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Evaluation of Four Commercial Systems for the Diagnosis of Epstein-Barr Virus Primary Infections▽

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To compare the performance of four diagnostic commercial systems for Epstein-Barr virus (EBV) serology (for IgM and IgG virus capsid antigen [VCA] and EBV nuclear antigen [EBNA] antibodies), a collection of 125 samples from clinically suspected infectious mononucleosis cases was studied. Indirect immunofluorescence (IIF) for VCA IgM and IgG antibodies and anticomplement immunofluorescence for EBNA antibodies (Meridian Bioscience Inc.) were used as reference methods. By these methods, the cases were classified EBV primary infection (presence of IgM to VCA or IgG to VCA in the absence of EBNA antibodies; $n = 82$), EBV past infection (presence of VCA IgG and EBNA antibodies in the absence of VCA IgM; $n = 26$), or no infection (negative for the three markers; $n = 17$). The following systems were tested: two chemiluminescent immunoassays (CLIAs; the Liason [CLIA-L; DiaSorin] and the Immulite 2000 [CLIA-I; Siemens]), immunofiltration (IF; All.Diag), and an enzyme-linked immunosorbent assay (ELISA; DiaSorin). In the IgM assays, sensitivities ranged from 67.1% (ELISA) to 92.2% (CLIA-L) and specificities ranged from 93.8% (CLIA-L) to 100% (IF). In the VCA IgG assays, sensitivities varied from 79.4% (IF) to 94.4% (CLIA-I) and specificities varied from 94.4% (IF and CLIA-L) to 100% (CLIA-I and ELISA). In EBNA assays, sensitivities ranged from 78.1% (IF) to 93.8% (CLIA-I) and specificities ranged from 32.3% (CLIA-L) to 91.4% (IF). In relation to EBV profiles, the corresponding figures for sensitivity (in detecting primary infection) for IF, CLIA-L, CLIA-I, and ELISA were 92.7%, 93.8%, 89%, and 89.6%, respectively, and those for specificity (to exclude primary recent infection) were 90.7%, 94.6%, 97.7%, and 95.2%, respectively. Although there were limitations in some individual markers, especially CLIA-L for EBNA IgG, the systems evaluated appear to be useful for diagnosis of EBV infection.

Infectious mononucleosis (IM) is a syndrome caused mainly by Epstein-Barr virus (EBV), although other infectious agents, including cytomegalovirus (CMV), human herpesvirus 6 (HHV-6), human immunodeficiency virus, adenovirus, herpes simplex virus, *Streptococcus pyogenes*, and *Toxoplasma gondii* (10), are also recognized to be potential etiological agents. Of these, the most frequent one is CMV, which can cause up to 7% of the cases of mononucleosis syndromes (4, 5, 12).

Infections by EBV can be diagnosed serologically by detecting heterophile antibodies (HAs). However, the sensitivity of this marker is low in both children (the age group with a high prevalence of infection) and adults, but in the latter case sensitivity is low mainly in the early stages of the disease. Detection of specific IgM, the tool of choice for most viral infections, is complicated in the case of infections by EBV due to the high degree of cross-reaction with other herpesviruses causing IM, such as CMV and HHV-6 (1). In addition, in cases of IM, the polyclonal stimulation of memory lymphocytes may be reflected as positive IgM broad reactivity (9). Thus, antibody profiles are needed to achieve effective serological diagnosis of infection by EBV. The main virus-specific markers for this purpose are IgM and IgG responses to virus capsid antigen (VCA) and antibodies to the EBV nuclear antigen (EBNA), following well-established criteria (13).

Currently, indirect immunofluorescence (IIF) techniques are recognized as being the "gold standard" for detection of IgG and IgM to VCA, as is the case for anticomplement immunofluorescence (ACIF) for antibodies to EBNA. Nevertheless, these are laborious techniques, since they cannot be sufficiently automated to achieve a good level of output, and there is a certain degree of subjectivity in interpreting results. Other assays in solid phase have been developed; however, their results depend on the use of a range of different antigens, which can produce different serological responses.

The objective of the study described here is the comparative evaluation of chemiluminescent immunoassay (CLIA), immunofiltration (IF), and enzyme-linked immunosorbent assay (ELISA) commercial systems to detect IgM to EBV, VCA IgG, and anti-EBNA antibodies in order to establish serological profiles for the diagnosis of EBV infection.

MATERIALS AND METHODS

A total of 142 serum samples (125 from clinically suspected cases of IM and 17 additional samples from cases of CMV recent primary infection) were studied.

Serological procedures. IIF for VCA IgM and VCA IgG (Merifluor EBV VCA IgM IFA and Merifluor EBV VCA IgG IFA, respectively; Meridian Bioscience Inc.) and ACIF for EBNA antibodies (Merifluor EBV nuclear antigen test; Meridian Bioscience Inc.) were used as reference methods. IIF for VCA

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IgM was tested after the IgG was removed from the sample using an anti-human IgG serum (RF Absorbens; Siemens, Germany).

IgM against CMV was measured by indirect ELISA (Enzygnost anti-CMV IgM; Siemens) and capture ELISA (CMV-IgM-ELA assay PKS; Medac, Germany). Measurements by indirect ELISA were taken after the IgG was removed from the sample (RF Absorbens; Siemens). Characterization of specific IgG avidity was done by ELISA using urea elution (cytomegalovirus IgG avidity EIA Well; Radim, Italy).

The commercial methods described in the following sections were evaluated.

Immunofiltration. The Immunoquick filtration IgM and Immunoquick filtration IgG methods (ALL.Diag, France) were studied. The IgM assay uses the protein BamHI Z Epstein-Barr activator (ZEBRA) as antigen. The assay for IgG simultaneously detects antibodies to VCA, which uses p18, and EBNA, which uses EBNA-1. Samples were assayed at a 1:61 dilution. Results were read by two different technicians.

Chemiluminescent immunoassays. Chemiluminescent immunoassays from two manufacturers were used.

(i) Liaison CLIA. We tested the Liason VCA IgM and Liason VCA IgG CLIA (CLIA-L; DiaSorin S.p.A, Italy) methods, which use the synthetic peptide p18 as antigen, and the Liason CLIA EBNA IgG, which uses a synthetic peptide, EBNA-1. All are indirect methods. The assay dilutions were 1:7.3 for the IgM assay and 1:40 for the VCA IgG and EBNA IgG assays. The assays were done on the Liaison platform.

The CLIA-L method enables the status of the infection to be established on the basis of jointly studying three markers to determine whether the patient (i) is negative for EBV infection (titers of all three markers <20 units [U]), (ii) has a suspected primary infection (VCA IgM ≥ 20 U, VCA IgG and EBNA IgG < 20 U), (iii) is in the acute phase of primary infection (VCA-IgM and IgG ≥ 20 U, EBNA IgG < 20 U), (iv) is in the transient phase (VCA IgM ≥ 40 U, VCA IgG ≥ 20 U, EBNA IgG ≥ 20 U), (v) had past infection (convalescent phase; VCA IgM < 40 U, VCA IgG ≥ 20 U, EBNA IgG ≥ 20 U), and (vi) had EBV past infection (VCA IgM < 20 U, VCA IgG ≥ 20 U, EBNA IgG ≥ 5 U). All the remaining profiles are considered unresolved. It should be noted that assays for EBNA IgG and for VCA IgM use two different cutoff values (5 and 20 for EBNA IgG and 20 and 40 for VCA IgM) to establish these profiles.

(ii) Immulite 2000 CLIA. The Immulite 2000 CLIA (CLIA-I; Siemens, Germany) EBV VCA IgM, Immulite 2000 EBV VCA IgG, and Immulite 2000 EBV EBNA IgG methods were studied. The assay for IgM is a capture assay that uses the synthetic peptide p18 as antigen, and the other two are indirect methods; the anti-VCA IgG assay uses gp125, and the anti-EBNA assay uses a recombinant of p72. Samples were assayed at a 1:20 dilution, and assays were undertaken on the Immulite 2000 platform.

ELISA. The ETI-EBV-M reverse assay (to determine anti-VCA IgM titers) and ETI-VCA-G assay (for anti-VCA IgG titers) were evaluated. Both use synthetic EBV VCA peptide p18, in addition to ETI-EBNA-G, for IgG antibodies to EBNA, using a synthetic peptide of EBNA-1. All assays were from DiaSorin S.p.A. The assay for IgM anti-VCA was based on the capture method, whereas the other two were indirect. The samples were assayed at a dilution of 1:101 and were processed in an ETI Max 3000 processor (DiaSorin S.p.A.).

To calculate the sensitivity and the specificity of each method evaluated, indeterminate results were considered the most adverse; i.e., the samples were classified as positive if a negative result was expected, and conversely, as negative if a positive one was expected.

Classification of EBV cases. Considering serological results by IIF as the reference criterion for anti-VCA IgG and IgM antibodies and the results by ACIF as the reference criterion for EBNA antibodies, the 125 cases with clinical suspicion of IM were classified, first, EBV primary recent infections (presence of VCA IgM or VCA IgG in the absence of EBNA antibodies; $n = 82$); second, EBV past infections (presence of VCA IgG and/or EBNA antibodies in the absence of VCA IgM; $n = 26$); and third, no infection (negative for the three markers; $n = 17$). This case classification was used to establish serological profiles for the assays evaluated for diagnosis of EBV infection. To calculate the specificity of every system (by combining results) for diagnosis of EBV primary infection, past infections and no infections were globally considered nonprimary infections.

CMV cases. The 17 remaining samples were taken from cases characterized as CMV primary infections, documented by detection of specific IgM by indirect ELISA and capture ELISA and by the presence of low-avidity IgG. These samples were used to evaluate the specificities of the assays in determining specific IgM as a marker of recent infection by EBV.

TABLE 1. Evaluation of assays for detection of IgM against EBV[a]

Assay and result	No. of samples with the following IIF result:		Sensitivity (%)	Specificity (%)	Correlation (%)
	Positive	Negative			
IF, IgM ZEBRA			74.0	100	84.0
Positive	57	0			
Indeterminate	12	0			
Negative	8	48			
CLIA-L, IgM p18			92.2	93.8	92.8
Positive	71	2			
Indeterminate	3	1			
Negative	3	45			
CLIA-I, IgM p18			77.9	95.8	84.8
Positive	60	2			
Indeterminate	4	0			
Negative	13	46			
ELISA, IgM p18[b]			67.1	95.8	78.5
Positive	49	1			
Indeterminate	5	1			
Negative	19	46			

[a] For calculating sensitivity and specificity, indeterminate results were considered the most adverse.

[b] For ELISA, data are for 121 results (73 IIF positive and 48 IIF negative).

RESULTS

Evaluation of assays for anti-EBV IgM. Results obtained by the assays under evaluation for measurement of IgM were compared with those of the reference technique (IIF) (Table 1). Concordance values between 78.5% (ELISA) and 92.8% (CLIA-L) were obtained, with sensitivities being between 67.1% (ELISA) and 92.2% (CLIA-L) and specificities being between 93.8% (CLIA-L) and 100% (IF).

When cases of primary infection by CMV were analyzed, concordantly positive results were obtained in all assays for two samples and concordantly negative results were obtained in all assays for three samples. In IIF, 9 (52.9%) samples were positive, in IF and CLIA-L, 13 (74.5%) samples were positive, in CLIA-I, 7 (42.1%) samples were positive, and in ELISA, 3 (17.6%) samples were positive.

Evaluation of assays for anti-VCA IgG. The general results obtained for each of the assays for anti-VCA IgG compared with the results obtained by IIF are shown in Table 2. The correlations ranged from 81.6% (IF) to 95.2% (CLIA-I). Sensitivities varied from 79.4% (IF) to 94.4% (CLIA-I), and specificities varied from 94.4% (IF and CLIA-L) to 100% (CLIA-I and ELISA).

Evaluation of assays for anti-EBNA antibodies. The general results of assays for anti-EBNA antibodies are shown in Table 3. The correlation ranged from 47.2% (CLIA-L) to 89.3% (ELISA). Sensitivities ranged from 78.1% (IF) to 93.8% (CLIA-L), and specificities ranged from 32.2% (CLIA-L) to 91.4% (IF).

Evaluation of antibody profiles for EBV. Overall results of the antibody profiles for EBV can be seen in Table 4. The

TABLE 2. Evaluation of assays for detection of IgG against EBV[a]

Assay and result	No. of samples with the following IIF result:		Sensitivity (%)	Specificity (%)	Correlation (%)
	Positive	Negative			
IF, IgG p18			79.4	94.4	81.6
Positive	85	0			
Indeterminate	15	1			
Negative	7	17			
CLIA-L, IgG p18			86.9	94.4	88.0
Positive	93	1			
Indeterminate	0	0			
Negative	14	17			
CLIA-I, IgG gp125			94.4	100	95.2
Positive	101	0			
Indeterminate	2	0			
Negative	4	18			
ELISA, IgG p18[b]			89.1	100	90.8
Positive	90	0			
Indeterminate	3	0			
Negative	8	18			

[a] For calculating sensitivity and specificity, indeterminate results were considered the most adverse.
[b] For ELISA, data are for 119 results (101 IIF positive and 18 IIF negative).

TABLE 3. Evaluation of assays for detection of IgG against EBNA[a]

Assay and result	No. of samples with the following ACIF result:		Sensitivity (%)	Specificity (%)	Correlation (%)
	Positive	Negative			
IF, IgG EBNA-1			78.1	91.4	88.0
Positive	25	2			
Indeterminate	1	6			
Negative	6	85			
CLIA-L, IgG EBNA-1			90.6	32.3	47.2
Positive	29	28			
Indeterminate	3	35			
Negative	0	30			
CLIA-I, IgG p72			93.8	79.6	83.2
Positive	30	15			
Indeterminate	1	4			
Negative	1	74			
ELISA, IgG EBNA-1[b]			87.5	89.9	89.3
Positive	28	7			
Indeterminate	1	2			
Negative	3	80			

[a] For calculating sensitivity and specificity, indeterminate results were considered the most adverse.
[b] For ELISA, data are for 121 results (32 ACIF positive and 89 ACIF negative).

sensitivities of the systems (combination of results) evaluated for their ability to correctly identify cases of recent infection were 92.7% (IF), 93.8% (CLIA-L), 89.0% (CLIA-I), and 89.6% (ELISA); the corresponding values for specificity (exclusion of recent infection) were 90.7%, 94.6%, 97.7%, and 95.2%.

With regard to the CLIA-L, classification of cases followed the manufacturer's criteria. Of the total, 117 cases were classified correctly; however, for the remaining 8 cases the results obtained were not included among the possible profiles (unresolved cases): 2 among the positives, 3 among the cases of past infection, and 3 among the negatives. In 75 out of 80 cases using reference criteria, it was possible to identify recent infection. Of these, 12 were suspected primary infection (showing VCA IgM $\geq$ 20 U, VCA IgG and EBNA IgG $<$ 20 U), 39 were classified acute-phase primary infections (showing IgM and IgG $\geq$ 20 U, EBNA IgG $<$ 20 U), and 24 were classified transient-phase infections (showing VCA IgM $\geq$ 40 U, VCA IgG $\geq$ 20 U, EBNA IgG $\geq$ 20 U). The remaining 5 cases were identified as past infections (showing VCA IgM $<$ 20 U, VCA IgG $\geq$ 20 U, and EBNA IgG $\geq$ 5 U). In 23 of the past infection cases, a definitive resolved profile was obtained; of these, past infection was confirmed in 20. Finally, a negative result was confirmed in 13 out of 14 negative cases where a resolved profile was obtained.

DISCUSSION

Determining profiles of IgG and IgM antibodies to VCA and EBNA, in order to ascertain the serological status of IM patients for infection by EBV, requires simple procedures. Given the different characteristics of each type of laboratory, the methodologies best adapted to each laboratory's operation must be chosen. The availability of a broad spectrum of assays for measuring antibodies to EBV (both manual and automatic assays and assays based on different methodologies) would make diagnosis possible in practically all laboratories. This study compares ELISA, IF, and CLIA methods, which represent the broad range of assays available for the virus, applied to the diagnosis of infections by EBV, using as benchmark assays IIF for IgG and anti-VCA IgM and ACIF for anti-EBNA.

From among the different assays for IgM, we compared methods using different antigens: ZEBRA for IF and the synthetic peptide and p18 for the remainder. For IF, the sensitivity obtained (74%) was lower than the sensitivity obtained in a previous evaluation (95.2%) and the specificity was higher (100% versus 97.3%) (3). These differences can be justified by the use of ELISA in the study mentioned, which used affinity-purified VCA (gp125) as a reference (3). In addition, the other three assays which use the peptide p18 showed variations in sensitivities from 67.1% (ELISA) to 92.2% (CLIA-L), although specificities were maintained at about 95% in all assays. Variations in sensitivity can be attributed to other specific characteristics of each assay which are different from the antigen used; in fact the two DiaSorin assays (CLIA-L and ELISA) are based on the use of the same antigen but show a difference in sensitivity of over 20%. Thus, other factors, such as the different dilutions used for analysis, may justify these differences. Recently, the two CLIAs evaluated in this study were compared, along with the Bioplex test, and the

TABLE 4. Evaluation of antibody profiles obtained by the methods compared

Test system and type of infection	No. of samples with the following result by reference method:			Sensitivity (%)[a]	Specificity (%)[a]
	Primary	Past	Negative		
IF				92.7	90.7
Primary	76	4	0		
Past	3	22	0		
Negative	3	0	17		
CLIA-L				93.8	94.6
Primary	75	2	0		
Past	5	20	1		
Negative	0	1	13		
Unresolved[b]	1	3	3		
CLIA-I				89.0	97.7
Primary	73	1	0		
Past	8	25	1		
Negative	1	0	16		
ELISA[c]				89.6	95.2
Primary	69	2	0		
Past	5	23	0		
Negative	3	0	17		

[a] Sensitivity and specificity for identification of recent infection.
[b] Unresolved samples were excluded from the calculations.
[c] For ELISA, data are results for 119 samples (77 primary infections, 25 past infections, and 17 negative result) determined by reference methods.

performance characteristics between the two CLIAs were found to be comparable (2).

Single positive results in IgM assays in acute cases of IM are possible, although this is very infrequent. In fact, of all the samples included in this study only one showed this profile in the reference assays. Nevertheless, in all the assays under evaluation, single positive results were obtained for IgM assays (3 in IF, 2 in CLIA-I, 10 in CLIA-L, and 2 in ELISA) (results not shown). These can be understood as being due to the low sensitivity in the corresponding IgG assays. This is especially true in the CLIA-L, which showed a sensitivity of 86.9% and where the highest number of cases with single IgM reactivity was detected.

As regards measurements of IgG, all assays used the p18 peptide, except CLIA-I, which used gp125. In this case, the greater sensitivity (about 95%) of this assay could, indeed, be related to the use of this antigen.

Single positive results for IgG in cases of IM are relatively frequent, especially in children under 5 years, and are considered to occur at a rate of about 5% (4, 6). Of the 82 cases of IM by EBV analyzed in this study, 5 showed the presence of anti-VCA IgG as the sole indicator of recent infection by the virus using the reference criteria. Only in the CLIA-L were no cases with this profile obtained.

A significant difficulty in comparisons of assays for detecting EBNA IgG is the fact that ACIF, used as a reference for this comparison, detects both IgG and IgM antibodies, and small amounts of EBNA IgM could cause positive ACIF results, since IgM fixes complement more efficiently than IgG. Despite this, the correlation with the reference assay was reasonable (over 83%) in all assays except CLIA-L, where it was 47.2%, due to an excessive lack of specificity, since 63 out of the 93 cases with a negative result for ACIF (Table 3) were identified to be positive (reactivity, over 5 U). The manufacturer considers two cutoff values for calculation of the positivity of the anti-EBNA assay (5 U and 20 U), establishing that cases in the transient phase and convalescent-phase cases must have values over 20 U and that only cases of past infection could have values over 5 U. In this study, it has been found that four EBV-negative cases gave a positive result (>20 U), nine were weak positives (>5 and <20 U), and only four were negative (<5 U) (data not shown).

Regardless of the lack of specificity of the anti-EBNA CLIA-L, sufficiently documented in this study, the presence of only anti-EBNA antibodies has been described to be a relatively frequent finding in laboratories (8), with this possibly being due fundamentally to some defect of sensitivity of VCA-IgG assays run simultaneously. In fact, in this assessment, this frequent pattern has often been obtained in the CLIA-L and is the one that shows the lowest level of sensitivity for anti-VCA IgG.

In EBNA IgG assays it is important to point out that more sensitivity for detecting antibodies implies a greater ability to identify past infections (more specificity for identifying primary recent infection). On the contrary, more specificity for detecting EBNA IgG is related to an increased ability to identify primary recent infection (more sensitivity for this clinical classification).

An important aspect to consider in infections by EBV is the heterologous reactivities detected when IgM is analyzed. These are due to either polyclonal stimulation of memory lymphocytes or cross-reaction to antigenically related pathogens. The most important reactivity from the point of view of diagnosing IM is the reactivity due to cross-reaction with CMV (5, 11), since this virus is the second most important agent producing a mononucleosis syndrome (5, 7, 12). All the assays compared, including IIF, which was used as the reference assay, showed positive results when well-documented cases of primary infection by CMV were analyzed (by detection of specific IgM and low-avidity IgG). The method that proved to be the most specific with this group of samples was ELISA.

In conclusion, although there were limitations in some individual markers, the systems evaluated appear to serve a useful purpose for diagnosis of EBV infections, by establishing EBV seroprofiles.

REFERENCES

1. **Balachandran, N., D. E. Oba, and L. M. Hutt-Fletcher.** 1987. Antigenic cross-reactions among herpes simplex virus types 1 and 2, Epstein-Barr virus, and cytomegalovirus. J. Virol. **61:**1125–1135.
2. **Berth, M., and E. Bosmans.** 2010. Comparison of three automated immunoassay methods for the measurement of Epstein-Barr virus-specific immunoglobulin M. Clin. Vaccine Immunol. **17:**559–563.
3. **Bravo, D., et al.** 2009. Evaluation of an immunofiltration assay that detects immunoglobulin M antibodies against the ZEBRA protein for the diagnosis of Epstein-Barr virus infectious mononucleosis in immunocompetent patients. Clin. Vaccine Immunol. **16:**885–888.
4. **De Ory, F., J. Antonaya, M. V. Fernández, J. M. Echevarría, and A. de la Loma.** 1993. Comparison of diagnostic criteria for Epstein Barr virus infection in children. Serodiagn. Immunother. Infect. Dis. **1:**46–48.
5. **De Ory Manchón, F.** 1994. Ph.D. thesis. Universidad Complutense, Madrid, Spain.
6. **De Paschale, M., et al.** 2009. Seroepidemiology of EBV and interpretation of the "isolated VCA IgG" pattern. J. Med. Virol. **81:**325–331.

7. **Evans, A. S.** 1978. Infectious mononucleosis and related syndromes. Am. J. Med. Sci. **276:**325–339.
8. **García, T., N. Tormo, C. Gimeno, and D. Navarro.** 2008. Assessment of Epstein-Barr virus (EBV) serostatus by enzyme immunoassays: plausibility of the isolated EBNA-1 IgG positive serological profile. J. Infect. **57:**351–353.
9. **Haukenes, G., et al.** 1994. Viral antibodies in infectious mononucleosis. FEMS Immunol. Med. Microbiol. **8:**219–224.
10. **Hurt, C., and D. Tammaro.** 2007. Diagnostic evaluation of mononucleosis like illnesses. Am. J. Med. **120:**911.e1–911.e8.
11. **Miendje Deyi, Y., P. Goubau, and M. Bodéus.** 2000. False positive IgM antibody assays for cytomegalovirus in patients with acute Epstein Barr virus infection. Eur. J. Clin. Microbiol. Infect. Dis. **19:**557–560.
12. **Nikoskelainen, J., J. Keikola, and E. Klemola.** 1974. IgM antibodies specific for Epstein Barr virus in infectious mononucleosis without heterophil antibodies. BMJ **2:**72–75.
13. **Sumaya, C. V., and Y. Ench.** 1985. Epstein Barr virus infectious mononucleosis in children. II. Heterophil antibody and viral specific responses. Pediatrics **75:**1011–1019.