 489 serum samples from 292 patients were available as follows: 135 samples from 49 patients in group 1; 69 samples from 27 women in group 2; 85 samples from 16 patients in group 3; and 200 samples from 200 women in group 4. These serum samples had also been stored at −20°C for 1 month to 13 years, and some had been thawed on several occasions.

Serology. IgG antibodies to CMV for pregnant women were measured with a commercially available ELISA (Cytomegelisa; Whittaker M. A. Bioproducts, Walkersville, Md.) (2, 15, 16). Procedures and interpretation of ELISA values were done according to the instructions of the manufacturer. Briefly, optical density values at 405 nm were measured for a single 1/50 dilution of serum in both CMV antigen-coated and control antigen-coated microwells after addition of alkaline phosphatase-conjugated rabbit antibody to human IgG, washing, and addition of substrate (*p*-nitrophenylphosphate). For each serum sample the ELISA value was calculated from a calibration curve.

Detection of IgM antibodies to CMV. All specimens from pregnant women were tested by an ELISA for CMV-specific IgM (ELISA-IgM) provided by Whittaker M. A. Bioproducts. Cord sera were tested on an experimental CMV-specific ELISA-IgM. To remove interfering RF and potentially competing IgG antibodies, all sera were absorbed with a modified staphylococcal protein A preparation (2, 12, 15). The protein A preparation was standardized to remove more than 95% of total IgG as measured by radial immunodiffusion. Controls for each assay included one negative and two positive calibrator sera and an RF-positive sera. For each serum sample the ELISA value was calculated from a calibration curve. The CMV ELISA-IgM was modified to increase the sensitivity for measuring CMV-specific IgM in cord sera. The CMV antigen was coated at a higher concentration, and concentration of protein A was altered to minimize the amount of IgM removal that occurs with protein A absorption. On the basis of preliminary data, corrected optical density values of ≥0.300 and ≥0.600 were considered positive for cord and adult sera, respectively. The 0.600 cut-off value for adults was chosen to enhance the distinction between recurrent and primary infection.

Ninety-two specimens of cord sera (47 from CMV-infected and 45 from uninfected control babies) and 209 specimens from pregnant women (94 from group 1, 65 from group 2, and 50 from group 3) had been previously tested for IgM antibodies to CMV by solid-phase RIA. The results obtained with the RIA in both study groups have been published (7, 8). Briefly the test was performed and the results were calculated as described by Kangro (10). Alkaline glycine-extracted, complement-fixing antigen and control antigen were obtained commercially (Calbiochem-Behring Corp., La Jolla, Calif.). They were diluted to contain 1.0 complement-fixing U/ml, and 50-μl volumes were Formalin-fixed to polyvinyl chloride microtiter plates (Dynatech Laboratories, Alexandria, Va.). Rabbit antihuman IgM serum (Calbiochem-Behring) was purified by affinity chromatography. This antiserum was labeled with [^{125}I]sodium iodide (New England Nuclear Corp., Boston, Mass.) by the iodogen method to a specific activity of 16 μCi/μg of protein for testing cord sera and 9 to 11 μCi/μg of protein when testing the other specimens.

Serum samples were initially screened for the presence of specific IgM antibodies by testing in duplicate at dilutions of 50, 100, 200, and 400 for cord sera and dilution of 1:100, 1:400, 1:1,600, and 1:6,400 for sera obtained from pregnant women (7, 8). To exclude the possibility of false-positive reactions being produced by RF, sera (10 μl) giving positive reactions at any of these screening dilutions were incubated for 1 h at 37°C with 500 μl of latex beads containing human IgG (Rapi/Tex; Calbiochem-Behring). After centrifugation at 4,000 × *g* for 30 min, doubling dilutions of the supernatant fluids were titrated in duplicate in one batch by RIA. In each assay for cord sera, the radiolabel was used within 24 h of iodination, as prior experiments had shown that such fresh radiolabel most readily detected low levels of the specific antibody (8). For maternal sera the labeled antiserum could be used over a period of 3 weeks (7).

Some technical differences between ELISA and RIA used in this study are worth mentioning. In ELISA-IgM tests, all sera are preabsorbed with Staffinoc (Whittaker M. A. Bioproducts), a preparation containing staphylococcal protein A and streptococci. This absorption effectively eliminates all nonspecific IgM reactions by removing all IgG, including IgG3 and IgA (12). This pretreatment of serum significantly increased the sensitivity of the specific IgM assay by removing most competing IgG. It also eliminated the risk of false-positive results due to RF (IgM antibodies to IgG). With the RIA-IgM only sera which were found IgM positive were subsequently absorbed with latex beads coated with IgG, which removed free RF but did not remove IgG antibodies.

Statistical analyses. The χ^2 test was used to assess observed differences in distributions. Student's *t* test and Duncan's multiple-range test were used to compare mean titers.

RESULTS

Study A: detection of IgM antibody to CMV in cord sera by ELISA. The results of testing 131 cord sera are illustrated in Table 1. The sensitivity of the ELISA for diagnosing congenital CMV infection was 69% (42 of 61 infants) when compared with virus isolation from the urine. The sensitivity

TABLE 1. CMV-specific IgM antibodies by ELISA and RIA in cord sera from congenitally infected infants and uninfected control subjects

Subjects	No. positive/total (%) by:	
	ELISA	RIA
Congenitally infected infants		
Symptomatic	10/11 (91)	3/3 (100)
Asymptomatic	32/50 (64)	38/44 (86)
After primary maternal infection	11/15 (73)[a]	8/10 (80)
After recurrent maternal infection	6/14 (43)	10/12 (83)
Unknown type of maternal infection	25/32 (78)	23/25 (92)
Total	42/61 (69)	41/47 (87)
Uninfected controls		
From seronegative mothers	0/8 (0)	0/6 (0)
With maternal seroconversion	0/8 (0)	0/4 (0)
From seropositive mothers	1/36 (3)	0/21 (0)
Raised total IgM level in cord	2/8 (25)	0/8 (0)
RF in cord serum	1/10 (10)	0/6 (0)
Total	4/70 (5.7)[b]	0/45 (0)

[a] Only one symptomatic infant.
[b] All specimens found positive by ELISA were negative by RIA.

of the assay was higher for symptomatic (91%) than for asymptomatic (64%) infants and for babies born to women who experienced a primary infection (73%) as opposed to a recurrent infection during pregnancy (43%). However, the differences between symptomatic and asymptomatic or primary and recurrent groups were not statistically significant (P = 0.08 and 0.09, respectively). The specificity of the assay was 94.3% since 4 of the 70 cord sera collected from uninfected infants were positive. As illustrated in Table 1, these false-positive results occurred in 4 of 34 specimens from uninfected infants born to seropositive mothers. Two of the positive samples were among eight samples from infants with raised total IgM levels in the cord, and 1 positive specimen was among 10 serum samples which contained RF. The cord blood ELISA-IgM values for babies with symptomatic and asymptomatic infections were compared. The mean ELISA value for symptomatic infants was 1.053, whereas the mean for asymptomatic infants was 0.743 (P = 0.18). A similar comparison was made between infants infected after primary and recurrent maternal CMV infections. The mean ELISA value (0.943) of cord sera taken from infants of the primary CMV infection group was significantly greater than the mean ELISA value (0.262) found in cord sera from infants born to women in the recurrent infection group (P = 0.02).

Forty-seven cord serum samples from the congenitally infected infants had been examined by RIA for IgM antibodies. The sensitivity of the RIA was 87%, and the specificity was 100%. In the congenitally infected group both ELISA and RIA results were positive in 36 specimens and negative in 5 specimens. One specimen was positive by ELISA and negative by RIA, whereas the reverse occurred in five specimens. All 45 specimens from the uninfected controls were negative by RIA, and only four gave a positive result by ELISA. The overall concordance of the two tests was 89%.

Study B: detection of specific IgM antibodies to CMV by ELISA in women with four types of maternal CMV infections. The results of testing 489 serum specimens from 292 women with four types of CMV infections are shown in Table 2. Overall IgM antibodies were demonstrated by ELISA-IgM in 35% of 135 serum specimens or in 73% of 49 women with proven primary infection during pregnancy. In contrast, only 3 of 69 specimens (4%) obtained from 3 of 27 (11%) women who experienced a recurrence of CMV infection which resulted in congenital infection contained this class of antibody. These three specimens were obtained 2.5, 3.5, and 4 years, respectively, after these women had been documented to be seropositive, and in all three patients earlier specimens had been negative for IgM antibodies. Specific IgM antibodies were also detected by ELISA in 3 of 85 (4%) specimens obtained from 2 of 16 women in the recurrent-without-transmission group. Two of the three positive specimens were obtained at a time these two women were shedding CMV. The third specimen was obtained 5 months after the patient had ceased excreting virus. These results show there was a highly significant difference (P = 0.00005) in the proportion of women with detectable IgM antibodies between primary and recurrent CMV infection. Finally, IgM antibodies were also demonstrated in 5 of 200 specimens obtained in early gestation from 200 random seropositive women. Two of these five patients had donated blood 5 and 23 months before conception and were seronegative at that time. Thus, it is conceivable they had a recent primary infection. In fact in the interim, one of them had miscarried at 12 weeks of gestation, followed by a mononucleosis-like syndrome within 2 to 5 months before the demonstration of IgM antibodies. Of the other four patients, one had a spontaneous abortion 2 weeks after IgM antibodies were first demonstrated. The products of conception were not examined. The other three women delivered uninfected, normal infants.

For women with proven primary CMV infection during pregnancy, the mean ELISA value for specimens obtained on the date seroconversion was confirmed was 1.490. In contrast, for women who underwent recurrent infections which resulted in congenital infection, women with recurrent CMV infection without transmission, and random seropositive women in early stages of pregnancy, the mean ELISA values were 0.2040, 0.3616, and 0.1410, respectively.

As illustrated in Table 2, the ability of the RIA to discriminate between primary and recurrent CMV infections during pregnancy was better than ELISA, inasmuch as 78% of the women (44% of the sera) with primary infection and none of the 19 women with proven recurrent infection with transmission had detectable IgM antibodies by RIA (including the three that had detectable antibodies by ELISA). In the group of women with recurrent CMV infection without transmission, only one specimen (ELISA-IgM negative) obtained from a patient in the early stages of gestation gave a positive RIA reaction for IgM antibodies.

The kinetics of the IgM class antibody responses were studied in women with primary infection by examining both the rate of seropositivity and the titer of IgM antibody detected by ELISA in serial specimens of sera. In 13 women with seroconversions localized to within 8 weeks, IgM antibodies were detected in 10 (77%). In the remaining 36 women the exact time at which seroconversions occurred was not well defined because the interval between the collection of their last seronegative and first seropositive (IgG class antibody) specimens was too long. The reason for the failure to detect specific IgM antibodies in 10 of these 36 women with proven primary CMV infections may in some cases be explained by the long intervals between serum specimens. However, of the 13 women who failed to demonstrate ELISA-IgM antibodies, five had a serum sample tested within 16 weeks of their seroconversions; two of these

TABLE 2. CMV-specific IgM antibodies by ELISA and RIA in serum specimens from women with four types of CMV infections

Type of infection[a]	No. positive/total (%) by ELISA			No. positive/total (%) by RIA	
	Patients	Sera	Mean (SE)	Patients	Sera
Primary	36/49 (73)	47/135 (35)	1.490 (0.159)	25/32 (78)	41/94 (44)
Recurrent with transmission	3/27 (11)	3/69 (4)	0.2040 (0.0244)	0/19 (0)	0/65 (0)
Recurrent without transmission	2/16 (12)	3/85 (4)	0.3616 (0.0761)	1/6 (17)	1/50 (2)
Random seropositive	5/200 (2.5)	5/200 (2.5)	0.1410 (0.060)	ND	ND

[a] Primary, women who underwent seroconversions during pregnancy; recurrent with transmission, women shown to possess IgG antibodies to CMV before conception and who were later delivered of infected infants; recurrent without transmission, women shown to possess IgG antibodies before conception and who excreted CMV, but whose infants were not infected in utero; random seropositive, women shown to possess IgG antibodies to CMV in the early stages of gestation.

five patients also failed to react by RIA. The sensitivity of the RIA for IgM to identify primary infections within 16 weeks of seroconversion was 86% (7).

The duration of the IgM response was also investigated postseroconversion. Table 3 shows the proportion of sera with demonstrable levels of IgM antibodies and the mean ELISA value at various time intervals after seroconversion. For each interval we considered only one specimen per patient. Seventy-three percent of the first seropositive (IgG) samples had IgM-specific antibodies. The percentage of reactive specimens remained at 70% at 1 to 8 weeks and then dropped to 23 and 24%, respectively, at 9 to 16 weeks and more than 17 weeks past the first seropositive specimen (P = 0.00003 and 0.00002, respectively). Mean optical density values also declined significantly from 1.490 to 0.377 over the same intervals (P = 0.0001). In only one patient did IgM antibodies become undetectable, and 2 weeks later they reappeared. The persistance of IgM beyond 17 weeks was carefully scrutinized. Overall 6 of 25 (24%) women had detectable IgM antibody by ELISA, and 3 of the 21 (14%) tested by RIA also had similar results. Only one patient had demonstrable levels with both tests. The longest time that IgM antibody was detectable was 30 weeks by ELISA and 28 weeks by RIA.

The results of testing for specific IgM antibodies by ELISA in the 49 women who had primary CMV infections during pregnancy were also analyzed according to whether the infection in congenital infection or not (Table 4). Overall, 78% of the 32 women who delivered infected infants and 65% of the 17 who delivered uninfected babies possessed IgM antibodies. Or, stated from the point of view of serological results, of the 36 women that possessed IgM antibodies, only 25 (69%) transmitted the infection in utero. Of the 13 women that had undetectable IgM antibodies, 7 (54%) delivered infected infants. For women with primary infections localized within 16 weeks, 83 and 71%, respectively, delivered infected and uninfected infants. The persistance of IgM antibodies to CMV between 1 and 16 weeks postseroconversion was documented in 16 (73%) of the mothers of infected infants, in contrast to only 4 (50%) of the women who were delivered of uninfected infants. None of these differences was statistically significant.

TABLE 3. Demonstration of IgM antibodies to CMV by ELISA and values for serum samples of women who seroconverted during pregnancy

Wk after first seropositive specimen	No. positive/ total (%)	Mean ELISA value (SE)
0	36/49 (73)	1.490 (0.159)
1–8	16/23 (70)	0.915 (0.161)
9–16	5/22 (23)	0.472 (0.098)
≥17	6/25 (24)	0.377 (0.086)

DISCUSSION

The results of this study showed that CMV-specific ELISA-IgM done with commercially prepared kits could correctly identify 69% of the infants with congenital CMV infections and 73% of the women with a recent primary CMV infection.

The results of the study on pregnant women showed that the commercially available ELISA-IgM was as sensitive as the RIA-IgM to identify recent primary CMV infections in pregnant women (73 versus 78%). Although not all patients were tested by both assays and the tests were performed at different times, the two assays performed comparably. It is unfortunate that even when serum samples were examined soon after seroconversion (within 8 weeks), these tests failed to demonstrate a specific IgM antibody response in 10 to 15% of the cases. Although the risk of transmission in utero was somewhat higher among women with detectable IgM antibodies (69%) as opposed to those whose tests remained negative (54%) after a primary infection the difference was not statistically significant. Only a small percentage of women with well-defined recurrent infections (reactivation or reinfection) during pregnancy had detectable levels of specific IgM antibodies in serum. Of 46 such patients, 5 were

TABLE 4. Specific IgM antibodies to CMV by ELISA in sera from women who did and did not transmit CMV infection in utero after a primary infection during pregnancy

Subjects	No. (%) of women[a] delivered of:	
	Congenitally infected infants	Uninfected infants
Women possessing IgM antibodies	25/32 (78)	11/17 (65)
Women possessing IgM antibodies with seroconversion localized to ≤16 wk	10/12 (83)	10/14 (71)
Women with persistent IgM antibodies between 1 and 16 wk postseroconversion	16/22 (73)	4/8 (50)

[a] The mean (± standard error) ELISA-IgM values for the first specimens containing IgM antibodies were 1.042 (0.140) and 1.024 (0.222) for women delivered of congenitally infected infants and uninfected infants, respectively.

identified as positive by ELISA-IgM, whereas only 1 of 25 patients reacted in the RIA-IgM; both assays were better than the immunofluorescence test for differentiating between primary and recurrent infections (7, 11). No difference could be detected between the ELISA-IgM values for mothers with recurrent infections that were transmitted to infants in utero and those for mothers with infections that were not. Although the ability of both ELISA- and RIA-IgM tests to discriminate between primary and recurrent infection was statistically significant, the possibility that 11% of individuals with recurrent infection may have a positive ELISA-IgM result and that 4% of individuals with similar infection may have a positive RIA-IgM result is a fact which limit the usefulness of the assays in the clinical setting.

The ability to distinguish primary from recurrent infection is of great importance, inasmuch as primary maternal infections have a greater pathological potential for the fetus (21, 23). Even though the majority of babies infected by the former route appear to develop normally, symptomatic and affected babies are generally the product of a maternal primary infection. Although the reason(s) for this difference remains ill defined, it is conceivable that a greater amount of virus might be transmitted to the fetus during the course of a primary infection, resulting in a more severe infection and greater antigenic stimulation (20).

Specific IgM antibodies were detected by ELISA only transiently after seroconversion. Overall 83% of the patients with primary infections localized to within 16 weeks and 70% of the patients examined between 1 and 8 weeks after the first seropositive specimen produced IgM antibodies that were detected by ELISA. Beyond 17 weeks the percentage dropped to 24%, whereas for RIA-IgM it was 14%. This persistence of IgM antibodies is worrisome if one of these tests is to be used to diagnose primary maternal infection in early pregnancy. The longest time that IgM antibody was detectable was 30 weeks by ELISA and 28 weeks by RIA. Consequently, a positive result with either test in a serum specimen obtained early in pregnancy, particularly if the titer is low, could represent CMV infection acquired before conception.

As a diagnostic tool for screening for congenital CMV infection, the experimental ELISA-IgM adapted for neonates with a sensitivity of 69% and a specificity of 94% was not as good as an in-house prepared RIA which had a sensitivity of 87% and specificity of 100% (8). However, both tests were much better than the indirect immunofluorescent test for IgM antibodies, which in earlier studies indentified only 45 to 80% of infected babies and showed false-positive reactions in 20 to 33% of sera from uninfected controls (8, 13, 22). The proportion of positive ELISA-IgM results and the mean ELISA-IgM values observed in cord sera were higher in clinically affected infants, a reflection of the greater antigenic stimulation sustained by symptomatic infants in utero. Also, the proportion of positive ELISA-IgM results and the mean ELISA-IgM values obtained in cord sera were higher for infants infected as a result of primary maternal infection, even though the majority were asymptomatic. Similar observations have been made with RIA-IgM and the immunofluorescent test for IgM (8). The lowest sensitivity of the ELISA-IgM occurred in the category of congenitally infected infants born to mothers with recurrent CMV infection (43%). The neonatal ELISA-IgM was least sensitive in detecting IgM in the group of infected infants that is at the lowest risk of disease or to develop sequelae at a later age (20). Although the ELISA-IgM compared favorably with the RIA-IgM and its ability to identify symptomatic infants and those infected after primary maternal infection, both techniques had lower sensitivity than isolation of virus (22). This procedure should remain the standard against which new diagnostic tests must be compared.

Although in these studies the ELISA-IgM test was slightly less sensitive and specific than the RIA-IgM, it has several technical advantages for a general clinical virology laboratory. The test is commercially available, has a long shelf life, and does not require radiolabeled probes. To efficiently detect low levels of specific IgM antibodies by RIA the radiolabel must be fresh. For instance, to detect CMV IgM antibodies in cord sera it must be used within 72 h of iodination, whereas to detect CMV-IgM antibodies in adult sera it must be no older than 3 to 4 weeks (7, 8). The ELISA-IgM uses protein A to remove IgG from all specimens before testing, which avoids false-positive results due to RF. All sera giving apparently positive reactions in RIA-IgM must either be fractionated or absorbed and then retested. Competing IgG antibodies which are not removed before testing may interfere with IgM antibodies to produce false-negative results in RIA-IgM, particularly when IgG titers are high and IgM titers are low. This may account for the differences seen between assays during recurrent infection.

The ELISA-IgM could be used in combination with a CMV-specific IgG test for monitoring pregnant women for primary infection. The first specimen would be screened for CMV-specific IgG antibody to determine immune status. The ELISA-IgM would be used to rule out primary infection occurring in early pregnancy or to confirm an infection at a later time. However, before this approach for patient management is adopted, it must be understood that there are limitations. The specificity and sensitivity of this and other assays that measure IgM antibodies are less than perfect, and consequently there is the risk of missing a recent primary infection or conversely of diagnosing as primary a recurrent CMV infection. Moreover, since in a small proportion of women with primary infection IgM antibodies assessed by either ELISA or RIA may be detectable for more than 16 weeks, a positive result obtained in the early phases of pregnancy would be difficult to interpret, particularly in attempting to estimate the risk to the fetus.

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