

Comparative Evaluation of Two Test Methods (Enzyme Immunoassay and Latex Fixation) for the Detection of Heterophil Antibodies in Infectious Mononucleosis

MINBAE KIM† AND MANOHER WADKE*

Department of Pathology, Muhlenberg Regional Medical Center, Plainfield, New Jersey 07061

Received 22 February 1990/Accepted 15 August 1990

The present study evaluated the Ventrescreen Mono enzyme immunoassay (Ventrex Laboratories) and Monolater (Wampole Laboratories) for detection of infectious mononucleosis-specific heterophil antibodies by comparing test results on sera of 247 symptomatic patients. The purpose of this study was to compare not only the two different test systems but also the two different methods they represent for accuracy in detecting infectious mononucleosis-specific heterophil antibodies. Discrepancies in the test results of the two kits were arbitrated by determining the Epstein-Barr virus antibody profiles of the samples. The study showed that the Ventrescreen Mono enzyme immunoassay is as good as the Monolater test for detecting heterophil antibodies. After discrepancies were resolved, the sensitivity and specificity of the Ventrescreen assay were 100 and 98%, respectively.

Infectious mononucleosis (IM) is an acute, self-limited, lymphoproliferative disease caused by the Epstein-Barr virus (EBV). Infection with EBV usually occurs early in life with no recognizable disease. When primary infection is delayed until young adulthood and adolescence, however, there is about a 50% chance that it will occur with the classic clinical manifestations associated with IM (2, 3).

The diagnosis of IM is usually based on evaluation of characteristic clinical, hematologic, and serologic changes (2, 8, 9). Because other diseases may mimic the clinical symptoms of IM, serologic testing is essential for the most accurate diagnosis. Serologic diagnosis of IM is demonstrated by the presence of heterophil and EBV antibodies in the sera of patients (2, 7-9).

It has been well established that most individuals exposed to EBV develop a heterophil antibody response. Heterophil antibodies make up a broad class of antibodies which are characterized by the ability to react with surface antigens present on erythrocytes (RBCs) of different mammalian species. There are heterophil antibodies which appear in IM that are referred to as IM-specific or IM heterophil antibodies, and they are of the immunoglobulin M (IgM) class. These antibodies do not react with EBV antigens, and it is unknown which specific antigen stimulates their production. IM heterophil antibodies do, however, react with beef, sheep, and horse RBCs. Most commercially available tests for IM heterophil antibody detection incorporate this principle into their test formats (1, 5, 6, 12).

It has been a common practice for physicians to use detection of IM heterophil antibodies in sera of patients as an aid in the diagnosis of IM. The purpose of this study was to evaluate comparatively a new solid-phase enzyme immunoassay (EIA), the Ventrescreen Mono test (Ventrex Laboratories, Portland, Maine), and a latex agglutination method, Monolater (Wampole Laboratories, Cranbury, N.J.), for detection of IM heterophil antibodies.

MATERIALS AND METHODS

Specimens. Sera obtained from adolescents and adults symptomatic for the mononucleosis syndrome were processed in our laboratory. These patients were seen at various clinics and physicians' offices in the local community. All specimens were tested within 3 days of receipt. Most of the specimens (214 [86%] of 247) were tested within 6 h after collection. Sera that could not be tested promptly were stored frozen at or below -20°C and subsequently tested within 3 days of receipt.

Test procedures. (i) **Ventrescreen Mono EIA.** The Ventrescreen Mono EIA is a solid-phase EIA. A purified antigen extracted from bovine RBCs is coated onto the wall of the reaction tube in which the assay is performed. There is no guinea pig kidney absorption step required because of the specificity of the beef RBC antigen for heterophil antibodies. Tests were performed in accordance with the manufacturer's instructions.

(ii) **Monolater.** The Monolater test is a rapid latex agglutination test. Specific IM antigen obtained from beef RBCs is coated onto latex beads. Absorption of the serum of a patient is not required in this test because of the specificity of IM antigen for heterophil antibodies. The tests were performed in accordance with the packaged instructions.

(iii) **EBV antibody test.** The EBV serology profile included tests for antibodies to viral capsid antigen (VCA) IgG and EBV VCA IgM, antibodies to EBV nuclear antigen, and diffused and restricted antibodies to EBV early antigen. The specimens for EBV serology testing were sent to a reference laboratory (EBV Laboratories, Children's Hospital of Philadelphia, Philadelphia, Pa.).

Statistics. Sensitivity, specificity, and predictive values were determined by the statistical methods of Galen and Gambino (5). Monolater was used as the reference method before resolution of discrepancies. The results were subsequently recalculated by using the EBV serology profile as the reference standard for resolution of discrepant results obtained with the two screening tests.

Resolution of test results. All specimens from patients were analyzed by using the two commercial kits in our laboratory. Whenever a discrepancy arose between results of the two

* Corresponding author.

† Present address: Department of Pathology, Jersey City Medical Center, Jersey City, NJ 07304.

TABLE 1. Resolution of discrepant specimens by EBV antibody profile

Specimen no.	Result of: ^a		Antibody titer to: ^b					Interpretation
	Monolatest	Ventrescreen Mono	VCA		EBNA	EA-D	EA-R	
			IgG	IgM				
4	-	+	1:160	<1:10	1:40	<1:10	1:40	Past infection
32	-	+	1:640	<1:10	1:40	<1:10	1:10	Past infection
46	-	+	1:2,560	<1:10	1:80	1:640	1:10	Past infection
50	-	+	1:1,280	1:160	<1:2	<1:10	1:10	Recent infection
52	-	+	<1:40	<1:10	<1:2	<1:10	1:10	No evidence of infection
93	+	-	1:320	<1:10	<1:2	1:40	1:10	Past infection
111	-	+	1:640	1:80	<1:2	1:40	1:10	Recent infection

^a +, Positive; -, negative.

^b As reported by EBV Laboratories, The Children's Hospital of Philadelphia. EBNA, Antibody to EBV nuclear antigen; EA-D, diffused antibody to EBV early antigen; EA-R, restricted antibody to EBV early antigen.

assays, the test was repeated with each to confirm the initial findings. All specimens with persistent discrepant results were sent to a reference laboratory for EBV serology profile determination. On the basis of the EBV antibody pattern, the results were classified as past, current, or no evidence of infection. The results were interpreted as follows. Recent infection was characterized by the presence of any level of VCA IgM (1:10 or greater). Absence of IgM (<1:10), along with an elevated VCA IgG level, was considered indicative of past infection, and absence of any EBV serology markers was considered no evidence of infection.

RESULTS

The study included 247 serum samples from symptomatic patients being screened for IM by Monolatest, our usual method, as the reference test, against which the Ventrescreen Mono test was compared. Both tests were positive in 44 cases and negative in 196 cases. Seven specimens were discrepant before resolution by EBV serology profiles: six that were negative by Monolatest and positive by Ventrescreen Mono and one that was positive by Monolatest and negative by Ventrescreen Mono. Table 1 shows the results of EBV serology profiles of the discrepant cases. Two of the specimens that were Monolatest negative and Ventrescreen Mono positive were considered positive by the EBV antibody profile, leaving four that were classified as false-positive. The one discrepant specimen that was positive by Monolatest and negative by Ventrescreen Mono was resolved as true-negative, leaving no false-negative results.

Although the EBV antibody profile can be considered the test of choice for determination of stages of IM, there are several problems with the method. Therefore, we chose to have the tests run and interpreted at a reference laboratory that specializes in the method (EBV Laboratories, Children's Hospital of Philadelphia).

Table 2 presents test results before and after resolution by EBV profile. Ventrescreen Mono manifested a sensitivity of 97.8% when Monolatest was used as the reference method and a sensitivity of 100% after resolution of discrepant samples. The specificities of Ventrescreen Mono when tested against Monolatest were 97% before and 98% after resolution. The positive predictive values of Ventrescreen Mono were 88% before resolution and 92% after resolution, and the negative predictive values were 99.5% before and 100% after resolution.

DISCUSSION

There are a number of commercially available methods for measuring heterophil antibodies in serum for laboratory

diagnosis of IM. Their features have been well discussed elsewhere (1, 4, 9, 11, 13). The objective of this study was to evaluate the accuracy of a recently introduced EIA with reference to a latex agglutination test for detection of IM heterophil antibodies. Despite the differing methods of the tests, they have common features. Both have IM-specific antigen in their test systems, eliminating the need for serum absorption of interfering heterophil antibodies, and both need no special equipment or skill, since all of the necessary testing material is included in the kit. The list price of the Ventrescreen Mono EIA is approximately \$3.00 per test, and that of Monolatest is \$1.50 per test.

Overall, the two tests were in agreement. When they differed, EBV serology profiles were able to provide a basis for presumptive and not necessarily firm diagnosis. EBV antibody profiles can be helpful in determining the presence and stage of the disease. However, a definitive EBV diagnosis is not a simple matter and antibody titers may be interpreted differently in different laboratories (10). The question of agreement of EBV profiles with heterophil screening tests when the tests agree (i.e., when both are positive or negative) was not pursued in this study but was addressed in an earlier study by Tilton et al. (13). In that study, there was complete agreement between the screen results and EBV profiles when a random number of samples from patients were studied.

The apparent high rate of false-positive results obtained with the Ventrescreen Mono test may be due to the greater sensitivity of EIA than latex agglutination. Latex endpoints are often subject to different interpretations by different technologists, as observed by those researchers. The weak reactions are often interpreted as negative or inconclusive. On the other hand, EIA endpoints based on color change are much easier to interpret, making this technology useful in the private practice setting. However, the relatively high rate of false-positive results obtained with the Ventrescreen Mono EIA could be a problem for physicians who use the test as the only laboratory test to establish the diagnosis of

TABLE 2. Comparison of Ventrescreen Mono EIA and Monolatest before and after resolution with EBV antibody profile

Ventrescreen Mono EIA result	No. of Monolatest results before/after resolution ^a	
	Positive	Negative
Positive	44/46	6/4
Negative	1/0	196/197

^a n = 247.

IM. In that case, a number of other causative agents, including toxoplasma, herpesvirus, cytomegalovirus, etc., that can mimic IM should be ruled out. The negative predictive value of Ventrescreen Mono demonstrates the high degree of agreement with the Monolatest test on this important measure of any screening test, which is to rule out the disease as the cause of the symptoms.

LITERATURE CITED

1. Cook, L., J. Midgett, D. Willis, B. Clinton, and J. D. Folds. 1987. Evaluation of latex-based heterophile antibody assay for diagnosis of acute infectious mononucleosis. *J. Clin. Microbiol.* **25**:2391-2394.
2. Davidsohn, I. 1937. Serologic testing of infectious mononucleosis. *J. Am. Med. Assoc.* **183**:289-295.
3. Evans, A. S. 1974. History of infectious mononucleosis. *Am. J. Med. Sci.* **267**:189-195.
4. Evans, A. S., and J. C. Niederman. 1982. EBV-IgA and new heterophile antibody tests in diagnosis of infectious mononucleosis. *Am. J. Clin. Pathol.* **77**:555-560.
5. Galen, R. S., and S. R. Gambino. 1975. *Beyond normality: the predictive value and efficiency of medical diagnosis.* John Wiley & Sons, Inc., New York.
6. Hoff, G., and S. Bauer. 1978. A new rapid slide test for infectious mononucleosis. *J. Am. Med. Assoc.* **194**:119-121.
7. Lennette, E., and W. Henle. 1987. Epstein-Barr virus infections: clinical and serologic features. *Lab. Manage.* **25**:23-26.
8. Paul, J. R., and W. W. Bunnell. 1932. The presence of heterophile antibodies in infectious mononucleosis. *Am. J. Med. Sci.* **183**:91-104.
9. Pochedley, C. 1987. Laboratory testing for infectious mononucleosis. *J. Postgrad. Med.* **81**:335-342.
10. Steeper, T. A., C. A. Horwite, W. H. Henle, and G. Henle. 1987. Selected aspects of acute and chronic infectious mononucleosis and mononucleosis-like illnesses for the practicing allergist. *Ann. Allergy* **59**:243-245.
11. Sumaya, C. 1985. Serologic testing for infectious mononucleosis. *J. Infect. Dis.* **15**:984-987.
12. Sumaya, C. 1986. Infectious mononucleosis and other EBV infections: diagnostic factors. *Lab. Manage.* **24**:37-45.
13. Tilton, R. C., F. Dias, and R. W. Ryan. 1988. Comparative evaluation of three commercial tests for detection of heterophile antibody in patients with infectious mononucleosis. *J. Clin. Microbiol.* **26**:275-278.