Evaluation of a Novel Dry Latex Preparation for Demonstration of Infectious Mononucleosis Heterophile Antibody in Comparison with Three Established Tests

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Received 6 July 1998/Returned for modification 13 August 1998/Accepted 8 October 1998

A new, dried antigen-coated latex preparation for the demonstration of infectious mononucleosis (IM) heterophile antibody (Dryspot IM kit; Oxoid, Ltd., Basingstoke, Hampshire, United Kingdom) was compared with the IM kit (a liquid latex reagent from the same source), an immunossay (ImmuNoCard Mono; Meridian Diagnostics), and an absorption test (Monospot; Meridian Diagnostics). The latter was used as a standard for initial statistical comparisons. Discrepancies were resolved by using Epstein-Barr virus serology. Of the 328 routine samples tested, 77 were positive and 222 were negative by all IM heterophile antibody-based kits. Twenty-nine samples gave discrepant results. Following resolution of discrepant results, the sensitivity and specificity values for the IM Dryspot kit were 87.0 and 98.7%, those for the Oxoid liquid latex IM kit were 83.0 and 99.6%, and those for the ImmunoCard Mono immunossay were 85.0 and 100.0%, respectively. The evaluation shows that the Dryspot kit, which is uniquely straightforward to use and may be stored at room temperature, is comparable in performance to other rapid heterophile tests for the confirmation of IM.

Infectious mononucleosis (IM) is an acute, self-limiting lymphoproliferative disease caused by the Epstein-Barr virus (EBV). It is usually spread by oral contact. The severity of the disease is variable, with symptoms that include lymphadenopathy, malaise, pharyngitis, sore throat, fever, and jaundice. Exceptionally, death may result through EBV infection-associated complications (1). The peak age distribution of IM is a band spanning 15 to 24 years (3). Other diseases, such as cytomegalovirus (CMV) and Toxoplasma gondii infection, may produce an illness clinically indistinguishable from IM, in which hematological indications (lymphocytosis with activated CD8 T lymphocytes, usually described as “atypical mononuclear cells”) may also be seen (7). Hence, the demonstration of characteristic antibodies in a blood sample is essential in the confirmation of a clinical diagnosis of IM.

It has been known since 1932 that in IM, an IM heterophile antibody (IMHA) is demonstrable that is capable of reacting with antigens expressed on the surface of the erythrocytes of a number of mammalian species (13). Absorption studies indicate that the antibody is heterogeneous; absorption with bovine erythrocytes removes all activity against sheep, horse, and goat cells, but absorption with sheep cells removes only some of the activity against bovine, horse, and goat cells (10). An immunoglobulin M (IgM) component is always present, IgA is found in 92% of the samples, IgE is found in 88% of the samples (5), and IgG is found in approximately 5% of them (6). The antibody does not react with EBV antigens, and the precise nature of its production and function (if any) remains unclear, although it is probably linked to the polyclonal stimulation of B lymphocytes following infection of these cells with EBV. A 92% agreement between IMHA-based and EBV-specific antibody tests has been demonstrated (12); in a few cases, IMHA is not produced despite convincing clinical, hematological, and other serological evidence of IM. The confirmation of IM by heterophile antibody methods is unreliable in children less than 10 years of age, because approximately 50% of such patients do not produce the antibody, although IM in this sector of the population is uncommon (4). Conversely, occasional cases showing persistence of IMHA for up to 4 years have been reported (8), in which case, in the absence of other indications, such results are probably of no clinical significance.

Methods for the diagnosis of IM based on the demonstration of IMHA continue to be popular because of the relative reliability of the antibody as a diagnostic indicator and the ease with which it can be demonstrated, and because the duration of the presence of the antibody usefully reflects the period of clinically significant disease in the sector of the population in which IM is most prevalent. Such methods will probably remain in widespread use in routine clinical laboratories until a comparably convenient and economical method for the detection of appropriate EBV-specific antibodies is produced. In a comparison between a rapid test for the demonstration of antibodies to EBNA-1 and conventional EBV-specific serology (viral capsid antigen [VCA] plus EBNA) (11), the rapid test gave a sensitivity of 71% and specificity of 95%. Monospot showed sensitivity and specificity of 77 and 92%, respectively, suggesting that IMHA-based kits are not an inferior method. One drawback for the specific anti-EBV antibodies is the duration of persistence of antibodies of different classes and specificities. IMHA, on the other hand, has a period of persistence that closely resembles that of clinical disease, and previous unpublished work by the principal author indicates that in enzyme-linked immunosorbent assay (ELISA)-based methodology, IgG components of IMHA, when present, do not significantly compete with the IgM component upon which IMHA immunossays rely. This is presumably a reflection of the relative avidities of the IgM and IgG IMHA components.

The demonstration of IMHA is straightforward. However, other heterophiles, such as Forssman and serum sickness an-
tibodies, must be differentiated from IMHA if the test is to be sufficiently specific. The original method of Paul and Bunnell (13) as modified by Davidsohn (2) is based on a differential absorption in which agglutination of intact horse erythrocytes following absorption with guinea pig kidney antigen indicates a positive IMHA result. Agglutination of the horse erythrocytes following absorption with bovine erythrocyte stroma indicates another, non-IM indicative heterophile antibody. Alternatively, some newer kits utilize extracted bovine erythrocyte antigen, purified to give specificity for IMHA. Purified antigen may be adsorbed to latex particles for use in agglutination tests or in the end point visualization in immunochromatographic assays. The purified antigen may also be used in solid-phase immunoassays producing a colored end point following the binding of labeled anti-human IgM to immobilized IMHA-heterophile antigen complex. Three of the products included in this evaluation (i.e., Dryspot IM kit, IM kit, and ImmunoCard Mono) use the purified antigen described above.

The aim of this study was to compare a new latex agglutination kit (Dryspot IM kit, Oxo, Ltd., Basingstoke, Hampshire, United Kingdom) with three other established commercial kits for the detection of IMHA. The evaluation was performed at two district general laboratories by using identical protocols.

MATERIALS AND METHODS

Specimens. A total of 328 specimens (175 serum samples and 153 EDTA plasma samples) were selected from samples submitted to the two hospital laboratories performing the evaluation for routine IM testing. A number of stored samples were also used in the study. These samples were previously confirmed as positive by the laboratory's routine IMHA method. These samples were stored at −20°C for no longer than 1 month prior to the commencement of the study. The number of positive results in this evaluation therefore does not reflect the frequency of these in the routine work of either participating laboratory. All samples were tested on the day of collection or frozen at −20°C and tested at a later date. Frozen samples underwent a maximum of one cycle of freeze-thawing before being tested. Four aliquots of all samples were frozen and stored in case further testing was required. In addition, 27 serum samples from patients known to have diseases thought to be potentially capable of giving rise to false-positive results due to cross-reactivity were tested, namely, CMV (3 samples), influenza (2 samples), hepatitis A (3 samples), hepatitis B (2 samples), hepatitis C (2 samples), human immunodeficiency virus (HIV) (2 samples), adenovirus (1 sample), rubella (4 samples), rheumatoid arthritis (6 samples), measles (1 sample), and HIV plus hepatitis C (1 sample).

(i) Dryspot IM kit (Oxo, Ltd.). The Dryspot kit is a rapid latex agglutination test using purified IMHA-specific bovine erythrocyte antigen adsorbed onto latex particles. The kit is supplied with the latex reagent presented as dried spots within discrete test zones on reaction cards. In use, the dry reagent is resuspended with patient serum or plasma at the time of testing. Control sera are presented as dried spots on strips of 10 tear-off single-use card sticks. All tests were performed in accordance with the manufacturer's instructions. Positive and negative controls were performed daily with all kits used.

(ii) Oxo IM kit (Oxo, Ltd.). The IM kit is a 2-min latex agglutination test. Reagent is supplied as a suspension of blue latex particles coated with purified IMHA-specific antigen.

(iii) Monospot (Meridian Diagnostics). The Monospot kit utilizes stabilized horse erythrocytes as indicator cells with a differential absorption stage using guinea pig kidney antigen and bovine erythrocyte stroma to ensure specificity for the IMHA.

(iv) ImmunoCard Mono (Meridian Diagnostics). ImmunoCard Mono is a solid-phase immunoassay using purified, immobilized IMHA. Following the binding of alkaline phosphatase-conjugated anti-human IgM to the heterophile antigen-antibody complex (if present), a blue color is formed by the action of the enzyme on a substrate.

(v) EBV serology. Samples giving anomalous results by the IMHA methods were sent to a third laboratory (Leeds Public Health Laboratory, Leeds, United Kingdom) for EBV serology. VCA IgG was tested by an in-house indirect fluorescence test. EBNA IgG and EBV IgM (EBNA-1:p72, EA-D:p54, and EA-p136) were tested with Biotest kits [Biotest (UK) Ltd.] utilizing recombinant antigens.

Statistics. Sensitivity, specificity, and predictive values were calculated from a 2 × 2 table analysis (9). Monospot was used as the initial reference method, because the method's traditional methodology and continuing popularity as a routine test.

Interpretation of test results. All samples were tested by the four IMHA-based methods. Results were graded for all agglutination-based kits from − (negative [no agglutination]) to ++++ (complete agglutination). Any agglutination including equivocal results (+/−) was interpreted as positive. ImmunoCard Mono results were not graded in this way because of the nature of the reaction. However, obviously pale reactions were recorded as weak by both trial centers.

Resolution of test results. In the event of one of the four IMHA test kits being discrepant, the sample was retested with the kit yielding the discrepancy. If two results were discrepant, the sample was retested with all four kits. If there was less than total agreement after retesting, samples were referred for EBV serology. Serology profiles were then used as the correct reference result. Serology results were interpreted according to the scheme shown in Table 1.

In the event of EBV serology failing to determine the presence of IM, the original Monospot result was used as the correct reference result.

RESULTS

Of 328 samples from patients suspected of having IM, 77 were positive and 222 were negative with all IMHA kits. Twenty-nine samples gave discrepant results. Similar proportions of samples giving discrepant results were found in both participating laboratories (7.9 and 10% of the samples tested). Any reaction interpreted as equivocal was designated positive for the purposes of this study. When compared with Monospot as the reference test, there were 8 false-positive and 14 false-negative Dryspot IM kit results, 1 false-positive and 13 false-negative IM kit results, and 2 false-positive and 13 false-negative ImmunoCard Mono results. There were two equivocal results (interpreted as weakly positive) with Monospot, four with the IM kit, and five with the Dryspot kit. No samples giving equivocal results by other methods gave correspondingly equivocal results by the other methods.

The EBV serology results for the 29 samples that gave discrepant results are shown in Table 2; the strength of agglutination reactions is not quantified. Hematological indications of IM are included, based on lymphocyte count and morphology. Lymphocytosis (>4.0 × 10^9 lymphocytes/liter) with the presence of atypical mononuclear cells was considered suggestive of IM, and neutropenia (<2.0 × 10^9 neutrophils/liter) or the presence of reactive lymphocytes without lymphocytosis was considered equivocal. hematological results were not available in all cases. Analysis of the EBV serology results shows that the numbers of discrepancies after resolution are 16 for the Dryspot IM kit (3 false positive, 13 false negative), 15 for ImmunoCard Mono (15 false negative), and 18 for the IM kit (1 false positive, 17 false negative). This includes three results that could not be resolved due to inconclusive EBV serology; in these cases, the original Monospot results were used.

Table 3 details the sensitivity, specificity, and positive and negative predictive values for the latex agglutination tests and ImmunoCard Mono before and after the resolution of the discrepant results by EBV serology. After resolution of the discrepant samples, both sensitivity and specificity increased for the Dryspot IM kit, sensitivity decreased and specificity remained the same for the IM kit, and sensitivity decreased but specificity increased for ImmunoCard Mono.

<table>
<thead>
<tr>
<th>Titer (interpretation)</th>
<th>IgG</th>
<th>IgM</th>
<th>Infection status</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1:10 (−)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1:10 (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1:10 (−)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥1:10 (+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Current primary +
Recent primary +
Past −
None −
Of the 27 disease-state samples tested, 26 gave negative results for IMHA by all methods. One of the CMV samples gave a positive result with both of the latex agglutination kits. This specimen had previously been tested for EBNA IgG; the result was interpreted as indicative of past EBV infection.

Table 2 also shows that seven negative and two positive Monospot results were changed to false-negative and false-positive results, respectively, by EBV serology.

**DISCUSSION**

When resolved data were used, the two Oxoid kits and the ImmunoCard Mono kit gave similar sensitivity, specificity, and predictive results.

The highest sensitivity was demonstrated by the Oxoid Dryspot IM kit (87%), followed by ImmunoCard Mono (85%) and the IM kit (83%). In the protocol for this evaluation, equivocal results were regarded as positive; if they had been regarded as negative, the sensitivity results would have been slightly lower for all kits.

The numbers of results for each kit at variance with EBV serology were 9 for the Monospot (2 false positive, 7 false negative), 14 for ImmunoCard Mono (14 false negative), 14 for the Oxoid Dryspot IM kit (2 false positive, 12 false negative), and 16 for the Oxoid IM kit (16 false negative), confirming the choice of Monospot as a reference. Since samples were referred for EBV serology on the basis of discrepancies between the four tests evaluated in this study, none of the differences are a consequence of the inclusion of IMHA nonproducers. The possibility of discrepancies between EBV serology and IMHA tests that were in complete agreement (i.e., all positive or all negative) was not addressed in this study. However, a previous study (14) found 100% agreement between IMHA tests and EBV serology when a random sample of nondiscordant results was studied. It was concluded that EBV testing of all samples in studies such as this one is not necessary, because reference test results would not be significantly changed.

The Dryspot IM kit gave equivocal results (graded +/-) for both of the samples in the “past infection” category for which all of the other test results were negative; both of the hematological results were nonindicative of IM. Also, there were two samples tested by the EBV panel that demonstrated no evidence of EBV infection. All tests gave negative results, except Monospot, which gave one positive result and one equivocal result. It has been reported (8) that samples may give persistent (up to 4 years) false-positive results when tested with Monospot despite no serological evidence of infection.

The only disease-state sample to suggest cross-reactivity was a specimen from a patient with a positive result with both of the latex agglutination kits. This result was confirmed by EBV serology.

**TABLE 3.** Sensitivity, specificity, and predictive values of three commercial IMHA tests compared with those of the Monospot hemagglutination test before and after resolution with EBV serology

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dryspot IM kit</td>
<td>85.4/87.0</td>
<td>96.6/98.7</td>
<td>91.1/96.7</td>
<td>94.1/94.5</td>
</tr>
<tr>
<td>IM kit</td>
<td>86.5/83.0</td>
<td>99.6/99.6</td>
<td>98.8/98.8</td>
<td>94.7/93.0</td>
</tr>
<tr>
<td>ImmunoCard Mono</td>
<td>86.5/85.0</td>
<td>99.1/100.0</td>
<td>97.7/100.0</td>
<td>94.7/93.8</td>
</tr>
</tbody>
</table>

* PPV, positive predictive value; NPV, negative predictive value.
CMV positive, also with EBV serology suggestive of past infection. This sample was positive by both latex kits but negative by Monospot and ImmunoCard Mono. The potential for false-positive IMHA results caused by antibodies produced during CMV infection should be investigated. With all IMHA-based kits, however, it is important that samples giving results inconsistent with clinical and hematological observations are referred for EBV serology before confirming or eliminating a diagnosis of IM.

Specificity values were generally greater than sensitivity values, reflecting the low incidence of false-positive results. ImmunoCard Mono gave the highest specificity (100%), followed by the Oxoid IM kit (99.6%) and the Dryspot IM kit (98.7%). Since the symptoms of IM may resemble malignant hematological disease, it is essential that methods for the confirmation of IM do not achieve sensitivity (the avoidance of false negatives) at the expense of specificity (the avoidance of false positives), since a delay in initiating treatment for malignant disease caused by a false-positive IM result may have serious clinical consequences. All of the methods evaluated were satisfactory in this respect.

The Oxoid Dryspot IM kit was found to be easy and convenient to use, with the added advantage that it may be stored at room temperature for up to 2 years. Kits may also be refrigerated, but as with all products tested in this evaluation, it is essential that all kits are allowed to reach room temperature before being removed from the packet, as stated in the manufacturer’s instructions.

If a number of samples are processed simultaneously by using the Dryspot kit, care needs to be taken that incubation times are accurate, since the time required to mix the dried latex and test plasma or serum is longer than that for conventional liquid reagents.

The development of methodologies for the demonstration of IMHA by using purified antigen rather than modified, intact erythrocytes has been driven by the greater ease of use and longer shelf life (up to 2 years in the case of Oxoid latex agglutination kits, compared with approximately 6 weeks for Monospot).

The results of our study show that the performance of the Oxoid Dryspot IM kit is comparable to those of other methods currently used for the confirmation of IM. It is particularly simple and convenient to use. The room temperature storage capability and 2-year shelf life are additional benefits.

ACKNOWLEDGMENTS
We are very grateful to R. Eglin of the Public Health Laboratory, Leeds, United Kingdom, for the EBV serology. This work was supported by Oxoid, Ltd.

REFERENCES