### Table 1: Clinical Sample Testing Arrangement

<table>
<thead>
<tr>
<th>Site</th>
<th>Finger Stick Blood</th>
<th>Venous Whole Blood</th>
<th>Serum/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>
</tr>
<tr>
<td>POL No. 2</td>
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</tr>
<tr>
<td>POL No. 3</td>
<td>6</td>
<td>42</td>
<td>0</td>
<td>48</td>
</tr>
<tr>
<td>POL No. 4</td>
<td>20</td>
<td>13</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>POL No. 5</td>
<td>31</td>
<td>31</td>
<td>0</td>
<td>62</td>
</tr>
<tr>
<td>POL No. 7</td>
<td>51</td>
<td>0</td>
<td>0</td>
<td>51</td>
</tr>
<tr>
<td>Reference Lab</td>
<td>17</td>
<td>17</td>
<td>0</td>
<td>34</td>
</tr>
<tr>
<td>In-house</td>
<td>27</td>
<td>27</td>
<td>0</td>
<td>54</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>152</td>
<td>280</td>
<td>144</td>
<td>576</td>
</tr>
</tbody>
</table>

Venous whole blood samples were tested with **Accutest® Rapid Mono**, and the corresponding serum/plasma samples were tested with a commercially available immunochromatographic heterophile antibody assay (Predicate) kit. When a finger stick blood sample was tested with **Accutest® Rapid Mono**, venous whole blood was drawn from the same patient at the same time. The plasma or serum was then prepared from each venous whole blood sample and run on a **Accutest® Rapid Mono** device.

### Table 2: Specifics

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

### Table 3: Whole Blood (Finger Stick and Venous)

<table>
<thead>
<tr>
<th><strong>Commercially available immunochromatographic heterophile antibody assay</strong></th>
<th><strong>Positive</strong></th>
<th><strong>Negative</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>77</td>
<td>349</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>349</td>
<td>77</td>
</tr>
</tbody>
</table>

### Table 4: Serum or Plasma Specimens

<table>
<thead>
<tr>
<th><strong>Commercially available immunochromatographic heterophile antibody assay</strong></th>
<th><strong>Positive</strong></th>
<th><strong>Negative</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>14</td>
<td>130</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>130</td>
<td>14</td>
</tr>
</tbody>
</table>

### References


Manufactured for: Jant Pharmaceutical Corporation
Encino, CA 91364, USA
Tel: (800) 676-5565

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### Accutest® Rapid Mono Test

#### For Whole Blood, Serum or Plasma

**Rapid Heterophile Antibody Test for Infectious Mononucleosis**

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

#### Jant Pharmaceutical Corporation

**Claudia Complexity**

<table>
<thead>
<tr>
<th>Serum/Plasma</th>
<th>Non- Waived</th>
<th>Whole-Blood Waived</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalog No.</td>
<td>ID516</td>
<td>25 Test Kit</td>
</tr>
</tbody>
</table>

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### Accutest® Rapid Mono One-Step antibody test for IM uses direct solid-phase immunochromatography technology for the qualitative detection of IM heterophile antibodies in human serum, plasma or whole blood. In the test procedure, 10 µl serum or plasma are added in the Sample Well (S) located below the result window. For finger stick whole blood, 2µl of blood is collected in a sample transfer pipette and spotted in the Sample Well (S). If any IM-specific heterophile antibody is present in the sample, it will be captured by the antigen band (bovine erythrocyte extract) impregnated in the test membrane. The developer solution is then added in Sample Well (S). As the specimen is brought by capillary action to the antigen band, the solution mobilizes the dye conjugated to anti-human IgM antibodies. Visualization of the antigen band at the Test position (T) is in a window which will only be true 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 another band located at the Control position (C), which gives a control band regardless of the presence of IM heterophile antibodies in the sample. Therefore, the presence of true control bands, one at the Test position (T) and another at the Control position (C), indicates a positive result; while the absence of a control band at the Test position (T) indicates a negative result.

### Reagents and Materials Provided

- **Accutest® Rapid Mono** 25 test devices containing a membrane strip coated with bovine erythrocyte extract and a pad impregnated with the monochromatic anti-human IgM antibody-dye conjugate in a protein matrix containing 0.1% sodium azide.
- **Developer Solution**: Phosphate salure buffer containing 0.1% sodium azide as preservative.
- **Negative Control**: Diluted in serum containing 0.1% sodium azide as preservative.
- **Positive Control**: Diluted in serum containing 0.1% sodium azide as preservative.
- **Packaging Insert**
- **Procedure card**
- **Procedure card**
- **25 (10µ) (black line) sample transfer pipettes for use with serum/plasma**
- **25 (25µ) (black line) sample transfer pipettes for use with whole blood**

### Materials required but not provided:

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

### Precautions

- The reagents in this kit contain sodium azide. Sodium azide may react with lead and copper plating to form highly explosive metal azides. Upon disposal, flush with large amount of water to prevent azide buildup.
- All patient samples should be handled as if they are capable of transmitting disease. Observe established precautions against microbial hazards throughout all procedures and follow the standard procedures for proper disposal of potentially infectious specimens.
- Human blood and its products are potentially infectious; handle with appropriate precautions.
- For in vitro diagnostic use: Do not use after expiration date.
- Do not interchange reagents from different kit lots or use beyond the expiration date. The reagent in each kit are tested by Quality Control to function as a unit to assure proper sensitivity and maximum accuracy.
- **Use Accutest® Rapid Mono test only in accordance with instructions supplied with the kit.**

### Storage and Stability

**Accutest® Rapid Mono** test kit should be stored at 2°- 30°C (36°- 86°F) in its sealed pack. Do not freeze. The storage conditions and stability dating were established under these conditions.
Whole Blood

- To add the Developer Solution, hold the dropper bottle in a vertical position.
- Label the device with the patient’s name or control number.
- Do not reuse a lancet.

Serum or Plasma

- Use serum or plasma obtained from blood collected acutely by venipuncture into a clean tube. If serum or plasma filter isolates are used, follow the manufacturer’s instructions.
- For serum, no anticoagulant should be used. For plasma, collect the whole blood specimen into a tube containing anticoagulant such as CPDA-1, heparin, or EDTA.
- For serum, blood should be allowed to clot at room temperature (18°-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.
- Remove the serum or plasma from the clot or red cells as soon as possible to avoid hemolysis. When possible, anticoagulated 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 particular 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 using the black tubing.
- 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 frozen and thawed. If specimens are to be mailed, they should be packed in appropriate shipping containers as currently described by the carrier services for handling of potentially infectious materials.

Test Procedure

- The test must be followed in order to achieve optimal test reactivity with specimens. Follow the assay procedure and always test the reagents under carefully controlled conditions.
- Allow Accutest® Rapid Mono test devices, reagents and specimens to warm to room temperature before testing.
- Accutest® Rapid Mono test device should remain in the sealed pouch prior to testing.
- Do not release a lancing.
- To avoid cross-contamination, use a new disposable sample transfer pipette for each device.
- 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 to skin or Accutest® Rapid Mono test device.
- Use accepted microbiological practices for proper disposal of potentially infectious test materials and of any contaminated equipment.
- After testing, dispose of Accutest® Rapid Mono test devices, sample transfer pipettes and specimens in approved biohazard containers.

Specimen Collection and Preparation

- Whole blood collected over CPDA-1, heparin or EDTA can be used. Mix whole blood by inversion and use the sample as tested in the outlined 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 the plasma.
- Caution: Do not freeze & thaw whole blood; hemolyzed blood cannot be used in this test.
- Fingerprick Blood
- For fingerprick blood, prick the finger and discard the first drop. Wipe the finger and collect blood from second drop in the sample transfer pipette up to the red fill line (25 µL). Follow the “Test Procedure.”

Interpretation of Results

Positive

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

Negative

- One pink-purple colored horizontal band at the Control position (C) should always appear. This is considered an internal control band and is diagnostic of an occult infection. 18, 19 In some of these patients, serological evidence for a diagnosis of cytomegalovirus infection, toxoplasmosis, or viral hepatitis, as well as others, have been found. 20-22

Interpretation of Results

- Using the 10µL (black line) sample transfer pipette for serum/plasma samples, follow the directions for sampling using the sample transfer pipette.
- Add 2-3 drops of Developer Solution into the lower area of the Sample Well (S).
- Read the results at 8 minutes. Do not read test after 15 minutes.

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 most patients will have a detectable heterophile antibody level within three weeks of the onset of illness, a minority patient with strong clinical signs of IM may take longer than three months to develop a detectable level. 6 Further testing is needed, 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. 8 EBV-specific laboratory diagnosis 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 may be detected in blood specimens taken more than one year after the onset of the illness. 7 Such false positive test results occurring in 3-5% of patients can be excluded by EBV-specific serology.
- The IM heterophile antibody has been associated with disease states other than IM, such as lymphoma, cytomegalovirus, Burkitt’s lymphoma, rheumatoid arthritis, adenovirus, viral hepatitis, and Toxoplasma gondii. 15 In primary infections of adults with clinically atypical diseases, EBV-specific laboratory diagnosis may also be helpful.
- Accutest® Rapid Mono for serum and plasma is classified as moderately complex under the CLIA ’88 regulations. Accutest® Rapid Mono for whole blood test is classified as waived under the CLIA ’88 regulations.
- Open or broken/damaged pouches may produce erroneous results due to instability from exposure to moisture and should be discarded. Do Not Use.

Expected Values

- In patients with symptoms indicating 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 quite rapid. In IM patients with high levels of heterophile antibodies are seen during the first month of illness and decrease rapidly after week four. 3, 4
- Positive test results may persist for months or even years due to the presence of persistent IM heterophile antibodies. 5, 7 This may occur with or without any clinical evidence of heterophile antibody or evidence of IM. 6, 9, 11,12 Conversely, a confirmed heterophile antibody test may indicate an occult infection. 15, 16 In some of these patients, serological evidence for a diagnosis of cytomegalovirus infection, toxoplasmosis, or viral hepatitis, as well as others, have been found. 20-22
- Some patients remain persistently negative, even though there may exist hematological and clinical evidence of IM. 17, 18 In some of these patients, serological evidence for a diagnosis of cytomegalovirus infection, toxoplasmosis, or viral hepatitis, as well as others, have been found. 17, 18

Performance Characteristics

- Specificity
- The following potentially interfering substances do not interfere with infectious mononucleosis heterophile antibody determinations in Accutest® Rapid Mono:
- Human Albumin 15 g/dL
- Bilirubin 60 mg/dL
- Hemoglobin 1 g/dL
- Triglycerides 1,300 mg/dL

- Proficiency Testing Results
- Venous blood was taken from 20 individuals. Five samples out of twenty were spiked with monoclonal heterophile antibodies and the remaining 15 samples were not spiked. All samples were tested with Accutest® Rapid Mono Kit. Both spiked and unspiked samples were provided to a clinical POC for blind testing. The results showed 100% correlation.

- Clinical Testing Results
- A total of 432 whole blood clinical samples (152 finger-stick and 280 venous blood) were tested at 7 different Physician Office Laboratory (POL) clinical sites, a reference laboratory, and in-house. Concurrently, serum or plasma samples from the same patients were obtained and tested at the same sites. In addition, a total of 144 serum/plasma samples were tested at a reference laboratory clinical site (Table 1).

Clinical Chemistry

- Serum Triglycerides 1,300 mg/dL
- Serum Triglycerides 1,300 mg/dL
- Serum Triglycerides 1,300 mg/dL