MICROGEN BIOPRODUCTS

REF

M120CE PathFlow™ MONONUCLEOSIS RAPID TEST CASSETTE

25

IVD

INTENDED USE

PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of Infectious Mononucleosis heterophile antibodies in whole blood, serum or plasma as an aid in the diagnosis of Infectious Mononucleosis.

SUMMARY

Infectious Mononucleosis (IM) is caused by the Epstein-Barr virus, which is a member of the herpesvirus family. Symptoms of IM are fever, sore throat and swollen lymph glands. In very rare cases, heart or central nervous system problems may occur. Diagnosis of IM is made based on the presence of heterophile antibodies. Infectious Mononucleosis heterophile antibodies belong to the IgM class. They are present in 80-90% of acute IM cases and can be detected in 60-70% of patients during the first week of clinical illness.[1, 2, 3, 4]

PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) is a simple test that utilises an extract of bovine erythrocytes to qualitatively and selectively detect Infectious Mononucleosis heterophile antibodies in whole blood, serum or plasma in five minutes.

PRINCIPLE

PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) is a qualitative, lateral flow immunoassay for the detection of IM heterophile antibodies in whole blood, serum or plasma. In this test, bovine erythrocyte extracted antigen is immobilized in the test line region of the strip. During testing, the specimen reacts with bovine erythrocyte extracted antigen coated particles that have been applied to the label pad. This mixture migrates chromatographically along the length of the test and interacts with the immobilized bovine erythrocyte extracted antigen. If the specimen contains IM heterophile antibodies, a coloured line will appear in the test line region, indicating a positive result. If the specimen does not contain IM heterophile antibodies, a coloured line will not appear in this region indicating a negative result. To serve as a procedural control, a coloured line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

KIT PRESENTATION

CASSETTE — M120a Mononucleosis Rapid Test - Cassette 25

Individually wrapped test devices. Each cassette contains one test strip with heterophile antigen coated membrane and coloured antibody pad.

BUFFER — M120b Mononucleosis Rapid Test Buffer 8ml

0.1M Phosphate buffered saline with additives and preserved with ProClin 300.

Control + — M120c Positive Control

Positive control (Diluted human plasma containing IM heterophile antibodies, ProClin 300).

Control - — M120d Negative Control

Negative control (Diluted human plasma, ProClin 300).

Disposable droppers

Instructions for use

Additional requirements

Timer
Specimen collection containers (for venepuncture whole blood)
Lancet (for finger-stick whole blood only)
Centrifuge
Heparinised capillary tubes and dispensing bulb (for finger-stick whole blood only)

WARNINGS AND PRECAUTIONS

1. The reagents supplied in this kit are for in vitro diagnostic use only.
2. All patients' samples should be treated as if capable of transmitting disease.
3. Haemolysed samples should not be used.

STORAGE AND STABILITY

Store as packaged in the seal pouch either at room temperature or refrigerated (2-30˚C).The test is stable through the expiration date printed on the sealed pouch. The test must remain in the sealed pouch until use. DO NOT FREEZE. Do not use beyond the expiration date.

PROCEDURE

1. The kit should be used in accordance with these Instructions for Use.
2. Do not use the test kit beyond the expiration date.
3. Ensure that all reagents and cassettes have reached room temperature (15 – 30°C) before use.
4. Do not mix reagents from different lots.
5. The use of the positive control is recommended to ensure proper procedure (see Quality Control).
6. Do not use if protective foil pouch is damaged.
7. Human plasma used in the Positive and Negative Controls was tested by ELISA for the presence of antibodies to Human Immunodeficiency Virus (HIV-1/HIV-2), Hepatitis B surface antigen (HBsAg) and Hepatitis C (HCV), and found to be negative. Nevertheless, caution should be used in handling and disposing of these items

SPECIMEN COLLECTION & PREPARATION

PathFlow™ Mononucleosis Rapid Test Cassette can be performed using whole blood, non-haemolysed serum or plasma. Serum or plasma should be separated from blood as soon as possible to avoid haemolysis. Testing should be performed immediately after specimen collection. Do not leave specimens at room temperature for extended periods. Serum and plasma samples may be stored at 2-8˚C for up to 3 days, for long term storage specimens should be kept at -20 ˚C .

Whole blood collected by venepuncture can be stored at 2-8 ˚C and used within 2 days. Whole blood collected by fingerstick should be used immediately. Do not freeze whole blood specimens.

Bring specimens to room temperature before testing. Frozen specimens should be thawed completely and mixed well prior to testing. Specimens must not be thawed and refrozen.

DIRECTIONS FOR USE

Allow the test, specimen, buffer, and/or controls to equilibrate to room temperature (15-30°C) prior to testing.

Remove the Test Cassette from the foil pouch and use it as soon as possible. Best results will be obtained if the assay is performed within one hour.

Place the Test Cassette on a clean and level surface.

For **<u>Serum or Plasma</u>** specimens:

Hold the dropper vertically and transfer **1 drop** of serum or plasma (approximately 25 μL) to the specimen well (S) of the Test Cassette, and add **1 drop** of buffer (approximately 55 μL), then start the timer. See illustration below.

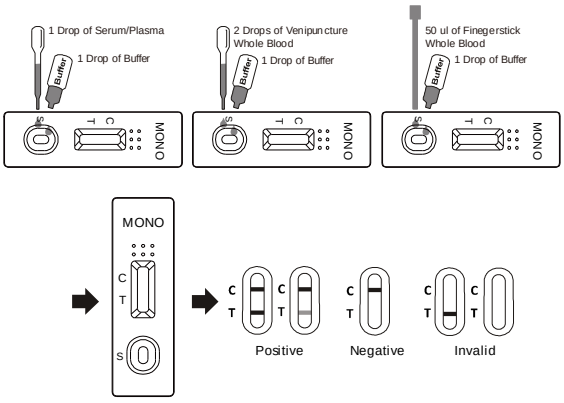
For **<u>Venipuncture Whole Blood</u>** specimens:

Hold the dropper vertically and transfer **2 drops** of whole blood (approximately 50 μL) to the specimen well (S) of the Test Cassette, and add **1 drop** of buffer (approximately 55 μL), then start the timer. See illustration below.

For **<u>Finger-stick Whole Blood</u>** specimens:

Using a capillary tube: fill the capillary tube and transfer approximately **50 μL** of finger-stick whole blood specimen to the specimen well (S) of the Test Cassette, then add **1 drop** of buffer (approximately 55 μL) and start the timer. See illustration below.

Wait for the coloured line(s) to appear. **Read results at 5 minutes.** Do not interpret the result after 10 minutes.



Note: Buffer must be discarded 6 months after initial opening.

INTERPRETATION OF RESULTS

(Please refer to the illustration above)

POSITIVE:* Two distinct coloured lines appear. One line should be in the control line region (C) and another line should be in the test line region (T).

***NOTE:** The intensity of the colour in the test line region (T) will vary depending on the concentration of IM heterophile antibodies present in the specimen. Therefore, any shade of colour in the test line region (T) should be considered positive.

NEGATIVE: One coloured line appears in the control line region (C). No apparent coloured line appears in the test line region (T).

INVALID: Control line fails to appear. Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure. Review the procedure and repeat the test with a new test. If the problem persists, discontinue using the test kit immediately and contact your local distributor.

QUALITY CONTROL

A procedural control is included in the test. A coloured line appearing in the control line region (C) is considered an internal procedural control. It confirms sufficient specimen volume, adequate membrane wicking and correct procedural technique.

In addition to your laboratory's standard quality control procedures, it is recommended that a positive and negative external control be tested at least once within each test kit and by each operator performing testing within a kit. This will verify that the reagents and test are working properly and the operator is able to correctly perform the test procedure. External positive and negative controls are supplied in the kit.

Procedure for External Quality Control Testing

1. Holding the bottle vertically, add 1 full drop (approximately 40 μL) of positive or negative control solution to the specimen well (S) of the Test Cassette, and add 1 drop of buffer (approximately 55 μL).
2. Wait for the coloured line(s) to appear. **Read results at 5 minutes.** Donot interpret the results after 10 minutes.
3. If the controls do not yield the expected results, do not use the test results. Repeat the test or contact your distributor.

LIMITATIONS

1. PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) is for *in vitro* diagnostic use only. The test should be used for the detection of Infectious Mononucleosis antibodies in whole blood, serum or plasma specimens only. Neither the quantitative value nor the rate of increase in Infectious Mononucleosis antibody concentration can be determined by this qualitative test.
2. PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) will only indicate the presence of Infectious Mononucleosis antibodies in the specimen and should not be used as the sole criteria for the diagnosis of Infectious Mononucleosis infection.
3. As with all diagnostic tests, all results must be interpreted together with other clinical information available to the physician.
4. If the test result is negative and clinical symptoms persist, additional testing using other clinical methods is recommended. A negative result does not at any time preclude the possibility of Infectious Mono nucleosis infection.
5. Use only clear, non-haemolysed specimens.
6. The haematocrit of the whole blood should be between 25% and 65%.

EXPECTED VALUES

Epstein-Barr virus (EBV) infection during adolescence or young adulthood causes Infectious Mononucleosis in 35% to 50% of reported cases.[1,5]

The incidence of EBV-associated Infectious Mononucleosis in the USA has been estimated at 45 per 100,000 and is highest in adolescent and young adults - about 2 out of 1,000. No seasonal pattern of EBV infection exists. The incubation period is 10 to 60 days, though 7 to 14 days is common for children and adolescents.

PERFORMANCE CHARACTERISTICS

Sensitivity

PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) has been evaluated with specimens confirmed positive or negative by a leading commercial slide agglutination test. The slide agglutination test served as the reference method for PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma). The result shows that the sensitivity of the PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) is >97.6% relative to the slide agglutination test.

Specificity

PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) uses an antigen that is highly specific for IM antibodies in whole blood, serum or plasma. The results show that the specificity of PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) is 97.8% relative to the slide agglutination test.

PathFlow™ Mononucleosis Rapid Test Cassette vs. Slide Agglutination (combined samples)

Method		Slide Agglutination		Total Results
Mononucleosis Rapid Test Cassette	Results	Positive	Negative	
	Positive	122	4	126
	Negative	3	176	179
Total Results		125	180	305

Relative Sensitivity: 97.6% (93.1%-99.5%)*
Relative Specificity: 97.8% (94.4%-99.4%)*

Relative Accuracy: 97.7% (95.3%-99.1%)*
* 95% Confidence Intervals

Precision

Intra-Assay

Within-run precision has been determined by using four replicates of three specimens: a negative, a low positive, middle positive and a high positive. The negative, low positive and middle positive values were correctly identified >99% of the time.

Inter-Assay

Between-run precision has been determined by 10 independent assays on the same three specimens: a negative, a low positive and a middle positive. Three different lots of PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) have been tested using negative, low positive and middle positive specimens. The specimens were correctly identified >99% of the time.

Cross-Reactivity

RF, HBsAg, HBeAg, HBcAb, HBeAb, HCV, TB, HIV and Syphilis positive specimens were tested with PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma). No cross-reactivity was observed, indicating that PathFlow™ Mononucleosis Rapid Test Cassette (Whole Blood/Serum/Plasma) has a high degree of specificity for human antibodies to IM.

Interfering Substances

The following potentially Interfering substances were added to MONO negative and positive specimens.

Ascorbic acid: 20mg/ml Hemoglobin: 1000mg/dl

Gentistic acid: 20mg/dl Oxalic acid: 60mg/dl

Bilirubin: 1000mg/dl Uric acid: 20mg/ml

Acetaminophen: 20mg/dl Aspirin: 20mg/dl

Methanol: 10% Creatine: 200mg/dl

Albumin: 2000mg/dl Caffeine: 20mg/dl

None of the substances at the concentration tested interfered with the assay.

BIBLIOGRAPHY

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TABLE OF SYMBOLS

SYMBOL	DEFINITION
LOT	Batch Number
REF	Catalogue reference
	Store at
	Expiry date
	Manufacturer
	Read the instructions for use

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