Table 3: Whole Blood (Finger Stick and Venous)

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercially available immunochromatographic heterogeneous antibody assay</td>
<td>77</td>
<td>0</td>
<td>77</td>
</tr>
<tr>
<td>Reference Assay</td>
<td>6</td>
<td>349</td>
<td>355</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>349</td>
<td>432</td>
</tr>
</tbody>
</table>

Table 4: Serum or Plasma Specimens

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercially available immunochromatographic heterogeneous antibody assay</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Reference Assay</td>
<td>0</td>
<td>130</td>
<td>130</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>130</td>
<td>144</td>
</tr>
</tbody>
</table>

References

Materials required but not provided:
- Centrifuge capable of separation of blood cells from plasma
- Lancet

Precautions
- The reagents in this kit contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Use splash-proof, flush with a large amount of water to prevent azide buildup.
- Human blood and its products are potentially infectious; handle with appropriate precautions.
- For in vitro diagnostic use
- Do not interchange reagents from different kit lots or use beyond the expiration date.
- Human blood and its products are potentially infectious; handle with appropriate precautions.
- Do not interchange reagents from different kit lots or use beyond the expiration date.

Storage and Stability
Status Mono test kit should be stored at 2°-30°C (36°-86°F) in its sealed pouch. Do not freeze. The storage conditions and stability dating given were established under these conditions.

Specimen Collection and Preparation
Whole Blood:
- a) Anticoagulated Blood
- Whole blood collected over CPDA-1, heparin or EDTA can be used. Mix whole blood with the test as outlined in the Test Procedure. Whole blood can be stored at 2°-8°C for 24 hours. If testing is anticipated in 24 hours, separate plasma, as outlined below, and freeze at or below -20°C.
- Caution: Do not freeze thaw whole blood; hemolysed blood can not be used in this test.

Principle
- Status Mono one-step antibody test for IM uses direct solid-phase immunochromatography technology for the qualitative detection of IM heterophile antibodies in serum, whole blood, or plasma. In the test procedure, 10 µl serum or plasma are added in the Sample Well (S) below the result window. For whole blood in the Sample Well (S), 25 µl washed erythrocyte extract is added. In the presence of IM heterophile antibody present in the sample, the result will be captured by the antibody bound to the anti-human IgM conjugated to the red membrane. The developer solution is then added into Sample Well (S). The specimen, followed by the developer, moves by capillary action to the antigen binding site, the solution mobilizes the dye conjugated to anti-human IgM antibodies. Visualisation of the antigen band at the Test strip will result in a positive result. If no antigen band is detected, no staining will occur at the Control position (C). The result window will only occur when the IM-specific heterophile antibody binds to the extracted antigen obtained from bovine erythrocytes. As the antibody-dye conjugate continues to move along the test membrane, it will bind to IM heterophile antibody located at the Control position (C) to generate a red band regardless of the presence of IM heterophile antibodies in the sample. This result window (S) indicates a positive result. If the antibody binding site at the Test position (T) is not detected, this indicates a negative result.

Reagents and Materials Provided
- Status Mono 25 test devices containing a membrane strip coated with bovine erythrocyte extract and a pad impregnated with the monoclonal anti-human IgM-heterophile-dye conjugate in a protein matrix containing 0.1% sodium azide.
- Developer Solution: Phosphate saline buffer containing 0.1% sodium azide as a preservative.
- Negative Control: Diluted serum containing 0.1% sodium azide as preservative.
- Positive Control: Diluted in serum containing 0.1% sodium azide as preservative.
- Storage Insert
- Procedure card
- 25 (10 µl) black line sample transfer pipettes for use with serum/plasma
- 25 (25 µl) red line) sample transfer pipettes for use with whole blood

Specimen Type
- Serum or Plasma
- Whole Blood

Specimen Type
- Serum or Plasma
- Whole Blood

LifeSign LLC

Immunoassay for the Qualitative Detection of Infectious Mononucleosis Heterophile Antibodies in Whole Blood, Serum or Plasma

For in vitro Diagnostic Use

For Whole Blood, Serum or Plasma

Rapid Heterophile Antibody Test for Infectious Mononucleosis

P-5216-J
room temperature. The serum should be separated as soon as possible and
maintained at room temperature before testing.

Status Mono test device should remain in the sealed pouch prior to testing.

Procedure

1. The test procedure must be followed in order to achieve optimal test
reactivity with specimen.

2. Follow the directions for sampling using the sample transferpipette.

3. The test device is designed for single use. Do not reuse the device.

4. Some individuals are reported to maintain a low but persistent level of
heterophile antibody.

5. The results obtained by this kit yield data which must be used only as adjunct
to other information available to the physician.

6. Allowing the first drop of blood to fall and mix the contents.

7. Any drop of blood collected will need to be released from the pipette
for each specimen.

8. DISCARD: If the reaction is negative, then the test is negative.

9. Finger stick venous whole sera samples can be obtained and tested at the
same sites, a reference laboratory, and in-house. Concurrently, serum or plasma
samples from the same patients were obtained and tested at the same sites.

Table 1: Clinical Sample Testing Arrangement

<table>
<thead>
<tr>
<th>Site</th>
<th>Finger Stick Blood</th>
<th>Venous Whole Blood</th>
<th>Serum</th>
<th>Plasma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>POL No. 1</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>POL No. 2</td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>POL No. 3</td>
<td>0</td>
<td>42</td>
<td>0</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>POL No. 4</td>
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<td>0</td>
<td>13</td>
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<td></td>
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<tr>
<td>POL No. 5</td>
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<td>POL No. 6</td>
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<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reference Lab</td>
<td>0</td>
<td>50</td>
<td>144</td>
<td>194</td>
<td></td>
</tr>
<tr>
<td>In-house</td>
<td>26</td>
<td>0</td>
<td>54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>250</td>
<td>144</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Total Specimens

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Venous Whole Blood</th>
<th>Commercially available immunochromatographic heterophile antibody assay devices</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>479</td>
<td>485</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>479</td>
<td>479</td>
</tr>
</tbody>
</table>

Table 3: Total Specimens

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Venous Whole Blood</th>
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<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>479</td>
<td>485</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>479</td>
<td>479</td>
</tr>
</tbody>
</table>

Table 4: Total Specimens

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Venous Whole Blood</th>
<th>Commercially available immunochromatographic heterophile antibody assay devices</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>479</td>
<td>485</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>479</td>
<td>479</td>
</tr>
</tbody>
</table>

Table 5: Total Specimens

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Venous Whole Blood</th>
<th>Commercially available immunochromatographic heterophile antibody assay devices</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>6</td>
<td>479</td>
<td>485</td>
</tr>
<tr>
<td>Negative</td>
<td>0</td>
<td>479</td>
<td>479</td>
</tr>
</tbody>
</table>
room temperature. The serum should be separated as soon as possible and may be tested immediately. Remove the serum as very high titers of antibody may produce an invalid test result. If serum is placed on ice or in the refrigerator, they should be stored at or below -20°C. Specimens should not be refrigerated or frozen. If specimens are to be mailed, they should be placed in appropriate shipping containers as currently described by the carrier services for handling of potentially infectious materials.

**Procedures**

**Procedural notes**
- The test protocol must be followed in order to achieve optimal test reactivity with specimen. Follow the directions for sampling using the sample transfer pipette and always perform the test under carefully controlled conditions.
- Allow Status Mono test devices, reagents and specimens to warm to room temperature before testing.
- Status Mono test device should remain in the sealed pouch prior to testing.
- Do not re-use a lancet.
- To avoid cross-contamination, use a new disposable sample transfer pipette for each specimen.
- Label the device with the patient’s name or control number.
- When collecting finger-tip blood, allow a free flow drop to form. Wipe away any smears from the tip and collect the drop. Do not squeeze the finger too hard. Follow instructions under “Specimen Collection and Preparation.”
- To add the Developer Solution, hold the dropper bottle in a vertical position above the LOWER END of the Sample Well (S) and dispense 2-3 drops in the well.
- Mildly hemolyzed whole blood specimens do not affect the test result, but may create an undesirable reddish background in the result window.
- The test is optimized to have a minimal prozone effect. Therefore, positive results are possible even in the presence of high-titered antibodies that may not be detectable in 80-85% of IM cases. Humoral responses to primary infections appear to be relatively variable and titers to high levels of heterophile antibodies are seen during the first month of illness and decrease rapidly after four weeks.

**Performing the test**

**Step 1**
Remove a test device from its pouch and place on a flat surface.

**Step 2**
Collect the sample using the appropriate sample transfer pipette according to volume of sample required.

**Step 3**
Add 2-3 drops of Developer Solution into the lower area of the Sample Well (S). Follow the directions for sampling using the sample transfer pipette.

**Step 4**
Read the results at 8 minutes. Do not read test after 15 minutes.

**Interpretation of Results**

**Positive**
One pink-purple colored horizontal band at each the Test position (T) and at the Control position (C) indicates that IM-specific heterophile antibodies have been detected.

**Negative**
A distinct colored horizontal band at the Control position (C) only indicates that the test device is working properly. This is considered a negative test result. The background in the result window will be clear, providing a distinct result.

**Limitations of the Procedure**

- The results obtained by this kit yield data which must be used only as adjunct to other information available to the physician.
- Although the first drop of serum or plasma will not affect the test result, it may create an undesirable reddish background in the result window. The test is optimized to have a minimal prozone effect. Therefore, positive results are possible even in the presence of high-titered antibodies that may not be detectable in 80-85% of IM cases. Humoral responses to primary infections appear to be relatively variable and titers to high levels of heterophile antibodies are seen during the first month of illness and decrease rapidly after four weeks.
- If a sample does not expel, hold the pipette vertically and place a finger over the vent hole. Then align the tip of the device over the upper area of the Sample Well (S) of the test device and squeeze the bulb.

**Table 1: Clinical Sample Testing Arrangement**

<table>
<thead>
<tr>
<th>Site</th>
<th>Finger Stick Blood</th>
<th>Venous Whole Blood</th>
<th>Serum</th>
<th>Plasma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>In-house</td>
<td>27</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>54</td>
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<tr>
<td>POL No. 1</td>
<td>3</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>53</td>
</tr>
<tr>
<td>POL No. 2</td>
<td>5</td>
<td>42</td>
<td>0</td>
<td>0</td>
<td>47</td>
</tr>
<tr>
<td>POL No. 3</td>
<td>13</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>26</td>
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<td>POL No. 4</td>
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<td>POL No. 5</td>
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<td>0</td>
<td>34</td>
</tr>
<tr>
<td>Reference Lab</td>
<td>0</td>
<td>50</td>
<td>144</td>
<td>194</td>
<td>344</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>284</td>
<td>144</td>
<td>524</td>
<td>1060</td>
</tr>
</tbody>
</table>

**Table 2: Total Specimens**

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercially available immunochromatographic heterophile antibody assay</td>
<td>479</td>
<td>485</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>479 576</td>
</tr>
</tbody>
</table>

**Specificities**

- Although most patients will have a detectable heterophile antibody level within three weeks of infection, occasionally a patient with strong clinical signs of IM may take longer than three months to develop a detectable level. If further testing is desired, collect additional specimens every few days.
- Some segments of the population who contract IM do not produce detectable heterophile antibodies long after their primary illness. Such children under 4 years of age who may test as IM heterophile antibody negative. EBV-specific laboratory diagnosis may be helpful in these cases.
- Some individuals are reported to maintain a low but persistent level of heterophile antibodies for years after the onset of the illness. Such false positive test results occurring in 2-3% of patients can be excluded by EBV-specific serology.
- The IM heterophile antibody test is associated with state diseases other than IM, such as leukaemia, cytomegalovirus, Burkitt's lymphoma, rheumatoid arthritis, adenosarcoma, viral hepatitis, and toxoplasmosis. In primary infections of adults with clinically atypical diseases, EBV-specific laboratory diagnosis may also be helpful.

**Expected Values**

- In patients with symptomatic IM, a positive heterophile antibody result is diagnostic, and no further testing is necessary. During the acute phase of illness, IM-specific heterophile antibodies are detectable in 80-85% of IM cases. Humoral responses to primary infections appear to be highly variable and titers to high levels of heterophile antibodies are seen during the first month of illness and decrease rapidly after four weeks.
- Positive test results may persist for months or even years due to the persistence of IM-specific heterophile antibodies. This may be helpful in establishing the diagnosis of IM. Conversely, a confirmed heterophile antibody test may indicate an occult infection. A subsequent test performed at the onset of clinical symptoms has been reported.
- Some patients remain persistently negative, even though there may exist humoral and clinical evidence of IM. Some of these patients, serological evidence for a diagnosis of cytomegalovirus infection, toxoplasmiasis, or viral hepatitis, as well as others, have been found.
Table 3: Whole Blood (Finger Stick and Venous)

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercially available immunochromatographic heterogeneous antibody assay</td>
<td>77</td>
<td>0</td>
<td>77</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>349</td>
<td>355</td>
</tr>
</tbody>
</table>

Table 4: Serum or Plasma Specimens

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercially available immunochromatographic heterogeneous antibody assay</td>
<td>14</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>130</td>
<td>130</td>
<td>260</td>
</tr>
</tbody>
</table>

References

Summary and Explanation
Infectious mononucleosis (IM) is an acute, self-limited, lymphoproliferative syndrome caused by the EBV. Infectious with EBV usually occurs only once in a human’s lifetime. 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.

The diagnosis of IM is usually based on the evaluation of characteristic hematologic and serologic changes. In most cases of IM, clinical diagnosis can be made from the characteristic triad of fever, pharyngitis, and lymphadenopathy. However, for those patients who require more definitive testing, hematologic features of IM include lymphocytosis, accelerated lymphocytosis, and lymphadenopathy. The diagnosis of IM is established by the presence of EBV antibodies in the sera of patients.

The assay detects infectious mononucleosis antibodies in human whole blood, serum or plasma specimens. This test is intended for use as an aid in the diagnosis of infectious mononucleosis.

For in vitro Diagnostic Use

CLIA Complexity:
CDC Analyte Identifier Code:
CDC Test System Identifier Code:

Intended Use

Status Mono test qualitatively detects infectious mononucleosis antibodies in whole blood, serum or plasma specimens. This test is intended for use as an aid in the diagnosis of infectious mononucleosis.

Storage and Stability

**Status Mono** test kit should be stored at 2°C to 8°C (36° F to 46° F) in its sealed pouch. Do not freeze. The storage conditions and stability dating given were based under these conditions.

Materials required but not provided:
- Centrifuge capable of separation of blood cells from plasma
- Lancet

Precautions
- The reagents in this kit contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with a large amount of water to prevent azide buildup.
- Human blood and its products are potentially infectious; handle with appropriate precautions.
- For in vitro diagnostic use
  - Do not change reagents from different kit lot or use beyond the expiration date. The reagent in each kit are tested by Quality Control at room function as a unit to assure proper sensitivity and maximum accuracy.
- Use **Status Mono** test only in accordance with instructions supplied with the kit.

**Status Mono** test kit should be stored at 2°C to 8°C (36° F to 46° F) in its sealed pouch. Do not freeze. The storage conditions and stability dating given were based under these conditions.

**Specimen Collection and Preparation**

**Whole Blood**
- a. Anticoagulated Blood: Whole blood collected over CPDA-1, heparin or EDTA can be used. Mix well for 20 minutes. Store at room temperature as outlined in the test Procedure. Whole blood can be stored at 2°C to 8°C for 24 hours. If testing is anticipated over 24 hours, separate plasma, as outlined below, and freeze at or below -20°C.
- b. Fingertip Blood: Do not freeze & thaw whole blood; hemorrhagic blood cannot be used in this test.
- c. Fingerprick Blood: For first blood specimen, prick the finger and collect blood in the sample transfer pipette up to the red line (5 µL). Follow the “Test Procedure” section.

**Serum or Plasma**

- Use serum or plasma obtained from blood collected asymptptomatically from a vein into a tube cleaned with aseptically with an anticoagulant. If serum or plasma filters are utilized, follow the manufacturer’s instructions for such filters.

- For serum, no anticoagulant should be used. For plasma, collect the whole blood in a tube containing anticoagulant such as CPDA-1, heparin or EDTA, and separate plasma, as outlined below, and freeze at or below -20°C.

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- For serum, no anticoagulant should be used. For plasma, collect the whole blood in a tube containing anticoagulant such as CPDA-1, heparin or EDTA, and separate plasma, as outlined below, and freeze at or below -20°C.

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**Status Mono** test kit should be stored at 2°C to 8°C (36° F to 46° F) in its sealed pouch. Do not freeze. The storage conditions and stability dating given were based under these conditions.

**Specimen Collection and Preparation**

**Whole Blood**
- a. Anticoagulated Blood: Whole blood collected over CPDA-1, heparin or EDTA can be used. Mix well for 20 minutes. Store at room temperature as outlined in the test Procedure. Whole blood can be stored at 2°C to 8°C for 24 hours. If testing is anticipated over 24 hours, separate plasma, as outlined below, and freeze at or below -20°C.
- b. Fingertip Blood: Do not freeze & thaw whole blood; hemorrhagic blood cannot be used in this test.
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