Comparative evaluation of seven commercial tests for detection of heterophile antibody in infectious mononucleosis

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M SKULNICK, DE LOW, AE SIMOR, M PATEL, P GEORGE, R CHUA. Comparative evaluation of seven commercial tests for detection of heterophile antibody in infectious mononucleosis. Can J Infect Dis 1991;3(1):23-26. Detection of heterophile antibodies in infectious mononucleosis is the most rapid and cost-effective method for confirming the clinical diagnosis of the disease. This study compared seven commercial test kits (the Oxoid Infectious Mononucleosis Kit [Oxoid Ltd], Immunoscan Im-Latex [Baxter Travenol], Mono-Latex [Wampole Laboratories], Monospot and Im Screen Test [Ortho Diagnostics], Immunoscan Im-RBC Test [Baxter Travenol], and Infectious Mononucleosis Test [NCS Diagnostics]) to the Davidsohn differential test. All of the kits were shown to be acceptable for use, with specificities and sensitivities greater than 96.5% and 95.5%, respectively.

Key Words: Epstein-Barr virus, Heterophile antibodies, Infectious mononucleosis

Evaluation comparative de sept tests commerciaux servant à la détection des anticorps hétérophiles dans la mononucléose infectieuse

RESUME: La détection des anticorps hétérophiles dans la mononucléose infectieuse est la façon la plus rapide et la plus économique de confirmer le diagnostic clinique. La présente étude a comparé sept tests commerciaux (Oxoid Infectious Mononucleosis Kit [Oxoid Ltd], Immunoscan Im-Latex [Baxter Travenol], Mono-Latex [Wampole Laboratories], Monospot et Im Screen Test [Ortho Diagnostics]) au test différentiel de Davidsohn. Tous les tests se sont avérés acceptables, avec des spécificités et sensibilités supérieures à 96,5 et à 95,5 %, respectivement.

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INFECTIONOUS MONONUCLEOSIS IS A COMMON AND USUALLY self-limiting disease caused by the Epstein-Barr virus. The disease is most commonly seen in adolescents and young adults. The diagnosis of infectious mononucleosis is usually made on the basis of clinical symptoms (such as pharyngitis, atypical lymphocytosis, rash, fever, lymphadenopathy and splenomegaly) and confirmed by the detection of infectious mononucleosis heterophile antibodies and/or antibodies to Epstein-Barr virus (1,2). However, certain adult patients may not produce infectious mononucleosis heterophile antibodies, and some children with acute infectious mononucleosis have no measurable heterophile antibodies with current rapid methods (3,4). Other heterophile antibodies such as the Forssman and serum sickness antibodies are produced in a variety of diseases such as rheumatoid arthritis, lymphoma and hepatitis, and must be differentiated from the infectious mononucleosis heterophile antibody (5). In response to this concern, Davidsohn (5) developed a confirmatory differential test which removes Forssman and sheep heterophile antibodies by absorption with beef erythrocytes and guinea pig kidney cells.

MATERIALS AND METHODS

A total of 318 consecutive sera obtained from non-hospitalized adult and adolescent patients seen in family physicians' offices and suspected of having infectious mononucleosis were tested with each of the commercial kits and compared to the results of the Davidsohn differential test (6). All testing was done in a blinded manner and comparative results were unknown until tabulation of all data was complete.

All sera with discrepant test kit results compared to with the Davidsohn differential test were repeated with the test kit or kits. In addition, specific Epstein-Barr serology using immunofluorescent techniques was performed. These tests were viral capsid antigen IgG (Granbio Inc, California), viral capsid antigen IgM (Gull Laboratories, Utah), early antigen IgG and Epstein-Barr nuclear antigen IgM. Acute infection was defined as the

presence of either viral capsid antigen IgM antibodies or antibody to early antigen (7). The two latter tests were performed at the Ministry of Health Laboratories using fluorescent antibody technique.

The kits tested in this study were a cross-section of those available on the market. Three were latex agglutination products (Oxoid Infectious Mononucleosis Kit [Oxoid Ltd, United Kingdom], Immunoscan Im Latex [Baxter Travenol Diagnostics, California] and Mono-Latex [Wampole Laboratories, New Jersey]), and four were red cell agglutination products (Monospot and Im Screen Test [Ortho Diagnostic Systems], Immunoscan Im-RBC test [Baxter Travenol Diagnostics, California] and Infectious Mononucleosis Test [NCS Diagnostics Corp, New York]). The latex agglutination kits all used latex polystyrene beads sensitized with purified bovine red blood cell heterophile antigen. This glycoprotein has been shown to be specific for the infectious mononucleosis heterophile antibody and, therefore, removed the need for procedures to absorb Forssman or serum sickness antibodies as with the monospot kit (8,9). The other four kits all used fresh or modified horse red blood cells.

RESULTS

Of the 318 sera tested, 89 (28%) were positive for heterophile antibodies using the Davidsohn differential test. A comparison of the test kit results is shown in Table 1. The results show that all kits had acceptable sensitivities and specificities greater than 95.5% and 96.5%, respectively.

The sera giving discordant results are shown in Table 2. The majority of sera were discordant against two or fewer kits. However, patient sera 153, 156, 200 and 259 gave discordant results against four test kits each. One serum, 213, positive for the Davidsohn test with a titre of 1:40, was negative against all test kits. However, the viral capsid antigen IgM test was less than 1:10 and the viral capsid antigen IgG and Epstein-Barr nuclear antigen were positive at greater than or equal to 1:640 and greater than or equal to 1:40, respectively. These results indicate that the Davidsohn test with this

TABLE 1
Heterophile antibody results for seven test kits compared with the Davidsohn differential test

Commercial kit	Antigen	No. of samples positive (% sensitivity)*		No. of samples negative (% specificity) [†]	
Latex reagents					
Oxoid	Bovine glycoprotein [‡]	86	(96.6)	228	(99.6)
Immunoscan	Bovine glycoprotein	87	(97.8)	226	(98.7)
Monolater	Bovine glycoprotein	85	(95.5)	225	(98.3)
Red cell reagents					
Monospot	Fresh horse red blood cells	87	(96.7)	223	(97.4)
Immunoscan	Modified horse red blood cells	88	(98.9)	221	(96.5)
Ortho IM	Modified horse red blood cells	86	(97.7)	225	(98.7)
NCS	Modified horse red blood cells and latex particles	85	(95.5)	223	(97.4)

*n=89. [†]n=229. [‡]The glycoprotein is derived from bovine red blood cells. See text for complete names of test kits

TABLE 2
Epstein-Barr virus serology results for discordant test kit results

Patient number	Davidsohn test results	Commercial kit with discordant test results	Antibody titre			
			VCA IgG	VCA IgM	EA	EBNA
21	-	IL	≥1:640	≤1:10	≤1:40	1:20
26	-	IM	≥1:640	≤1:10	≤1:40	≥1:40
58	-	IR	≥1:640	≤1:10	≤1:40	≥1:40
81	+	IL, IR	1:80	≥1:10	≤1:40	≤1:40
123	-	IT	1:320	≤1:10	≤1:40	≤1:40
140	-	IM, IR	1:320	≤1:10	≤1:40	≥1:40
153	-	IM, MS, IT, IR	1:640	≤1:10	≤1:40	≥1:40
156	-	IM, MS, IT, IR	≥1:640	≤1:10	≤1:40	≥1:40
171	-	OX, MS	1:40	1:10	≤1:40	≤1:10
173	-	MS, ML	1:320	NS	NS	≥1:40
200	+	OX, IM, IT, ML	1:320	≤1:10	≤1:40	≥1:40
201	-	IR	1:60	≤1:10	≤1:40	≥1:40
211	-	MS, ML, IR	1:640	≥1:10	≤1:40	≤1:10
213	+	All kits	≥1:640	≤1:10	≤1:40	≥1:40
218	-	IT, IL, IR	1:640	≥1:10	≤1:40	≥1:10
259	-	MS, ML, IL, IR	1:640	≥1:10	≤1:40	≤1:10
275	+	IM, IT, IR	≥1:640	≤1:10	≤1:40	≤1:10
280	-	ML	1:640	≤1:10	≤1:40	1:20

EA Early antigen; EBNA Epstein-Barr nuclear antigen; IL Immunoscan-Latex; IM IM Screen Test; IR Immunoscan RBC; IT Infectious Mononucleosis Test (NCS); ML Mono-Latex; MS Monospot; NS Nonspecific; OX Oxoid Infectious Mononucleosis Kit; VCA Viral capsid antigen

specimen was a false positive. In most cases the Epstein-Barr virus serology was in agreement with the 'gold standard'.

Analysis of discrepant results showed that in all but two patients Epstein-Barr serology was consistent with no acute infection. The two other sera showed evidence of acute infection and could, therefore, represent false negative results of the Davidsohn differential test.

DISCUSSION

There are many commercial test kits available on the market for detection of infectious mononucleosis heterophile antibody. Several authors have compared many of these kits and have shown that there is no significant statistical advantage concerning sensitivity and specificity (10-12). The present results agree with published reports. In the present study, the erythrocyte-based kits, although equal in sensitivity to the latex kits, appeared to be less specific. However, the difference was not statistically significant. This result is also comparable to the study by Hoiby et al (11) which showed an increased number of false positives in erythrocyte-based tests. The Ortho monospot test, which is widely used, has been shown to give false positive reactions occasionally with leukemia, rubella, malaria and several other diseases (13,14). In the present study the monospot test demonstrated a specificity comparable to that of other erythrocyte-based tests. However, the monospot had the disadvantage of an absorption step that was not required in any other test.

In general the latex kits were technically simpler to perform and the endpoint agglutination was easier to

read. All tests were rapid, ranging from 2 mins for the NCS test to 4 mins for the Immunoscan latex test. The costs for kits are comparable and would vary from institution to institution depending on the volume of tests.

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