Evaluation of Two Slide Agglutination Tests and a Novel Immunochromatographic Assay for Rapid Diagnosis of Infectious Mononucleosis

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Infectious mononucleosis (IM) is an acute, self-limiting disease characterized by fever, lymphadenopathy, and the presence of atypical mononuclear cells in peripheral blood. Although Epstein-Barr virus (EBV) causes 80 to 90% of the cases of clinically apparent IM, other infectious agents can produce a similar syndrome (13, 14). In rare cases, it may cause serious complications and death (3, 11). Consequently, the accurate diagnosis and follow-up, particularly of severe cases of IM, cannot be overemphasized.

The serodiagnosis of IM is based on either the demonstration of specific antibodies to EBV antigens or the detection of heterophile antibodies (HA) in serum (1, 7, 10, 12). The serologic methods based on the detection of HA are the most rapid and cost-effective, but 10 to 20% of adults and more than 50% of children less than 12 years old lack a HA response to EBV infection (4, 15). Moreover, HA can also be found in patients with diseases other than IM, and the test can give positive results for 6 to 12 months after the acute infection. Therefore, the precision of the diagnosis of primary EBV infection based on these methods only is limited.

The purpose of this study was to assess the accuracy of BIFA-MI-Tira (BIFA-MI) (Bifa kit; Sumilab, Madrid, Spain), Monoslide Test (Bio Merieux, Lyon, France), and IM Absorption Kit (Microgen Bioproducts, Innogenetics, Camberley, United Kingdom) three tests based on HA detection, for the diagnosis of primary EBV infection, defined by EBV-specific serology. A total of 350 serum specimens were collected from 350 patients (198 males and 152 females between 1 and 80 years of age; median age, 26 years) with clinically suspected IM. All the specimens were either tested immediately, stored in 4°C, and tested within 48 h or frozen at −20°C and tested later. BIFA-MI, Monoslide Test, IM Absorption, and EBV-specific serology were performed in all the cases.

BIFA-MI is an immunochromatographic rapid test for the detection of HA. It uses a nitrocellulose strip with a capture site to show the result for the patient and a procedure control site to confirm the validity of the assay. It is based on the principle of binding the HA present in the serum with a bovine erythrocyte extract. This reaction involves a conjugate of murine anti-human immunoglobulin M (IgM) antibodies mobilized by the specimen across the strip to the region containing the immobilized bovine erythrocyte extract to form a sandwich. During the procedure, the strip is placed in 150 to 200 μl of buffered serum. The fluid moves across the strip to the erythrocyte region, and a positive result is indicated by the formation of a red line within 5 min. Monoslide Test and IM Absorption Kit are two agglutination methods that use stabilized horse erythrocytes as indicator cells, with a differential absorption stage using guinea pig kidney antigen and bovine erythrocyte to ensure the specificity of the assay. EBV-specific serology was performed by immunofluorescence assays for IgG and IgM antibodies to EBV-viral capsid antigen (EBV-VCA) (Guil Laboratories) and IgG antibody to EBNA-1 by enzyme-linked immunoassay (Wampole, Innogenetics). This assay used a purified recombinant EBNA-1 antigen (the carboxy-terminal tail of EBNA-1 genome, representing roughly 200 codons). Positive IgG and IgM VCA results with negative or very weak antibody response to EBNA-1 was considered as indicative of primary EBV infection. Negative results by all tests indicated susceptibility to EBV infection. Negative IgM VCA results with positive EBNA-1 and IgG VCA results indicated past infection. Sensitivity, specificity, and predictive values were calculated from a 2 × 2 table analysis (6).

A total of 350 samples were studied, 98 of which had serologic evidence of primary EBV infection (Table 1). The sensitivities, specificities, and positive and negative predictive values of the three methods are summarized in Table 2. In the population older than 13 years, the sensitivities of the slide agglutination kits were 59 and 80% for the Monoslide Test and IM Absorption Kit, respectively, and it was 61% for BIFA-MI. On the other hand, in the population less than 13 years old, the sensitivities were 15 and 32% for the Monoslide Test and IM Absorption Kit, respectively.

<table>
<thead>
<tr>
<th>Classification</th>
<th>Patient age (yr)</th>
<th>No. of serum samples</th>
</tr>
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<tbody>
<tr>
<td>Primary EBV infection</td>
<td>≤12</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>≥13</td>
<td>52</td>
</tr>
<tr>
<td>Past EBV infection</td>
<td>≤12</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>≥13</td>
<td>184</td>
</tr>
<tr>
<td>Susceptible to EBV</td>
<td>≤12</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>≥13</td>
<td>4</td>
</tr>
</tbody>
</table>

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Absorption Kit, respectively, and 30% for BIFA-MI. The IM Absorption Kit had the highest sensitivity but the lowest specificity. BIFA-MI did not give better results than the other agglutination kits in any age group and cost more (Table 2). The positive predictive values were more than 95% by Monoslide Test in the population less than 13 years old and by BIFA-MI in the population older than 13 years. The negative predictive values of these kits ranged from 62 to 66% for patients in the youngest age group. Therefore, the probability of primary EBV infection in spite of a negative test result was more than 30%, irrespective of the kit used. In contrast, in the population older than 13 years, that probability was approximately 10%.

In primary health care and also in many microbiological laboratories, the clinical diagnosis of IM is confirmed by a HA test. Most of the commercially available rapid kits rely on this classical method. In the present study, we compared three rapid IM kits with EBV-specific serology. We found that these kits, including a new immunochromatographic test, have low sensitivities, especially in children. As not all patients (80 to 90% of adults and less than 50% of young children) develop HA after primary EBV infection, these results were not entirely unexpected.

Linderholm et al. (8) evaluated nine kits for the rapid diagnosis of IM, including the IM Absorption Kit (but not Monoslide Test or BIFA-MI). The results obtained with the IM Absorption Kit were similar to ours in both age groups. Farhat et al. (2) evaluated a immunochromatographic assay (Cards O.S. Mono test), obtaining a sensitivity and specificity of 91 and 100%, respectively. However, there were relevant methodological differences. They compared the results of the test with those of the Paul-Bunnel-Davidsohn and latex agglutination test, obtaining a sensitivity and specificity of 91 and 100%, respectively. Therefore, the probability of primary EBV infection in spite of a negative test result was more than 30%, irrespective of the kit used. In contrast, in the population older than 13 years, that probability was approximately 10%.

With these data in mind, it is reasonable to perform EBV-specific serology in order to rule out the diagnosis of primary EBV infection in children. Also, it should be considered in adults when there is a high probability of a primary EBV infection despite a negative HA-based result. IgM VCA has been considered the most valuable single test for the diagnosis of primary EBV infection. However, IgM VCA false-positive results occur, despite corrections for the rheumatoid factor, when the patient has other herpesvirus infections (9; T. Krech and U. Krech, Abstr., Group Meet., Turku, Finland, August 1983, Newsl. 6, Eur. Group Rapid Viral Diagn., 1983). It is therefore recommended to combine IgG and IgM VCA with EBNA antibodies for the diagnosis of primary EBV infection.

Since the presence of EBNA antibodies excludes primary EBV infection, a reliable test for IgG EBNA-1 antibodies can be used as a screening test. Antibodies against other EBV antigens should be analyzed if EBNA-1 IgG antibodies are absent or present at low titer (5).

In summary, we found that the sensitivity of the three rapid HA kits was low, particularly in the population less than 13 years old, and therefore, EBV-specific serology should be performed in children and in HA-negative cases. Testing for IgG EBNA-1 antibodies was more reliable for the screening of primary EBV infection than testing for HA.

### REFERENCES