P-52150-A

BioSign® 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 with DXpress™ Reader

PBM

Catalog No.	BSP-410-35	35 Test Kit
	BSP-410-10	10 Test Kit

Intended Use

BioSign® Mono test qualitatively detects infectious mononucleosis antibodies in human whole blood, serum or plasma specimens. This test is intended for use with the DXpress™ Reader 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. **BioSign® 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

BioSign® 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 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

- **BioSign® Mono** 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.
- Sample transfer pipette: 10 µl (black line) for use with serum/plasma; 25 µl (red line) for use with whole blood.
- Package insert

Materials required but not provided:

- Centrifuge capable of separation of blood cells from plasma
- Micropipette (precision pipette)
- Lancet
- DXpress™ Reader

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 **BioSign® Mono** test only in accordance with instructions supplied with the kit.

Storage and Stability

BioSign® 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 discard the first drop. Wipe the finger and use a sample transfer pipette to collect 25 µl of blood from the second drop. 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 result 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.

Procedure

Test Procedure Summary

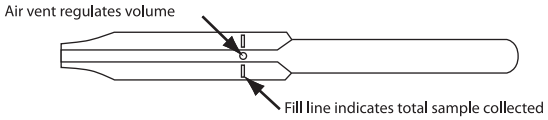
The procedure consists of adding the specimen and Developer Solution to the sample well in the device, inserting the device into the DXpress™ Reader and following the instructions to get the result.

Procedural Notes

- Allow the dropper to fill with sample without air bubbles.
- Handle all specimens as if capable of transmitting disease.
- After testing, dispose of the **BioSign**® device, and the specimen dispenser following good laboratory practices. Consider each material that comes in contact with specimen to be potentially infectious.

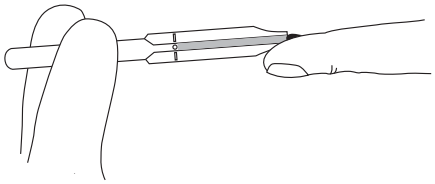
Directions for Use of Sample Transfer Pipette

The sample transfer pipette has an air vent positioned on the sidewall of the pipette to provide automatic air venting and sample volume control.



NOTE: Once the specimen is drawn into the sample transfer pipette, the pipette will not leak; the pipette will hold the specimen until the bulb of the pipette is squeezed.

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



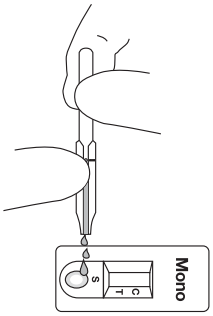
STEP 1

Hold the sample transfer pipette horizontally and touch the tip of the pipette to the sample. DO NOT SQUEEZE the pipette. 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 SLOWLY squeeze the bulb until a hanging drop forms and touch this drop to the sample pad.

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 SLOWLY squeeze the bulb until a hanging drop forms and touch this drop to the sample pad.



Using Dxpress™ Reader

For complete instructions, including installation and start up, refer to the DXpress™ Reader User Manual. Operators must consult the DXpress™ Reader User Manual prior to use and become familiar with the processes and quality control procedures.

Performing Self Check

Each time the DXpress™ Reader is turned on, Self Check is automatically performed and the operator may then proceed to Calibration QC. If the DXpress™ Reader is left on or in power save mode, the operator should perform Self Check daily, as follows:

From the Main Menu, select:	[2] RUN QC
Then select	[1] SELF CHECK

Self Check takes about 15 seconds. PASS or FAIL results will be displayed/printed when testing is completed. All Self Check items should pass before testing patient samples.

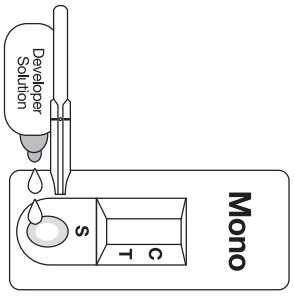
Note: Perform Calibration QC in accordance with your laboratory procedures. Consult the DXpress™ Reader User Manual for more information.

Testing Patient Samples

Patient samples may be tested using the DXpress™ Reader Scheduler mode, as described below. To use other modes (batch mode or read-now mode) consult the DXpress™ Reader User Manual.

1. Open the pouch and remove the test device.
- Write the patient ID on the test device.
- Place the test device on a level surface.

2. Enter test information in the DXpress™ Reader:
- From the Main Menu, select [1] RUN PATIENT.
- Scan the lot number barcode.
- Confirm test device information and lot number as displayed on the screen and press ENTER.
- Scan or enter the Operator ID.
- Scan or enter the Patient ID.
- From the Incubation Time window, select SCHEDULER.
- SCHEDULER asks the user to add sample.



3. Collect 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.
4. Add 2 to 3 drops of Developer Solution into the LOWER AREA of the Sample Well (S).
5. Place the device on the tray and press ENTER.
6. After 8 minutes of incubation the DXpress™ Reader will automatically display/print the results.
- At this point the test device may be removed and appropriately discarded.

Interpretation of Results

Positive

The instrument will automatically determine the result as positive.

Negative

The instrument will automatically determine the result as negative.

Invalid

The instrument will automatically determine if a procedural error has occurred by confirming that the control line is not present (invalid test).

If the result is invalid, the sample should be retested with a new device. If the problem persists, contact your local distributor of PBM.

Quality Control

Internal Control: Each **BioSign® Mono Test** device has a built-in control. The Control line is an internal positive procedural control. A distinct reddish-purple Control line should appear at the C position, indicating an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the reagents at the Control line and the conjugate-color indicator are reactive. In addition, the clearing background in the Result window, by providing a distinct readable result, may be considered an internal negative procedural control. If background color appears in the Result window, which interferes with the result interpretation of the reader, then the result is invalid. If the problem persists, contact PBM for technical assistance.

External Control: External controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested before using a new lot or a new shipment of kits as good laboratory testing practice and that users follow federal, state, and local guidelines for quality control requirements. For information on how to obtain controls, contact PBM Technical Services.

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.
- **BioSign® Mono** for serum and plasma is classified as moderately complex under the CLIA '88 regulations. **BioSign® 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 (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

Specificity

The following potentially interfering substances do not interfere with infectious mononucleosis heterophile antibody determinations in **BioSign® 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 **BioSign® 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 **BioSign® 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 **BioSign® 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 **BioSign® Mono** device. **BioSign® 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 **BioSign® 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). **BioSign® Mono** demonstrated a relative specificity of 98.8% (479/485) and a relative sensitivity of >99.9% (91/91). The results obtained with the **BioSign® Mono** test correlated well to the results obtained with the commercially available immunochromatographic heterophile antibody assay test.

Table 2: Total Specimens

		BioSign® 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)

		BioSign® 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

		BioSign® 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

	Manufactured by
CE	CE Mark
EC REP	Authorized Representative
IVD	*In Vitro* Diagnostic Medical Device
REF	Catalog Number
	Consult Instructions for Use
LOT	Batch Code
EXP YYYY-MM-DD	"Use By" date in year-month-day format
2°C (35°F) Min / 30°C (86°F) Max	Temperature Limitation
Σ n	Contains sufficient for <n> tests
	Do not reuse
CONT	Contents
DEV	Test Device
SOL	Developer Solution
PIP	Sample Transfer Pipette
IFU	Instructions for Use
TEST MON	Infectious Mononucleosis Test

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