P-5218-F

Status Mono

For Whole Blood

Rapid Heterophile Antibody Test for Infectious Mononucleosis

For *in vitro* Diagnostic Use

Immunoassay for the Qualitative Detection of Infectious Mononucleosis Heterophile Antibodies in Whole Blood

LifeSign LLC

CLIA Complexity: Waived
CDC Analyte Identifier Code: 2809
CDC Test System Identifier Code: 37143

Stock No.	84W10	10 Test Kit
	84W30	30 Test Kit

Intended Use

Status Mono whole blood test qualitatively detects infectious mononucleosis antibodies in human whole blood 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 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 syndrome[4] 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]

Principle

Status Mono whole blood one step antibody test for IM uses a solid-phase immunochromatographic assay technology for the qualitative detection of IM heterophile antibodies in whole blood. For finger-tip or whole blood, 25 µl of blood are collected in a sample transfer pipette and added to the Sample Well (S). 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. 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. Visualization of the antigen band at the Test position (T) in the result 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 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.

Reagents and Materials Provided

- ***Status Mono*** whole blood 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.
- Package insert
- Procedure card
- 10 (25 µL) sample transfer pipettes (84W10)
- 30 (25 µL) sample transfer pipettes (84W30)

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 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.

Storage and Stability

Status Mono whole blood test kit should be stored at 2°- 30°C (35°- 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

a). Anticoagulated Venous Blood:

Venous 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.

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 pipettes up to the red fill line (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”.

Storage of specimens - Whole blood can be stored between 2°- 8°C for 24 hours. 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.

Procedure

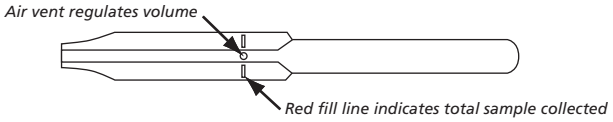
Procedural notes

- 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.

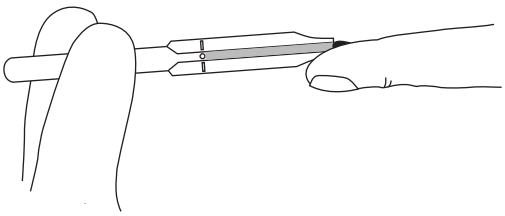
- Allow ***Status Mono*** whole blood test devices, reagents and specimens to warm to room temperature before testing.
- ***Status Mono*** whole blood test device should remain in the sealed pouch prior to testing.
- To avoid cross-contamination, use a new disposable sample transfer pipette for each specimen.
- Label the device with the patient's name or control number.
- To avoid contamination, do not touch the tip of the Developer Solution dropper bottle to skin or ***Status Mono*** whole blood test device.
- Use accepted microbiological practices for proper disinfection of potentially infectious test materials and contaminated equipment disposal.
- After testing, dispose of ***Status Mono*** whole blood test devices, micropipet and specimens 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.

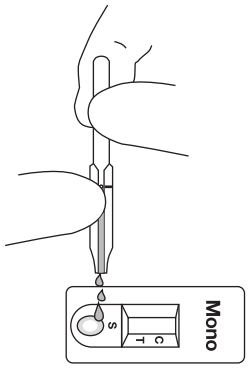


CAUTION: Filling is automatic: Do not squeeze the sample transfer pipette while filling. Avoid air bubbles.



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 fingerstick. Capillary action will automatically draw up the correct volume to the red 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

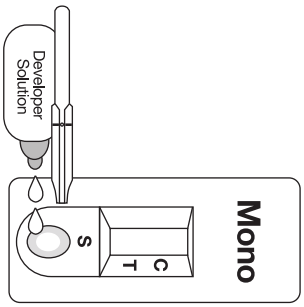
Remove a test device from its pouch and place on a flat surface.

STEP 2

Collect the sample using the **25µL sample transfer pipette for whole blood**. 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).



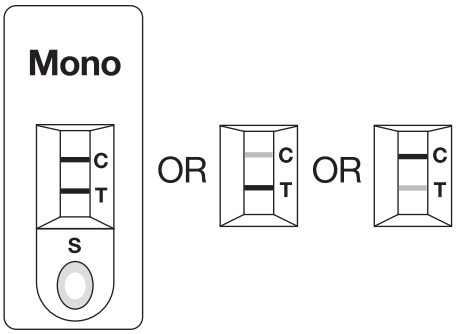
STEP 4

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

Interpretation of Results

Positive

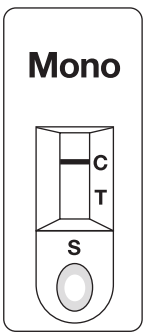
One pink-purple colored horizontal band each at the Test position (T) and at the Control position (C) indicates that IM-specific heterophile antibodies have been detected.



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).

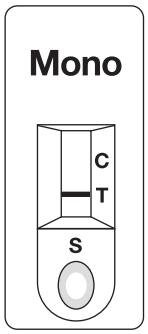
Negative

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).



Quality Control

There are two internal control features in ***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 control materials to ensure proper kit performance. Positive and Negative controls are available from LifeSign. Positive and negative controls should be run in place of blood according to the Test Procedure.

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.

Limitations of the Procedure

- ***Status Mono*** whole blood 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.
- 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 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.
- 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.[11] 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 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]
- 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.

Expected Values

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]

Performance Characteristics

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)

The agreement between the two tests was 98.6% (426/432). ***Status Mono*** whole blood test demonstrated a relative sensitivity of >99.9%. (77/77) and a relative specificity of 98.3% (349/355). The results obtained with ***Status Mono*** whole blood test correlated well with the results obtained with ***Status Mono*** (serum/plasma) test.

Table 1: Total Samples (Finger-Tip and Venous)

	Status Mono **whole blood**		
Status Mono (serum/plasma)	Positive	Negative	Total
Positive	77	0	77
Negative	6	349	355
Total	83	349	432

Also Available From LifeSign

Description	Kit Size	Item #
Status Mono Moderately Complex	30	84M30
Status Mono Waived & Moderate	25	68364
Status hCG Urine/Serum	35	35035
Status hCG Urine Only	35	35135
Status Strep A Strip Waived	30	34130
Status AccuStrep A - Moderate	25	34125
Status H. pylori WB Waived - Serum/Plasma Moderate	30	37030

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Symbols Key

Instructions For Use (Read)	Do Not Reuse
REF Catalog Number	IVD For *In Vitro* Diagnostic Use
2°C 30°C Store At	LOT Lot Number
Expiration Date	Manufacturer
CONT Contents	MF Manufactured For
SOL Developer Solution	EC REP Authorized Representative
DEV Test Device	CONT + Positive Control
IFU Instructions For Use	CONT - Negative Control
PRO Procedure Card	PIP Sample Transfer Pipette
	CE CE Mark

Printed in U.S.A.
P-5218-F
397-6/15/12

CE

EC REP

MT Promedt Consulting GmbH
Altenhofstrasse 80
66386 St. Ingbert
Germany
+49-68 94-58 10 20

MF Manufactured for:

lifeSign

A PBM Group Company
85 Orchard Road
Skillman, NJ 08558
800-526-2125, 732-246-3366
www.lifesignmed.com

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