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, but disease incidence increases with age; disease is not known which specific antigen stimulates their production. It has been postulated that IM heterophile antibodies are raised in response to the presence of surface antigens present on erythrocytes of different mammalian species. It is of interest that rabbit, bovine, and feline erythrocytes are the sources of the antigen used in the diagnosis of IM heterophile antibodies in the sera of patients. Therefore, the presence of two colored bands, one at the Test position (T) and the other at the Control position (C), indicates a positive result. The appearance of a colored band at the Test position (T) indicates a negative result.

**References**


**Table 1: 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</td>
<td>77</td>
<td>77</td>
<td>154</td>
</tr>
<tr>
<td>Immunochromato graphic heterophile antibody assay</td>
<td>36</td>
<td>349</td>
<td>485</td>
</tr>
</tbody>
</table>

**Table 3: 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</td>
<td>130</td>
<td>130</td>
<td>140</td>
</tr>
<tr>
<td>Immunochromato graphic heterophile antibody assay</td>
<td>14</td>
<td>14</td>
<td>28</td>
</tr>
</tbody>
</table>

**Table 2: Total Specimens**

<table>
<thead>
<tr>
<th>Status Mono</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercially available</td>
<td>97</td>
<td>476</td>
<td>573</td>
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<tr>
<td>Immunochromato graphic heterophile antibody assay</td>
<td>4</td>
<td>482</td>
<td>486</td>
</tr>
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**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</td>
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<tr>
<td>Immunochromato graphic heterophile antibody assay</td>
<td>14</td>
<td>14</td>
<td>28</td>
</tr>
</tbody>
</table>

**Symbols Key**

- **For Use As (Read)**
- **Catalog Number**
- **Store At**
- **Expiration Date**
- **Test Device**
- **Normal Range**
- **Test Procedure**
- **Sample Transfer Pipette**
- **CE Mark**

**Intended Use**

**Status Mono** test qualitatively 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.

**Summary and Explanation**

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, but disease incidence increases with age. EBV may be transmitted by oral-oral contact or by respiratory droplets. The virus infects tonsil and adenoid lymphocytes, and can also infect B lymphocytes and other lymphoid tissues. The virus has been shown to be present in saliva, tears, nasal secretions, and urine. The virus is present in the blood of patients as an aid in the diagnosis of IM heterophile antibodies.

**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°- 8°C (36°- 46°F) in its sealed pack. Do not freeze. The storage conditions and stability dating given were established under these conditions.

**Storage and Stability**

**Materials May Required but Not Provided:**

- Centrifuge capable of separation of blood cells from plasma
- Lancet

**Specimen Collection and Preparation**

**Whole Blood:**

- Use whole blood.

**Whole blood collected over CPDA-1, heparin or EDTA can be used.** Mix whole blood by inversion and use in the test as outlined in the Test Procedure. Whole blood can be stored at 2° - 8°C for 24 hours. If testing is anticipated after 24 hours, separate plasma, as outlined below, and freeze at or below -20°C. Carefully thaw plasma & thaw whole blood; hemolyzed blood can not be used in this test.

**Finger Tip Blood:**

- For finger tip blood, prick the finger and collect blood in the sample transfer pipette up to the red fill line (25 µl). Follow the “Test Procedure”.

**Serum or Plasma:**

- Use serum or plasma obtained from blood collected aseptically by venipuncture into a clean tube. If serum or plasma filters isolates are used, follow manufacturers’ instructions.

**For serum, no anticoagulant should be used.** For plasma, collect the whole blood sample, then separate serum by allowing anticoagulated blood to clot at room temperature (19°C-24°C) and then centrifuged at 1500 x g for ten minutes at room temperature. The serum should be separated as soon as possible and may be tested immediately.

**Materials and Preparations Provided:**

- **Status Mono** test kit contains sodium azide as preservative.
- **Packaging:**
  - **50 µl (black) line sample transfer pipettes for use with serum/plasma.
  - **25 (µl) red line sample transfer pipettes for use with whole blood.

**Material**
Remove the serum or plasma from the clot of red cells as soon as possible to avoid hemolysis. When possible, clear, nonhemolyzed specimens should be used. Mildly hemolyzed specimens do not affect the test result, but may create an undesirable reddish background in the result window. Specimens containing any particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to testing. Collect the serum or plasma in the sample transfer pipette up to the black fill line (T & C). Follow the test procedure.

Storage of specimens - Refrigerate all specimens at 2-8°C until ready for testing. If serum or plasma specimens will not be tested within 48 hours of collection, they should be stored at -20°C. Specimens should not be repeatedly frozen and thawed. If specimens are to be mailed, they should be packaged in appropriate shipping containers as currently specified by the carrier services for handling of potentially infectious materials.

Procedure

Procedural notes

- The test protocol must be followed in order to achieve optimal test results and facilitate comparison between tests.
- Follow the assay procedure 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 lancets.
- 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 the first drop and collect the second 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 3-5 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.
- To avoid contamination, do not touch the tip of the Developer Solution dropper bottle. The dropper tip should not be reused for a different device.
- Use accepted microbiological practices for proper disposal of potentially infectious material and disinfection of contaminated equipment.
- After testing, dispose of Status Mono test devices, sample transfer pipettes and specimen in approved biohazard containers.

Directions For Use Of Sample Transfer Pipette

The sample transfer pipette has an air vent positioned on the sidewall of the pipette to provide automatic air venting and sample volume control.

STEP 1
Hold the sample transfer pipette horizontally and touch the tip of the pipette to the fill line and stop. If a sample does not expel, hold the pipette vertically and place a finger over the vent hole. Then align the pipette tip over the upper area of the Sample Well (S) of the test device and squeeze the bulb.

NOTE: If a sample does not expel, hold the pipette vertically and place a finger over the vent hole. Then align the pipette tip over the upper area of the Sample Well (S) of the test device and squeeze the bulb.

Test Procedure

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 the volume of sample required.

Use the 25l (ted mark) sample transfer pipette for whole blood or the 10µL, blunt tip sample transfer pipette for serum/plasma samples.

Follow the directions for sampling using the sample transfer pipette.

STEP 3
Add 2-3 drops of Developer Solution into the lower area of the Sample Well (S).

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 the Test position (T) and at the Control position (C) indicates that IM-specific heterophile antibodies have been detected.

NOTE: A positive test result may be read as soon as a distinct pink-purple colored band appears at the Test Position (T) and at the Control Position (C). Any shade of pink-purple colored horizontal band at the Test Position (T) should be reported as a positive result. The intensity of the colored band at the Control Position (C) may be different from the intensity of the band at the Control Position (C).

Negative

One pink-purple colored horizontal band at the Control position (C) indicates that IM-specific heterophile antibodies have not been detected.

Invalid

A distinct colored horizontal band at the Control position (C) indicates that test is invalid if no such band forms at the Control Position (C). The test is invalid if any such band forms at the Control Position (C).

Status Mono test is optimized to have a minimal prozone effect. Therefore, specimens containing a very high titer of antibody may produce a somewhat weaker signal but will still produce a positive result. The test does not require any specimen dilution, but it is recommended that the specimen be diluted and then retested if the result is in a positive range and the sample is suspected. The test should be used only for the qualitative detection of heterophile antibody.

Quality Control

There are two internal control features in Status Mono test. A colored band will always appear at the Control Position (C) if the test has been performed correctly and if the device is working properly. This is considered an internal positive procedural control. A clear background in the result window is considered an internal negative procedural control, if the test has been performed correctly and Status Mono device is working properly, the background in the result window should always be a normal faint background color, indicates the device is working properly. Good laboratory practice recommends the periodic use of external control materials to verify test performance. The Status Mono test is performed correctly and if the device is working properly. Good laboratory practice recommends the periodic use of external control materials to verify test performance. The Status Mono test is performed correctly and if the device is working properly.

Before testing, observe the Control Position (C) for a pink-purple colored band. A pink-purple colored band at the Control Position (C) should always appear. The test is invalid if no such band forms at the Control Position (C). The test is invalid if any such band forms at the Control Position (C).

Limitations of the Procedure

The results obtained by this kit yield data which must be used only as an aid to diagnosis and referral to the physician.

Although most patients will have a detectable heterophile antibody level within three weeks of infection, occasionally a patient with direct clinical signs of IM may take longer than three months to develop a detectable level.12,13 Further testing is desired, collect additional specimens every few days and retest.

Some segments of the population who contract IM do not produce detectable heterophile antibodies. Approximately 5% of children under 4 years of age who have IM may test as IM heterophile antibody negative. In this case, serology test results may be helpful in these cases.

Some individuals are reported to maintain a low but persistent level of heterophile antibodies long after their primary illness. Heterophile antibody levels seen in such patients tend to fall faster than seen in typical patients.12,14 Therefore, the test should be repeated in 4-6 weeks to confirm an antibody negative test in such cases.

The IM heterophile antibody has been associated with disease states other than IM, such as sarcoidosis, tuberculosis, Behçet’s syndrome, rheumatoid arthritis, adenovirus, viral hepatitis, and Toxoplasma gondii.17 In non-immunocompromised patients with clinically typical disease, EBV-specific laboratory diagnosis may also be helpful.

Status Mono for serum and plasma is classified as moderately complex utilizing Clinical Pathology Laboratory Processing guidelines. Status Mono test is classified as waived under the CLIA ’88 regulations.

Open or broken/damaged pouches may produce erroneous results due to kit instability from exposure to moisture and should be discarded - Do Not Use.
Finger stick. Capillary action will automatically draw up the correct volume to

**Procedure**

**Procedural notes**
- The test protocol must be followed in order to achieve optimal test
  - Follow the assay procedure 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 be in the sealed pouch prior to testing.**
- **Do not reuse 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 the first drop and collect the second 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.**
- **To avoid contamination, do not touch the tip of the Developer Solution dropper bottle.**
- Use accepted microbiological practices for proper disposal of potentially infectious materials.
- **NOTE:**
  - **When using Status Mono test device and squeezing the bulb, place the device and squeeze the bulb away from the field of view.**
  - **Do not squeeze the device to skin or away from the field of view.**

**Test Site**

- The finger stick blood sample was tested with a commercially available immunochromatographic heterophile antibody assay (Dako Cytomation). When a finger stick blood sample was tested with **Status Mono**, venous whole blood was drawn from each patient on the same day as the finger stick. The plasma or serum was then prepared from each venous whole blood sample and run on a **Status Mono** device.

**Limitations of the Procedure**

- The results obtained by this kit yield data which may be used only as an adjunct to other information available to the physician.
- 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.**
-** Although further testing is desired, collect additional specimens every few days and retest.
- **Some segments of the population who contract IM do not produce measurable levels of heterophile antibody.** Approximately 50% of children under 4 years of age who have IM may test as IM heterophile antibody negative. Therefore, clinical signs of IM may be helpful in these cases.
- **Some individuals are reported to maintain a low but persistent level of heterophile antibodies long after their primary illness.** Heterophile antibodies may be detectable in serum samples taken more than one year after the onset of the illness.**
- **The IM heterophile antibody has been associated with disease states other than IM, such as malaria, cryptosporidiosis, Burkitt’s lymphoma, rheumatic fever, adenovirus, hepatitis, and toxoplasma.**
- **IM-specific laboratory diagnosis may also be helpful.**
- **Status Mono** for serum and plasma is classified as moderately complex under CLIA ’88 regulations.
- Open or broken/pierced pouches may produce erroneous results due to kit instability from exposure to moisture and should be discarded. Do Not Use.
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 and is asymptomatic. When primary infection is delayed until adulthood, or in the presence of underlying immunosuppression, it may be complicated by splenomegaly, hepatitis, pericarditis, or central nervous system involvement. IM may be complicated by mononucleosis-associated lymphoproliferative disorders. The diagnosis of IM is usually based on the evaluation of clinical, hematologic, and serologic abnormalities. In most cases of IM, the lymphoproliferative syndrome is self-limited and the diagnosis can be confirmed by serologic testing for the associated EBV antibody response. Heterophile antibodies make up a broad group of antibodies that are directed against surface antigens present on erythrocytes of different mammalian species. It is not known which specific antigen stimulates their production. It has been established that the presence of these antibodies in the serum supports the diagnosis of infectious mononucleosis. Heterophile antibodies are usually first detected in the serum 2-3 weeks after the onset of a pharyngeal infection.

The diagnosis of IM is usually based on the evaluation of characteristic clinical, hematologic, and serologic abnormalities. In most cases of IM, the lymphoproliferative syndrome is self-limited and the diagnosis can be confirmed by serologic testing for the associated EBV antibody response. Heterophile antibodies make up a broad group of antibodies that are directed against surface antigens present on erythrocytes of different mammalian species. It is not known which specific antigen stimulates their production. It has been established that the presence of these antibodies in the serum supports the diagnosis of infectious mononucleosis. Heterophile antibodies are usually first detected in the serum 2-3 weeks after the onset of a pharyngeal infection.