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Evaluations of Enzyme-Linked Immunosorbent Assay Procedure for Determining Specific Epstein-Barr Virus Serology and of Rapid Test Kits for Diagnosis of Infectious Mononucleosis

MICHAEL A. GERBER,[1]* EUGENE D. SHAPIRO,[2] RAYMOND W. RYAN,[3] AND GILLIAN L. BELL[4]

Department of Pediatrics, University of Connecticut School of Medicine, Connecticut Children's Medical Center, Hartford,[1] Department of Pediatrics and Epidemiology, Yale University School of Medicine, New Haven,[2] and Department of Laboratory Medicine[3] and Department of Pediatrics,[4] University of Connecticut Health Center, Farmington, Connecticut

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Using the results of Epstein-Barr virus-specific immunofluorescence serology as the "gold standard," we found that the sensitivities of five rapid test kits varied from 78 to 84% and specificities varied from 89 to 100%. Enzyme-linked immunosorbent assay-determined specific Epstein-Barr virus antibody profiles had a sensitivity and specificity of 98.6 and 95.5%, respectively.

Infectious mononucleosis, an acute infectious disease that occurs primarily in older children and young adults, is characterized by fever, pharyngitis, generalized lymphadenopathy, and splenomegaly. Although Epstein-Barr virus (EBV) causes 80 to 95% of the cases of clinically apparent infectious mononucleosis, the disease is sometimes caused by other infectious agents such as cytomegalovirus, toxoplasmas, adenovirus, rubella virus, and hepatitis A virus (12, 13).

In patients with the typical clinical findings of infectious mononucleosis, a complete blood cell count and a test for heterophile antibodies can usually establish the diagnosis of an EBV infection (11). However, approximately 20% of adults with EBV-associated infectious mononucleosis will not have detectable heterophile antibodies (1). Young children may have an EBV infection without the characteristic clinical findings of infectious mononucleosis, and more than 50% of those less than 4 years of age will not have detectable heterophile antibodies (3, 14). For these patients, and for patients with either unusual clinical manifestations or complications of infectious mononucleosis, performance of specific EBV serology is required for diagnosis.

The purpose of this study was to assess the accuracy of EBV-specific antibody profiles measured by a new enzyme-linked immunosorbent assay (ELISA) procedure in comparison with the traditional EBV-specific antibody profiles as measured by immunofluorescence (IF). In addition, the sensitivities and specificities of five rapid tests for the diagnosis of EBV-associated infectious mononucleosis were determined. Specific EBV serology as measured by IF was used as the "gold standard" with which the results of these tests were compared.

From 1 February to 30 April 1994, acute serum and plasma samples were obtained from students who presented to the student health service at either the University of Connecticut (Storrs, Conn.) or Yale University (New Haven, Conn.) with signs and symptoms consistent with infectious mononucleosis. All specimens were transported to the University of Connecticut Health Center, where they were stored at −70°C and then tested simultaneously for antibodies to EBV-specific antigens (EBV-viral capsid antigens [VCA]-immunoglobulin M [IgM], EBV-VCA-IgG, anti-early antigens [EA], and anti-EBV nuclear antigens [EBNA]) using both an ELISA procedure (EBV ELISA; Incstar Corp., Stillwater, Minn.) and an IF procedure (Organon-Teknika, Durham, N.C.). IF-determined antibody titers of ≥1:10 for VCA-IgM, VCA-IgG, and EA and titers of ≥1:2 for EBNA were considered positive. In addition, a subgroup of specimens were also tested simultaneously with four rapid heterophile antibody tests (MONOSPOT [Meridian Diagnostics, Cincinnati, Ohio], MONO-LEX [Gull Laboratories, Salt Lake City, Utah], and MONO-plus and MONO-LATEX [Wampole Laboratories, Cranbury, N.J.]) and one rapid ELISA kit for antibodies to EBNA-1 (MONOLERT [Meridian Diagnostics, Cincinnati, Ohio]). However, some specimens could not be tested in this manner because of insufficient volume collected. All commercial tests were performed with both positive and negative controls, using the instructions provided by the manufacturer. Laboratory evidence of a current EBV infection, a recent EBV infection, a past EBV infection, a reactivated EBV infection, and susceptibility to EBV were defined as shown in Table 1 (5, 6). Using the results of the EBV-specific IF serology as the gold standard, we determined the sensitivity and specificity of the EBV-specific ELISA serology as well as the sensitivities and specificities of the five rapid test kits.

Over a 3-month period, serum and plasma specimens were collected from 512 patients. Based on the results of the IF-determined specific EBV antibody titers, the sensitivities and specificities, respectively, of the ELISA-determined specific EBV antibody titers were as follows: VCA-IgG (100%, 100%), VCA-IgM (100%, 96%), EA (78%, 99%), and EBNA (100%, 99%). A comparison of the results of the ELISA-determined and IF-determined specific EBV antibody profiles is shown in Table 2. The overall agreement between the ELISA-determined and IF-determined EBV-specific antibody profiles was 96.3%. Using patients with IF-determined profiles of current or recent infections to calculate sensitivity and patients with IF-determined profiles of past infections or susceptibility to calculate specificity, we found that the sensitivity and specificity of the ELISA-determined specific EBV antibody profiles were 98.6 and 95.5%, respectively. Of the 512 specimens tested, 37 (7.2%) had indeterminate antibody profiles with both the IF and ELISA procedures, while 19 (3.7%) had indeterminate results with only the ELISA procedure. In 17 of the 19 specimens with indeterminate antibody profiles with only the ELISA procedure, VCA-IgG and EBNA antibodies were identified with both procedures and VCA-IgM antibodies were identified with ELISA but not with IF.

* Corresponding author. Mailing address: Connecticut Children's Medical Center, 282 Washington St., Hartford, CT 06106.

TABLE 1. Serologic profiles of EBV-induced infectious mononucleosis

Antibody-antigen	Antibody present at stage:							
	Susceptible	Current	Recent	Recent	Past	Reactivated	Indeterminate	Indeterminate
IgM-VCA	−	+	−	+	−	−	−	+
IgG-VCA	−	+	+	−	+	+	+	+
anti-EA	−	+	+	+	−	+	−	−
anti-EBNA	−	−	−	−	+	+	−	+

In addition, a subgroup of 447 specimens were also tested with a battery of rapid heterophile antibody kits and a rapid ELISA kit for anti-EBNA-1. The sensitivities and specificities of these kits were calculated as described above using IF-determined specific EBV antibody profiles as the gold standard (Table 3).

The IF procedure for determining EBV-specific antibody profiles was felt to be difficult to perform, required 5 to 6 h, and had unclear endpoints. In contrast, the ELISA procedure for determining the same antibody profiles was felt to be easy to perform, required 4 to 5 h, and had clear endpoints.

The rapid test kits all required ≤5 min to complete and were very easy to perform except for the MONOLERT, which required 20 min to complete and was considerably more difficult to perform than the other tests. The kits ranged in price from approximately $1.70 to $4.60 per patient, with the MONO-plus and the MONOLERT being the most expensive.

Although many rapid heterophile antibody kits are commercially available, little research has been performed to determine the accuracy or utility of these tests (2, 4, 15). In a recent comprehensive investigation, Linderholm and coworkers evaluated five slide agglutination assays and four solid-phase immunoassays for heterophile antibodies and compared the accuracy of these kits with IF-determined specific EBV antibody profiles (9). The sensitivities and specificities of the slide agglutination kits ranged from 71 to 84% and 84 to 98%, respectively; the sensitivities and specificities of the solid-phase immunoassays ranged from 63 to 70% and 95 to 100%, respectively. The authors noted that there was a considerable variation in the performance of the kits and that all of the kits had low sensitivities, particularly when children were tested.

An ELISA kit that detects IgG and IgM antibodies to a specific peptide sequence (p62) of EBNA-1 that appears early in acute EBV infections (MONOLERT; Meridian Diagnostics) was recently developed as a rapid diagnostic test for infectious mononucleosis. However, several investigators have found the accuracy of the MONOLERT to be unsatisfactory and the kit unsuitable for the diagnosis of infectious mononucleosis (7, 8, 10).

TABLE 2. Comparison of ELISA-determined specific EBV antibody profiles with IF assay-determined specific EBV antibody profiles

ELISA-determined antibody profile	IF assay-determined antibody profile:				
	Current or recent	Past	Susceptible	Reactivated	Indeterminate
Current or recent	71				
Past		330			
Susceptible			54		
Reactivated				1	
Indeterminate	1	17	1		37

TABLE 3. Accuracy of five rapid test kits compared with IF assay-determined specific EBV serology

Test	Sensitivity (%)	Specificity (%)
MONOSPOT	81	100
MONO-plus	84	98
MONO-LATEX	83	99
MONO-LEX	78	99
MONOLERT	82	89

In this investigation, we found that the accuracies of the four rapid heterophile antibody kits we evaluated were comparable and similar to those previously reported (9). We also found that the specific EBV antibody profiles produced by the Incstar ELISA procedure were comparable to the profiles produced by the IF procedure. However, the ELISA procedure produced slightly more indeterminate results than the IF procedure, and this could lead to unnecessary additional testing. Nevertheless, because the ELISA procedure is considerably faster and easier to perform than the IF procedure, in those situations in which specific EBV serology is required, the ELISA procedure should be employed.

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