lifeSign

CLSI PROCEDURE

Product Name: **Status Mono**	
Item Number: 68364 and 84M30	**Waived for Whole Blood**

Institution:	
Prepared By:	Date:
Title:	

Accepted By:	Date:
Title:	

Accepted By:		Date:	

Discontinued By	Date:

CONTENTS

SECTION 1 - TEST NAME

Status Mono

CLIA Complexity: Moderate For Serum/Plasma; Waived For Whole Blood

SECTION 2 - INTENDED USAGE

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.

SECTION 3 - SUMMARY AND EXPLANATION OF TEST

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 with no recognizable disease. 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.[1,2]

The diagnosis of IM is usually based on the evaluation of characteristic clinical, hematological, and serological changes. In most cases of IM, clinical diagnosis can be made from the characteristic triad of fever, pharyngitis, and cervical lymphadenopathy, lasting for 1 to 4 weeks. IM may be complicated by splenomegaly, hepatitis, pericarditis, or central nervous system involvement.[3] Rare fatal primary infections occur in patients with histiocytic hemophagocytic syndrome4 or with a genetic X-linked lymphoproliferative syndrome.[5] Hematologic features of IM include lymphocytosis with prominent atypical lymphocytes. Because other diseases may mimic the clinical and hematological symptoms of IM, serological testing is essential for the most accurate diagnosis. Serological diagnosis of IM is demonstrated by the presence of heterophile and EBV antibodies in the sera of patients.[2, 6, 7]

It has been well established that most individuals exposed to EBV develop a heterophile antibody response. Heterophile antibodies make up a broad class of antibodies which are characterized by the ability to react with surface antigens present on erythrocytes of different mammalian species. It is not known which specific antigen stimulates their production. It has been a common practice for physicians to use the detection of IM heterophile antibodies in the blood of patients as an aid in the diagnosis of IM. **Status Mono** whole blood assay utilizes an extract of bovine erythrocytes which gives a greater sensitivity and specificity than similar extracts prepared from sheep and horse erythrocytes. The Forssman antibody interference has been known to be minimized by using the bovine erythrocyte extract.[8,9]

SECTION 4 - PRINCIPLE OF TEST

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 result window. For finger-tip or whole blood, 25 µL 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 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 at the Test position (T) in the result window will occur only 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) to generate a colored band regardless of the presence of IM heterophile antibodies in the sample. 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, while the absence of a colored band at the Test position (T) indicates a negative result.

SECTION 5 - KIT CONTENTS AND STORAGE

MATERIALS PROVIDED:

- **Status Mono** 25 (68564), or 30 (84M30) test devices containing 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.
- Developer Solution: Phosphate saline buffer containing 0.1% sodium azide as preservative.
- Negative Control: Diluted serum containing 0.1% sodium azide as preservative.
- Positive Control: Diluted in serum containing 0.1% sodium azide as preservative.
- Package insert
- Procedure card
- 25 (68564), or 30 (84M30) 10 µL (black line) sample transfer pipettes for use with serum/plasma
- 25 (68564), or 30 (84M30) 25 µL (red line) sample transfer pipettes for use with whole blood.

STORAGE REQUIREMENTS:

The **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.

SECTION 6 - MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge capable of separation of blood cells from plasma
- Lancet
- Timer
- Latex Gloves

SECTION 7 - WARNINGS AND 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. Decontamination procedures for azide contaminated plumbing are available upon request from LifeSign Technical Services.
- 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. The reagents in each kit are tested by Quality Control to function as a unit to assure proper sensitivity and maximum accuracy.
- Use **Status Mono** whole blood test only in accordance with instructions supplied with the kit

SECTION 8 - PATIENT PREPARATIONS AND SPECIMEN COLLECTION

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 below, and freeze at or below -20°C.

Caution: Do not freeze & thaw whole blood; hemolyzed blood cannot 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 sample transfer pipette up to the red mark (25 µl). Immediately transfer the blood onto 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 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 (10µL). 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 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.

SECTION 9 - QUALITY CONTROL AND ASSURANCE

There are two internal control features in the **Status Mono** test. A colored control 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 will be clear, providing a distinct result.

Good laboratory practice recommends the periodic use of external control materials to ensure proper kit performance. The included positive and negative controls can be run in place of serum or plasma according to the test procedure for this purpose. Use the 10µL (black fill line) transfer pipette when testing external controls and serum/plasma proficiency survey samples.

If the controls do not perform as expected or the colored control band does not appear at the Control position (C), contact LifeSign LLC Technical Services immediately for assistance at 1-800-526-2125.

SECTION 10 - TEST PROCEDURE

Procedural Notes

- The test protocol must be followed in order to achieve optimal test reactivity with specimens. 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 reuse a lancet.
- To avoid cross-contamination, use a new disposable sample transfer pipette for each specimen.
- 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 **Status Mono** test device.

- Use accepted microbiological practices for proper disposal of potentially infectious test materials and disinfection of contaminated equipment.
- After testing, dispose of **Status Mono** test devices, sample transfer pipettes and specimens in approved biohazard containers.
- The sample transfer pipette has an air vent positioned on the sidewall of the pipette to provide automatic air venting and sample volume control.
- **CAUTION:** Filling is automatic: Do not squeeze the sample transfer pipette while filling. Avoid air bubbles.

Directions for use of Sample Transfer Pipette

STEP 1
Hold the sample transfer pipette horizontally and touch the tip of the pipette to the sample. The specimen can be obtained from Vacutainer, test tube or finger stick. Capillary action will automatically draw up the correct volume to the fill line and stop.

STEP 2
To expel sample, align the tip of the pipette 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 **25µL (red line) sample transfer pipette for whole blood** or the **10µL (black line) 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.

SECTION 11 - INTERPRETATION OF RESULTS

Positive:
One pink-purple colored horizontal band each 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 Test position (T) 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), with no distinct colored horizontal band at the Test position (T) other than the normal faint background color, indicates the IM-specific heterophile antibodies have not been detected.

Invalid:
A distinct colored horizontal band at the Control position (C) should always appear. The test is invalid if no such band forms at the Control position (C).

SECTION 12 LIMITATIONS

1. 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.
2. The results obtained by this kit yield data which must be used only as adjunct to other information available to the physician.
3. 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 longer than three months to develop a detectable level.[10] If further testing is desired, collect additional specimens every few days and retest.
4. 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.
5. 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]
6. 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.
7. S**tatus Mono** for serum and plasma is classified as moderately complex under the CLIA '88 regulations. S**tatus Mono** for whole blood test is classified as waived under the CLIA '88 regulations
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.

SECTION 13 EXPECTED RESULTS

1. 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. Moderate to high levels of heterophile antibodies are seen during the first month of illness and decrease rapidly after week four.[3]
2. Positive test results may persist for months or even years due to the presence of persistent IM heterophile antibodies.[14] This may occur with or without any clinical symptoms or hematological evidence of IM.[12, 15-17] Conversely, a confirmed heterophile antibody test may indicate an occult infection.[18, 19] In fact, detection of IM prior to onset of clinical symptoms has been reported.[20, 21]
3. Some patients remain persistently negative, even though there may exist hematological and clinical evidence of IM.[13, 22] In some of these patients, serological evidence for a diagnosis of cytomegalovirus infection, toxoplasmosis, or viral hepatitis, as well as others, have been found.[13, 23]

SECTION 14 PERFORMANCE CHARACTERISTICS

Specificity

The following potentially interfering substances do not interfere with infectious mononucleosis heterophile antibody determinations in **Status Mono** Assay up to the levels show below:

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 mononucleosis positive serum. Plasma was separated from these samples to test with Status Mono kit. These spiked and unspiked samples were provided to a clinical POL site for blind testing. The results showed 100% correlation.

Clinical Results Testing

A total of 432 whole blood clinical samples (152 finger-tip and 280 venous blood) were tested at seven physician office laboratory (POL) sites, a clinical reference laboratory, and in-house. Concurrently, serum or plasma samples from the same patients were obtained and tested at the same sites. The venous whole blood samples were tested with the Status Mono whole

blood test kit and the corresponding serum/plasma samples were tested with Status Mono (serum/plasma) kit. Status Mono whole blood test results were compared with the Status Mono (serum/plasma) results. (Table 1)

Table1: Clinical Sample Testing Arrangement

Site	Finger Stick Blood	Venous Whole Blood	Serum/Plasma	Total
POL #1	0	50	0	50
POL #2	0	50	0	50
POL #3	6	42	0	48
POL #4	20	13	0	33
POL #5	31	31	0	62
POL #6	51	0	0	51
POL #7	17	17	0	34
Reference Lab	0	50	144	194
In-House	27	27	0	54
Total	152	280	144	576

Venous whole blood samples were tested with **Status Mono** and the corresponding ser/plasma samples were tested with a commercially available immunochromatographic heterophile antibody assay (Predicate) kit. When a finger stick blood sample was tested with **Status 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 **Status Mono** device. **Status Mono** results were compared with the commercially available immunochromatographic heterophile antibody assay (Predicate) test results (Table 3). In the case of serum/plasma samples, each sample was run on both heterophile antibody assay devices, and the results were compared (Table 4). Table 2 combines both results shown in Tables 3 and 4.

Table 2 shows that the agreement between the two tests was 99.0% (570/576). **Status Mono** demonstrated a relative specificity of >98.98%. (479/485) and a relative sensitivity of >99.9% (91/91). The results obtained with Status Mono whole blood test correlated well with the results obtained with the commercially available immunochromatographic heterophile antibody assay test.

Table 2: Total Specimens

		Status Mono		
Commercially available immunochromatographic heterophile antibody assay		Positive	Negative	Total
	Positive	91	0	91
	Negative	6	479	485
	Total	97	479	576

Table 3: Whole Blood (Finger Stick and Venous)

		Status Mono		
Commercially available immunochromatographic heterophile antibody assay		Positive	Negative	Total
	Positive	77	0	77
	Negative	6	349	355
	Total	83	349	432

Table 4: Serum or Plasma Specimens

		Status Mono		
Commercially available		Positive	Negative	Total

immunochromatographic heterophile antibody assay	Positive	14	0	14
	Negative	0	130	130
	Total	14	130	144

SECTION 15 REFERENCECES

1. Davidson I. Serologic Testing of Infectious Mononucleosis. J. Am. Med. Assoc. 183:289, 1937
2. Evans, A.S. History of Infectious Mononucleosis. Am J Med Sci 267:189, 1974
3. Lennette, E.T. Epstein-Barr Virus. Manual of Clinical Microbiology, 5th ed., Balows, A., et al (ed.) American Society for Microbiology, Washington DC, pp. 847-852, 1991.
4. Grierson, H. and Purtillo, D.T. Epstein-Barr Virus infections in Males with X-linked Lymphoproliferative Syndrome. Ann Intern Med 106:538, 1987.
5. Wilson, E.R., et al. Fetal Epstein-Barr Associated Hemophagocystic Syndrome J Pediatr 98:260, 1981.
6. Paul J.R. and Bunnel, W.W. The Presence of Heterophile Antibodies in Infectious Mononucleosis. Am J. Med Sci 183:91, 1932.
7. Lennette, E. and Henle, W. Epstein-Barr Virus Infections: Clinical and Serological features. Lab Manager 25:23, 1987.
8. Baily, G.H. and Raffel, S. Hemolytic Antibodies for Sheep and Ox Erythrocytes in Infectious Mononucleosis. J. Clin Invest 14:228, 1935.
9. Fletcher, M.A. and Woodfolk, B.J. Immunological Studies of Infectious Mononucleosis: Isolation and Characterization of Heterophile Antigens from Hemoglobin-free Stroma. J. Immunol 107:842, 1971.
10. Penman, H.G. Seronegative Glandular Fever. J. Clin Path 21;50, 1968.
11. Fleisher, G.R. Textbook of Human Virology, Belshe, R.B. (ed) Littleton, Mass., PSG Publishing Co., pp 853-886, 1984.
12. Evans, A.S., et al. A prospective Evaluation of Heterophile and Epstein-Barr Virus-Specific IgM Antibody tests in Clinical and Subclinical Infectious Mononucleosis: Specificity and Sensitivity of Tests and Persistence of Antibody. J Infect Dis 132:546, 1975.
13. Chin, T.D.Y. Diagnostic Criteria and Differential Diagnosis: Infectious Mononucleosis, 2nd ed. Schlossberg, D. (ed) Springer-Verlag, New York, 1990.
14. Henle, W.G., et al. Infectious Mononucleosis and Epstein-Barr Virus Associated Malignancies: Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections, 5th ed. Lennette, E. H. and Schmidt, N.J. (ed) American Public Health Association, Inc., Washington D.C. 1979.
15. Henle, G., et al. Relation of Burkitt's Tumor Associated Herpes-type Virus to Infectious Mononucleosis. Proc Natl Acad Sci U.S.A. 59:94, 1968.
16. Askinazi, C., et al. Positive Differential Heterophile Antibody Test. Persistence in a Symptomatic Patient. J Am Med. assoc 236:1492, 1976.
17. Horwitz, C.A., et al. The Specificity of Heterophile Antibodies in Patients and Healthy Donors with No or Minimal Signs of Infectious Mononucleosis. Blood 47:91, 1976.
18. Hallee, T.J., et al. Infectious Mononucleosis at the United States Military Academy: A Prospective Study of a Single Class Over Four Years. Yale J Biol Med 3:182, 1974.
19. Infectious Mononucleosis and Its Relationship to EB Virus Antibody. A Joint Investigation by University Health Physicians and P.H.L.S. Laboratories. Br. Med J 11:643, 1971.

20. Bauer, S. and Holf, G. Test Detects Mononucleosis in Incubation Period. Annual Meeting of ASCP and CAP, Chicago, Illinois, October 15-23, 1965.
21. Baehner, R.L and Schuler, S.E. Infectious Mononucleosis in Childhood. Clinical Expressions, Serologic Findings, Complications, Prognosis. Clin Pediatr 6:393, 1967.
22. Henle, G. and Henle, W. Epstein-Barr Virus and Infectious Mononucleosis. N Engl J Med 288:263, 1964.
23. Cameron, D. and McBean, L.M. A Clinical Study of Infectious Mononucleosis and Toxoplasmosis. Baltimore, The Williams and Wilkins Company, pp. 24-27, 1973.

SECTION 16 TECHNICAL ASSISTANCE

For technical assistance, contact LifeSign Technical Service Department at 1-800-526-2125

Helpful CLIA brochure links to explain Clinical Laboratory Improvement Amendments (CLIA) regulation requirements

Individualized Quality Control Plan- IQCP
http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIAbrochure11.pdf

Proficiency Testing

https://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/downloads/CLIAbrochure8.pdf

Proficiency Testing Providers

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/ptlist.pdf

Personnel Competency Assessment

http://www.cms.gov/Regulations-and-Guidance/Legislation/CLIA/Downloads/CLIA_CompBrochure_508.pdf

Corrective Action Form

Problem /Error	Corrective Action

Laboratory Technologist: _____ Date: _____

Laboratory Director: _____ Date: _____

Test Validation Form

Account Name: _____

Address: _____

_____

Telephone: _____

Test Name: _____ Lot # : _____

Start Date: _____

Supervisor Signature: _____

Sample Number	Expected Results	Test Result	Tester's Initials	Comments

Reviewed by: _____

Certification of Training

This is to verify that personnel responsible for running _____ test at _____ have been thoroughly in-serviced on the test and the test procedure(s).

This has included:

Review of the package insert
Demonstration of the product assay
Successful performance of the test and interpretation of results

Names of the personnel who have been trained with the above test and are responsible for reporting patient results:

Print Name	Signature	Date

Signature(s) of those responsible for personnel and testing:

_____ _____
Signature Date

_____ _____
Signature Date

_____ _____
Signature Date

lifeSign

Quality Control

Name of Facility _____

Use this cover sheet with each new shipment and/or with each new kit lot

Product _____ Lot# _____ Exp Date _____

Date Received _____ Rec'd By _____

	Date	Positive Control	Negative Control	Initials
Initial QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				
Additional QC				

Reviewed by _____ Date _____

Testing Personnel Competency Assessment

Test _____

Procedure	Satisfactory	Unsatisfactory	Not Applicable	Comments/Corrective Action(s)
Observation of Test performance				
Patient Sample Preparation				
Specimen Handling/Processing				
Testing				
Recording/Reporting Results				
Assessment of Test Performance Using Known Samples				
Review of Records				
Patient/Quality Control Log Sheet Records				
Proficiency Testing Records				
Assessment of Problem Solving Skills				

(Attach all supporting documents)

Evaluator: _____ Date: _____

Testing Personnel: _____ Date: _____