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Evaluation of Microparticle Enzyme Immunoassays for Immunoglobulins G and M to Rubella Virus and *Toxoplasma gondii* on the Abbott IMx Automated Analyzer

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The ability of the Abbott IMx automated analyzer to detect immunoglobulin G (IgG) and IgM antibodies to rubella virus and to *Toxoplasma gondii* was compared with the abilities of RUBAZYME, RUBAZYME-M, ABBOTT TOXO-G enzyme immunoassay, and ABBOTT TOXO-M enzyme immunoassay, respectively. Specimens that produced discordant results were evaluated by RUBACELL II, Behring Enzygnost-Rubella enzyme-linked immunosorbent assay, Behring Enzygnost Toxoplasmosis/IgG, and bioMerieux Toxo-ISAGA (immunosorbent agglutination assay), respectively. After resolution of discordant results, IMx Rubella IgG, IMx Rubella IgM, IMx Toxo IgG, and IMx Toxo IgM antibody assays had sensitivities of 99.9, 100, 98.0, and 100%; specificities of 98.9, 99.0, 97.5, and 98.7%; and accuracies of 99.8, 99.3, 97.8, and 98.8%, respectively.

In utero infection with either rubella virus or *Toxoplasma gondii* can severely damage the fetus, causing congenital rubella syndrome (including low birth weight, cataracts, deafness, congenital heart disease, and mental retardation) (9) or congenital toxoplasmosis (including neurologic disease, ocular involvement, auditory impairment, and intellectual defects) (3).

A number of tests are available to determine the presence of immunoglobulin G (IgG) and IgM antibodies to rubella virus or *T. gondii* in human serum (18). IgG tests are used to document immunity or past infection by the presence of protective levels of IgG antibodies. IgM tests are used to detect IgM antibodies produced as a result of recent infection. The more commonly used tests include indirect immunofluorescence (26), fluoroimmunoassay (4, 25), enzyme immunoassay (EIA) (2, 28), latex agglutination (19), passive hemagglutination (15), hemagglutination inhibition (13), radioimmunoassay (11), reverse passive latex agglutination (18), and hemolysis-in-gel (21). These methods require one or more of the following: reagent preparation, stepwise manual addition of reagents, manipulation of test specimens following one or more lengthy incubations, and reading and recording of test results. Although most methods demonstrate excellent sensitivity and specificity, the speed with which a result can be obtained and the simplicity with which a method may be performed remain important considerations in its selection for routine use in the clinical laboratory. A procedure that provides highly accurate results and requires minimal technician interaction would be a significant improvement over the often labor-intensive, technique-dependent test methods currently available.

The Abbott IMx, an automated immunoassay system, was previously reported to provide automation of microparticle capture enzyme immunoassays (MEIAs) for high-molecular-mass analytes (7). The purpose of this study was to evaluate the ability of the IMx to detect IgG and IgM antibodies to rubella virus and IgG and IgM antibodies to *T. gondii*. Methods of comparison were RUBAZYME, RUBAZYME-M, ABBOTT TOXO-G EIA, and ABBOTT TOXO-M EIA, respectively. We present the results from 865 specimens tested for rubella IgG, 283 specimens tested for rubella IgM, 625 specimens tested for *T. gondii* IgG, and 339 specimens tested for *T. gondii* IgM antibodies.

MATERIALS AND METHODS

Specimens. Serum specimens were collected in England, France, Germany, Italy, Scotland, Spain, and the United States from healthy individuals and from those seeking medical attention. All specimens were received frozen at Abbott Laboratories, thawed, mixed well, split into two aliquots, sequentially numbered, and refrozen. One frozen aliquot of each specimen was sent blinded to the clinical site for evaluation, while the second remained at Abbott for comparison testing, also done blinded. Specimens were stored at −20 to −70°C and underwent at least two freeze-thaw cycles.

IMx procedures. Prior to the evaluation of clinical specimens, each laboratory performed testing on duplicate panel members of various analyte concentrations to establish assay reproducibility. At the start of the study and at specified intervals throughout the study, calibration curves were performed to assess stored-curve stability. Each of three sites evaluated two of the four IMx assays. Levels of IgG antibodies to rubella virus and *T. gondii* were determined by using IMx Rubella IgG antibody and IMx Toxo IgG antibody assays, respectively. Once specimens were loaded into disposable reaction cells and placed into the IMx carousel, the instrument automatically performed all steps required for their analysis. Briefly, both assays involved antigen-coated microparticles which, when added to a serum specimen previously diluted by the IMx and containing the specific IgG antibody being measured, formed an antigen-antibody complex. This complex was then further diluted, allowed to pass over a glass fiber matrix, and washed with

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buffer. A conjugate of anti-μ or anti-γ antibody coupled to alkaline phosphatase was added to form an antigen-antibody-conjugate complex. This complex was washed with buffer to remove any unbound conjugate, and a substrate of 4-methylumbelliferyl phosphate was added. The rate of conversion of the substrate to a fluorescent product was expressed in international units of antibody per milliliter by point-to-point extrapolation from a calibration curve. A calibration curve (stable for at least 2 weeks) had been previously prepared by using six calibrators at concentrations across the assay range and was stored in memory. Slight run-to-run variation was adjusted for by incorporating a calibrator in the first position on each specimen carousel. Results for each test specimen were calculated off the adjusted, stored calibration curve. Positive and negative controls were included with each carousel. Specimens found to contain ≥10 IU of IgG antibodies to rubella or ≥6 IU of IgG antibodies to *T. gondii* per ml were considered positive; specimens with values below the positive cutoff were considered negative for IgG antibodies.

Detection of IgM antibodies was performed in a similar manner by using IMx Rubella IgM antibody assay and IMx Toxo IgM antibody assay, except that instead of determining specimen values in international units per milliliter, an IgM index was calculated for each specimen. The IMx index calibrator was placed in the first carousel position, and the IMx calculated a ratio (specimen index) of the specimen value to the index calibrator value. A specimen with an index of ≥0.900 for rubella or ≥0.600 for *T. gondii* was considered reactive for IgM antibodies. A specimen with an index between 0.800 and 0.899 for rubella or between 0.500 and 0.599 for *T. gondii* was considered equivocal. A specimen with an index below the equivocal zone was considered negative. All specimens found reactive for IgM antibodies were adsorbed with IMx rheumatoid factor neutralization reagent (human gamma globulin-coated microparticles) and retested. Rheumatoid factor neutralization was necessary to avoid the false-reactive test results that would occur should rheumatoid factor become bound to IgG specifically reactive to the antigen-coated microparticle. Following adsorption, any specimen with an index of ≥0.900 for rubella or ≥0.600 for *T. gondii* was considered positive for IgM antibodies. Any specimen with an index between 0.800 and 0.899 for rubella or between 0.500 and 0.599 for *T. gondii* was considered equivocal. Any specimen with an index below the equivocal zone was considered negative.

Comparison testing procedures. Comparison testing with RUBAZYME, RUBAZYME-M, ABBOTT TOXO-G EIA, and ABBOTT TOXO-M EIA was performed blind by Abbott according to the instructions of the manufacturer.

Resolution of discordant results. A result was considered negatively discordant if the comparison testing result was positive and the IMx result was negative; a positively discordant result was one in which the comparison testing result was negative and the IMx result was positive. Discordant rubella IgG results were resolved by using RUBACELL II, rubella IgM results were resolved by using Behring Enzygnost-Rubella enzyme-linked immunosorbent assay, *T. gondii* IgG results were resolved by using Behring Enzygnost Toxoplasmosis/IgG, and *T. gondii* IgM results were resolved by using bioMerieux Toxo-ISAGA (immunosorbent agglutination assay). Specimens that remained discordant following this further analysis were considered false-positive (IMx positive and discordant-result resolution method negative) or false-negative (IMx negative and discordant-result resolution method positive).

TABLE 1. Summary of reproducibility and curve storage data

Assay	Panel IU or index	CV (%)[a]		No. of:		Curve storage time (days)
		Within site	Between site	Runs	Sites	
IMx Rubella IgG (IU/ml)	0	NA[b]	NA	32	3	35
	10	4	6			
	34	5	3			
	75	7	1			
	160	9	7			
	360	11	8			
IMx Rubella IgM (index)	0.306	11	3	19	2	20–30
	0.527	11	4			
	1.441	7	1			
	2.632	7	2			
IMx Toxo IgG (IU/ml)	0	NA	NA	36	3	35–48
	8	7	5			
	33	3	2			
	66	5	3			
	92	5	5			
	221	7	1			
IMx Toxo IgM (index)	0.247	8	15	20	2	19–21
	0.404	4	2			
	1.509	3	1			
	2.687	2	0			

[a] CVs rounded off to the nearest whole integer.
[b] NA, Not available.

Formulae. Values were calculated as follows: (i) sensitivity = [number correctly identified as positive by IMx/(number correctly identified as positive by IMx + number incorrectly identified as negative by IMx)] × 100; (ii) specificity = [number correctly identified as negative by IMx/(number correctly identified as negative by IMx + number incorrectly identified as positive by IMx)] × 100; (iii) accuracy = [(number correctly identified as positive by IMx + number correctly identified as negative by IMx)/total number of specimens tested] × 100.

RESULTS

Assay reproducibility and curve storage. Within-site reproducibility for the four IMx antibody assays ranged from 2 to 11% coefficient of variation (CV), whereas between-site reproducibility ranged from 0 to 15% CV. Stored curves for the two IMx IgG antibody assays remained stable over the 35- to 48-day period during which they were evaluated. Results of assay reproducibility and curve storage testing done at four sites on panel members in duplicate at various concentrations are presented in Table 1.

IMx Rubella IgG antibody assay. A total of 748 specimens were positive by both IMx Rubella IgG antibody and RUBAZYME assays, while 90 specimens were negative by both assays. One specimen was negatively discordant, whereas 26 were positively discordant. After discordant-specimen resolution, one false-negative and one false-positive remained.

IMx Rubella IgM antibody assay. A total of 59 specimens were positive by both IMx Rubella IgM antibody and RUBAZYME-M assays, and 207 specimens were negative by both assays. Four specimens were positively discordant. Five specimens were IMx positive and RUBAZYME-M equivocal; two were equivocal by both assays; one was IMx negative and RUBAZYME-M equivocal; five were IMx

TABLE 2. Performance characteristics of four MEIAs on the Abbott IMx[a]

IMx MEIA	% Sensitivity	% Specificity	% Accuracy
Rubella IgG antibody	99.9 (773/774)	98.9 (90/91)	99.8 (863/865)
Rubella IgM antibody	100.0 (61/61)	99.0 (207/209)	99.3 (268/270)
Toxo IgG antibody	98.0 (296/302)	97.5 (315/323)	97.8 (611/625)
Toxo IgM antibody	100.0 (24/24)	98.7 (299/303)	98.8 (323/327)

[a] Equivocal results obtained using the IMx, comparison testing methods, and discordant-specimen resolution methods were not included in assay performance calculations. For formulae used to obtain results, see Materials and Methods.

equivocal and RUBAZYME-M negative. No further evaluation of specimens testing equivocal in either assay was carried out. After discordant-specimen resolution, two false-positives remained.

IMx Toxo IgG antibody assay. A total of 289 specimens were positive by both IMx Toxo IgG antibody and ABBOTT TOXO-G EIA, and 315 specimens were negative by both assays. Six specimens were negatively discordant, whereas 15 were positively discordant. After discordant-specimen resolution, six false-negatives and eight false-positives remained.

IMx Toxo IgM antibody assay. A total of 22 specimens were positive by both IMx Toxo IgM antibody and ABBOTT TOXO-M EIA, and 299 were negative by both assays. Six specimens were positively discordant. One specimen was IMx positive and EIA equivocal; one was equivocal by both assays; three were IMx negative and EIA equivocal; seven were IMx equivocal and EIA negative. No further evaluation of specimens testing equivocal in either assay was carried out. After discordant-specimen resolution, four false-positives remained.

A summary of the performance characteristics of the four IMx MEIAs evaluated is presented in Table 2.

DISCUSSION

In studies that compare the performances of various methods to detect IgG and IgM antibodies to rubella virus, it has been shown that no one method is perfect (1, 5, 6, 12–14, 17, 20, 22, 29). The same is true for methods used to detect IgG and IgM antibodies to *T. gondii* (8, 10, 16, 23, 24, 26, 27). Such studies have shown that certain sera yield discordant results. Sera collected during various stages of infection and convalescence often give different results, since methods have different sensitivities, measure different immunoglobulin classes of antibodies, or measure antibodies to different viral components (1). One of the reasons for these test differences may be the different antigens coated on the solid phase of each assay and the resulting ability of each assay to detect these immunoglobulin classes and viral components. Specimens that yielded discordant test results following evaluation by IMx Rubella IgG, IMx Toxo IgG, IMx Toxo IgM, and their respective comparison methods may be explained by such differences in solid-phase coating. When the IMx Rubella IgG assay with a sensitivity of 10 IU/ml is considered, it is not surprising that 26 serum specimens were IMx positive and RUBAZYME negative, as the sensitivity of RUBAZYME is approximately 15 IU/ml. Evaluation of these 26 specimens by using RUBACELL II resulted in 25 IgG antibody-positive specimens. The majority of these specimens yielded RUBAZYME test results just below the 1.000 index-positive cutoff.

Reproducibility reported previously for the assay of alpha-fetoprotein on the IMx ranged from 2.6 to 6.3% CV (7). Similar reproducibilities, well within acceptable laboratory limits, are demonstrated by these four IMx assays. Stored curves, stated to be stable for at least 2 weeks, demonstrated no instability when evaluated for 35 to 48 days. Curve stability such as this eliminates the costly and time-consuming recalibrations common with some analyzers.

The IMx is an automated system that requires only that the technician add specimens, place the unitized reagent pack into the instrument, and press "RUN." Times from run initiation to result printout for a full carousel of specimens evaluated for IgG or IgM antibodies, a partial carousel of specimens evaluated for IgG antibodies, and a partial carousel of specimens evaluated for IgM antibodies were 35, 30, and 18 min, respectively. Assays can be performed on the IMx on all laboratory shifts because of the ease of use of the instrument. Specimens previously sent to outside test laboratories because of assay complexity can be performed in-house on the IMx. Shorter run times and automated IMx methods allow low-volume tests to be performed at less cost and with shorter turnaround in the clinical laboratory.

This study indicates that IMx Rubella IgG antibody assay, IMx Rubella IgM antibody assay, IMx Toxo IgG antibody assay, and IMx Toxo IgM antibody assay are sensitive and specific methods for IgG and IgM antibody detection and that rapid and accurate results are obtainable by using these reagents with the IMx automated immunoassay system.

LITERATURE CITED

1. **Chernesky, M., D. DeLong, J. Mahony, and S. Castriciano.** 1986. Differences in antibody responses with rapid agglutination tests for the detection of rubella antibodies. J. Clin. Microbiol. **23**:772–776.
2. **Cremer, N. E., S. J. Hagens, and R. Fukuchi.** 1983. Improved serological diagnosis of rubella. J. Clin. Microbiol. **18**:743–744.
3. **Desmonts, G., and J. Couvreur.** 1974. Congenital toxoplasmosis. A prospective study of 378 pregnancies. N. Engl. J. Med. **290**:1110–1116.
4. **Echevarria, J. M., F. de Ory, and R. Najera.** 1985. Fluoroimmunoassay for detection of rubella-specific immunoglobulin M: comparison with indirect enzyme immunoassay and μ-chain capture. J. Clin. Microbiol. **22**:428–434.
5. **Enders, G., and F. Knotek.** 1986. Detection of IgM antibodies against rubella virus: comparison of two indirect ELISAs and an anti-IgM capture immunoassay. J. Med. Virol. **19**:377–386.
6. **Field, P., and C. Gong.** 1984. Diagnosis of postnatally acquired rubella by use of three enzyme-linked immunosorbent assays for specific immunoglobulins G and M and single radial hemolysis for specific immunoglobulin G. J. Clin. Microbiol. **20**: 951–958.
7. **Fiore, M., J. Mitchell, T. Doan, R. Nelson, G. Winter, C. Grandone, K. Zeng, R. Haraden, J. Smith, K. Harris, J. Leszczynski, D. Berry, S. Safford, G. Barnes, A. Scholnick, and K. Ludington.** 1988. The Abbott IMx™ automated benchtop immunochemistry analyzer system. Clin. Chem. **34**:1726–1732.
8. **Fuccillo, D., D. Madden, N. Tzan, and J. Sever.** 1987. Difficulties associated with serological diagnosis of *Toxoplasma gondii* infections. Diagn. Clin. Immunol. **5**:8–13.
9. **Hanshaw, J., and J. Dudgeon.** 1978. Viral diseases of the fetus and newborn. Major Probl. Clin. Pediatr. **17**:17–96.
10. **Herbrink, P., A. van Loon, J. Rotmans, W. van Knapen, and W. van Dijk.** 1987. Interlaboratory evaluation of indirect enzyme-linked immunosorbent assay, antibody capture enzyme-linked immunosorbent assay, and immunoblotting for detection of immunoglobulin M antibodies to *Toxoplasma gondii*. J. Clin. Microbiol. **25**:100–105.

11. **Kalimo, K., O. Meurman, P. Halonen, B. Aiola, M. Viljanaen, K. Granfors, and P. Toivanen.** 1976. Solid-phase radioimmunoassay of rubella virus immunoglobulin G and immunoglobulin M antibodies. J. Clin. Microbiol. **4**:117–123.
12. **Kyriatzis, D., I. Kampa, and S. Garner.** 1984. Comparison of three diagnostic test kits for rubella. Lab. Med. **15**:199–201.
13. **Lennette, E., N. Schmidt, and R. Magoffin.** 1967. The hemagglutination inhibition test for rubella: a comparison of its sensitivity to that of neutralization, complement fixation and fluorescent antibody tests for diagnosis of infection and determination of immunity status. J. Immunol. **99**:785–793.
14. **Mahony, J., S. Castriciano, and M. Chernesky.** 1987. Cost and performance analysis of hemagglutination inhibition, passive hemagglutination, radial haemolysis, and enzyme immunoassay for measuring rubella antibody. J. Virol. Methods **18**:133–142.
15. **Meurman, O.** 1977. Antibody responses in patients with rubella infection determined by passive hemagglutination, hemagglutination inhibition, complement fixation, and solid-phase radioimmunoassay tests. Infect. Immun. **19**:369–372.
16. **Naot, Y., and J. Remmington.** 1980. An enzyme-linked immunoassay for detection of IgM antibodies to *Toxoplasma gondii*; use for diagnosis of acute acquired toxoplasmosis. J. Infect. Dis. **142**:757–766.
17. **Safford, J., G. Abbott, and C. Deimler.** 1985. Evaluation of a rapid passive hemagglutination assay for anti-rubella antibody: comparison to hemagglutination inhibition and a vaccine challenge study. J. Med. Virol. **17**:229–236.
18. **Sever, J.** 1985. TORCH tests and what they mean. Am. J. Obstet. Gynecol. **152**:495–498.
19. **Sever, J., N. Tzan, I. Shekarchi, and D. Madden.** 1982. Rapid latex agglutination test for rubella antibody. J. Clin. Microbiol. **17**:52–54.
20. **Skenzel, L., K. Wilcox, and D. Edson.** 1983. Evaluation of assays for the detection of antibodies to rubella. A report based on data from the College of American Pathologists surveys of 1982. Am. J. Clin. Pathol. **80**:594–598.
21. **Stannegard, O., L. Grillner, and I. Lindberg.** 1975. Hemolysis-in-gel test for demonstration of antibodies to rubella virus. J. Clin. Microbiol. **1**:491–494.
22. **Vaananen, P., V.-M. Haiva, P. Koskela, and O. Meurman.** 1985. Comparison of a simple latex agglutination test with hemolysis-in-gel, hemagglutination inhibition, and radioimmunoassay for detection of rubella virus antibodies. J. Clin. Microbiol. **21**: 793–795.
23. **van Loon, A., J. van der Logt, F. Heessen, and J. van der Veen.** 1983. Enzyme-linked immunosorbent assay that uses labeled antigen for detection of immunoglobulin M and A antibodies in toxoplasmosis: comparison with indirect immunofluorescence and double-sandwich enzyme-linked immunosorbent assay. J. Clin. Microbiol. **17**:997–1004.
24. **Violand, S., T. Mitchell, and K. Kleeman.** 1982. Comparison of an enzyme-linked immunoassay and a quantitative indirect fluorescent-antibody test with the conventional indirect fluorescent-antibody test for detecting antibodies to *Toxoplasma gondii*. J. Clin. Microbiol. **16**:341–344.
25. **Walls, K. W., and E. R. Barnhart.** 1978. Titration of human serum antibodies to *Toxoplasma gondii* with a simple fluorometric assay. J. Clin. Microbiol. **7**:234–235.
26. **Walton, B., B. Benchoff, and W. Brooks.** 1966. Comparison of the indirect fluorescent antibody test and methylene blue dye test for detection of antibodies to *Toxoplasma gondii*. Am. J. Trop. Med. Hyg. **15**:149–152.
27. **Wielaard, F., H. van Gruijthuijsen, W. Duermeyer, A. Joss, L. Skinner, H. Williams, and E. van Elven.** 1983. Diagnosis of acute toxoplasmosis by an enzyme immunoassay for specific immunoglobulin M antibodies. J. Clin. Microbiol. **17**:981–987.
28. **Wilson, M., D. Ware, and K. Walls.** 1987. Evaluation of commercial serodiagnostic kits for toxoplasmosis. J. Clin. Microbiol. **25**:2262–2265.
29. **Wittenburg, R., M. Roberts, L. Elliott, and L. Little.** 1985. Comparative evaluation of commercial rubella virus antibody kits. J. Clin. Microbiol. **21**:161–163.