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Development and Clinical Evaluation of a Recombinant-Antigen-Based Cytomegalovirus Immunoglobulin M Automated Immunoassay Using the Abbott AxSYM Analyzer

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A new microparticle enzyme immunoassay (MEIA), the Cytomegalovirus (CMV) Immunoglobulin M (IgM) test, was developed on the Abbott AxSYM analyzer. This test uses recombinant CMV antigens derived from portions of four structural and nonstructural proteins of CMV: pUL32 (pp150), pUL44 (pp52), pUL83 (pp65), and pUL80a (pp38). A total of 1,608 specimens from random volunteer blood donors (n = 300), pregnant women (n = 1,118), transplant recipients (n = 6), and patients with various clinical conditions and disease states (n = 184) were tested during development and evaluation of this new assay. In a preliminary clinical evaluation we tested specimens collected prospectively from pregnant women (n = 799) and selected CMV IgM-positive archived specimens from pregnant women (n = 39). The results from the new CMV IgM immunoassay were compared to the results of a consensus interpretation of the results obtained with three commercial CMV IgM immunoassays. The results for specimens with discordant results were resolved by a CMV IgM immunoblot assay. The relative sensitivity, specificity, and agreement for the AxSYM CMV IgM assay were 94.29, 96.28, and 96.19%, respectively, and the resolved sensitivity, specificity, and agreement were 95.83, 97.47, and 97.37%, respectively. We also tested serial specimens from women who experienced seroconversion or a recent CMV infection during gestation (n = 17) and potentially cross-reactive specimens negative for CMV IgM antibody by the consensus tests (n = 184). The AxSYM CMV IgM assay was very sensitive for the detection of CMV IgM during primary CMV infection, as shown by the detection of CMV IgM at the same time as or just prior to the detection of CMV IgG. Specimens from individuals with lupus (n = 16) or parvovirus B19 infection (n = 6) or specimens containing hyper IgM (n = 9), hyper IgG (n = 8), or rheumatoid factor (n = 55) did not cross-react with the AxSYM assay. One specimen each from individuals infected with Epstein-Barr virus (n = 26), measles virus (n = 10), herpes simplex virus (n = 12), or varicella-zoster virus (n = 13) infection, one specimen from an influenza vaccinee (n = 14), and one specimen containing antinuclear antibody cross-reacted with the assay. The overall rate of cross-reactivity of the specimens with the assay was 3.3% (6 of 184). The AxSYM CMV IgM assay is a sensitive and specific assay for the detection of CMV-specific IgM.

Human cytomegalovirus (CMV) is a herpesvirus which is ubiquitously distributed in the human population. Although rarely pathogenic in immunocompetent individuals, the virus poses a significant health threat to immunocompromised individuals and is a significant cause of morbidity and mortality in organ allograft and bone marrow transplant recipients (7, 23, 29). Pregnant women are also a risk group for this virus as CMV is the most common cause of congenital infection. Since infections with CMV either are asymptomatic or are accompanied by symptoms not specific for CMV, laboratory diagnostic methods are used to diagnose CMV infection. Diagnosis of CMV infection can be accomplished by detection of virus in several body fluids such as blood, urine, or saliva or indirectly through serology. Serological tests are used to diagnose primary CMV infection by the detection of antibodies in a previously seronegative individual. In the absence of seroconversion, CMV-specific immunoglobulin M (IgM) is a sensitive and specific indicator of active or recent CMV infection, while it is very often produced during viral reactivation in immunocompromised individuals (1, 19).

Detection of CMV-specific IgM is most commonly done by using preparations of the virus or viral lysate in an enzyme-linked immunosorbent assay (ELISA) (11, 30). Poor agreement among these tests has been found (13, 14), presumably due to the different viral preparations used in the various commercial kits. The key serological targets for detection of CMV-specific IgM comprised both the structural pUL32 (pp150), pUL83 (pp65), and pUL80a (pp38) (8, 9, 10) viral proteins and the nonstructural pUL57 (p130) and pUL44 (pp52) (24, 31) viral proteins. Variations in the relative amounts of these antigens produced during growth and purification of the virus can result in different relative compositions of the structural and nonstructural viral antigens used in the

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various IgM tests. The use of nonstandardized viral antigens to capture CMV IgM can contribute to interassay variation. In contrast, purified recombinant proteins and peptides can be consistently manufactured and optimized to capture CMV-specific IgM, which can improve CMV assay standardization (5, 12, 32). In this work we describe the development and preliminary clinical evaluation of the first fully automated, commercially available, recombinant antigen-based CMV IgM immunoassay.

(A portion of this work was presented at the Abbott-sponsored symposium entitled New Developments in the Diagnosis of CMV, Toxo, and Rubella Infection, held in Venice, Italy, May 1998.)

MATERIALS AND METHODS

Cloning and expression of CMV genes. All CMV gene fragments that encode antigens were obtained by PCR amplification with PCR primers designed to amplify specific nucleotide sequences. These gene fragments were cloned into a modified *Escherichia coli* CKS (CTP:CMP-3-deoxy-D-manno-octulosonate cytidylyl transferase) epitope-embedding expression vector (G. Maine, unpublished results). Plasmids that encode recombinant proteins 4, 9, and 26 (12) were used as template DNA to generate the CKS expression plasmids pCMV-27, pCMV-28, and pCMV-29, respectively, which express the recombinant proteins rp27, rp28, and rp29 fused to CKS, respectively. Portions of the following CMV antigenic regions were contained in three recombinant antigens: rp27 (pUL32 [pp150] and pUL44 [pp52]), rp28 (pUL83 [pp65]), and rp29 (pUL80a [pp38]). The DNA sequences of all cloned CMV genes were determined and confirmed. Bacterial clones that express the fusion proteins were grown in rich media, and the synthesis of the fusion proteins was induced as described previously (26). After postinduction, the cells were harvested and the cell pellets were stored at −80°C until protein purification.

Purification of recombinant fusion proteins. Insoluble fusion proteins (rp27, rp28, and rp29) were purified after lysis by a combination of detergent washes and then solubilization in 1% sodium dodecyl sulfate (26). After solubilization, the fusion proteins were purified by Sephacryl S-300HR chromatography (Pharmacia Biotech, Piscataway, N.J.), dialyzed, and stored at −80°C until coating of microparticles.

Recombinant antigen-coated microparticles. Purified fusion proteins were coated onto polystyrene microparticles (Polysciences, Inc., Warrington, Pa.). After coating, uncoated antigen was removed by diafiltration and the microparticles were resuspended in a microparticle diluent buffer containing Tris buffer with protein (bovine) stabilizers and antimicrobial agents. The microparticle diluent buffer also contains *E. coli* CKS to competitively block the binding of anti-CKS antibodies to the solid phase. After equilibration, the microparticles were diluted to their final concentration, and two pp150 peptides, A1C2 (20mer) and F3 (43mer) (AnaSpec, Inc., San Jose, Calif.), were added. These peptides contain the identical amino acid sequence of pp150 present in the 1A (12) and rp27 fusion proteins and are used to competitively modulate the immunoreactivity of the pp150 amino acid sequences present on the microparticles.

Other reagents. Purified goat anti-human IgM (μ-chain specific; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, Md.) conjugated with alkaline phosphatase (Boehringer Mannheim, Indianapolis, Ind.) (33) was used to detect CMV IgM bound to the microparticles as described previously (28). The instrument was calibrated with an index calibrator prepared with anti-CMV IgM (human) prepared in human serum.

Instrumentation. The AxSYM and IMx instruments (Abbott Laboratories, Abbott Park, Ill.) are automated immunoassay analyzers that use microparticle enzyme immunoassay technology. Details of these instruments are given elsewhere (4, 28).

Human serum samples used for assay cutoff determination and preliminary performance evaluation. (i) Specimens from blood donors and pregnant women. Specimens from random volunteer whole-blood donors (n = 300; Interstate Blood Bank, Memphis, Tenn.) and specimens randomly selected from U.S. and European populations of pregnant women (n = 199; Bartek Associates, Inc., Barrington, Ill.; University of Nantes, Nantes, France) were used to evaluate assay specificity.

(ii) Selected positive specimens. Specimens from pregnant women and heart transplant recipients (n = 73) positive for CMV IgM antibody as determined with the Enzygnost anti-HCMV/IgM kit (Behring AG, Marburg, Germany) or by the CMV IgM immunoblot assay (16, 17) were used to evaluate assay sensitivity. Due to the low natural prevalence of CMV IgM antibody in the general population, it was necessary to run selected positive specimens.

Human serum samples used for clinical evaluation. Specimen testing at the Eastern Virginia Medical School was approved after review of the clinical protocol by the Internal Review Board (compliance no. X97-016), with signed consent from donors obtained when appropriate. Specimen testing at Dianalab did not require special approval of the Internal Review Board. Specimen testing at Abbott Laboratories, Eastern Virginia Medical School, and Dianalab with AxSYM CMV IgM assay investigational reagent lots was conducted by an Abbott Laboratories-approved clinical protocol. Frozen specimens were centrifuged prior to testing.

(i) Specimens from pregnant women. Fresh maternal serum specimens were collected prospectively from pregnant women from Swiss (n = 599; Dianalab S.A., Geneva, Switzerland) and U.S. (n = 200; Eastern Virginia Medical School, Norfolk, Va.) populations. The average age of the women in the Swiss population was 31.1 years, with 53.1, 29.9, and 17.1% of the specimens drawn during the first, second, and third trimesters, respectively. The average age of the women in the U.S. population was 25.9 years, with 40.0 and 60.0% of the specimens drawn during the first and second trimesters, respectively. These specimens were tested with the AxSYM instrument prior to freezing and were used to evaluate assay specificity.

(ii) Selected positive specimens. Selected frozen serum specimens (n = 39) from a Swiss population of pregnant women positive for CMV IgM antibody as determined by the IMx CMV IgM assay (Abbott Laboratories) were used to evaluate assay sensitivity. Three of these specimens overlap with the specimens listed below under Selected serial specimens.

(iii) Selected serial specimens. A total of 17 serial specimens from three suspected CMV IgM-positive women were tested to evaluate the kinetics of appearance and disappearance of CMV-specific IgM. These women were pregnant during the evaluation. These specimens were also tested by the IMx CMV IgM and AxSYM CMV IgG assays.

(iv) Potentially cross-reactive specimens. Potentially cross-reactive specimens, i.e., specimens known to be seropositive for a variety of specific infections and/or medical conditions, were tested to determine potential cross-reactivity in the assay. The potentially cross-reactive specimens were positive for antinuclear antibody (n = 15; Gamma Dynamics, Inc., Pompano Beach, Fla.; Boston Biomedica, Inc., West Bridgewater, Mass.), systemic lupus erythematosus (n = 16; QCP, Inc., Pompano Beach, Fla.), rheumatoid factor (RF); n = 55; (QCP, Inc.), Epstein-Barr virus (n = 26; Boston Biomedica, Inc.; BioClinical Partners, Inc., Sharon, Mass.; BioMedical Resources, Hatboro, Pa.), parvovirus B19 (n = 6), measles virus (n = 10), herpes simplex virus (n = 12), varicella-zoster virus (n = 13) (Boston Biomedica, Inc.), Hyper IgM (n = 9; Bartek Associates, Inc., Barrington, Ill.; BioClinical Partners, Inc.), and Hyper IgG (n = 8; BioClinical Partners, Inc.) or were from influenza vaccinees (n = 14; Cash Blood Bank, Pompano Beach, Fla.). These specimens were characterized by the vendor by the appropriate methodologies to verify the clinical condition or disease state. RF neutralization reagent (Abbott Manufacturing Inc., Abbott Park, Ill.) was used to neutralize RF antibodies.

(v) Precision panels. Serum and plasma panels were prepared to evaluate the precision of the AxSYM assay. Four panel members were negative for CMV IgM, four panel members were low positive (index values, ≤1.000) for CMV IgM, and four panel members were positive for CMV IgM. The low-positive and positive serum and plasma panel members were prepared artificially by spiking CMV-negative serum or plasma with CMV IgM-positive serum.

CMV antigen detection. The method of Wunderli et al. (34) was used for the detection of the immediate-early antigen in human embryo fibroblasts.

Commercial CMV IgM assays and consensus interpretation. The assay cutoff and the relative performance characteristics of the AxSYM assay were determined by testing all specimens with three commercial tests (consensus result) for the detection of CMV IgM: the Gull (Salt Lake City, Utah) CMV IgM ELISA, the Trinity Biotech/Centocor (Jamestown, N.Y., and Malvern, Pa.) CAPTIA CMV-M, and the Abbott Laboratories CMV-M EIA. The results obtained by each of the three commercial assays were interpreted according to the manufacturer's guidelines. A specimen interpretation was based upon a consensus result (two of three) of the assays. If the assays had three different results (positive, negative, and equivocal) a consensus specimen interpretation was not possible and the interpretation "none" was used. The consensus interpretation was chosen for this performance evaluation as it had been shown to agree reasonably well with the CMV IgM immunoblot assay result (16, 17) (data not shown). Specimens that were positive or negative by the AxSYM assay and discordant by the consensus interpretation were further resolved by CMV IgM immunoblot testing (17).

Statistical methods. Sensitivity and specificity were calculated as described by Griner et al. (6). Agreement was calculated as follows: $(TP + TN)/(TP + TN + FP + FN) \times 100$, where TP is the number of true-positive specimens, TN is the number of true-negative specimens, FP is the number of false-positive specimens, and FN is the number of false-negative specimens. The 95% confidence interval (CI) determined for relative sensitivity, specificity, and agreement was based on the binomial distribution by using the STATXACT-3 software (SAS Institute, Inc., Cary, N.C.) (21). A receiver operator characteristic (ROC) analysis was used to assist the determination of the preliminary cutoff for the AxSYM assay (25, 35). The precision of the AxSYM assay was determined by use of National Committee for Clinical Laboratory Standards protocol EP5-T2 as a guideline (22). The standard deviation (SD) and percent coefficient of variance (CV) were determined by a variance component analysis for a random-effects model (2, 27). Negative variance components were set equal to zero.

TABLE 1. AxSYM CMV IgM ROC classification summary

Cutoff	No. of specimens				% Sensitivity	% Specificity	Distance[a]
	Consensus positive and AxSYM CMV IgM:		Consensus negative and AxSYM CMV IgM:				
	Positive	Negative	Positive	Negative			
0.300	76	5	63	408	93.83	86.62	14.73
0.350	74	7	45	426	91.36	90.45	12.88
0.400	72	9	28	443	88.89	94.06	12.60
0.450	69	12	17	454	85.19	96.39	15.24
0.500	68	13	14	457	83.95	97.03	16.32
0.550	65	16	11	460	80.25	97.66	19.89
0.600	64	17	10	461	79.01	97.88	21.10
0.650	64	17	9	462	79.01	98.09	21.08
0.700	61	20	8	463	75.31	98.30	24.75

[a] Distance is an index of overall assay performance in terms of sensitivity and specificity. A value of zero would result if there was 100% sensitivity and 100% specificity.

RESULTS

Determination of assay cutoff and preliminary performance evaluation. An assay cutoff was established by testing 572 specimens from the following categories: 199 specimens from pregnant women, 300 specimens from random volunteer whole-blood donors, and 73 suspected positive specimens from heart transplant recipients and pregnant women. These specimens were tested by the AxSYM CMV IgM assay and by three other commercial assays (Gull CMV IgM ELISA, Trinity Biotech/Centocor CAPTIA CMV-M, and Abbott CMV-M EIA). The results from the AxSYM assay were then compared to the consensus interpretation. ROC analysis was used to assist in the determination of the preliminary cutoff. ROC analysis depicts the overlap between the negative and positive distributions by tabulating sensitivity and specificity over a range of cutoff values. Specimens with a consensus interpretation of none or equivocal were excluded from the ROC analysis. Specimens that were tested by the AxSYM assay and that had index values greater than or equal to the cutoff were classified as positive, and specimens that had index values less than the cutoff were classified as negative. The ROC classification summaries for the AxSYM assay are presented in Table 1. As shown in Table 1, the ROC profile indicates a minimum distance at a cutoff of 0.400 as the optimum point where both sensitivity and specificity were maximized. In order to further optimize the assay cutoff subsequent to the ROC analysis, the cutoff was raised to 0.500 and a normal approximation of proportions statistical *z* test was applied to determine if raising the cutoff would improve assay specificity without negatively affecting assay sensitivity. Comparison of the sensitivity of the AxSYM assay at a 0.500 versus a 0.400 index value cutoff indicated no statistically significant difference in assay sensitivity within a 95% CI ($z = 0.917$; $P > 0.05$). However, a statistically significant difference in assay specificity was observed at this cutoff within a 95% CI ($z = 2.210$; $P \leq 0.05$). On the basis of these analyses, the optimum cutoff for the assay was set at an index value of 0.500.

To further improve the separation between the negative and positive populations, an equivocal zone with index values from 0.400 to 0.499 was introduced. Specimen results were then interpreted as follows. Specimens with index values less than 0.400 were considered negative for CMV IgM antibody. Specimens with index values in the range of 0.400 to 0.499 were considered equivocal. Specimens interpreted as equivocal may contain very low levels of CMV IgM antibody. Specimens with index values equal to or greater than 0.500 were considered positive for CMV IgM antibody. All specimens were then tested by the three commercial assays. The relative sensitivity, specificity, and agreement for the AxSYM CMV IgM assay are shown in Table 2. There were approximately 4 SDs from the mean for the negative population to the assay cutoff of 0.500 (data not shown). Specimens that had positive and negative results by the AxSYM CMV IgM assay but that were discordant by the consensus interpretation were tested by the CMV IgM immunoblot assay. Of the 23 discordant specimens, 21 were tested by the CMV IgM immunoblot assay. In addition, one of the six specimens which was negative by AxSYM and none by the consensus interpretation was also tested by the

TABLE 2. Comparison of AxSYM CMV IgM assay results to consensus and resolved interpretations[a]

Interpretation	Serum sample group (no. of specimens)	No. of specimens with the following interpretation:				% Sensitivity (95% CI)	% Specificity (95% CI)	% Agreement (95% CI)
		Pos	Eqv	Neg	None			
Consensus	Pos (93)	68	0	14	11	88.31 (78.97–94.51)		
	Eqv (21)	4	0	15	2		96.93 (94.90–98.31)	
	Neg (458)	9	1	442	6			95.68 (93.60–97.25)
Resolved	Pos (93)	76	0	6	11	97.44 (91.04–99.69)		
	Eqv (21)	4	0	15	2		98.68 (97.16–99.52)	
	Neg (458)	2	1	450	5			98.50 (97.07–99.35)

[a] Consensus assays: Abbott CMV-M EIA, Gull CMV IgM ELISA, and Trinity Biotech/Centocor CAPTIA CMV-M. Abbreviations: Pos, positive; Eqv, equivocal; Neg, negative; None, no interpretation. Sensitivity, specificity, and agreement are relative values for the consensus interpretation and resolved values for the resolved interpretation.

TABLE 3. Comparison of AxSYM CMV IgM assay results to consensus and resolved interpretations[a]

Population and test	Serum specimen group (no. of specimens)	No. of specimens							
		Consensus interpretation				Resolved interpretation			
		Pos	Eqv	Neg	None	Pos	Eqv	Neg	None
Pregnant women (n = 799)[b]									
AxSYM	Pos (36)	6	1	27	2	16	0	19	1
CMV	Eqv (34)	2	0	27	5	2	0	27	5
IgM	Neg (729)	1	2	722	4	2	0	727	0
Suspected CMV IgM-positive pregnant women (n = 39)[c]									
AxSYM	Pos (30)	27	0	1	2	30	0	0	0
CMV	Eqv (5)	2	0	2	1	2	0	2	1
IgM	Neg (4)	1	0	3	0	0	0	4	0

[a] Consensus assays: Abbott CMV-M EIA, Gull CMV IgM ELISA, and Trinity Biotech/Centocor CAPITA CMV-M. Abbreviations: Pos, positive; Eqv, equivocal; Neg, negative; None, no interpretation.
[b] Relative specificity = 96.40% (95% CI = 94.80 to 97.61%); resolved specificity = 97.45% (95% CI = 96.05 to 98.46%).
[c] Relative sensitivity = 96.43% (95% CI = 81.65 to 99.91%); resolved sensitivity = 100.00% (95% CI = 88.43 to 100.00%).

immunoblot assay. The resolved sensitivity, specificity, and agreement for the AxSYM CMV IgM assay are shown in Table 2.

The AxSYM CMV IgM and IMx CMV IgM version 2.0 assays were developed in parallel, and both use recombinant CMV antigen-coated microparticles. The main difference between these assays is that the AxSYM and IMx assay reagents are run on their respective instruments. With samples (n = 572) from the same patient population described above, we compared the performance of the AxSYM assay to that of the IMx version 2.0 assay. The relative agreement between these assays was calculated to be 99.26% (535 of 539), with the results for four of the 572 specimens tested being discordant. In contrast, 26 specimens had discordant results between the IMx version 2.0 assay and the consensus interpretation (data not shown).

Evaluation of assay precision. The precision of the AxSYM assay was determined by National Committee for Clinical Laboratory Standards Protocol EP5-T2 (22) as a guideline. The precision panels were run twice daily for 21 days. The SD and percent CV were determined by a variance component analysis for a random-effects model (2, 27). The total CVs for the AxSYM assay ranged from 7.3 to 13.7%. The assay precision near the cutoff with the low-positive panels ranged from 7.9 to 9.7%.

Clinical evaluation of assay with samples from pregnant women. The performance characteristics of the AxSYM CMV IgM assay were determined in part by the prospective evaluation of random serum specimens from pregnant women. All three trimesters of pregnancy were represented by this population. The specimens were tested by the AxSYM CMV IgM assay (prior to freezing) and the three commercial CMV IgM assays (Gull CMV IgM ELISA, Trinity Biotech/Centocor CAPTIA CMV-M, and Abbott CMV-M EIA) over a 2-month period as the specimens arrived in the laboratory. A total of 599 specimens from a Swiss population and 200 specimens from a U.S. population were tested by the AxSYM assay, and the results were compared to the consensus interpretation. The relative specificity for the AxSYM CMV IgM assay is shown in Table 3. The CMV IgM positive reactivity rates for the U.S. and Swiss populations as measured by the AxSYM assay were 4.0 and 4.7%, respectively. The CMV IgM positive reactivity rates for the AxSYM, Gull, Captia, Abbott EIA, and consensus interpretation for both populations were 4.5, 3.3, 3.0, 1.3, and 1.1%, respectively. There were approximately 4 SDs from the mean for the negative population to the assay cutoff. Specimens that had positive and negative results by the AxSYM CMV IgM assay (n = 37) but that were discordant by the consensus interpretation (positive, negative, equivocal, or none) were tested by the CMV IgM Immunoblot assay. The resolved specificity for the AxSYM CMV IgM assay is shown in Table 3. The performance characteristics of the AxSYM CMV IgM assay were also determined in part by the retrospective evaluation of selected CMV IgM-positive specimens from pregnant women. In the first part of this retrospective evaluation, 39 specimens from individual pregnant women positive for CMV IgM by the IMx CMV IgM assay were tested by the AxSYM CMV IgM assay and by the three commercial assays. The relative sensitivity for the AxSYM CMV IgM assay is shown in Table 3. Specimens that had positive and negative results by the AxSYM CMV IgM assay (n = 4) but that were discordant by the consensus interpretation (positive, negative, or none) were tested by the CMV IgM immunoblot assay. The resolved sensitivity for the AxSYM CMV IgM assay is shown in Table 3. By combining the results presented in Table 3, the relative sensitivity, specificity, and agreement for the AxSYM CMV IgM assay for this population of pregnant women (n = 838) were 94.29% (80.84 to 99.30%), 96.28% (94.67 to 97.52%), and 96.19% (94.61 to 97.42%), respectively. The 95% CIs are indicated in parentheses. Following resolution of the results for discordant specimens by the CMV IgM immunoblot assay, the resolved sensitivity, specificity, and agreement were 95.83% (85.75 to 99.49%), 97.47% (96.07 to 98.47%), and 97.37% (96.01 to 98.36%), respectively. The 95% CIs are indicated in parentheses.

In the second part of the retrospective evaluation, 17 serial specimens from three pregnant women were evaluated to examine the kinetics of the appearance and disappearance of CMV IgM as measured by the AxSYM CMV IgM assay relative to those measured by the IMx CMV IgM assay and the three commercial CMV IgM assays. The titer of CMV IgG was also monitored in these individuals and was compared to the kinetics of CMV IgM. Patient 1 and 2 experienced a seroconversion to CMV IgG positivity during gestation. Detection of CMV IgM antibodies by the AxSYM and IMx CMV IgM assays occurred at the same time (patient 1) or before (patient 2) detection of CMV IgG antibodies by the AxSYM CMV IgG assay. Following seroconversion to CMV IgG positivity, the CMV IgG titer increased and the CMV IgM antibody titer declined, with the CMV IgM titer declining faster as measured

by the AxSYM CMV IgM assay in patient 2. There were no clinical indications of congenital CMV infection in either pregnancy (as determined by ultrasound) or during the subsequent 2 years of postnatal follow-up. Patient 3 experienced a recent CMV infection as indicated by the presence of CMV IgM (as tested only by the IMx assay) antibodies at week 6 of gestation. The first specimen that was tested by the AxSYM CMV IgM assay was positive for CMV IgM at week 7 of gestation. This pregnancy was terminated at week 8 of gestation, and at autopsy the fetus was found to be congenitally infected with CMV (CMV early antigen positive). During a second pregnancy 5 months later, patient 3 remained positive for CMV IgM as measured by the IMx assay but negative for CMV IgM as measured by the AxSYM assay. The faster kinetics of disappearance of CMV IgM as measured by the AxSYM assay relative to that measured by the IMx assay has been subsequently confirmed with six of eight patients who experienced a recent CMV infection (data not shown).

Evaluation of assay cross-reactivity. Potentially cross-reactive specimens, i.e., specimens known to be seropositive for a variety of specific infections and/or medical conditions, were tested to determine potential cross-reactivity in the assay (n = 184). All specimens tested were negative for CMV IgM antibody by all three commercial assays (consensus). Cross-reactivity was indicated if the specimen was positive by the AxSYM CMV IgM assay. For RF-positive specimens, cross-reactivity was indicated if the result for the specimen by the AxSYM CMV IgM assay changed from positive to negative following neutralization of the specimen with RF neutralization reagent. Specimens from individuals with systemic lupus erythematosus or parvovirus B19 infection or specimens containing Hyper IgM, Hyper IgG, or RF did not cross-react with the AxSYM assay. One specimen each from individuals infected with Epstein-Barr virus, measles virus, herpes simplex virus, or varicella-zoster virus infection, one specimen from an influenza vaccinee, and one specimen containing antinuclear antibodies cross-reacted with the assay. The overall rate of cross-reactivity of the specimens with the assay was 3.3% (6 of 184).

DISCUSSION

One of the problems that the diagnostic laboratory has faced over the past 10 years is the lack of agreement between commercial tests for the detection of CMV-specific IgM (13, 14). This lack of agreement has its roots in the different viral preparations used to detect IgM antibodies to CMV. Since detection of the humoral IgM response is improved by including both structural and nonstructural viral proteins (8, 9, 10, 24, 31), the performance of the viral antigen-based tests is directly dependent on how the virus is grown and how the viral antigens are purified. Our group and others have shown that a balanced cocktail of highly purified recombinant antigens (12, 18) or peptides (5), which contain both structural and nonstructural viral antigens, can replace the virus for detection of CMV-specific IgM. In this report we describe the development of the first automated, commercially available, recombinant antigen-based CMV IgM immunoassay for the detection of CMV-specific IgM.

One of the challenges that we faced with the development of a recombinant antigen-based test is that the results of this new test would likely not agree with those of the other commercial CMV IgM tests whose results disagree with one another. A CMV IgM serological reference standard was needed to define the "truth" for a specimen with respect to the presence of CMV-specific IgM apart from virus detection. During development of the recombinant antigen-based test, we also developed two versions of a CMV IgM immunoblot assay which can be considered a reference test for CMV IgM serology (13, 15, 16, 17). The CMV IgM immunoblot assay (16, 17) was used as a benchmark for development of this recombinant antigen-based CMV IgM test on the AxSYM and IMx immunoassay analyzers. This blot was also used to select the three commercial assays (Abbott CMV-M EIA, Gull CMV IgM ELISA, CAPTIA CMV-M) which, as a consensus, were used to determine the assay cutoff and performance characteristics. The optimal cutoff established for the assay (Tables 1 and 2) was further examined during the clinical evaluation of the assay with samples from a population of pregnant women (Table 3). The resolved sensitivity and specificity for the assay presented in Table 3 are similar to those presented in Table 2, thus validating the cutoff for the assay. Statistical analysis of the precision of the assay near the cutoff indicates that the precision of the assay is sufficient to withstand false-positive or false-negative results on the basis of mere measurement variability within a 95% CI (data not shown).

The sensitivity and specificity of the AxSYM assay were examined further by testing characterized specimens. The sensitivity of the assay was examined by testing serial specimens from pregnant women who experienced seroconversion or a recent CMV infection during gestation. Our results indicate that the AxSYM CMV IgM assay can detect early seroconversion at a rate comparable to that for the viral lysate-based commercial CMV IgM assays. The sensitivities and concomitant positive reactivity rates for various commercial assays for the detection of CMV-specific IgM have been shown to vary widely (13, 14). In this study the AxSYM assay had a higher positive reactivity rate than the three commercial tests. Several studies have shown that anywhere from 5 to 15% of CMV-seropositive women excrete the virus during gestation, with higher rates of viral excretion observed in women of advanced gestational age (20, 29). The positive reactivity rate of the AxSYM assay is consistent with this percentage of women undergoing active CMV infection, as indicated by excretion of the virus. The specificity of the AxSYM assay was further evaluated by testing potentially cross-reactive specimens. Low levels of cross-reactivity were observed for the assay. Treatment of CMV IgM-positive or equivocal specimens with RF neutralization reagent was found to be unnecessary. In conclusion, the new recombinant antigen-based AxSYM CMV IgM assay is a sensitive and specific test for the detection of CMV-specific IgM.

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