Evaluation of Three Commercial Enzyme-Linked Immunosorbent Assays and Two Latex Agglutination Assays for Diagnosis of Primary Epstein-Barr Virus Infection

ANITA SVAHN,[1]* MONA MAGNUSSON,[1] LENA JÄGDAHL,[2] LOTTIE SCHLOSS,[2]
GUNNAR KAHLMETER,[1] AND ANNIKA LINDE[2]

*Clinical Microbiological Laboratory, Central Hospital, Växjö,[1] and Swedish Institute for Infectious
Disease Control, Stockholm,[2] Sweden*

Three commercially available enzyme-linked immunosorbent assays (ELISAs) from Gull, Biotest, and Behring (Enzygnost) and two latex agglutination tests for heterophile antibodies (Monolatex [Biotest] and Mono-Lex [Trinity Laboratories]) were evaluated for the diagnosis of primary Epstein-Barr virus (EBV) infection and EBV seropositivity. Two hundred fourteen consecutive samples from 197 patients with symptoms of primary EBV infection were analyzed by the five assays at a clinical microbiology laboratory. The samples were also analyzed independently by immunofluorescence methods at a reference laboratory. According to the reference methods, 37 patients (40 serum samples) had primary EBV infections, 120 patients (127 serum samples) had had past EBV infections, 33 patients (36 serum samples) were seronegative, and 7 patients (11 serum samples) exhibited atypical reactions. The respective sensitivities and specificities for the diagnosis of primary EBV infection were 95 and 100% for the Gull assays, 100 and 94% for the Biotest assays, and 100 and 89%, for the Enzygnost assays. The Monolatex and Mono-Lex methods showed similar sensitivities and specificities (78 to 85% and 100 to 99%, respectively) for the diagnosis of primary EBV infection. This study demonstrates the usefulness of commercially available assays for the rapid diagnosis of primary EBV infection, but also the importance of large-scale testing of routine samples before choosing an assay.

Epstein-Barr virus (EBV) is a human herpesvirus. It infects epithelial cells and B lymphocytes (10). EBV is frequently acquired during early childhood, and the infection is often asymptomatic. A clinical case of infectious mononucleosis (IM) often occurs when adults and adolescents are infected (23). Once acquired, EBV establishes a latent infection that persists throughout the lifetime of the patient (24). Immunocompromised patients can develop atypical symptoms during primary infection, and symptomatic reactivation of previous disease can also occur. IM-like symptoms can also be caused by cytomegalovirus (CMV), *Toxoplasma gondii*, adenovirus, human immunodeficiency virus, herpes simplex virus, and hepatitis virus and also by malignancies (13, 14, 17, 19). A laboratory-verified diagnosis is therefore of great value for the correct care of the patient.

Traditionally, EBV-associated mononucleosis is diagnosed by assays for heterophile antibodies. These are immunoglobulin M (IgM) antibodies directed to antigens on erythrocytes from various animals and occur during primary EBV infection in 80 to 85% of patients (8). The Paul-Bunnell-Davidsohn test, traditionally used for the measurement of heterophile antibodies, is often replaced by latex agglutination tests and enzyme-linked immunosorbent assays (ELISAs) (13). These assays give a very rapid diagnosis. However, children below the age of 12 years develop heterophile antibodies at a lower frequency than adolescents (3). Heterophile antibodies can also be found in patients with diseases other than IM, and the test can remain positive for 6 to 12 months after IM.

If heterophile antibodies are absent or further confirmation of the EBV diagnosis is necessary for a heterophile-positive patient, specific EBV serology should be performed. Antibodies against viral capsid antigen (VCA), early antigens (EAs), and EBV nuclear antigens (EBNAs) are usually measured. VCA is a structural protein located inside the virus. There are two forms of EA: diffuse (EA-D) and restricted (EA-R). Both are enzymes that are expressed in infected cells and that are involved in virus replication. There are six EBNAs, denoted EBNAs 1 to 6 (6, 15), and these are located in the nucleus of the EBV-infected B cell. EBNA-1 is involved in the control of viral DNA, and EBNA-2 is involved in the transformation of infected B cells. Usually, antibodies against EBNA-1 and sometimes EBNA-2 are measured in serological evaluations for EBV status.

The serological response to the various EBV antigens is characteristic for different stages of the infection. Early during primary infection, IgM and IgG antibodies against VCA (VAC IgM and VCA IgG, respectively) and EA-D, but not against EBNA, can be detected. Antibodies against EA-R reach their maximum 3 to 6 months after the EBV infection (10). During convalescence, the EA-D antibodies and VCA IgM disappear. Antibodies against EBNA appear after 1 to 6 months (first EBNA-2 antibodies and then EBNA-1 antibodies) (12). VCA IgG and EBNA antibodies normally persist throughout a person's lifetime (9, 18). Sometimes, low titers of antibodies against EA-R remain (9).

Immunofluorescence (IF) is the most commonly used method for EBV serology. However, it is labor-intensive and time-consuming, and it is difficult to use routinely on a large scale. Commercially available ELISA methods are available, but thorough evaluation of their sensitivities and specificities are needed before they can be accepted for routine use, since there are many pitfalls in EBV serology (9, 12). In this study, three selected commercially available ELISAs for EBV-specific antibodies and two tests for heterophile antibodies were

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* Corresponding author. Mailing address: Clinical Microbiological Laboratory, Central Hospital, S-351 85 Växjö, Sweden. Phone: (46) 470-88670. Fax: (46) 470-39638. E-mail: anita.svahn@swipnet.se.

TABLE 1. Number of positive samples at different times after onset of IM caused by EBV in the Biotest assays when evaluated with a reference panel at SIIDC

Assay	No. of positive samples/total no. tested (%) on the following days after onset of symptoms:						
	0	14	28	90	180	720	1080
EA IgG	34/39 (87)	16/19 (84)	18/20 (90)	36/38 (95)	35/39 (90)	11/14 (79)	7/11 (64)
EA IgM	36/39 (92)	16/19 (84)	17/20 (85)	20/38 (53)	17/39 (44)	5/14 (36)	3/11 (27)
EA IgA	38/39 (97)	19/19 (100)	19/20 (95)	28/38 (74)	20/39 (51)	12/14 (86)	5/11 (45)
EBNA IgG	0/39	0/19	0/20	9/38 (24)	26/39 (67)	14/14 (100)	11/11 (100)

compared with a reference IF method (7) for the diagnosis of primary EBV infection (6, 12). The analyses were performed with consecutive serum samples from patients with suspected EBV infection.

MATERIALS AND METHODS

A total of 214 consecutive samples from 197 patients with symptoms evaluated by the respective clinicians to be compatible with primary EBV infection (IM) were analyzed at the Clinical Microbiology Laboratory, Växjö, Sweden, and at the Swedish Institute for Infectious Disease Control (SIIDC), Stockholm. The patients were 4 to 84 years old (median age, 18 years). The sera were analyzed by three different ELISAs, two latex agglutination tests for heterophile antibodies, and the reference IF method at SIIDC. The commercially available tests were performed in accordance with the manufacturers' instructions.

The commercially available assays were selected on the basis of their performance when previously tested at SIIDC with panels of sera with various EBV antibody profiles (as established by the reference IF method). The number of samples examined varied slightly, depending on the assays available for preliminary testing, and consisted of 40 to 60 samples from patients with primary EBV infection, 10 to 20 EBV-seronegative serum samples, 8 to 10 samples from patients with very low EBV titers (mainly passively transferred antibodies), and 15 to 17 samples from patients with primary CMV infection. A series of samples drawn up to 3 years after the primary EBV infection (12) was also evaluated by the Biotest assays (Table 1). Altogether, nine commercially available assays or assays under development have been examined with these serum panels. The high sensitivities and specificities (>95%) of the EBNA assay (Biotest) and the EBV VCA IgG and IgM assays (Gull) were the reasons for the inclusion of these assays in the evaluation of routine performance. The Enzygnost assay was included, despite a lack of specificity in the IgM assay when the serum panels were tested, due to its optimal performance in two external quality control panels, distributed by the authority for External Quality Assurance in Laboratory Medicine in Sweden (Equalis). In these panels samples with rheumatoid factor, samples with anti-nuclear antibodies (ANAs), and samples from patients with CMV infections were also included. These samples were assigned a correct EBV status only by the laboratories that used one of the three commercially available assays or the IF method used in this study. In a previous publication, the Monolatex assay was recommended for use in the measurement of heterophile antibodies (13). In this study, the Monolatex assay was compared with another latex agglutination assay, Mono-Lex, which was not included in the previous study.

Reference methods at SIIDC. The reference methods performed at SIIDC were as follows (9). VCA IgG and IgM were determined by indirect immunofluorescence (IIF) with acetone-fixed P3HR-1 cells. The EBNA immunoglobulin status was determined by an anticomplement IF (ACIF) test with NC37 cells swelled in hypotonic solution and fixed in acetone-methanol. Molt cells treated in the same way were used as controls for ANAs. The P3HR-1 cell line originated from Herbert Schmitz, Bernard-Nocht-Institute für Schiffs- und Tropenkrankheiten, Hamburg, Germany, and the cells used in this study were received by SIIDC in 1988. The NC37 and Molt cell lines, used for detection of EBNA antibodies and as controls, respectively, were received from the Department of Tumor Biology, Karolinska Institute, Stockholm, Sweden, in 1988. After a few passages at SIIDC, the cell lines are frozen in aliquots and stored in liquid nitrogen. New aliquots are taken up each half year. The new stock is assayed for mycoplasmas before being introduced into the routine. The cells used in the routine are split weekly, and staining is always performed with freshly made slides (P3HR-1 cells) or larger, frozen batches (NC37 and Molt cells). The serum samples used in this study were sent to and examined at the reference laboratory weekly. The P3HR-1 cells used during the study were obtained between passages 13 and 32, and the NC37 cells were from passage 8 after thawing. The preparation of slides and control of the number of antigen-expressing cells on the slides are performed by an accredited procedure with well-defined serum samples. Sera are diluted fourfold from 1/20 to 1/1,280 in phosphate-buffered saline with 0.5% bovine serum albumin and 0.05% Tween 20 (ELISA buffer) for the VCA examinations and from 1/5 to 1/320 in NaCl for the EBNA examination. The details of the staining procedure for the IF examinations have been described previously (9). The EBNA-1 status was screened by an ELISA for IgG and IgM against a peptide from the glycine-alanine repeat of EBNA-1 (p107). The peptide was produced by Ferring AB, Malmö, Sweden, by 9-fluorenylmethoxycarbonyl chemistry on a Milligen synthesizer and was purified on a Kromasil C8 semipreparative column. The purity was evaluated by high-pressure liquid chromatography and was 75%. The concentration used for coating was 0.005 mg/ml. The serum samples were examined in the ELISA at the 1/80 dilution also used for the VCA examinations. The ELISA procedure and the evaluation of results have previously been described in detail (12). The only modification to that procedure is a change of the batch of antigen. The new batch has a higher purity than the one used previously, but the performance of the test with the two batches were identical when the batches were run in parallel. The performance of the p107 ELISA is continuously monitored by an accredited procedure. VCA IgG and IgM were examined in fourfold dilutions from 1/20, EBNA antibodies were determined in fourfold dilutions from 1/5 to 1/320, and p107 ELISA IgG and IgM were determined at a dilution of 1/80. In the IF assays, results are given as titers.

Interpretation of results. Primary EBV infection was indicated by VCA IgM and IgG antibody titers of ≥20, a p107 ELISA IgG absorbance of <1, and a <p107 ELISA IgM absorbance higher than that for p107 IgG and/or EBNA titer of <5. Previous EBV infection was indicated by a VCA IgG antibody titer IgG of ≥20, a p107 ELISA IgG absorbance of >0.2, and a >p107 ELISA IgM absorbance lower than that for p107 IgG and/or EBNA titer of >5 (>2). Seronegativity was indicated by no detectable antibodies.

Commercially available ELISAs for EBV antibodies. (i) Gull ELISA. Determination of VCA IgM and IgG and EBNA IgG was performed by separate ELISAs according to the manufacturer's instructions. The antigens used were purified EBV VCA (gp125 from infected P3HR-1 cells) and recombinant EBNA-1 without glycine-alanine copolymer. A specimen diluent containing rheumatoid factor absorbent was used for the IgM ELISA.

Interpretation of the results was performed as described by the manufacturer and were as follows: primary infection, positive result for IgM VCA and negative result for EBNA; previous infection, positive result for EBNA. A positive result for VCA IgG in combination with a positive result for VCA IgM strengthens the diagnosis of primary infection, and a positive result for IgG in combination with a positive result for EBNA IgG strengthens the diagnosis of previous infection.

(ii) Biotest ELISA. Anti-EBV recombinant antigens for the detection of EA IgM, IgA, and IgG and EBNA IgG were provided in separate kits. The following recombinant antigens were used as described by the manufacturer: recombinant EBNA-1 p72 without glycine-alanine copolymer and EAs (recombinant EA-D p54, the dominant antigen of the EA-D complex, and recombinant EA-p138, the major DNA-binding protein).

Interpretation of the results was as follows: primary infection, positive result for EA IgM or positive result for EA IgM and EA IgG and negative result for EBNA; previous infection, positive result for EBNA.

A positive result for EA IgA strengthens the diagnosis and/or a positive result for EA IgG in combination with a positive result for EA IgM strengthens the diagnosis of primary EBV infection. A positive result for EA IgG in combination with a positive result for EBNA IgG strengthens the diagnosis of previous infection.

(iii) Enzygnost ELISA. IgG and IgM against a mixture of VCA, EBNA, and EA-D antigens were measured by the Enzygnost (Behring) assay. Rheumatoid factor absorbent was used in the IgM test.

Interpretation of the results was as follows: primary infection, positive result for IgM or positive result for IgM and IgG; previous infection, negative result for IgM and positive result for IgG. Samples with equivocal results should be retested. For an equivocal IgM result, if the sample is positive after retesting, a positive result is reported; if it is negative after retesting, a negative result is reported. If an equivocal classification is confirmed after repeating the test for IgM, a sample collected no less than 7 days after the first sample was collected must be tested in parallel with the first sample.

For an equivocal IgG result, if the sample is positive after retesting, a positive result is reported; if it is negative after retesting, a negative result is reported. If the result is still equivocal after retesting, it can be assessed as a negative finding for a person at risk of infection and as a positive finding for an organ transplant donor. A second sample drawn no less than 7 days later should be tested together with the first sample.

Latex agglutination tests. Both the Monolatex (Biokit) and the Mono-Lex (Trinity Laboratories) kits consist of a suspension of latex particles coated with purified Paul-Bunnell-Davidsohn antigen from bovine erythrocyte membranes.

TABLE 2. Interpretation of EBV status of patients by
reference methods

EBV status	No. of patients	No. of serum samples
Primary infection	37	40
Past infection	120	127
Seronegative	33	36
Atypical reaction	7[a]	11[a]
Total	197	214

[a] Three patients (three serum samples) from whom follow-up samples could not be obtained were excluded from further analysis. The remaining four patients are discussed separately.

The degree of purity of the antigen is such that the Monolatex and Mono-Lex tests should react only with heterophile antibodies from patients with IM, and differential absorption is therefore not necessary. The assays were performed with serum according to the manufacturers' instructions. The presence of agglutination indicates the presence in serum of heterophile antibodies from patients with IM.

RESULTS

Interpretation of results of reference methods. The EBV status of 190 of 197 patients could be determined by the reference method (Table 2). For seven patients, atypical reactions, mainly a lack of EBNA antibodies but no other signs of primary EBV infection, were found (see below). The pattern was identical with follow-up samples from four patients, and those patients are discussed separately. Follow-up samples could not be obtained from three patients with atypical serologies, and these were excluded from the study. In 10 patients VCA IgM was detected, but it was not regarded as a sign of primary EBV infection, since the sera contained EBNA antibodies at titers of ≥ 20.

(i) Gull assay. According to the manufacturer's instructions (positive VCA IgM and negative EBNA IgG, characteristic of primary EBV infection), the sensitivity and specificity of the Gull assays were 95 and 100%, respectively (Table 3). This was also the case when VCA IgG was included. The Gull assay for VCA IgM alone had a sensitivity for primary EBV infection of 98% and a specificity of 99%. The test for EBNA had a sensitivity of 74% for past infection (Table 4), whereas the VCA IgG test used alone had a high sensitivity and a high specificity (99 and 97%, respectively) for past infection.

(ii) Biotest assays. The sensitivities of the Biotest assays for primary EBV infection were high, but the low specificity of the IgM assay (64%) reduced the positive predictive value. The cutoff value was recalculated in the same way as for the Enzygnost IgM assay (see below). However, the sensitivity

TABLE 3. Performance of three commercially available ELISAs[a]
when used with consecutive samples for routine diagnosis of primary
EBV infection

Assay	No. of samples[b]	Sensitivity (%)	Specificity (%)	Positive PV (%)[c]	Negative PV (%)
Enzygnost	179[d]	100	89	73	100
Biotest	203	100	94	80	100
Gull	203	95	100	100	99

[a] All assays were performed and the results were interpreted in accordance with the manufacturers' instructions.
[b] The 11 atypical samples were excluded from the 214 samples.
[c] PV, predictive value.
[d] Twenty-four of the 203 samples were excluded in accordance with the manufacturer's recommendation. Twenty-three samples gave borderline IgM values and 1 sample gave borderline IgG values.

TABLE 4. Performance of three commercially available ELISAs
when serial samples for routine testing were assayed for past EBV
infection and results were interpreted according to the
manufacturers' recommendations

Assay	No. of samples	Sensitivity (%)	Specificity (%)	Positive PV (%)[a]	Negative PV (%)
Enzygnost	139[b]	88	100	100	71
Biotest	163	97	100	100	90
Gull	163	74	100	100	52

[a] PV, predictive value.
[b] The 24 serum samples giving borderline values were excluded from the analysis of data.

dropped to 78% with the alternative cutoff value. Alternative calculations indicated that the combination of positive EA IgM and EA IgA results and a negative EBNA IgG result had the best predictive values for primary EBV infection (positive predictive value, 100%; negative predictive value, 98%). EA IgA and EA IgM in combination had a higher sensitivity and specificity for primary EBV infection than those of each test separately. The Biotest assay for EBNA IgG had a sensitivity of 97% and a specificity of 100% in relation to the EBNA ACIF test results (Table 4).

(iii) Enzygnost assays. In the Enzygnost assay kit, the cutoff values for IgM and IgG were such that repeated borderline values were obtained for 24 of 203 (12%) of the samples. For borderline results, the manufacturer recommends testing of follow-up samples. Follow-up samples were not available from these patients and were not indicated in this study since all the other assays in the study were diagnostic. The borderline samples have therefore been regarded as nonevaluable (according to the manufacturer) and were excluded from the evaluation. The primary evaluation of Enzygnost is therefore based on 179 and 139 samples for the data presented in Tables 3 and 4, respectively. The low specificity for the diagnosis of primary EBV infection and the low sensitivity for the diagnosis of past EBV infection are both explained by the false-positive IgM reactions, despite the exclusion of borderline samples. A new cutoff for IgM was calculated as the mean + 2 standard deviations of the absorbance values for all patients without primary EBV infection. By using this cutoff, the results were improved, and a sensitivity of 95% and a specificity of 99% were obtained. The sensitivity and specificity of the Enzygnost IgG assay for the demonstration of EBV seropositivity were 99 and 100%, respectively.

(iv) Latex agglutination for heterophile antibodies. The results of the latex agglutination tests are presented in Table 5. Both the Monolatex and Mono-Lex tests have high specificities. There were nine false-negative serum samples by the Monolatex test and six false-negative serum samples by the Mono-Lex test. Two false-positive results were obtained by the Mono-Lex test. No false-positive results were obtained by the

TABLE 5. Performance of heterophile antibody agglutination tests
for diagnosis of primary EBV infection[a]

Heterophile antibody agglutination test	Sensitivity (%)	Specificity (%)	Positive PV (%)[b]	Negative PV (%)
Monolatex	78	100	100	95
Mono-Lex	85	99	94	96

[a] All assays were performed and the results were interpreted in accordance with the manufacturers' instructions.
[b] PV, predictive value.

Monolatex test. The patients with false-negative agglutination test results were of the same age as those with positive test results.

Optimal assay combination for diagnosis of primary EBV infection. We evaluated combinations of assays from different manufacturers for use in the diagnosis of primary EBV infection. The combination of the VCA IgM and VCA IgG assays or only the VCA IgM assay from Gull and the EBNA IgG assay from Biotest proved to have a sensitivity of 98% and a specificity of 100% for primary EBV infection.

Patients with atypical EBV serology. In four patients the EBV status could not be definitely determined by the reference methods, despite the testing of follow-up samples. These patients are described below.

(i) Patient 1. Patient 1 was a 24-year-old woman with fever and a sore throat of 6 weeks' duration when the acute-phase sample was drawn. A second sample was drawn 5 months later, and the patient was then in good health. In both samples, IgG titers to VCA were 320 and IgM to VCA was lacking. The EBNA ACIF test result was positive, but the patient lacked p107 antibodies completely. The Biotest assay results were all negative. By the Gull assays, only the VCA IgG was positive, and the same result was obtained by the Enzygnost test. The most likely interpretation is that the patient had had an EBV infection in the past but could not develop EBNA-1 antibodies.

(ii) Patient 2. Patient 2 was a 33-year-old woman. She had a suspected case of tonsillitis, but penicillin treatment had had no effect. She had enlarged cervical lymph nodes and pain at the site of the liver. Serum samples were drawn 2 weeks apart and gave identical results by the reference methods. The VCA IgG titer was 320, there was no VCA IgM, and the EBNA immunoglobulin measured by ACIF was <2, while the p107 IgG assay result was positive. IF for EA antibodies was negative (9). IgM, but not IgG (either to EA or to EBNA), were found by the Biotest assays with the acute-phase sample, but EA IgG appeared in the second sample. Both samples were positive only for VCA IgG by the Gull assays, and only IgG was found by the Enzygnost test. Whether the patient's symptoms were related to EBV cannot be firmly determined, but the complete lack of IgM and EA antibodies by the reference method argues against EBV-related disease.

(iii) Patient 3. Patient 3 was a 7-year-old girl with enlarged cervical lymph nodes and tonsillitis. In samples drawn 6 months apart, she had an extremely low IgG VCA titer (20), a VCA IgM titer of 20 to 40, and negative EA IgG assay, EBNA ACIF test, and p107 IgG assay results. By the Biotest assays, only EA IgM was found in both samples. The Gull assay was weakly positive for VCA IgG in the first sample but was negative for VCA IgG in the second sample. The Enzygnost assay for IgM was positive for the first sample and gave a borderline value for the second sample. Streptococcus group C was cultivated from a throat swab specimen. The girl was asymptomatic upon examination after 6 months, and the role of EBV in her acute disease cannot be judged.

(iv) Patient 4. Patient 4 was an 18-year-old woman with fever and a sore throat of 2 days' duration. A high IgG VCA titer was found in her acute-phase sample, and traces of VCA IgM and EA-D IgG were found, but EBNA antibodies were also present. She had a low p107 IgG absorbance which, however, was still higher than that for IgM. Four months later the VCA IgG titer was still high, VCA IgM and p107 had disappeared completely, but there were no significant changes in EBNA immunoglobulin or p107 IgG. Both samples gave positive results by the Biotest EA IgG and EBNA IgG assays and in the Gull VCA IgG assay. The acute-phase sample was positive and the convalescent-phase sample was borderline by the Enzygnost IgM assay, while the assay was negative for IgG in both

samples. At the first visit, a throat swab culture indicated *Streptococcus pyogenes* infection. This patient had probably had a recent primary EBV infection unrelated to the acute tonsillitis.

DISCUSSION

When a laboratory diagnosis of primary EBV infection is indicated, specific EBV serology should always be performed when the heterophile antibody test is negative and for heterophile-positive patients with atypical symptoms or laboratory findings. According to the manufacturers, the commercially available EBV-specific ELISAs could also be of help in the diagnosis of reactivated EBV infection. However, evaluating the clinical importance of reactivated infection is difficult, since serology indicative of EBV reactivation can be seen in patients with autoimmune diseases and malignancies and patients receiving a variety of medical treatments. There is no reliable reference method for the diagnosis of symptomatic, reactivated EBV infection. Therefore, we evaluated the performance of the commercially available assays only for the diagnosis of primary EBV infection and EBV seropositivity.

Assay for IgM VCA has often been used as the single assay for the diagnosis of primary EBV infection. An IgM VCA assay result can, however, be falsely positive, despite correction for the rheumatoid factor if the patient has other herpesvirus infections and also sometimes for patients with other virus infections, especially if the patient is EBV seropositive (12). Ten false-positive IgM VCA reactions were found by the IIF assay in this study. It is therefore recommended that a test for EBNA antibodies be combined with assays for VCA IgG and/or IgM for the diagnosis of primary EBV infection. A reliable test for EBNA antibodies can be used as a screening test, since the presence of EBNA antibodies excludes primary EBV infection. Antibodies against other EBV antigens must then be analyzed only if EBNA antibodies are absent (4, 11).

Different manufacturers use different antigens. In the assays used here antibodies against VCA are determined by IF by the reference method and the Gull ELISAs. The Gull ELISA for VCA IgM and IgG performed excellently, with a high sensitivity and a high specificity, which were also found in a study by Wiedbrauk and Bassin (22). However, the test for EBNA provided by Gull had a low sensitivity and needs improvement.

The Biotest assays determine EA-D IgM, IgA, and IgG and EBNA IgG. The Biotest assay for EBNA performed well, but the EA IgM assay lacked specificity. The Biotest assay for EA IgA had a better specificity than the Biotest assay for EA IgM for the detection of primary EBV infection and they had equal sensitivities; the Biotest assay for EA IgA could perhaps be used instead of an assay for EA IgM or in combination with an assay for EA IgM. A higher sensitivity and a higher specificity were obtained when assays for both EA IgA and EA IgM were performed and the results were evaluated together than when each test was performed and its results were evaluated separately. Moderately elevated EA IgG and IgM titers have no diagnostic value for primary EBV infection since they can be seen in patients with many conditions affecting the immune system (11, 21), and antibodies to EA IgA have also been associated with nasopharyngeal carcinoma (11, 15, 21). The sensitive assay for EBNA included in the Biotest system is therefore of great value for the diagnosis of primary EBV infection. In a study by Färber et al. (2) the sensitivity and specificity of the Biotest assays for EA IgM, EA IgG, and EBNA IgG for the diagnosis of primary EBV infection were 99.2 and 98.8%, respectively, which are somewhat higher than those that we found.

The Enzygnost assays contain a mixture of different anti-

gens: VCA, EA, and EBNA-1. Since VCA IgG and EBNA IgG are measured in the same test, the valuable information obtained when patients have a positive VCA IgG result and a negative EBNA result is lost. The Enzygnost IgM assay exhibited many equivocal results, which was also shown in a previous study (1, 21) and in the preliminary study by SIIDC. To correct for this, we recalculated the cutoff level, after which the assay performed similarly to the others used in this investigation. Recalculation of cutoff levels for the Biotest did not have the same positive effect, since sensitivity was lost.

The assays from Gull were easy to use and had reagents with different colors. A disadvantage was the use of different dilutions for the assay for VCA IgM than for VCA IgG and EBNA IgG. The Biotest assays were also easy to use but have the disadvantage that a water bath is recommended for the incubation. The assays provided by Enzygnost were more time-consuming than the other tests because of complicated dilution steps.

In a study conducted by Linderholm et al. (13), nine kits for the rapid diagnosis of IM were evaluated. Tests with purified (bovine) heterophile antigens had the highest specificity. The Monolatex and Cards OS Mono assays were recommended for use in the rapid diagnosis of primary EBV infection. The Mono-Lex kit was not evaluated in that study. In another study, the Monolatex test was rapid, technically simple, and more sensitive than the Paul-Bunnell-Davidsohn test (5). Because the Monolatex and Mono-Lex kits are based on the same principle, the similar results obtained in this study are not surprising. Both can be recommended for the rapid diagnosis of EBV-related IM, but the disadvantages of these tests for the detection of heterophile antibodies must always be taken into account.

Although the specific assays used in this study had previously been evaluated at SIIDC with stored sera, it was considered important to evaluate them with consecutive routine samples from patients with suspected primary EBV infection. The criteria for evaluation of clinical tests have been described previously (16). These criteria have been followed in this study, in contrast to most other studies, in which the use of selected serum panels may have given results which are not relevant for routine use. The samples were analyzed by the commercial assays in the routine work flow at the microbiological laboratory, and all serum samples were then independently analyzed by the reference methods at SIIDC. No information about other serological results, age, symptoms, etc., was given to the reference laboratory before the EBV status of the patient was determined on the basis of the results obtained by the reference methods. Likewise, the result of each commercial assay was initially interpreted without knowledge of the results of the other assays.

Despite the use of three different ELISA systems, two latex agglutination tests, the reference methods, and examination of both acute and follow-up sera, the EBV status for eight serum samples (from four patients) could not be definitely established. This underlines the importance of the clinical evaluation of the patient and consideration of the causes of mononucleosis-like symptoms other than EBV in patients with serological results that are not unequivocally diagnostic of primary EBV infection. Also, two diseases may be present in patients with characteristic serology. Some patients, especially those with immunodeficiency disorders, may also have aberrant antibody responses and demonstrate either unusually low or unusually high antibody titers to EBV-specific antigens (15, 20) without obvious EBV-related disease.

In this study, the tests for VCA IgM and IgG from Gull in combination with the test for EBNA from Biotest proved to be an excellent combination, with a sensitivity of 98% and a spec-ificity of 100% for the diagnosis of primary EBV infection. The IgG test from Enzygnost had a high sensitivity and specificity for EBV seropositivity, but the antigen mixture used for IgG prevents the discrimination between recent primary or past infection obtained when separate assays for VCA and EBNA are used.

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