Comparative evaluation of tests for detection of parvovirus B19 IgG and IgM

FERNANDO DE ORY,1 TEODORA MINGUITO,1 JUAN EMILIO ECHEVARRÍA,1 MARÍA DEL MAR MOSQUERA1 and ANTONIO FUERTES2

1Instituto de Salud Carlos III, Centro Nacional de Microbiología, Majadahonda; and 2Servicio de Microbiología, Hospital 12 de Octubre, Madrid, Spain

The aim of this study was to evaluate enzyme immunoassays (EIA) (Euroimmun, Lübeck, Germany) and chemiluminiscent immunoassays (CLIA) (Diasorin, Saluggia, Italy) in their application to detect B19V-IgM and -IgG. For this purpose, one hundred and ninety samples were studied. Of them, 101 came from recent infection cases (B19V-specific IgM (86) and/or PCR (87), 42 from past infections, 18 from non-infected, and 29 from other viral recent infections (Epstein-Barr virus, measles, and rubella). Samples were characterized by capture (for IgM), or indirect (for IgG) EIA (Biotrin, Dublin, Ireland); indeterminate samples were classified by indirect immunofluorescence (IIF) (Biotrin). All the samples were used for testing IgM assays, and all but the cases from other viral infections were used for IgG tests. For IgM, CLIA, and EIA identified 76 and 62 of 86 IgM positives, respectively (sensitivity 88.4% and 72.1%). Considering B19V IgM negative samples, negative result was obtained in 95 and 92 of 104, being the specificity values of CLIA and EIA 91.3% and 88.5%, respectively. For IgG, CLIA and EIA identified correctly 114 and 115 of the 122 positive samples (sensitivity 93.4% and 94.3%, respectively), and 39 and 36 of 39 negative samples (specificity 100% and 92.3%). As conclusion, CLIA methods can be used in clinical laboratories as adequate alternatives to the well-established Biotrin EIAs.

Key words: B19V; enzyme immunoassay; chemiluminescent immunoassay.

Fernando de Ory, Servicio de Microbiología Diagnóstica, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda 28220, Spain. e-mail: fory@isciii.es

Human parvovirus B19 (B19V) (genus Erythrovirus, family Parvoviridae) is a widely distributed human virus that causes a diverse range of clinical conditions. The classic erythema infectiosum (fifth disease) usually affects school children, causing a red ‘slapped-cheek’ appearance accompanied by widespread rash on the trunk and limbs. Arthralgia, arthritis, and persistent or recurrent swelling of the joints are the clinical manifestations in adults, and are more common in women than in men. In pregnant women, the infection can lead to severe complications, and may cause fetal anemia, spontaneous abortion, and hydrops fetalis. Infection in patients with underlying chronic hemolytic disorders may result in transient aplastic crisis without any visible rash, and can be fatal. Finally, B19V infection in immunocompromized patients can lead to persistent infection, resulting in anemia (1).

Efficient etiologic characterization of B19V infections can be achieved by direct assays, such as polymerase chain reaction (PCR), in addition to serology assays that detect IgM in serum or plasma. Combined detection of B19V-DNA and antibodies improves the sensitivity of viral diagnosis (2). The use of multiplex PCR, which includes detection of
other viruses such as rubella and measles, is especially suitable for differential diagnosis (3). There are several appropriate serologic methods for viral infection diagnosis, and these have been the subject of a number of comparative studies. On the basis of these reports, it has been established that a μ-capture enzyme immunoassay (EIA) that utilizes B19V recombinant VP2 capsids for the detection of specific IgM is the most satisfactory method (4–6).

MATERIALS AND METHODS

The aim of this study was to compare assays for the detection of, firstly, specific IgM against B19V (indirect EIA and capture chemiluminescence immunoassay (CLIA)), using a capture EIA as the reference method, and secondly for the detection of specific IgG (indirect EIA and CLIA), using an indirect EIA as reference. Equivocal results from the reference methods in both cases were characterized by means of an indirect immunofluorescent (IIF) assay.

Serum samples

One hundred and ninety sera were studied. These were grouped as follows:

Panel i. Seventy-two samples showing positive IgM and PCR results (59 IgG positive and 13 IgG negative).
Panel ii. Five cases with single PCR positive results.
Panel iii. Ten samples, PCR and IgG positive, IgM negative.
Panel iv. Fourteen cases resulting IgM positive and PCR negative (3 IgG negative and 11 IgG positive).
Panel v. Forty-two specimens from cases of past infection, as characterized by negative IgM and PCR results, and a positive result for IgG-specific B19V.
Panel vi. Eighteen samples with no evidence of previous contact with the virus, that is to say, with a negative result for IgG, IgM, and PCR.
Panel vii. Twenty-nine specimens from patients with recent infection due to other viruses, such as Epstein-Barr virus (EBV) (10 samples), measles (9 specimens), and rubella (10 samples).

The clinical pictures in samples from panels i to vi were related to B19V recent infection and were sent to our laboratory for B19V diagnosis. They were used in this study for testing both IgM and IgG assays. Panel vii specimens were sent for diagnosis of the three above-mentioned viruses and used to evaluate IgM tests only.

Reference methods

The characterization of cases by B19V IgM antibodies was done with a capture EIA that utilizes a baculovirus-expressed VP2 protein (Biotrin, Dublin, Ireland). All positive and equivocal results were retested in a second aliquot by an IIF technique that utilizes recombinant VP1 protein expressed on insect cells (Biotrin); all the samples were confirmed. IgG characterization of specimens was by indirect EIA which uses the VP2 protein (Biotrin), as well as an IIF (Biotrin) in the case of equivocal results. A multiplex PCR that simultaneously detects rubella, measles, and B19 viruses was used for nucleic acid detection (3). Rubella and measles cases were characterized by specific IgM using indirect EIA (Siemens Healthcare, Marburg, Germany). Specific IgM against EBV was detected by IIF (Meridian, Cincinnati, OH, USA).

Methods under evaluation

The compared methods were as follows: firstly, capture CLIA (for IgM); secondly, indirect CLIA (for IgG) (Liaison, DiaSorin, Saluggia, Italy), which uses recombinant baculovirus-expressed VP2; and thirdly indirect EIA, for both isotypes (EuroImmun, Lübeck, Germany), which utilizes a recombinant VP2 antigen expressed in yeast. Indirect EIAs were performed on the BEPIII platform (Siemens Healthcare). All discrepant results were confirmed by retesting. To calculate sensitivity and specificity, equivocal results were considered in the most adverse conditions, that is, when the reference result was negative, an equivocal result was considered positive, and when the reference result was positive, the equivocal result was considered negative.

RESULTS

The results obtained with the four tests evaluated are shown in Table 1.

IgM assays

In recent infection specimens (panel i), following the reference criteria, the CLIA test identified 68 positives of 72 (94.4%), whereas the EIA test identified 59 positives (81.9%). Neither of the two tests evaluated detected any positive IgM result in PCR positive, IgM negative samples (panels ii and iii). When testing
IgM positive, PCR negative samples (panel iv), 8 samples by CLIA, and 3 by EIA were detected as positive. In B19V past infection cases (panel v), 35 samples of 42 (83.3%) were negative for CLIA and 36 of 42 (85.7%) were negative for EIA. For B19V negative cases (panel vi), both assays identified 16 of 18 negative specimens (88.9%). Finally, in patients with other viral infections (panel vii), all samples were negative for CLIA, whereas 25 of 29 were negative in the case of EIA (86.2%).

Given this data, the sensitivity and specificity of CLIA according to the reference criteria were 88.4% and 91.3%, respectively, while the sensitivity and specificity of EIA was 72.1% and 88.5%, with concordance 90% and 81.1% (Table 2).

Table 2. Results of CLIA and EIA for IgM, according to reference criteria

| Assay | Reference result | Correlation | Sensitivity | Specificity |
|-------|------------------|-------------|-------------|-------------|
|       |                  | Positive    | Indeterminate | Negative    |
|       |                  |             | 90%         | 88.4%       | 91.3%       |

IgG assays

Among the samples from panels i to iv, the CLIA test correctly identified 73 of 80 (91.3%) positive samples and 21 of 21 negative samples, 41 of 42 (97.6%) samples from past infections (panel v), and all negative samples (panel vii) (Table 1). Consequently, concordance, sensitivity and specificity values for the CLIA test were 95, 93.4, and 100%, respectively (Table 3). The EIA test identified as positive 74 of 80 (92.5%) and 20 of 21 (95.2%)
amongst cases from panels i to iv, while in past infection cases (panel v) 41 of 42 (97.6%) were identified as positive, and in negative cases (panel vi) 16 of 18 (88.9%) were identified as negative. Hence, concordance, sensitivity and specificity values for the EIA test were 93.8, 94.3, and 92.3%, respectively (Table 3).

Specimens showing discrepant results are listed in Table 4. For IgM assays, false negative results, with regard to the reference criteria, were obtained in cases of recent infection by both IgM assays (4 discrepant samples for CLIA and 13 for EIA of the 72 samples included in panel i). The values in the samples showing false negative result in CLIA are close to the cut-off; 3 of them came from samples with indeterminate result in EIA from Biotrin, confirmed by IIF. In PCR negative IgM positive cases (panel iv), negative result was obtained in 6 and 11 cases in CLIA and EIA, respectively; five samples showed negative results in both compared methods. With regard to IgM negative samples, false positive result was obtained in seven and six cases in CLIA and EIA, respectively, in past infections (panel v), in two cases each assay in negative patients (panel vi), and in 4 samples from other infection patients in EIA (panel vii).

In relation to IgG assays, false negative results were mainly obtained in samples with low reactivity according to the reference criteria (samples 14-20, panel i; sample 1, panel ii; and sample 11, panel iv).

DISCUSSION

The detection of antibodies against B19V is a useful approach for the diagnosis of acute infections caused by this virus, by means of IgM determination. Similarly, IgG detection is the method of choice for the determination of immunity status. For these purposes, commercial assays are available. It has been shown that the EIA assays used in this report for specimen classification (Biotrin) have the correct sensitivity and specificity characteristics when compared with other commercial assays (4–6), giving less equivocal results and thus more efficient specimen classification (7).

In the last few years, several CLIAs have been developed for the Liaison platform for the detection of IgG and IgM against a number of antigens and these, according to our experience, are adequately comparable with other well-established procedures (8, 9). In this comparison, both IgM and IgG CLIA have shown adequate performance characteristics (sensitivity 88.4% and 93.4%; specificity 91.3% and 100%, respectively, for IgM and IgG), improving on those obtained in the EIA from Euroimmun (sensitivity 72.1% and 94.3%; specificity 88.5% and 92.3%, respectively, for IgM and IgG). A possible cause for the discrepancies in the figures in sensitivity and specificity could be the use of different antigens in the compared assays, as described (7), or the use of different methodology. In the case of IgM assays, both EIA from Biotrin and CLIA for IgM employ the same antigen and the same procedure (a μ-chain capture), different from those of EIA from Euroimmun.

In the absence of a gold standard for IgG and IgM antibodies for B19V, we have characterized the samples using a well-established EIA and an IIF, accompanied by PCR detection. However, some samples are difficult to classify because a single positive in PCR or in IgM reference method was obtained. For this, the samples showing markers of B19V recent...
Table 4. Samples showing discrepant results in the methods in evaluation*

| Samples | PCR | IgM assays | IgG assays |
|---------|-----|------------|------------|
|         |     | Reference1 | CLIA2 | EIA1 | Reference1 | CLIA2 | EIA1 |
| i-1     | Pos | Pos 1.32   | Pos 7.4 | Neg 0.74 | Neg 0.3   | Neg <0.1 | Neg 0.33 |
| i-2     | Pos | Ind 0.96/Pos | Neg 0.7 | Neg 0.6 | Pos 46 | Pos 45.4 | Pos 5.63 |
| i-3     | Pos | Ind 1.05/Pos | Neg 0.8 | Neg 0.55 | Pos 8.8 | Pos 44.5 | Pos 5.72 |
| i-4     | Pos | Ind 1.06/Pos | Neg 0.8 | Neg 0.67 | Pos 6.1 | Pos 45.4 | Pos 5.79 |
| i-5     | Pos | Pos 1.74 | Neg 0.6 | Neg 0.16 | Pos 5.4 | Pos >46 | Pos 6.37 |
| i-6     | Pos | Ind 1.05/Pos | Pos 1.5 | Neg 0.53 | Pos 4.4 | Pos 39.2 | Pos 6.02 |
| i-7     | Pos | Pos 7.09 | Pos 15.0 | Ind 0.9 | Pos 5.7 | Pos 31.8 | Pos 5.2 |
| i-8     | Pos | Pos 5.57 | Pos 9.1 | Ind 0.83 | Pos 5.2 | Pos 31.4 | Pos 5.32 |
| i-9     | Pos | Pos 1.54 | Pos 2.4 | Neg 0.4 | Pos 6.6 | Pos 33.4 | Pos 6.03 |
| i-10    | Pos | Pos 2.49 | Pos 2.3 | Neg 0.21 | Pos 6.9 | Pos 45.7 | Pos 7.39 |
| i-11    | Pos | Pos 1.63 | Pos 1.4 | Neg 0.76 | Pos 5.4 | Pos 32.7 | Pos 5.9 |
| i-12    | Pos | Pos 2.12 | Pos 5.3 | Neg 0.53 | Pos 5.0 | Pos 40.5 | Pos 6.17 |
| i-13    | Pos | Pos 1.56 | Pos 1.2 | Neg 0.12 | Pos 6.0 | Pos >46 | Pos 6.95 |
| i-14    | Pos | Pos 15.0 | Pos >48 | Pos 2.9 | Ind 1/0/Pos | Neg 0.7 | Neg 0.6 |
| i-15    | Pos | Pos 10.4 | Pos >48 | Pos 3.36 | Ind 1/0/Pos | Ind 1.0 | Ind 1.06 |
| i-16    | Pos | Pos 8.33 | Pos >48 | Pos 1.58 | Pos 1.5 | Pos 1.4 | Ind 1.0 |
| i-17    | Pos | Pos 15.5 | Pos >48 | Pos 2.02 | Pos 2.0 | Neg 0.8 | Pos 2.23 |
| i-18    | Pos | Pos 2.37 | Pos 11.3 | Pos 1.18 | Ind 1/0/Pos | Pos 2.2 | Neg 0.82 |
| i-19    | Pos | Pos 9.76 | Pos >48 | Pos 4.41 | Pos 1.2 | Neg 0.8 | Pos 1.16 |
| i-20    | Pos | Pos 9.61 | Pos >48 | Pos 5.9 | Pos 1.2 | Neg 0.7 | Pos 1.16 |
| ii-1    | Pos | Neg 0.39 | Neg 0.8 | Neg 0.24 | Ind 1/0/Pos | Ind 0.9 | Ind 0.96 |
| iv-1    | Neg | Pos 1.24 | Neg 0.7 | Pos 1.66 | Neg 0.3 | Neg <0.1 | Neg 0.31 |
| iv-2    | Neg | Pos 1.29 | Pos 4.4 | Neg 0.22 | Neg 0.5 | Neg <0.1 | Pos 1.21 |
| iv-3    | Neg | Pos 1.13 | Pos 1.5 | Neg 0.23 | Neg 0.3 | Neg <0.1 | Neg 0.44 |
| iv-4    | Neg | Pos 1.11 | Neg <0.1 | Neg 0.4 | Pos 6.5 | Pos 35.7 | Pos 5.15 |
| iv-5    | Neg | Pos 1.14 | Neg 0.3 | Neg 0.21 | Pos 4.6 | Pos 31.5 | Pos 4.77 |
| iv-6    | Neg | Pos 1.43 | Neg 0.2 | Neg 0.63 | Pos | Pos 31.4 | Pos 5.04 |
| iv-7    | Neg | Pos 1.11 | Neg 0.5 | Neg 0.43 | Pos 6.0 | Pos 34.7 | Pos 5.59 |
| iv-8    | Neg | Pos 1.29 | Neg 0.8 | Neg 0.09 | Pos 5.3 | Pos 23.1 | Pos 5.11 |
| iv-9    | Neg | Pos 1.14 | Pos 10.3 | Pos 3.5 | Pos 5.2 | Pos 14.1 | Pos 3.92 |
| iv-10   | Neg | Pos 1.14 | Pos 4.0 | Neg 0.38 | Pos 6.2 | Pos 16.4 | Pos 4.58 |
| iv-11   | Neg | Pos 1.27 | Pos 1.3 | Neg 0.17 | Ind 1/0/pos | Neg 0.7 | Ind 1.05 |
| iv-12   | Neg | Pos 2.12 | Pos 3.2 | Ind 0.8 | Pos 6.1 | Pos 24.5 | Pos 6.16 |
| v-1     | Neg | Neg 0.34 | Pos 1.2 | Pos 1.2 | Pos 6.6 | Pos 36.4 | Pos 6.09 |
| v-2     | Neg | Neg 0.85 | Pos 15.8 | Neg 0.07 | Pos 1.9 | Pos 1.6 | Pos 1.85 |
| v-3     | Neg | Neg 0.68 | Pos 2.7 | Neg 0.11 | Pos 6.7 | Pos 29.6 | Pos 6.59 |
| v-4     | Neg | Neg 0.62 | Pos 2.1 | Neg 0.29 | Pos 2.6 | Pos 5.6 | Pos 3.46 |
| v-5     | Neg | Neg 0.57 | Pos 1.2 | Neg 0.32 | Pos 5.3 | Pos 37.2 | Pos 5.66 |
| v-6     | Neg | Neg 0.56 | Pos 6.0 | Neg 0.18 | Pos 6.4 | Pos 32.1 | Pos 6.34 |
| v-7     | Neg | Neg 0.41 | Pos 1.4 | Neg 0.75 | Pos 5.3 | Pos 17.5 | Pos 5.55 |
| v-8     | Neg | Neg 0.79 | Neg 0.7 | Ind 0.95 | Pos 5.8 | Pos >46 | Pos 6.86 |
| v-9     | Neg | Neg 0.39 | Neg 0.6 | Ind 1.02 | Pos 5.2 | Pos 22.3 | Pos 5.07 |
| v-10    | Neg | Neg 0.38 | Neg <0.1 | Ind 0.83 | Pos 7.1 | Pos 40.6 | Pos 6.79 |
| v-11    | Neg | Neg 0.36 | Neg 0.4 | Pos 1.24 | Pos 3.5 | Pos 3.9 | Pos 3.8 |
| v-12    | Neg | Neg 0.34 | Neg 0.2 | Pos 1.27 | Pos 2.0 | Pos 3.6 | Pos 2.1 |
| v-13    | Neg | Neg 0.72 | Neg <0.1 | Neg 0.01 | Pos 1.8 | Neg <0.1 | Neg 0.01 |
| vi-1    | Neg | Neg 0.48 | Ind 1.3 | Pos 1.5 | Neg 0.6 | Neg 0.1 | Pos 1.6 |
| vi-2    | Neg | Neg 0.33 | Pos 1.3 | Neg 0.71 | Pos 0.9 | Neg <0.1 | Neg 0.27 |
| vi-3    | Neg | Neg 0.31 | Neg 0.7 | Pos 1.26 | Neg 0.5 | Neg 0.1 | Neg 0.38 |
| vi-4    | Neg | Neg 0.34 | Neg <0.1 | Neg 0.16 | Neg 0.5 | Neg 0.7 | Pos 1.33 |
| vii-1   | (RV) | Neg | Neg 0.34 | Neg 0.4 | Pos 1.53 | n.d. | n.d. | n.d. |
| vii-2   | (MV) | Neg | Neg 0.45 | Neg 0.3 | Ind 0.96 | n.d. | n.d. | n.d. |
| vii-3   | (EBV) | Neg | Neg 0.46 | Neg 0.3 | Ind 0.81 | n.d. | n.d. | n.d. |
| vii-4   | (EBV) | Neg | Neg 0.18 | Neg 0.1 | Ind 1.1 | n.d. | n.d. | n.d. |

n.d., not determined; RV, rubella virus; MV, measles virus; EBV, Epstein-Barr virus.

*Discordant results are highlighted in grey.

1Results expressed as sample absorbance/cut-off.

2Results expressed as index.
infections were classified as recent infections if showed positive PCR and IgM (panel i), as window samples if only PCR positive result was obtained (panel ii), as coming from a prolonged PCR detection accompanied by a negative IgM result (panel iii), and as having specific B19V IgM in absence of nucleic acid detection (panel iv). Accordingly, only samples included in panel i (PCR and B19V IgM positive) and panel ii (PCR positive, IgM negative) can be unequivocally classified as B19V recent infections, considering that negative IgM result in samples from panel ii could be caused by the presence of specific antibodies-B19V immunocomplexes, as has been described previously (2). Samples from panel iv, characterized as having B19V IgM in the absence of PCR could either represent a clinically inappropriate, due to a polyclonal stimulation of B lymphocytes, or an analytically correct, clinically prolonged response, or a PCR false negative. A PCR false negative result could be caused by the inability of a concrete PCR assay in detecting different B19V genotypes; in fact the PCR technique used here (3) was designed before B19V genotypes were described. We made the alignment of the B19V primers used in our assay (3) with the prototype strains of the genotypes 1A, 1B, 2, 3A, and 3B used in different reports (10, 11), and we found that only some strains, mainly belonging to genotype 3, a non-predominant genotype in Europe, showed a mismatch in second position of 3'end of nested primers. Anyway, bearing these limitations in mind, samples 2, 3, 4, and 5 from panel i (Table 4), in which an IgM exclusive positive result was obtained by the reference approach, could be classified as clearly positive on the basis of PCR detection, probably reflecting a higher sensitivity in the reference. Other discrepancies obtained in the methods being evaluated are, however, difficult to justify. The three cases showing an IgM positive result in the absence of both PCR and IgG (samples 1, 2 and 3 from panel iv, Table 4), could be considered as recent B19V infections, as they showed positive result in two IgM assays (one of them, iv-1, in both EIAs, and the other two, iv-2 and iv-3, in Biotrin EIA and CLIA); in light of this, it could be suggested that the three samples came from true B19V recent infections, in the absence of both specific IgG and PCR. Five additional samples from panel iv (samples 4 to 8, Table 4) showed single, low-positive results in the Biotrin EIA for IgM, being negative in EIA from Euroimmun and CLIA. Due to the low reactivity of these samples in the reference assay, we cannot rule out them as false positive in the reference criteria. Conversely, a couple of samples (−1 from panel v and −1 from panel vi, Table 4) could be considered as false negative with regard to the reference, as a single negative result was obtained by EIA-Biotrin.

The differential diagnosis of B19V and other exanthematic or febrile diseases, as rubella, measles, or EBV infection, is something that requires thought in a clinical context, especially in relation to the plans for the elimination of measles and rubella currently being implemented in many countries. No IgM positive results were obtained in CLIA when samples from the infections in question were tested, thus ensuring its specificity in the differential diagnosis, as previously described (5). However, B19V-induced IgM positivity with bacteria (Borrelia, Campylobacter, and Salmonella) (12) seems to be a cause of misdiagnosis regarding some cases of arthritis or arthropathy.

An important aspect to be considered is the number of indeterminate results. In this evaluation, both IgG and IgM CLIA seem to have a well-defined cut-off that makes it possible to discriminate between positive and negative results, as a lower number of samples showing indeterminate results was obtained, compared with EIA.

As conclusion, CLIA methods can be used in clinical laboratories as adequate alternatives to the well-established Biotrin EIAs. On the other hand, EIA from Euroimmun seems to be useful for detecting IgG antibodies, with some limitation in its application to B19V IgM.

REFERENCES

1. Broliden K, Tolfvenstam T, Norbeck O. Clinical aspects of parvovirus B19 infection. J Intern Med 2006;260:285–304.
2. Bredl S, Plenzt A, Wenzel JJ, Pfister H, Möst J, Modrow S. False negative serology in patients with acute parvovirus B19 infection. J Clin Virol 2011;51:115–20.
3. Mosquera M, de Ory F, Moreno M, Echevarria JE. Simultaneous detection of measles virus, rubella virus, and parvovirus B19 using multiplex PCR. J Clin Microbiol 2002;40:111–6.

4. Butchko AR, Jordan JA. 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. J Clin Microbiol 2004;42: 3191–5.

5. De Ory F, Guisasola ME, Téllez A, Domingo CJ. Comparative evaluation of commercial methods for the detection of parvovirus B19 specific immunoglobulin M. Serodiagn Immunother Infect Dis 1996;8:117–20.

6. Sloots T, Devine PL. Evaluation of four commercial enzyme immunoassays for detection of immunoglobulin M antibodies to human parvovirus B19. Eur J Clin Microbiol Infect Dis 1996;15:758–61.

7. Jordan JA. Comparison of a baculovirus-based VP2 enzyme immunoassay (EIA) to an Escherichia coli-based VP1 EIA for detection of human parvovirus B19 immunoglobulin M and immunoglobulin G in sera of pregnant women. J Clin Microbiol 2000;38:1472–5.

8. De Ory F, Guisasola ME, Sanz JC, García-Bermejo I. Evaluation of four commercial systems for the diagnosis of Epstein Barr Virus primary infections. Clin Vaccine Immunol 2011;18:444–8.

9. Guisasola ME, Ramos B, Sanz JC, García-Bermejo I, De Ory-Manchón F. Comparison of IgG avidity assays in the confirmation of the diagnosis of cytomegalovirus primary infection. APMIS 2010;118:991–3.

10. Hübschen JM, Mihneva Z, Mentis AF, Schneider F, Aboudy Y, Grossman Z, et al. Phylogenetic analysis of human parvovirus B19 sequences from eleven different countries confirms the predominance of genotype 1 and suggests the spread of genotype 3b. J Clin Microbiol 2009;47:3735–8.

11. Corcoran C, Hardie D, Yeats J, Smuts H. Genetic variants of human parvovirus B19 in South Africa: cocirculation of three genotypes and identification of a novel subtype of genotype 1. J Clin Microbiol 2010;48:137–42.

12. Tuuminen T, Hedman K, Söderlund-Venermo M, Seppälä I. Acute parvovirus B19 infection causes nonspecificity frequently in Borrelia and less often in Salmonella and Campylobacter serology, posing a problem in diagnosis of infectious arthropathy. Clin Vaccine Immunol 2011;18:167–72.