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Comparative Evaluation of Nine Kits for Rapid Diagnosis of Infectious Mononucleosis and Epstein-Barr Virus-Specific Serology

MATS LINDERHOLM,[1,2]* JENS BOMAN,[1] PER JUTO,[1] AND ANNIKA LINDE[3]

Departments of Virology[1] and Infectious Diseases,[2] University Hospital of Northern Sweden, S-901 85 Umeå, and Department of Virology, National Bacteriological Laboratory, S-105 21 Stockholm,[3] Sweden

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Rapid diagnosis of Epstein-Barr virus (EBV)-associated infectious mononucleosis was compared by using nine kits and EBV-specific serology. Specific antibodies indicative of primary EBV infection were detected in 46 of 108 (43%) serum samples of infectious mononucleosis patients. The sensitivities and specificities of the rapid kits varied from 63 to 84% and 84 to 100%, respectively.

Infectious mononucleosis (IM) is most frequently encountered among children and young adults. The predominant etiology is Epstein-Barr virus (EBV), but other infectious agents such as cytomegalovirus, human immunodeficiency virus, and *Toxoplasma gondii* can cause mononucleosis-like diseases (5). Hematological malignancy is another important differential diagnosis.

The diagnosis of IM is based on clinical, hematological, and serological findings (7). The first serological test described was the heterophile antibody assay, developed by Paul and Bunnell (11). The assay was later modified by Davidsohn (1) by introducing a guinea pig kidney absorption to prevent interference of the Forssmann-type antibodies. After the isolation of EBV (5), specific serological tests were developed for demonstration of antibodies against, e.g., virus capsid antigen (VCA) and Epstein-Barr nuclear antigen (EBNA). The diagnostic value of these assays is well documented (6, 8, 9).

Most commercially available IM kits detect heterophile antibodies by agglutination of heterologous erythrocytes (RBC) on a test slide. The heterophile tests have been further developed by applying purified bovine heterophile antigens to a solid phase. The presence of immunoglobulin M (IgM)-type heterophile antibodies in the specimen is indicated by agglutination of antigen-coated latex beads on a slide or by a color reaction on a membrane when anti-human IgM, conjugated to enzyme or blue latex, binds to the heterophile antigen-antibody complex. At present, only a few rapid kits can detect an EBV-specific antibody response.

Most of the previous IM kit evaluations used the classical Paul-Bunnell test as the reference method (3, 13). Our aim was to evaluate nine commercially available kits for the rapid diagnosis of EBV-associated IM compared with EBV-specific immunofluorescence assays and enzyme immunoassays by determining antibodies to VCA and EBNA.

TABLE 1. Description of IM kits by the manufacturers' data

Trade name and manufacturer	Antigen	Guinea pig kidney absorption
Slide agglutination assays		
i.m. absorption; Mercia Diagnostics (Guildford, United Kingdom)	Horse RBC	Yes
Monospot; Ortho Diagnostic Systems (Raritan, N.J.)	Horse RBC	Yes
Monosticon Dri-Dot; Organon Teknika (Durham, N.C.)	Horse-sheep RBC	Yes
Mono-Plus; Dominion Biologicals (Dartmouth, Nova Scotia, Canada)	Horse RBC	Yes
Monolatex; Biokit, sa (Barcelona, Spain)	Latex beads coated with purified bovine RBC extract	No
Solid-phase immunoassays		
Cards Mono; Pacific Biotech, Inc. (San Diego, Calif.)	Purified bovine RBC extract	Yes
Cards OS Mono; Pacific Biotech, Inc.	Purified bovine RBC extract	No
Preview Mono; Lecco (Southfield, Mich.)	Purified bovine RBC extract	No
Monolert; Ortho Diagnostic Systems	EBNA-1 peptide (p62)	No

A total of 108 blood samples from 103 patients (56 males and 47 females between 2 and 60 years of age; median, 19 years) with clinically suspected IM were included. Twenty serum samples (19%) were from patients of ≤12 years of age, and 10 serum samples (9%) were from patients of ≥30 years of age. Blood samples were collected 1 to 60 days (median, 10 days) after onset of the disease: 31 (29%) were collected within 7 days of onset and 3 (3%) were collected 30 to 60 days after onset. Two-thirds of the serum samples were sent from general practitioners; the remaining were from various

* Corresponding author. Mailing address: Department of Infectious Diseases, University Hospital of Northern Sweden, S-901 85 Umeå, Sweden. Phone: 46 90 10 23 08. Fax: 46 90 13 30 06.

TABLE 2. Classification of sera according to the EBV-specific serology test results

Classification	No. of serum samples	Antibody titer to:		
		VCA		EBNA
		IgM	IgG	
Primary EBV infection	42	≥1:20	≥1:20	<1:5
	4	≥1:20	≥1:20	≤1:5[a]
Past EBV infection	54	<1:20	≥1:20	≥1:5
Susceptible to EBV	8	<1:20	<1:20	<1:5

[a] These sera with weak responses to EBNA were also analyzed by p107 enzyme immunoassay: all had IgG/IgM ratios of <1 and IgG optical density titers of <1.0, results compatible with a primary EBV infection (10).

hospital clinics. One portion of each serum sample was immediately used in the nine IM kits; another was frozen (−18°C) and subsequently analyzed by the reference methods. Characteristics of the IM kits are compiled in Table 1. There were four slide agglutination kits with whole RBC heterophile antigen, one slide agglutination and three solid-phase immunoassay kits with purified heterophile antigen, and finally, one solid-phase immunoassay kit with EBV-specific antigen. Tests were performed and evaluated as described in the manufacturers' instructions by the same experienced laboratory technician.

For the reference methods (National Bacteriological Laboratory, Stockholm, Sweden), we used an indirect immunofluorescence assay with P3 HR1 cells as antigen for determination of IgG and IgM antibodies to VCA (9) and an anticomplement immunofluorescence assay with NC-37 cells for determination of antibodies to all EBNAs (9). Only specimens containing specific IgM to VCA after absorption with rheumatoid factor absorbent (Behringwerke, Marburg, Germany) (15) were considered positive. In addition, in cases with a weak response to EBNA by the anticomplement immunofluorescence assay, specific IgG and IgM to an EBNA-1 peptide (p107) were determined by enzyme immunoassay (10). Sera were classified as shown in Table 2. Detection of IgG and IgM to VCA with no or a very weak antibody response to EBNA in a dilution of 1:5 was considered indicative of a primary EBV infection. In the case of a weak response to EBNA, a p107 IgG/IgM ratio of <1 with an optical density titer of IgG of <1.0 was additionally required (10). Demonstration of antibodies to EBNA and IgG to VCA, without detectable IgM to VCA, was interpreted as indicative of a past EBV infection. If no antibodies to VCA or EBNA were detected, the patient was considered susceptible to EBV.

In total, 46 of 108 serum samples (43%) were from patients with primary EBV infection, as determined by the reference methods (Table 2). Six of eight serum samples without detectable antibodies to VCA or EBNA were positive by one or two rapid kits. The sensitivities of the slide agglutination kits and of the solid-phase immunoassay kits, compared with the reference methods, ranged from 71 to 84% and 63 to 71%, respectively (Table 3). Exclusion of sera collected in the first week after onset of the disease did not improve the sensitivity (data not shown). In the population under the age of 13 years, the sensitivity was 25 to 50% (Table 3).

The highest specificity was obtained by kits with purified bovine heterophile antigens (Monolatex, Cards Mono, Cards OS Mono, and Preview Mono) and ranged from 95 to 100%, whereas whole RBC agglutination kits had a lower specificity, ranging from 84 to 95% (Table 3). The sensitivity and specificity of the EBV-specific kit (Monolert) were not higher than those of kits with heterophile antigen, neither early after onset of the disease nor in the youngest age group (Table 3).

A positive predictive value of more than 95% was obtained by two kits, Monolatex and Cards OS Mono (Table 3). The negative predictive values of the kits varied from 78 to 88%; i.e., the probability of EBV disease in spite of a negative test result was more than 10%, irrespective of the IM kit used.

In primary health care and also in many microbiological laboratories, the clinical diagnosis of IM is still confirmed by the demonstration of heterophile antibodies. Most of the commercially available rapid kits rely on this classical method. In the present study, we compared nine rapid IM kits to EBV-specific serology (VCA and EBNA). We found that all kits, particularly the solid-phase immunoassays, had low sensitivities, especially when children were tested. This result is also expected when heterophile antibody-detecting methods are used, since it has been reported that only 80 to 90% of adults and <50% of young children develop heterophile antibodies (12). The EBNA-based kit was, however,

TABLE 3. Sensitivity, specificity, and predictive values of nine kits for diagnosis of EBV-associated IM compared with EBV-specific serology (VCA and EBNA)[a]

Kit	Sensitivity (%)			Specificity (%)			Predictive value (%)		Ease of[b]:		Estimated time (min)[c]
	0–12 yr[d] (n = 8)	≥13 yr[d] (n = 38)	Total (n = 46)	0–12 yr[d] (n = 12)	≥13 yr[d] (n = 50)	Total (n = 62)	Positive	Negative	Performance	Reading	
i.m. absorption	38	79	71	92	82	84	77	80	+	++	5
Monospot	38	86	77	100	90	92	88	85	+	++	5
Monosticon Dri-Dot	50	82	76	100	94	95	92	84	++	+	5
Mono-Plus	50	91	84	92	86	85	80	88	+	++	5
Monolatex	38	84	76	100	98	98	97	85	+++	+++	5
Cards Mono	25	71	63	100	94	95	91	78	++	++	5
Cards OS Mono	25	74	65	100	100	100	100	80	+++	++	5
Preview Mono	25	79	70	100	96	97	94	81	++	++	5–10
Monolert	50	75	71	92	96	95	91	82	+	+++	10

[a] In total, 108 serum samples were tested.
[b] A subjective estimation of the ease of performance and reading: +, less easy; ++, easy; +++, very easy (mainly based on the number of reagents and steps in the procedure and on ease in discriminating between positive and negative test results).
[c] Estimated time to perform a single analysis.
[d] Patient age.

not more sensitive for diagnosing IM in children (Table 3). Specimens drawn in the early phase of the disease, when the heterophile antibody titer is lower (8), did not help explain the low sensitivity.

Kits with heterophile antigens purified from bovine RBC had a higher specificity than whole-RBC agglutination kits and the EBNA-specific kit. The data support that guinea pig kidney absorption is not needed when purified antigen is used. Positive rapid kit results in the absence of active EBV infection can be explained by the long-term persistence of heterophile antibodies (several months) (2) or might be caused by hematological and rheumatic disorders (4) or other infections. Thus, a false-positive IM kit result indicating EBV infection could delay the use of appropriate measures in, e.g., primary HIV mononucleosis (14) or an undiagnosed hematological malignancy. A high specificity of the rapid kits should therefore be given a higher priority than a high sensitivity. The kit should also be easy to perform and read to attain valid results in daily use (Table 3).

In summary, there was a considerable variation in the performance of the IM kits. Kits with purified heterophile antigen had the highest specificities, and Monolatex and Cards OS Mono should be especially useful in confirming a primary EBV infection. EBV-specific serologies are needed when the rapid result by the IM kit is negative or, particularly, in all cases for which a confirmation of the diagnosis is important, e.g., for patients with atypical symptoms or laboratory findings.

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