

Procedures: Status® Mono

Prepared by	Date Adopted	Supersedes Procedure #

Review Date	Revision Date	Signature

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PRINCIPLE:

The **Status® Mono** One Step Antibody Test for IM uses direct solid-phase immunoassay 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 **Test Window (T)**. For finger-tip or whole blood, 25 µl of blood is collected in a capillary tube 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 extracts) impregnated in the test membrane. The developer solution is then added in **Sample Well (S)**. As the specimen followed by the developer moves by capillary action to the antigen band, the solution mobilizes the dye conjugated to anti-human IgM antibodies. Visualization of the antigen band in the test window will occur only when the antibody-dye conjugate binds to the IM-specific heterophile antibody which has been bound 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 in the control window to generate a colored band regardless of the presence of IM heterophile antibodies in the sample. Therefore, the presence of two colored bands, one in the **Test Window (T)** and the other in the **Control Window (C)**, indicates a positive result, while the absence of a colored band in the **Test Window (T)** indicates a negative result.

SPECIMEN:

Whole Blood:

a). Anticoagulated Blood:

Whole blood collected over CPDA-1, heparin or EDTA can be used in this test. 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 above, and freeze at or below -20°C.

Caution: Do not freeze & thaw whole blood; hemolyzed blood can not be used in this test.

b). Fingertip Blood:

For fingertip blood, prick the finger and discard the first drop. Wipe the finger and collect the second drop in the glass capillary tubes up to the mark (25 µl). Immediately transfer the blood on to the upper end of the **Sample Well (S)** of the test device as outlined in 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 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, clear, nonhemolyzed specimens should be used. Mildly hemolyzed specimens do not affect the test result, but may create an undesirable reddish background in the Test Window. Specimens containing any particulate matter may give inconsistent test results. Such specimens should be clarified by centrifugation prior to testing.

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 or below -20°C. Specimens should not be repeatedly 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.

Warning: Specimens are potentially infectious; handle with appropriate precautions.

$$**g = 1.118 \times 10^5 \text{rn}^2$$

EQUIPMENT AND MATERIALS:

Materials:

Provided in the kit:

Status[®] Mono 25 test devices: contains a membrane strip coated with bovine erythrocyte extract and a pad impregnated with the monoclonal mouse anti-human IgM antibody-dye conjugate in a protein matrix containing 0.1% sodium azide.

Status[®] Mono Developer Solution 6ml: contains phosphate saline buffer and 0.2% sodium azide as preservative.

Status[®] Mono Negative Control 0.5 ml: Diluted (human) serum; contains preservative: 0.1% sodium azide.

Status[®] Mono Positive Control 0.5 ml: Diluted (human) serum; contains preservative: 0.1% sodium azide.

Status[®] Mono package insert
Capillary tubes and bulb

Materials required but not provided:

· Centrifuge capable of 1500 x g**

$$**g = 1.118 \times 10^5 \text{m}^2$$

R = centrifuge arm-sample holder length, cm (angular radius of centrifuge head). n = revolutions per minute (RPM). Consult centrifuge manufacturer for details.

Storage Requirements:

The **Status[®] Mono** test kit should be stored at 2°–30°C (36°–86°F) in its sealed pouch. The storage conditions and stability dating given were established under these conditions.

QUALITY CONTROL:

There are two internal control features in the **Status[®] Mono** test. A colored control band will always appear in the **Control Window (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 **Test Window (T)** is considered an internal negative procedural control. If the test has been performed correctly and the **Status[®] Mono** device is working properly, the background in the **Test Window (T)** will be clear, providing a distinct result.

If the controls do not perform as expected or the colored control band does not appear in the **Control Window (C)**, contact LifeSign, LLC Technical Services immediately for assistance, 800-526-2125.

Good Laboratory practice recommends the use of external control materials to ensure proper kit performance. Two levels of control are recommended to initially test each shipment of product, every 30 days thereafter, and if the product is stored under condition

outside the manufacturer's recommendations. Run the Positive and Negative Controls provided with this kit according to the Test Procedure for Serum or Plasma.

The Positive or Negative internal Controls contained in this product satisfy the requirements of testing a Positive Control or a Negative Control on a daily basis.

PROCEDURE - STEPWISE:

A. For Serum or Plasma:

Step 1. Holding the capillary tube with the red line on the bottom, place the tip of the capillary tube into the serum or plasma sample and collect the sample up to the red 10 µl mark. Add the serum or plasma to the upper end of the **Sample Well (S)** by touching and tapping the capillary to the membrane. (Alternately, the included pipet bulb may be used to expel the specimen).

Step 2. Add 2-3 drops of Developer Solution into the lower end of the **Sample Well (S)**.

Step 3. Read test results at 8 minutes. A strong positive result may appear in less than 3 minutes. Waiting 8 minutes is required to report a negative result. Results are stable up to 15 minutes after the addition of the Developer Solution.

B. For Whole Blood:

Step 1. Holding the capillary tube with the red line on the bottom, collect blood into the capillary tube up to the 25 µl black mark from a blood collection tube or a drop of finger stick blood. Add the blood at the upper end of the **Sample Well (S)** of the device (Figure 1) by touching and tapping the capillary to the membrane. (Alternately, the included pipet bulb may be used to expel the specimen).

Follow *Step 2 and 3* as specified above.

REPORTING RESULTS:

Positive:

One pink-purple colored horizontal band each in the **Test Window (T)** and in the **Control Window (C)** indicate 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 in the **Test Window (T)** and in the **Control Window (C)**. Any shade of pink-purple colored horizontal band in the **Test Window (C)** should be reported as a positive result. The intensity of the colored band in the **Test Window (T)** may be different than the intensity of the band in the **Control Window (C)**.

Negative:

One pink-purple colored band in the **Control Window (C)**, with no distinct colored horizontal band in the **Test Window (T)** other than the normal faint background color, indicates the IM-specific heterophile antibodies have not been detected.

Invalid:

A distinct colored horizontal band in the **Control Window (C)** should always appear. The test is invalid if no such band forms in the **Control Window (C)**.

The **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 would still produce a positive result. The test does not require any specimen dilution, but it is recommended that the specimen be diluted and retested to confirm the result in case a prozone effect is suspected. The test should be used only for the qualitative detection of heterophile antibody.

LIMITATIONS OF THE PROCEDURE:

1. As is the case with any diagnostic procedure, the results obtained by this kit yield data which must be used only as adjunct to other information available to the physician.
2. Although most patients will have a detectable heterophile level within three weeks of infection, occasionally a patient with strong clinical signs of IM may take as long as three months to develop a detectable level (10). If further testing is desired, collect additional specimens every few days and retest.
3. 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 negative (11). EBV-specific laboratory diagnosis may be helpful in these cases.
4. Some individuals are reported to maintain a low but persistent level of heterophile antibodies long after their primary illness. Heterophile antibodies have been detected in blood specimens taken more than one year after the onset of the illness (12). Such false positive test results occurring in 2-3% of patients can be excluded by EBV-specific serology (3).
5. The IM heterophile antibody has been associated with disease states other than IM, such as leukemia, cytomegalovirus, Burkitt's lymphoma, rheumatoid arthritis, adenovirus, viral hepatitis, and *Toxoplasma gondii* (13). In primary infections of adults with clinically atypical diseases, EBV-specific laboratory diagnosis may also be helpful.
6. **Status® Mono** is classified as moderately complex under the CLIA '88 regulations.
7. Very high positive samples may produce weakened test signal due to prozone effect.
8. Open or broken/damaged pouches may produce erroneous results due to kit instability from exposure to moisture and should be discarded - Do Not Use.

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ALTERNATIVE METHOD:

List alternative method to be used or reference laboratory should the Status Mono kit become unavailable.

Alternative Method:

Reference Laboratory

Name:

Address:

Address:

Phone:

Contact:

CRITERIA FOR REFERRAL OF SPECIMENS:

When referring specimens for outside testing, the following procedures are recommended:

1. Verify that the testing laboratory possesses a valid CLIA certification authorizing performance of the referred test.
2. Follow laboratory guidelines for specimen shipment.
3. Report results exactly as received (no alteration or revision either of results or interpretive information provided by the testing laboratory).
4. Permit direct test report from the testing laboratory to the authorized person or entity that ordered the test.
5. Retain or be able to produce an exact duplicate of each testing laboratory's report.
6. Provide the name and address where the test was performed and indicate this information on the test report.

TECHNICAL ASSISTANCE:

Technical assistance is available from distributor of Status Mono, LifeSign, LLC, Somerset, New Jersey, between the hours of 8:30 a.m. and 4:45 p.m. E.S.T.

1-800-526-2125