Table 2: Total Specimens

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

Table 3: Whole Blood (Finger Stick and Venous)

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

Table 4: Serum or Plasma Specimens

		Status Mono		
		Positive	Negative	Total
Commercially available immunochromatographic heterophile antibody assay	Positive	14	0	14
	Negative	0	130	130
	Total	14	130	144

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Symbols Key

	Instructions For Use (Read)		Do Not Reuse
REF	Catalog Number	IVD	For *In Vitro* Diagnostic Use
2°-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-5219-G
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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

Manufacturer
PBM
Princeton BioMeditech Corporation
4242 U.S. Hwy 1, Monmouth Jct.
New Jersey 08852, U.S.A.
1-732-274-1000 www.pbmc.com

P-5219-G

Status Mono

For Whole Blood, Serum or Plasma

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, Serum or Plasma

LifeSign LLC

	Serum/Plasma	Whole-Blood
CLIA Complexity:	Moderate	Waived
CDC Analyte Identifier Code:	2809	2809
CDC Test System Identifier Code:	37122	37143

Stock No. 84M30 30 Test Kit

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 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*** 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 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 are 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.

Reagents and Materials Provided

- ***Status Mono*** 30 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
- 30 (10 µL) (black line) sample transfer pipettes for use with serum/plasma
- 30 (25 µL) (red line) sample transfer pipettes for use with whole blood

Materials Maybe Required but Not Provided:

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

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 reagent in each kit are tested by Quality Control to function as a unit to assure proper sensitivity and maximum accuracy.
- Use ***Status Mono*** test only in accordance with instructions supplied with the kit.

Storage and Stability

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.

Specimen Collection and Preparation

Whole Blood:

a). Anticoagulated 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.

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 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 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 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 (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.

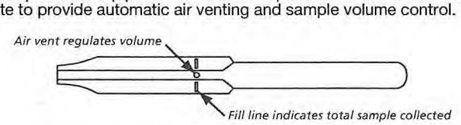
Procedure

Procedural notes

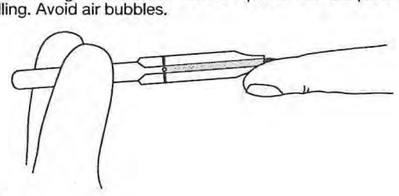
- The test protocol must be followed in order to achieve optimal test reactivity with specimens. Follow the assay procedure and always perform the test under carefully controlled conditions.
- Allow ***Status Mono*** test devices, reagents and specimens to warm to room temperature before testing.
- ***Status 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.
- 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 ***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.

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.



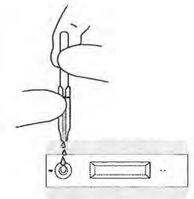
STEP 1

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 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.

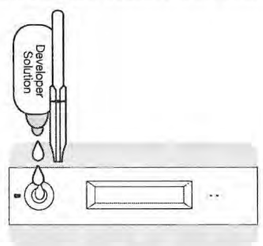
STEP 2

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.

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).

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.

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 external control materials to ensure proper kit performance. Using the positive or negative external controls in place of a patient sample, dispense **1 drop** of control solution into the upper end of the sample well(s) of the device. Then proceed to Step 3 in the Test Procedure section.

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

- 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.
- ***Status Mono*** for serum and plasma is classified as moderately complex under the CLIA '88 regulations. ***Status 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 kit instability from exposure to moisture and should be discarded - Do Not Use.

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.[16, 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

Specificity

The following potentially interfering substances do not interfere with infectious mononucleosis heterophile antibody determinations in ***Status Mono*** Assay up to the levels shown 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 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).

Table 1: Clinical Sample Testing Arrangement

Site	Finger Stick Blood	Venous Whole Blood	Serum /Plasma	Total
POL No. 1	0	50	0	50
POL No. 2	0	50	0	50
POL No. 3	6	42	0	48
POL No. 4	20	13	0	33
POL No. 5	31	31	0	62
POL No. 6	51	0	0	51
POL No. 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 serum/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 ***Status Mono*** and the commercially available immunochromatographic 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 two tests was 99.0% (570/576). ***Status Mono*** demonstrated a relative specificity of 98.8% (479/485) and a relative sensitivity of >99.9% (91/91). The results obtained with the ***Status Mono*** test correlated well to the results obtained with the commercially available immunochromatographic heterophile antibody assay test.