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Evaluation of Abbott CMV-M Enzyme Immunoassay for Detection of Cytomegalovirus Immunoglobulin M Antibody

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The Abbott CMV-M enzyme immunoassay (EIA) for the qualitative determination of immunoglobulin M (IgM) antibody to cytomegalovirus in human serum was compared with the indirect fluorescent-antibody (IFA) test on 338 human serum specimens. Discordant specimens were evaluated by IFA following isolation of IgM fractions. Discordant specimens remaining after IFA testing were evaluated by an IgM-specific EIA (CYTOMEGELISA M; M.A. Bioproducts). After resolution of discordant specimens, the CMV-M EIA was 94.7% sensitive and 99.1% specific.

An important part of the diagnosis of disease caused by cytomegalovirus (CMV) is the evaluation of patient serum for antibody produced in response to the virus. Antibody response in the normal host with a primary infection typically involves an initial immunoglobulin M (IgM) rise, followed by a rise in IgG level, a decline in IgM, and a subsequent persistence of IgG. IgM usually remains detectable for about 16 weeks (3), although in some clinical situations IgM may persist for many months (7). The presence of IgM antibody indicates primary infection with CMV or occasionally reinfection or reactivation of a latent infection (5). In most cases, CMV infection in the normal host is generally mild and subclinical (1), although in some cases CMV disease is apparent. Neonates and immunocompromised patients represent the populations at highest risk of developing clinically significant disease. Since primary maternal infection can be transmitted in utero and most primary infections are asymptomatic and not detected (7), the presence of IgM antibody to CMV is of clinical importance. The purpose of this study was to evaluate the ability of the Abbott CMV-M enzyme immunoassay (EIA) to detect IgM antibody to CMV. The indirect fluorescent-antibody test (IFA) was chosen as the method of comparison because IFA assays have typically been used to detect IgM antibody to CMV. Detection of IgM by IFA, however, may lead to inaccurate diagnosis, as IgG present in the serum may compete with IgM, causing an apparent reduction in IgM titer (2) or incorrect interpretation due to subjectivity in reading. We present the results with 338 serum specimens analyzed for the presence of IgM antibody to CMV by both CMV-M EIA and IFA.

MATERIALS AND METHODS

Populations. One hundred serum specimens from 100 healthy pregnant females were drawn at random points during gestation and evaluated. Thirty-one serum specimens from 25 individuals who received the Towne live attenuated CMV vaccine (6) were tested. These 25 included 21 vaccine recipients who had blood taken approximately 4 weeks postvaccination, 2 vaccine recipients who had blood drawn once prior to and once after vaccination, and 2 vaccine recipients who had blood drawn once prior to and twice after vaccination. Seventy-six serum samples from 32 immunocompromised patients or transplant recipients were tested. Two to three serum specimens were drawn from 1 week and 11 months apart from each immunocompromised person or transplant recipient. One hundred thirty-one serum specimens from 71 individuals who had experienced virologically or serologically confirmed primary, recurrent, or reinfectious CMV infection were tested. Serum specimens were stored at −20 to −70°C and underwent at least one freeze-thaw cycle.

Serology. Levels of IgM antibody to CMV were determined for all 338 specimens with the Abbott CMV-M EIA (Abbott Diagnostics Division, Abbott Park, Ill.) and by IFA. Briefly, the Abbott CMV-M EIA is a solid-phase EIA for the detection of IgM antibody to CMV in human serum. The sample to be tested for IgM antibody was incubated for 2 h at 37°C with a polystyrene bead coated with CMV antigen. After aspiration of unbound material and washing, anti-human IgM antibody conjugated to horseradish peroxidase was added to detect CMV-specific IgM. After incubation for 2 h at 37°C, unbound conjugate was aspirated and the bead was washed. Next, *o*-phenylenediamine solution containing hydrogen peroxide was added to the bead and incubated for 30 min at room temperature. The reaction was stopped with 1 N H_2SO_4, the resulting yellow-orange color being proportional to the amount of IgM antibody to CMV bound to the bead. The A_{492} value of the sample was determined spectrophotometrically. A specimen index (specimen absorbance value divided by low positive control absorbance value) of <0.500 was considered negative for IgM antibody to CMV. A specimen index of 0.500 to 0.799 was considered equivocal. Equivocal results were not included in the calculation of final resolved assay performance. A specimen index of ≥ 0.800 was considered positive for IgM antibody to CMV.

IFA was performed by placing 10 μl of each specimen onto CMV substrate slides (Bion Enterprises, Park Ridge, Ill.). The preparation was incubated for 1 h at 37°C in a moist chamber and then washed three times with a gentle stream of phosphate-buffered saline, allowing each washing to remain on the preparation for 5 min. Following air drying, 10 μl of

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TABLE 1. Clinical data comparing the Abbott CMV-M EIA with IFA, IFA-IgM, and CYTOMEGELISA M by population[a]

Population	No. in group	CMV-M EIA results			Positive predictive value	Negative predictive value
		% Sensitivity	% Specificity	% Agreement		
Healthy pregnant females	100	100.0 (1/1)	100.0 (94/94)	100.0 (95/95)	100.0 (1/1)	100.0 (94/94)
Vaccine recipients	31	100.0 (22/22)	87.5 (7/8)	96.7 (29/30)	95.7 (22/23)	100.0 (7/7)
Immunocompromised persons, transplant recipients	76	91.7 (22/24)	92.7 (38/41)	92.3 (60/65)	88.0 (22/25)	95.0 (38/40)
Recently exposed and actively infected persons	131	100.0 (26/26)	100.0 (80/80)	100.0 (106/106)	100.0 (26/26)	100.0 (80/80)

[a] CMV-M EIA equivocal results were not included in assay performance calculations.

conjugate (Wellcome Research Laboratories, Beckenham, England) was added to each specimen, incubated for 1 h at 37°C in a moist chamber, and washed twice for 5 min with phosphate-buffered saline. Evan blue was used to stain the preparation for 30 s, followed by a 5-min wash with phosphate-buffered saline and air drying. The slide was then mounted and examined for fluorescent CMV inclusions.

In brief, the CYTOMEGELISA M (M.A. Bioproducts) EIA was done by attaching CMV antigen to a polystyrene well. Patient sera were pretreated with a staphylococcal and streptococcal preparation to remove IgG and eliminate false-positive reactions caused by the presence of rheumatoid factor. Pretreated patient sera were then added to test wells. If present, CMV IgM antibody bound to the attached CMV antigen. Unbound antibody was removed by washing. Enzyme conjugate was added which bound to the antibody-antigen complex. Washing removed any unbound enzyme conjugate. Enzyme substrate was added next and incubated for a specific period. The reaction was stopped with sodium hydroxide. The A_{405} of the substrate was measured spectrophotometrically. The absorbance value of the control antigen reaction of each serum specimen was subtracted from the absorbance value of the antigen reaction of the corresponding serum specimen. Plotting these differences against a calibration curve constructed from the three calibrators provided in the kit gave the CYTOMEGELISA M EIA value for each specimen. A CYTOMEGELISA M EIA value of ≥ 0.60 was considered positive for IgM antibody to CMV, whereas a value of <0.60 was considered negative.

Discordant-specimen testing. A result was considered negatively discordant if the IFA result was positive and the CMV-M EIA result was negative; a positively discordant result was one in which the IFA result was negative and the CMV-M EIA result was positive. Discordant results were analyzed by IFA testing after IgM fraction isolation with the IgM/IgG Isolation System II (Isolab, Akron, Ohio) according to the instructions of the manufacturer. Serum specimens remaining discordant between IgM-IFA and CMV-M EIA were further analyzed by CYTOMEGELISA M.

Specimens remaining discordant after analysis by CYTOMEGELISA M were considered false-positive (CMV-M EIA positive/IFA, IgM-IFA, and CYTOMEGELISA M negative) or false-negative (CMV-M EIA negative/IFA, IgM-IFA, and CYTOMEGELISA M positive) results.

Abbott CMV-M EIA testing of sera was performed at the Joseph Stokes, Jr., Research Institute (for samples from transplant and immunocompromised patients and vaccinees), the University of Alabama at Birmingham (for samples from healthy pregnant women and recently exposed persons), and Abbott Laboratories (for samples from recently exposed persons). All sera were sent blinded to Abbott Laboratories for IFA testing. Discordant specimen testing by IgM-IFA and CYTOMEGELISA M was performed blind by Abbott Laboratories.

Formulas. Values were calculated as follows: (i) sensitivity = (no. positive by CMV-M EIA, IFA, IgM-IFA, or CYTOMEGELISA M/no. positive by IFA, IgM-IFA, or CYTOMEGELISA M) × 100; (ii) specificity = (no. negative by CMV-M EIA, IFA, IgM-IFA, or CYTOMEGELISA M/no. negative by IFA, IgM-IFA, or CYTOMEGELISA M) × 100; (iii) agreement = (no. positive + no. negative by CMV-M EIA, IFA, IgM-IFA, or CYTOMEGELISA M/total no. of specimens tested) × 100; (iv) positive [negative] predictive value = (no. positive [negative] by CMV-M EIA, IFA, IgM-IFA, or CYTOMEGELISA M/no. positive [negative] by CMV-M EIA) × 100.

RESULTS

Healthy pregnant females. Ninety-one serum specimens were negative by CMV-M EIA and by IFA. Three specimens were negative discordant, one was positive discordant, and five were CMV-M EIA equivocal and IFA negative. After discordant-specimen resolution, five specimens remained CMV-M EIA equivocal and IFA negative.

Vaccine recipients. Twenty-one serum specimens were positive by CMV-M EIA and IFA, and four were negative by both assays. Three specimens were negative discordant, two were positive discordant, and one was CMV-M EIA equivocal and IFA positive. After discordant-specimen resolution, one specimen remained CMV-M EIA equivocal and IFA positive.

Immunocompromised patients and transplant recipients. Seventeen serum specimens were positive by CMV-M EIA and IFA, while 26 were negative by both methods. Fourteen specimens were negative discordant, 8 were positive discordant, 9 were CMV-M EIA equivocal and IFA positive, and 2 were CMV-M EIA equivocal and IFA negative. Following discordant-specimen resolution, three specimens were CMV-M EIA equivocal and IFA positive, while eight remained CMV-M EIA equivocal and IFA negative.

Individuals with primary, recurrent, or reinfectious CMV. Twenty-five serum specimens were positive by both CMV-M EIA and IFA; 37 specimens were negative by both assays. Forty-three specimens were negative discordant, while one specimen was positive discordant. Fifteen specimens were CMV-M EIA equivocal and IFA positive, and 10 were CMV-M EIA equivocal and IFA negative. After discordant-specimen resolution, six specimens were CMV-M EIA equivocal and IFA positive, and 19 were CMV-M EIA equivocal and IFA negative. Results are presented in Table 1.

Results for samples from four pregnant women who experienced primary CMV infection are illustrated in Table 2. In

TABLE 2. Results for serial specimens from four pregnant women with primary CMV infection

Subject no.	Specimen date (mo/day/yr)	IgM-IFA	Abbott CMV-M EIA (specimen index)	CYTOMEGELISA M (EIA value)
1	8/09/83	+	+ (>2.302)	+ (2.23)
	12/15/83	+	E[a] (0.513)	− (0.10)
	3/04/84	+	− (0.318)	− (0.10)
2	3/22/85	+	+ (1.213)	+ (1.21)
	7/18/85	+	− (0.251)	− (0.25)
	9/16/85	+	− (0.293)	− (0.15)
3	2/02/86	−	− (0.088)	− (0.07)
	8/21/86	+	+ (>2.285)	+ (1.57)
	9/16/86	+	+ (2.103)	+ (1.28)
	12/11/86	+	− (0.494)	− (0.47)
4	1/04/84	−	− (0.090)	− (0.09)
	4/18/84	+	+ (1.812)	+ (1.91)
	5/04/84	+	+ (0.908)	+ (0.96)
	5/17/84	+	+ (1.256)	+ (1.19)
	6/01/84	+	+ (1.690)	+ (1.63)
	7/13/84	+	+ (0.997)	+ (1.05)
	10/08/84	+	E (0.689)	− (0.57)
	5/07/85	+	− (0.370)	− (0.29)

[a] E, Equivocal result.

each case, the results of IFA testing of serial specimens remained positive past the point at which the CMV-M EIA and CYTOMEGELISA M indicated negative results.

DISCUSSION

CMV-M EIA was found to be a sensitive and specific assay. A number of specimens in this study (63 of 338) were initially IFA positive and CMV-M EIA negative. After these 63 specimens were retested by IFA following IgM isolation and by CYTOMEGELISA M, only 2 gave false-negative reactions. Potential causes for these test differences are (i) nonspecific substances present in the non-IgM fraction of some sera detectable by IFA and (ii) the detection by IFA of very low levels of IgM antibody to CMV which either persist into the late stages of primary infections or are produced in response to recurrent or reinfectious CMV. The inability of IFA to differentiate high levels of IgM antibody from low levels is indicated by the 33 serum specimens from individuals experiencing primary, recurrent, or reinfectious CMV which tested CMV-M EIA negative and IgM-IFA positive. CYTOMEGELISA M found these 33 specimens negative for IgM antibody. Further evidence is provided by patient histories showing positive IFA testing across multiple serial specimens throughout the course of infection and convalescence. CMV-M EIA testing of these sera gave negative results for specimens obtained prior to infection, a rise in specimen index values during the acute stage, and a return to negative results.

A number of specimens in this study (8 of 338) were IgM-IFA negative and CMV-M EIA positive. A potential cause of these test differences is that the IgM fraction isolation technique, done to remove IgG and its potentially interfering substances, has been reported to be low in sensitivity, as the recovery of IgM is poor (2). Four of these specimens resulted in CMV-M EIA false-positive results following evaluation by IFA, IgM-IFA, and CYTOMEGELISA M.

The proportion of sera (12.4%) giving equivocal CMV-M EIA results was similar to that reported previously for radioimmunoassay methods (4).

Seven of 338 (2.1%) specimens were not assayable by IFA (due to nonspecific fluorescence or to destruction of the cell monolayer on the glass slide), highlighting a shortcoming of the IFA method. From 5 to 6% of specimens received in the laboratory have been reported as not being assayable by IFA (2).

This study indicates that the Abbott CMV-M EIA is a sensitive and specific method for detection of IgM antibody to CMV.

LITERATURE CITED

1. **Adler, S. P.** 1986. Nosocomial transmission of cytomegalovirus. Pediatr. Infect. Dis. **5**:239–246.
2. **Elder, B. L., and T. F. Smith.** 1986. Evaluation of CYTOMEGELISA™ immunoglobulin M assay and comparison with indirect fluorescent antibody testing of QAE-Sephadex® A50-treated sera. Am. J. Clin. Pathol. **87**:230–235.
3. **Kangro, H. O.** 1980. Evaluation of a radioimmunoassay for IgM-class antibodies against cytomegalovirus. Br. J. Exp. Pathol. **61**:512–520.
4. **Kangro, H. O., J. C. Booth, T. M. F. Bakir, Y. Tryhorn, and S. Sutherland.** 1984. Detection of IgM antibodies against cytomegalovirus: comparison of two radioimmunoassays, enzyme-linked immunosorbent assay and immunofluorescent antibody test. J. Med. Virol. **14**:73–80.
5. **Pass, R. F., P. D. Griffiths, and A. M. August.** 1983. Antibody response to cytomegalovirus after renal transplantation: comparison of patients with primary and recurrent infections. J. Infect. Dis. **147**:40–46.
6. **Plotkin, S. A., J. Farquhar, and E. Hornberger.** 1976. Clinical trials of immunization with the Towne-125 strain of human cytomegalovirus. J. Infect. Dis. **134**:470–475.
7. **Rasmussen, L., D. Kelsall, R. Nelson, W. Carney, M. Hirsch, D. Winston, J. Preiksutis, and T. C. Merigan.** 1982. Virus-specific IgG and IgM antibodies in normal and immunocompromised subjects infected with cytomegalovirus. J. Infect. Dis. **145**:191–199.