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Evaluation of Four Commercial Enzyme Immunoassays for Detection of Immunoglobulin M Antibodies to Human Parvovirus B19

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Four commercial enzyme immunoassays (EIAs) for the detection of parvovirus B19-specific immunoglobulin M (IgM) antibodies [Biotrin Parvovirus B19 IgM (Biotrin International, Ireland); Parvoscan B19 IgM (Euro-Diagnostica, Sweden); Parvovirus IgM (Immunobiological Laboratories [IBL], Germany); and human parvovirus B19 IgM (Hillcrest Biologicals, USA)] were compared to indirect immunofluorescence assay (IFA) and polymerase chain reaction (PCR). Using IFA as the reference test, high sensitivities (≥ 97%) were observed with all four EIAs, though the specificities of the Biotrin and IBL EIAs (99% and 96% respectively) were significantly higher than those of the Hillcrest and Euro-Diagnostica EIAs (81% and 79% respectively).

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Parvovirus B19 is the causative agent of *Erythema infectiosum* or fifth disease, a common childhood disease (1). Parvovirus infection can have serious consequences for patients with pre-existing haemolytic disorders, such as sickle cell anaemia, in whom a transient aplastic crisis may develop. Although parvovirus infection during pregnancy is uncommon, infection may result in foetal hydrops and spontaneous abortion or stillbirth (2).

Immunoglobulin M (IgM) antibodies to parvovirus B19 may be detected in the serum of some patients as early as two days following infection and may persist for more than 60 days (3). Acute B19 infection may be diagnosed by the presence of IgM antibodies, whereas earlier infection can be diagnosed only by the detection of virus or viral products using such techniques as the polymerase chain reaction (PCR). A period of overlap occurs when both IgM and viral DNA may be detected in the patient (4). The inability to propagate sufficient numbers of the virus for diagnostic purposes limited the development of serological assays for parvovirus B19 until synthetic peptide and recombinant viral antigens became available (5–7). A number of commercial assays were subsequently developed, four of which were compared with indirect immunofluorescence assay (IFA) and PCR in the present study.

Materials and Methods. The serum samples (n = 102) tested in this study were submitted to the Clinical Virology Research Unit of the Royal Children's Hospital, Queensland, Australia. Included were sera submitted for parvovirus B19 testing (n = 69) from patients suspected of having *Erythema infectiosum* infection (n = 30); from patients with haematological disease, including hereditary spherocytosis, leukaemia, and chronic anaemia (n = 17); from pregnant women with foetal loss as a result of an undiagnosed viral infection (n = 10); and from patients with acute arthropathy (n = 12). Also included were sera (n = 17) from patients with evidence of acute infection other than parvovirus B19, including three positive for rubella IgM; three positive for *Toxoplasma gondii* IgM; three positive for cytomegalovirus (CMV) IgM; two positive for Ross

River virus (RRV) IgM; one positive for both CMV and Ross River virus IgM; three positive for measles IgM; and two positive for mumps IgM. In addition, acute/convalescent serum specimens (n = 16) obtained from four children with clinically proven *Erythema infectiosum* infection (PCR positive and IgM positive for B19 by IFA) were also included.

Biotrin Parvovirus B19 IgM enzyme immunoassay (EIA) (Biotrin International, Ireland); Parvoscan B19 IgM (Euro-Diagnostica, Sweden); Parvovirus IgM EIA [Immunobiological Laboratories (IBL), Germany]; and human parvovirus B19 IgM EIA (Hillcrest Biologicals, USA) were compared with IFA and PCR. All assays were performed according to the manufacturers' instructions. All were indirect EIAs except the Biotrin assay, which was in the mu-capture format. The Parvoscan assay used a synthetic peptide antigen; the other assays used recombinant viral capsid antigens.

Parvovirus B19 IgM antibodies were also detected by IFA as described (8). Slides containing insect cells expressing both parvovirus structural proteins VP1 and VP2 were kindly donated by Dr. C. Brown (9). Polymerase chain reaction amplification of B19 DNA was performed using primers and conditions described (10, 11).

Results and Discussion. All EIAs showed acceptable coefficients of variation (CVs) of < 10% in control sera (n = 10). The Biotrin test gave a particularly low CV in the positive control (1.3%). The higher CV observed in the negative control (8.5%) likely reflects the lower absorbance of the negative control sera in the Biotrin test relative to the other tests.

All four EIAs and the IFA gave the same result in 78 of 102 serum samples tested (47/67 negative and 31/35 positive samples). Using IFA as the reference test, high sensitivities (≥ 97%) were observed with all four EIAs, though the specificities of the Biotrin and IBL EIAs (99% and 96%, respectively) were significantly higher than those of the Hillcrest and Euro-Diagnostica EIAs (81% and 79%, respectively) (Table 1).

Table 1: Comparison of sensitivity and specificity of the four parvovirus assays using indirect immunofluorescence assay as the reference test.

Test	Sensitivity (IFA positive) No. (%)	Specificity (IFA negative) No. (%)
IBL	34/35 (97)	64/67 (96)
Hillcrest	34/35 (97)	54/67 (81)
Parvoscan	34/35 (97)	53/67 (79)
Biotrin	35/35 (100)	66/67 (99)

The Hillcrest and Parvoscan EIAs showed elevation more often in sera from patients confirmed to have an IgM response to other infections (rubella, toxoplasmosis, cytomegalovirus, Ross River virus, mumps or measles; n = 17). The Parvoscan EIA was positive in nine of these sera; Hillcrest in seven; IBL in five; and Biotrin in three. The Parvoscan assay was the only test to give false-positive results in two of three rubella IgM-positive sera. The cross-reactivity of this test with the rubella sera is of concern because rubella is often clinically indistinguishable from parvovirus B19 infection. Cross-reactivity between rubella and parvovirus B19 IgM has been reported previously (12–14). The Parvoscan test also showed false positivity in five of ten sera collected from pregnant women suspected of having parvovirus infection. The Parvoscan test uses a synthetic peptide antigen, whereas the other EIAs use recombinant parvovirus antigens. The peptide antigen, based on parvovirus structural protein VP2 and the part of VP1 that overlaps VP2, may be the cause of higher cross-reactivity in sera from patients with nonparvovirus infections. It is of interest that the Hillcrest EIA which also uses an antigen based on VP1 and VP2 showed lower specificity than assays that use only VP2 antigen (Biotrin and IBL). Furthermore, differences between assays could be due to the purity of antigens used, the coating and blocking procedures used, the serum diluents used, or posttranslational modification of the recombinant antigens that lead to differences in the epitopes that are expressed or the conformation of synthetic peptide and recombinant antigens.

It is also of interest that all four EIAs were positive in three CMV IgM-positive sera. Immunoglobulin M IFA for parvovirus was also positive in two of these sera, and parvovirus was detected by PCR in one. This finding indicates that these sera may have been from parvovirus infection that showed a false-positive result in the CMV EIA used. Cytomegalovirus cross-reactivity in parvovirus IgM EIA has been reported previously (14). Similarly, infection with Epstein-Barr virus has also been reported to lead to high cross-reactivity in parvovirus IgM EIA, but sera from patients with acute infection due to varicella zoster and herpes simplex virus did not show significant cross-reactivity (14, 15).

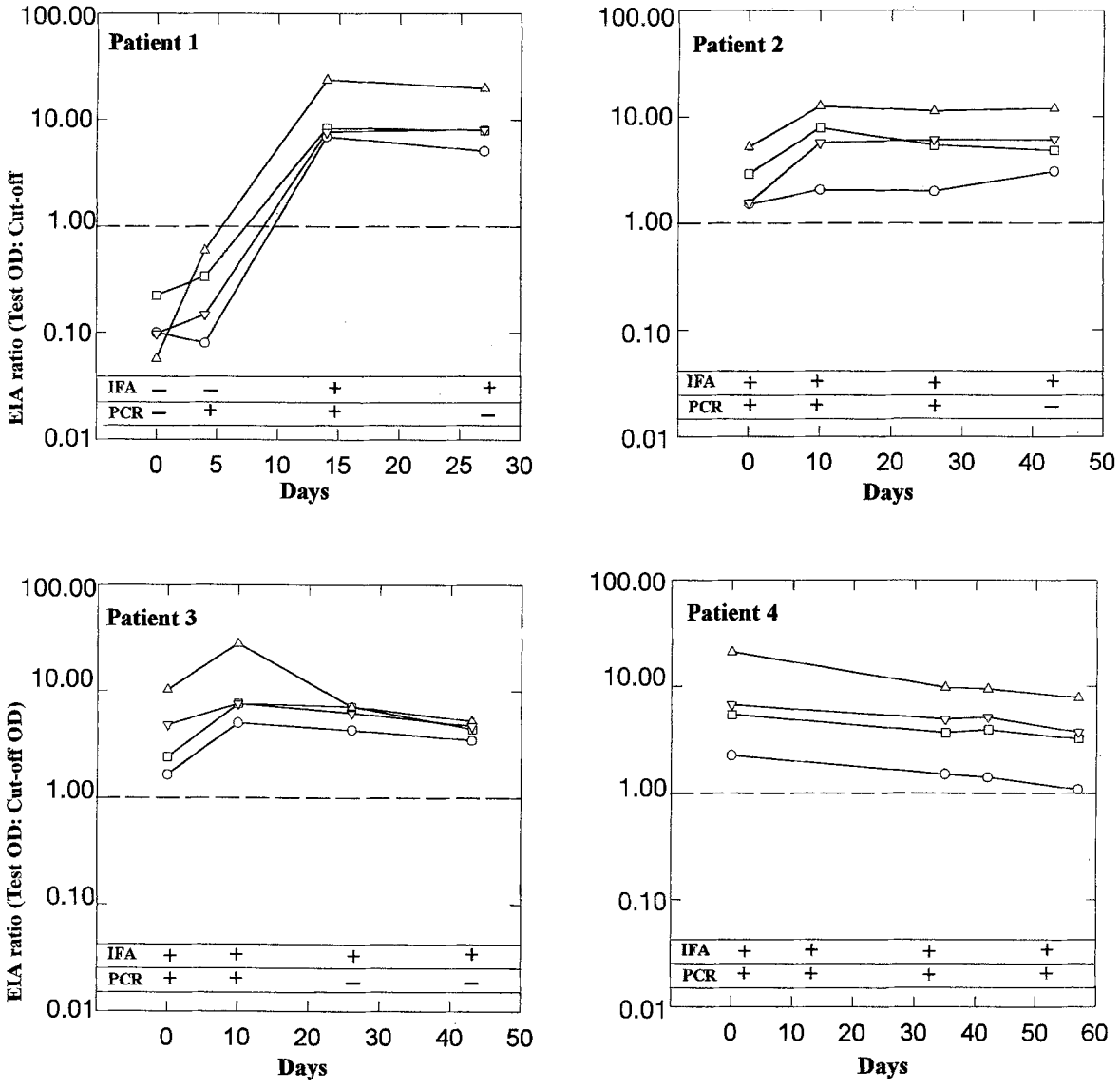

Figure 1: Parvovirus-specific IgM profiles in patients with confirmed parvovirus infection using commercial enzyme immunoassays for the detection of B19-specific IgM antibodies. □, Hillcrest; ▽, Biotrin; △, Euro-Diagnostica; and ○, IBL. The horizontal axis represents the day of sera collection relative to the first sera collected. The vertical axis displays EIA results, expressed as the ratio of sample OD to cut-off OD for each test. Results of IgM indirect immunofluorescence assay (IFA) and polymerase chain reaction (PCR) are also shown.

Despite differences in sensitivity and specificity of the four EIAs used in this study, all showed similar profiles when used to monitor IgM response in patients with *Erythema infectiosum*; these reflected the results of IFA for parvovirus-specific IgM (Figure 1). It is of interest to note the differences between serology and PCR for parvovirus. In one patient PCR detected parvovirus in the second sera tested, while IgM EIA and IFA were negative. Ten days later both the PCR and IgM assays were positive. At 25 days, parvovirus was no longer detected by PCR, but the IgM assays were still positive. Similarly, in two other patients, parvovirus-specific IgM was detected for a longer period than the viral products. This relationship between the detection of anti-viral IgM and viral DNA has been described previously (4).

We conclude that the Biotrin and IBL EIAs provide a suitable test method for determination of parvovirus-specific IgM in a routine clinical laboratory, particularly since IFA has certain disadvantages: it lacks sensitivity, requires an experienced operator to interpret results, and has a variable end-point dilution. In addition, these EIAs can

easily accommodate a large number of samples and give a quantitative result that is less dependent on operator interpretation than IFA.

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Inoculum Effect and Bactericidal Activity of Cefditoren and Other Antibiotics against *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*

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The inoculum effect on minimum inhibitory and minimum bactericidal concentrations of cefditoren, benzylpenicillin, ampicillin, cefotaxime, ceftriaxone, and meropenem against six clinical isolates each of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* was studied using inocula of approximately 10^4 to 10^5 and 10^7 to 10^8 cfu/ml. Vancomycin was also studied against *Streptococcus pneumoniae*. The inoculum effect was observed only with benzylpenicillin and ampicillin against five of six strains of *Haemophilus influenzae*. All antibiotics tested were bactericidal.

Cefditoren is a parenteral cephalosporin from which a pivoxyl derivative for oral administration has been developed (1). This antibiotic has an excellent in vitro activity against most leading bacteria involved in meningitis, namely *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* (1, 2), including strains with diminished susceptibility or resistance to penicillin (3). Antibiotics suitable for treatment of bacterial meningitis must not only inhibit pathogens by low concentration of the drugs but also be active against high bacterial inocula and have demonstrated bactericidal activity (4). We therefore stu-

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