JOURNAL OF CLINICAL MICROBIOLOGY, July 2004, p. 3191–3195 Vol. 42, No. 7
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.7.3191–3195.2004

Comparison of Three Commercially Available Serologic Assays Used To Detect Human Parvovirus B19-Specific Immunoglobulin M (IgM) and IgG Antibodies in Sera of Pregnant Women

Allyson R. Butchko[1] and Jeanne A. Jordan[1,2]*

Department of Pathology, University of Pittsburgh,[1] and Magee-Women's Research Institute,[2] Pittsburgh, Pennsylvania 15213

Received 5 January 2004/Returned for modification 26 February 2004/Accepted 3 April 2004

A split-sample study was conducted to evaluate the performances of three enzyme immunoassays (EIAs) utilizing one or more conformational antigens to detect human parvovirus B19 (B19V)-specific immunoglobulin M (IgM) or IgG in the sera of 198 pregnant women. We compared EIAs available from Biotrin International, Inc. (Dublin, Ireland), Medac Diagnostika (Wedel, Germany), and Mikrogen (Martinsried, Germany). Specimens with discordant results were analyzed further using an immunofluorescence assay (Biotrin). Equivocal data accounted for close to half of all the discrepant results for both IgM and IgG, with 7 of 15 discrepant results from the Medac and Mikrogen kits involving equivocal data and the Biotrin kit giving a single equivocal result. For each specimen, a consensus was established from the four test results if agreement occurred among at least three of four results. Overall, the highest percentage of agreement with the consensus results was seen when Biotrin kits were used; 194 (100%) of 194 and 194 (99.5%) of 195 results for IgM and IgG, respectively, agreed with the consensus results. When Medac kits were used, 189 (97.4%) of 194 and 191 (97.9%) of 195 results for IgM and IgG, respectively, agreed with the consensus, and when Mikrogen kits were used, 179 (92.3%) of 194 and 193 (99%) of 195 results for IgM and IgG, respectively, agreed with the consensus. Given the consensus results, the Medac EIA appeared to generate presumed false-positive results for IgM and the Mikrogen EIA appeared to generate presumed false-positive results for IgG and IgM. In summary, the Biotrin EIAs produced far fewer equivocal results than the other assays and results of the Biotrin EIAs agreed more often with the consensus results than did those of the other commercially available EIAs for detecting B19V-specific IgM and IgG antibodies.

During pregnancy, congenital infection with human parvovirus B19 (B19V) can be associated with poor outcome, including miscarriage, fetal anemia, and nonimmune hydrops (1, 6, 8, 9, 13, 18). Diagnosis of acute B19V infection in a pregnant woman, as defined by detection of measurable levels of B19V-specific immunoglobulin M (IgM) or a ≥4-fold rise in levels of B19V-specific IgG, can precipitate weekly ultrasonographic monitoring for a minimum of 8 to 10 weeks (3). Because of the high cost both financially and emotionally to the woman, it is critical that the physician be provided with the most accurate clinical data regarding the woman's immune status when significant exposure to B19V has been documented or infection with B19V is suspected (11, 12).

The average incubation period for B19V infection in an immunocompetent individual is 7 to 10 days, after which time virus can be detected within respiratory secretions and blood of the infected individual (2). The peak of viremia, which is short lived yet involves high titers, occurs prior to the appearance of specific clinical symptoms and before measurable production of B19V-specific Ig.

If a pregnant woman has detectable B19V-specific IgM but no detectable B19V-specific IgG, one assumes that she was infected within the past 7 days. If her serum contains detectable IgM and IgG, she acquired the infection within the last 7 to 120 days, indicating recent or acute infection. In contrast, levels of circulating B19V-specific IgG to conformational antigens remain elevated for years and their presence in the absence of detectable B19V-specific IgM usually indicates prior exposure or previous infection. The absence of both B19V-specific IgM and IgG indicates lack of infection and infers immune susceptibility status (2).

During acute infection with B19V, specific antibodies to the virion capsid proteins VP1 and VP2 as well as to B19V's nonstructural protein, NS1, are produced (2, 10). Circulating antibodies recognize both linear and conformational epitopes of the capsid proteins. Numerous investigators have demonstrated that B19V-specific IgG antibodies recognizing linear epitopes disappear around 6 months after infection, leaving only circulating antibodies that recognize conformational epitopes (4, 7, 15, 17, 20). Therefore, the nature of the viral antigen(s) used in the B19V-specific serologic assay is an important variable to consider in evaluating analytical test performance.

Assay design is another important feature to take into consideration when evaluating a commercially available assay. Capture enzyme immunoassays (capture EIAs) employing native or recombinant antigens are excellent choices for measuring B19V Ig (5, 15, 19). Systems utilizing either *Escherichia coli*-expressed or baculovirus-expressed B19V antigens or both in combination have been described. Although not all expressed *E. coli* antigens produce linear epitopes, the one expressing B19V-specific antigens does. This contrasts with the

* Corresponding author. Mailing address: Magee-Women's Research Institute, 204 Craft Ave., Pittsburgh, PA 15213. Phone: (412) 641-4104. Fax: (412) 641-6156. E-mail: rsijaj@mwri.magee.edu.

TABLE 1. Summary of features and test principles of B19V-specific EIAs[a]

Manufacturer	Antibody detected	B19V antigen(s)	Microwell coating	HRP conjugate	Total incubation time, temp
Biotrin	IgM	Baculovirus-expressed biotinylated VP2	Rabbit anti-human IgM[b]	Streptavidin	2 h 10 min, RT
Medac	IgM	Baculovirus-expressed VP1 and VP2	Mouse anti-human IgM[b]	Mouse anti-B19V Ig	3 h 30 min, RT
Mikrogen	IgM	*E. coli*-expressed VP1, baculovirus-expressed VP2	VP1 and VP2[c]	Anti-human IgM	2 h, RT and 37°C
Biotrin	IgG	Baculovirus-expressed VP2	VP2[c]	Rabbit anti-human IgG	1 h 40 min, RT
Medac	IgG	Baculovirus-expressed VP1 and VP2	VP1 and VP2[c]	Goat anti-human IgG	2 h 30 min, RT
Mikrogen	IgG	*E. coli*-expressed VP1, baculovirus-expressed VP2	VP1 and VP2[c]	Anti-human IgG	2 h, RT and 37°C

[a] Abbreviations: HRP, horseradish-peroxidase; RT, room temperature.
[b] Mu capture EIA.
[c] Antigen capture EIA.

baculovirus-based expression vectors, which produce conformational antigens. Some baculovirus expression systems also provide posttranslational modifications unavailable in prokaryotic systems that can affect antigenicity. In fact, baculovirus-expressed B19V capsid proteins (VP2 alone or in combination with VP1) can self-assemble into empty capsids with physical and immunogenic properties similar to those of native B19V virions (14).

Capture EIAs incorporating conformational antigens are superior to EIAs utilizing denatured, linear antigens; a previous split-sample study demonstrated significantly fewer equivocal results from baculovirus-based VP2 EIAs than from *E. coli*-based VP1 EIAs for IgM and IgG, with results from the former assays correlating more closely with the results from confirmatory baculovirus-expressed VP1 immunofluorescence assays (IFAs) than those from the latter (12).

The rationale for the present study was to compare the analytical performances of three commercially available EIAs for B19V-specific IgM and IgG. The designs of these EIAs differ from one another but are similar in that they all incorporate one or more conformational B19V antigens, with or without linear B19V antigens. All specimens with discordant results were further tested using B19V-specific IFAs that incorporated a conformational VP1 antigen. Instead of comparing assay performance to a "gold standard," we generated a consensus value from the results of these four assays to evaluate the individual assays.

MATERIALS AND METHODS

Study population. Discarded serum samples collected from 198 individual pregnant women over a 1-year period were tested with the three commercially available EIAs for B19V-specific IgM and IgG antibodies. The original serological testing of these women for B19V antibodies had been ordered by a physician as a screening test (40%) or as a result of known or suspected exposure to B19V (50%) or the appearance of symptoms consistent with B19V infection (i.e., fever, rash, and/or arthralgia; 10%).

Serum samples. Ten-milliliter volumes of whole blood were drawn from patients and collected in red-top tubes. The serum fractions were allowed to clot at room temperature prior to centrifugation. Each volume of serum was transferred aseptically to a sterile plastic tube and stored at 2 to 8°C for up to 1 week until the physician-ordered testing occurred. The remaining discarded portions of sera were stored at −20°C until comparison testing was initiated for this study. Approval was granted by the Magee-Women's Hospital Institutional Review Board for use of the discarded sera in this study.

Commercial B19V-specific EIAs used for detection of IgM and IgG. Biotrin International (Dublin, Ireland) B19V-specific EIAs for B19V-specific IgM and IgG are the only Food and Drug Administration-cleared assays for detecting B19V-specific antibodies. Both EIAs use a baculovirus-expressed VP2 conformational antigen. The B19V-specific IgM assay is a mu capture EIA, while the IgG assay is an antigen capture EIA.

Medac Diagnostika (Wedel, Germany) B19V-specific EIAs for IgM and IgG utilize both VP1 and VP2 antigens. The packet inserts accompanying the Medac kits do not state the precise nature of the expression vector(s) used to produce

TABLE 2. Summary of required controls, calculations, and test interpretations used in determining B19V EIA serology results

Manufacturer	Required controls (no.)	Calculations and performance criterion requirements[a]	Patient sample test interpretations
Biotrin	C (2), negative control (2)	COV = C × LSC	Positive, >(COV × 1.1); negative, <(COV × 0.9); equivocal, ≥(COV × 0.9) and ≤(COV × 1.1)
Medac	Positive control (1), weak-positive control (2), negative control (1), blank (1)	Blank OD_{450} value subtracted from all other OD_{450} values; mean OD of weak-positive control, >0.15 and <0.6; OD_{450} of negative control/OD_{450} of weak-positive control, <0.6; OD_{450} of positive control/OD_{450} of weak-positive control, >1.5	OD_{450} of patient sample/OD_{450} of weak-positive sample: ≥1.0, positive; 0.8, negative; ≥0.8 and <1.0, equivocal
Mikrogen	Positive control (1), cutoff control (2), negative control (1)	Duplicates must agree within 20% of the mean value; OD_{450} of negative control, <0.15; OD_{450} of cutoff control − OD_{450} of negative control > 0.05; OD_{450} of positive control − OD_{450} of cutoff control > 0.3	Positive, >(COV × 1.2); negative, <COV; equivocal, COV − (COV × 1.2)

[a] Abbreviations: C, calibrator; COV, cutoff value; LSC, lot-specific constant; OD_{450}, optical density at 450 nm.

TABLE 3. Comparison of Biotrin and Medac EIAs for detecting B19V-specific IgM[a]

Medac IgM EIA result	No. of specimens with Biotrin IgM EIA result:			No. of specimens tested
	Positive	Negative	Equivocal	
Positive	19	9	0	28
Negative	0	163	0	163
Equivocal	0	7	0	7
Total				198

[a] Results show 91.9% agreement (n = 182) and 8.1% disagreement (n = 16) between the Biotrin and Medac EIAs.

TABLE 5. Comparison of Biotrin and Mikrogen EIAs for detecting B19V-specific IgM[a]

Mikrogen IgM EIA result	No. of specimens with Biotrin IgM EIA result:			No. of specimens tested
	Positive	Negative	Equivocal	
Positive	14	0	0	14
Negative	4	176	0	180
Equivocal	1	3	0	4
Total				198

[a] Results show 96.0% agreement (n = 190) and 4.0% disagreement (n = 8), between the Biotrin and Mikrogen EIAs.

the B19V antigens. The B19V-specific IgM assay is a mu capture EIA, while the IgG assay is an antigen capture EIA.

Mikrogen (Martinsried, Germany) B19V-specific EIAs for IgM and IgG both use a combination of an *E. coli*-expressed VP1 antigen (linear) and a baculovirus-expressed VP2 antigen (conformational). The assay designs for both B19V-specific IgM and B19V-specific IgG EIAs utilize antigen capture platforms.

Biotrin IFAs for B19V-specific IgM and IgG utilize a VP1 antigen expressed from a baculovirus-based expression system (conformational) within *Spodoptera frugiperda* cells. To prevent interference from rheumatoid factor and to reduce IgG competition in the IgM IFA, serum samples were pretreated with an adsorbent reagent prior to testing. Two individuals, blinded to the results of the three different EIAs for B19V-specific IgM and IgG, each read and interpreted the IFA results independently. Agreement between the two readers for the B19V-specific IgM and IgG IFA results was 100% for the specimens with discordant results.

Each commercially available EIA and IFA was run according to the instructions on the manufacturer's packet insert. Each specimen was analyzed singly for each EIA and, if required, for the IFA. A summary of the assay features and test principles for each commercial EIA is given in Table 1. The necessary controls, calculations, and interpretations used to determine patient results for the various EIAs were carried out precisely as outlined in the package inserts and are described in Table 2.

RESULTS

Comparison of three commercially available EIAs for detecting B19V-specific IgM or IgG antibodies. The 198 serum samples obtained from 198 pregnant women were evaluated in a split-sample study for the detection of B19V-specific IgM and IgG antibodies by using Biotrin, Medac, and Mikrogen EIAs. Tables 3, 4, 5, and 6 indicate percent agreement among the various B19V-specific EIAs for detecting IgM (91.9% agreement between Medac and Biotrin and 96% agreement between Mikrogen and Biotrin) and IgG (97.5% agreement between Medac and Biotrin and 97% agreement between Mikrogen and Biotrin).

Sixteen (8%) of 198 pairs of results from Medac and Biotrin EIAs and 8 (4%) of 198 pairs of results from Mikrogen and Biotrin EIAs for B19V-specific IgM were discordant. Five (2.5%) of 198 pairs of results from Medac and Biotrin EIAs and 6 (3%) of 198 pairs of results from Mikrogen and Biotrin EIAs for B19V-specific IgG were discordant. Close to half of the discrepancies for IgM (46%) and IgG (40%) were due to equivocal results, with Medac and Mikrogen EIAs each producing seven equivocal results and the Biotrin IgG EIA producing one.

Resolution of discordant results. Specimens whose results lacked complete agreement among the three commercially available EIAs were further tested using B19V-specific IFAs for IgM and IgG antibodies. The results are illustrated in Tables 7 and 8, respectively. After IFA testing, a consensus result was generated if three of four results agreed. Outcomes for specimens whose results lacked this level of agreement could not be resolved and lacked consensus. Consensus was achieved for 20 (83.3%) of 24 discordant results for IgM and for 7 (70%) of 10 discordant results for IgG.

For the specimens with discrepant results, the results of the Biotrin IgM and IgG EIAs agreed with the consensus results in 20 (100%) of 20 and 6 (86%) of 7 cases, respectively, while the Medac results for IgM and IgG agreed with the consensus in 5 (25%) of 20 and 5 (71%) of 7 cases, respectively, and the Mikrogen results for IgM and IgG agreed with the consensus in 15 (75%) of 20 and 3 (43%) of 7 cases, respectively.

B19V-specific PCR testing was also performed as previously described (13) on the eight specimens listed in Table 7 whose results for B19V-specific IgM were presumed to be false positive based on the consensus result. None of these eight specimens had detectable levels of B19V DNA. Analysis was also performed to rule out the presence of inhibitors within the

TABLE 4. Comparison of Biotrin and Medac EIAs for detecting B19V-specific IgG[a]

Medac IgG EIA result	No. of specimens with Biotrin IgG EIA result			No. of specimens tested
	Positive	Negative	Equivocal	
Positive	117	2	0	119
Negative	2	76	1	79
Equivocal	0	0	0	0
Total				198

[a] Results show 97.5% agreement (n = 193) and 2.5% disagreement (n = 5) between the Biotrin and Medac EIAs.

TABLE 6. Comparison of Biotrin and Mikrogen EIAs for detecting B19V-Specific IgG[a]

Mikrogen IgG EIA result	No. of specimens with Biotrin IgG EIA result			No. of specimens tested
	Positive	Negative	Equivocal	
Positive	114	0	0	114
Negative	2	78	1	81
Equivocal	3	0	0	3
Total				198

[a] Results show 97% agreement (n = 192) and 3% disagreement (n = 6) between the Biotrin and Mikrogen EIAs.

TABLE 7. B19V-specific IgM EIA and IFA and consensus results for specimens with discrepant outcomes[a]

Specimen no.	Biotrin EIA result	Medac EIA result	Mikrogen EIA result	Biotrin1 IFA result	Consensus result
129	Positive	Positive	Negative (FN)	Positive	Positive
172	Positive	Positive	Negative (FN)	Positive	Positive
174	Positive	Positive	Negative (FN)	Positive	Positive
30	Negative	Negative	Equivocal	Negative	Negative
164	Negative	Negative	Equivocal	Negative	Negative
154	Negative	Equivocal	Negative	Negative	Negative
43	Negative	Equivocal	Negative	Negative	Negative
49	Negative	Equivocal	Negative	Negative	Negative
78	Negative	Equivocal	Negative	Negative	Negative
79	Negative	Equivocal	Negative	Negative	Negative
190	Negative	Equivocal	Negative	Negative	Negative
207	Negative	Equivocal	Negative	Negative	Negative
69	Negative	Positive (FP*)	Negative	Negative	Negative
142	Negative	Positive (FP*)	Negative	Negative	Negative
143	Negative	Positive (FP*)	Negative	Negative	Negative
148	Negative	Positive (FP*)	Negative	Negative	Negative
152	Negative	Positive (FP*)	Negative	Negative	Negative
167	Negative	Positive (FP*)	Negative	Negative	Negative
169	Negative	Positive (FP*)	Negative	Negative	Negative
192	Negative	Positive (FP*)	Negative	Negative	Negative
120	Positive	Positive	Equivocal	Negative	No consensus
139	Positive	Negative	Negative	Positive	No consensus
163	Positive	Positive	Negative	Negative	No consensus
144	Negative	Positive	Equivocal	Negative	No consensus

[a] FP, presumed false-positive result based on consensus; FN, presumed false-negative result based on consensus; *, specimen repeatedly found to be IgM positive but negative by PCR for B19V DNA.

specimens. To that end, 50 ng of B19V DNA (pYT110) was added to each specimen. Inhibition was not demonstrated in any of these eight specimens tested by PCR for B19V DNA (data not shown).

DISCUSSION

It is generally accepted that B19V-seronegative women are susceptible to infection and, as such, are at risk for adverse fetal outcome if they become infected during pregnancy. While the majority of pregnant women presenting with B19V infection go on to deliver healthy full-term infants, approximately 5 to 9% of the pregnancies may end in fetal death (9, 16). Although poor outcome is infrequent, pregnant women lacking B19V-specific antibodies or demonstrating measurable levels of B19V-specific IgM will likely be monitored weekly by ultrasound for 8 to 10 weeks. Consequently, it is critical that an accurate assessment be made of the B19V-specific IgM and IgG antibody status of pregnant women who are thought to be at risk for B19V infection or who may be infected with the virus following exposure.

It is now accepted that the most reliable indicator of past infection with B19V is the presence of IgG antibodies recognizing conformational VP2 epitopes (11, 12, 15). In a previous report, an *E. coli*-based linear VP1 expression system was compared to Biotrin's baculovirus-based conformational VP2 expression system. This comparison illustrated improved accuracy with the latter EIA in measuring B19V-specific IgM and IgG antibodies (12). The rationale for the present study was to compare the performances of three commercially available EIAs, all of which utilize one or both conformational B19V capsid antigens (VP1 and/or VP2) in various assay designs. It was therefore not surprising that the results of these three commercially available assays agreed more closely with one another than those of the two assays compared in the previous study where the antigens utilized in the two kits were so dramatically different; one assay used a linear VP1 antigen while the other used a conformational VP2 antigen (12).

TABLE 8. B19V-specific IgG EIAs and IFA and consensus results for specimens with discrepant outcomes[a]

Specimen no.	Biotrin EIA result	Medac EIA result	Mikrogen EIA result	Biotrin IFA result	Consensus result
151	Positive	Positive	Equivocal	Positive	Positive
98	Positive	Negative (FN)	Positive	Positive	Positive
176	Positive	Positive	Negative (FN)	Positive	Positive
188	Positive	Positive	Negative (FN)	Positive	Positive
189	Positive	Positive	Negative (FN)	Positive	Positive
70	Equivocal	Negative	Negative	Negative	Negative
97	Negative	Positive (FP)	Negative	Negative	Negative
22	Positive	Positive	Negative	Negative	No consensus
42	Positive	Negative	Equivocal	Positive	No consensus
117	Positive	Positive	Equivocal	Negative	No consensus

[a] FP, presumed false-positive result based on consensus; FN, presumed false-negative result based on consensus.

From this limited data set, it would appear that baculovirus-expressed (conformational) VP2 antigen alone was sufficient for accurate B19V serologic determination. In contrast to the conformational dependence of long-term IgG reactivity for VP2, which is indisputable, IgG immunoreactivity for VP1 is disputed in the literature. Lastly, the Biotrin EIAs produced fewer equivocal results ($n = 1$) than either the Medac EIAs ($n = 7$) or the Mikrogen EIAs ($n = 7$) and no false-positive and false-negative results.

In the cases of those specimens for which consensus was achieved, the overall percent agreement was highest for the Biotrin results, with 194 (100%) of 194 and 194 (99.5%) of 195 of the results for IgM and IgG, respectively, agreeing with the consensus, followed by Mikrogen, with 189 (97.4%) of 194 and 191 (97.9%) of 195 of the results for IgM and IgG, respectively, agreeing with the consensus, and Medac, with 179 (92.3%) of 194 and 193 (99%) of 195 of the results for IgM and IgG, respectively, agreeing with the consensus.

The performances of the three B19V-specific EIAs were assessed by agreement via consensus rather than by use of a "gold standard" for comparison. This approach can be problematic if, for example, there is one assay that is significantly more sensitive than the others; it may result in the former assay's appearing to give more false-positive results, especially with specimens containing low levels of specific antibodies. However, all specimens with discordant results were retested for the presence of antibodies and produced the same results (data not shown). Additionally, the specimens presumed to be false positive for IgM were also analyzed by B19V-specific PCR and found to lack detectable B19V DNA. These data strongly suggest that the presumed false-positive results for IgM were indeed false positives and were not due to the use of a more sensitive assay in this case.

Although this comparison was limited in the number of specimens evaluated, certain trends became apparent (Tables 7 and 8). First, both the Medac (7 of 198 results; 3.5%) and Mikrogen (4 of 198 results; 2%) IgM EIAs generated more equivocal results than the Biotrin IgM EIA (0 of 198 results; 0%). In our experience, equivocal results are rarely seen when the Biotrin IgM and IgG EIA kits are used. Over the past 5 years, the numbers of equivocal results generated using the Biotrin IgM and IgG EIAs were 6 of 1,067 (0.6%) and 2 of 1,067 (0.2%), respectively.

Secondly, the results of the Medac IgM and IgG EIAs lacked agreement with the consensus results, and these assays had the tendency to generate false-positive results (9 of 198; 4.5%). To a lesser extent, the Mikrogen IgM and IgG EIAs also generated presumed false-negative results (6 of 198; 3%). In contrast, the Biotrin IgM and IgG EIAs displayed neither of these trends in this study. In fact, from these results one may consider this Food and Drug Administration-cleared assay to be the "gold standard."

From the clinician's viewpoint, an equivocal result translates into additional patient visits, extra blood sampling, and repeated serology testing. At best, equivocal data are not helpful to the physician, and at worst they are misleading. If the woman's immune status remains unresolved, her clinician may decide to initiate costly fetal ultrasonographic monitoring. For the clinician, a false-positive result for IgM and/or a false-negative result for IgG would also negatively impact upon patient care, increasing the health system's costs due to unnecessary repetition of serologic tests and ultrasonography.

In summary, the accuracy of a serologic assay for detecting B19V-specific IgM or IgG antibodies is critical for the physician faced with making decisions on the extent to which follow-up care is appropriate. Ultimately, a serology test that produces far fewer equivocal or inaccurate results than other assays will be more cost-effective overall for the health care system.

ACKNOWLEDGMENT

This work was supported in part by Biotrin International, Inc., Dublin, Ireland.

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