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NOTES

Development and Evaluation of Capture Immunoglobulin G and M Hemadherence Assays by Using Human Type O Erythrocytes and Recombinant Parvovirus B19 Antigen

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The capacity of human parvovirus B19 to agglutinate human type O erythrocytes was used to develop immunoglobulin G and M antibody capture hemadherence assays. When results of these assays were compared with those of corresponding antibody capture enzyme immunoassays using a well-characterized panel of 125 serum specimens, a 96.8% overall agreement was obtained between the two methods.

Human parvovirus B19 is the etiologic agent of erythema infectiousum, an important cause of transient aplastic crisis in persons with underlying hemolytic disorders, and has been associated with a wide spectrum of other clinical diseases (8). Because a convenient culture system has not yet been identified for B19, antibody detection remains the preferred method for laboratory diagnosis of B19 infection in the immunologically normal host. Antibody capture enzyme immunoassays (EIAs) are generally regarded as the preferred method for B19 antibody detection (1, 6, 7) but require B19-specific antigen and monoclonal antibody reagents that can be difficult to obtain for some laboratories.

Recently, several groups (3, 4) have taken advantage of the ability of B19 antigen to agglutinate primate erythrocytes (2) by using these cells instead of monoclonal antibodies in capture hemadherence assays. These assays proved to be sensitive and specific for antibodies to B19. Because human type O erythrocytes also possess the P-antigen receptor, will agglutinate in the presence of B19 antigen (2), and are more readily available than primate erythrocytes, we chose to evaluate these cells in hemadherence assays for detection of B19-specific immunoglobulin G (IgG) and IgM antibodies.

Human type O erythrocytes obtained from five unrelated blood donors were prepared as previously described (3). Cells were stored at 4°C until use or for up to 1 week before being discarded. Hemagglutination was evaluated by a modification of the method of Brown and Cohen (2). Briefly, serial twofold dilutions of baculovirus recombinant B19 VP1/VP2 antigen (MedImmune, Inc., Gaithersburg, Md.) were prepared in DGA buffer (0.5% glucose, 0.03% gelatin, and 0.2% bovine serum albumin in 0.1 M phosphate-buffered saline [PBS], pH 5.8) in 50-μl volumes in Linbro U-bottom microplates (ICN, Costa Mesa, Calif.). The same volume of a 0.25% erythrocyte suspension in DGA buffer was then added to all wells, and the plate was left undisturbed at 4°C for 3 h. The end point was scored at 50% hemagglutination. Hemagglutination titers were comparable for the five erythrocyte samples, ranging from 1,280 to 2,560.

Serum samples had been previously analyzed for specific IgG and IgM antibodies to B19 by EIA (1) modified to use baculovirus recombinant B19 VP1/VP2 antigen (5). The hemadherence assays, as modified from the original method of Hilfenhaus et al. (4), were performed identically to the EIA through the addition of recombinant VP1/VP2 antigen (MedImmune, Inc.). Briefly, 96-well Immulon II microtiter plates (Dynatech Laboratories Inc., Alexandria, Va.) were coated with 75-μl volumes of either anti-human IgG or IgM antibodies (Tago Inc., Burlingame, Calif.) diluted 1:500 in 0.01 M PBS, pH 7.2, and incubated for 1 h at 37°C. After washing with PBS containing 0.05% Tween 20, serum specimens diluted 1:100 in PBS with 0.5% gelatin and 0.15% Tween 20 (PBS/G/T) were added in 75-μl volumes to four contiguous wells and incubated for 1.5 h at 37°C. After washing, 50-μl volumes of VP1/VP2 antigen diluted 1:2,000 in PBS/G/T with 0.1% chicken ovalbumin were added and incubated overnight at room temperature; for each serum specimen, two wells received antigen and two wells received only diluent to serve as the antigen control. The following day, unbound antigen was washed away and 75 μl of a 0.25% erythrocyte suspension in DGA buffer was added to each well and left undisturbed for 3 h at 4°C. Scoring was performed by visual inspection as previously described (4); results were scored from 4 (complete hemadsorbance) through 0 (tight button of settled erythrocytes). Scores of 4 and 3 were considered positive; a score of 2 was considered equivocal, requiring repeat testing; and scores of 1 and 0 were considered negative.

To evaluate our hemadherence assays, 125 serum specimens were selected from a well-characterized serum panel specifically designed to represent a broad spectrum of B19 cases and controls with a wide range of B19 IgG and IgM antibody levels. All specimens had been previously tested by EIA a minimum of three times to ensure reproducibility of assay results. Specimens included (i) 70 from persons with one or more signs consistent with B19 infection—rash, arthropathy, polyarthralgia, transient aplastic crisis, and pregnancy with associated

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TABLE 1. Comparison of results of hemadherence assay and enzyme immunoassay for detection of B19-specific IgG and IgM antibodies in human sera

Hemadherence assay result	No. of EIA result		
	Positive	Negative	Total
IgG antibodies			
Positive	84	0	84
Negative	1	40	41
Total	85	40	125
IgM antibodies			
Positive	47	0	47
Negative	3	75	78
Total	50	75	125

fetal hydrops or fetal demise; (ii) 37 from clinically healthy pregnant women who were contacts of persons with B19 infections; (iii) 7 from persons with unknown clinical histories; (iv) 10 from clinically healthy blood donors with no detectable B19 IgG and IgM antibodies used to establish the cutoff value for the EIA; and (v) 1 with high-titered rheumatoid factor. Of these specimens, 85 were positive and 40 were negative for B19-specific IgG antibodies and 50 were positive and 75 were negative for IgM antibodies by EIA. Among 60 randomly selected serum specimens from this panel, B19 DNA was detected by PCR assay in 11 of 23 (48%) positive for B19-specific IgM antibodies and in none of 37 that were IgM negative. All specimens were coded, and the hemadherence assays were performed in a blinded manner.

Results obtained with the hemadherence assay as compared with our EIA are shown in Table 1. For the 125 specimens tested, 121 (96.8%) results were in agreement between the two methods. Intermediate results (score of 2) were obtained for 46 (37%) and 10 (8%) of the specimens tested for specific IgG and IgM antibodies, respectively. Identical or higher scores were obtained on repeat testing of these specimens, and they were therefore judged positive. Only one (<1%) and five (4%) specimens tested for these respective antibodies gave a faint reaction (score of 1), and identical or lower scores were obtained on repeat testing. All other specimen results were unambiguous. For routine testing, it would be appropriate to include both negative and weakly positive specimens in each run to monitor assay performance and serve as a reference for making scoring judgments.

The sensitivity, specificity, and predictive values of the positive (PVP) and negative (PVN) were calculated for the hemadherence assays as compared with our EIA. For IgG antibodies, the sensitivity was 99%, specificity was 100%, PVP was 100%, and PVN was 98%. For IgM antibodies, the sensitivity was 94%, specificity was 100%, PVP was 100%, and PVN was 96%. Nonconcordant results were obtained with four specimens, three IgM-positive specimens and one IgG-positive specimen negative by the hemadherence assay. These specimens all gave weak absorbance values by EIA (lower 10th percentile of all positive EIA values), and therefore, we attribute these discrepancies to the slightly lower sensitivity of the hemadherence assays.

Our results show that human type O erythrocytes can be used in antibody capture assays as an indicator system for detection of B19-specific antibodies. We found the hemadherence assays to be surprisingly sensitive and specific and easy to interpret. We therefore conclude that in settings where reagent and equipment costs limit the use of EIA, hemadherence assays offer a simple and cost-effective alternative for parvovirus B19 diagnostics.

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