

Clearview[®] MONO-plus II

For the qualitative detection of infectious mononucleosis heterophile antibodies in human whole blood

CLIA COMPLEXITY: Waived
For Whole Blood or Fingerstick

INTENDED USE

The Clearview Mono-plus II Test is a rapid test for the visual, qualitative detection of heterophile antibodies specific to Infectious Mononucleosis (IM) in human whole blood. This test kit is intended as an aid in the diagnosis of IM in patients with characteristic clinical symptoms, and is intended for professional and laboratory use only.

SUMMARY

Infectious Mononucleosis is an acute, self-limiting disease caused by the Epstein-Barr virus (EBV) (ref.2,5,9). Infection with EBV in early life usually is asymptomatic (ref.1,6). However, up to 50% of infection occurring in young adulthood and adolescence will develop clinical manifestations associated with IM.

Diagnosis of IM is based on the evaluation of characteristic clinical symptoms and serological changes. Serological diagnosis of IM has been demonstrated by the detection of heterophile and EBV specific antibodies (ref.3-5). The heterophile antibody is detectable at some point during IM in most adults. It is a widely accepted practice among physicians to use the detection of heterophile antibodies as an aid in the diagnosis of IM (ref.1,3,5,6,8). The Clearview Mono-plus II Test utilizes bovine erythrocyte extract which has a higher sensitivity and specificity than extracts from other species (ref.1).

PRINCIPLE

The Clearview Mono-plus II Test is a chromatographic immunoassay (CIA) for the rapid, qualitative determination of IM heterophile antibodies in human whole blood. The test device contains a membrane strip which is precoated with heterophile antigens on the test line region and goat anti-mouse antibody on the control line region. The anti-human IgM antibody-colloidal gold conjugate pad is placed at the end of the membrane. A mixture of colloidal gold conjugate together with the sample and developer buffer will move along the membrane chromatographically by capillary action. When the IM heterophile antibodies are present in the patient sample, the mixture will migrate to the test line region and form a visible line as the antibody complexes with the heterophile antigen. Therefore, the presence of a colored line on the test line region indicates a positive result. When IM heterophile antibodies are absent from the sample, no visible color line will form on test line region. Therefore, the absence of a colored line in the test region (T) indicates a negative result. A colored line will always appear at the control region. This control line serves as a procedural indicator for the proper performance of the test and the device.

REAGENTS AND MATERIALS SUPPLIED

- Thirty (30) individually pouched test devices, each containing one disposable transfer pipette.
- Developer Buffer (8 ml): 0.1 M Phosphate Buffered Saline, with additives and 0.1% sodium azide.
- Negative Control (0.4 ml): Normal human plasma or serum diluted in saline solution with 0.2% sodium azide.
- Positive Control (0.4 ml): IM heterophile antibody positive human plasma or serum diluted in saline solution with 0.2% sodium azide.
- Two directional inserts: one waived and one moderately complex.
- One procedure card.

MATERIALS REQUIRED BUT NOT PROVIDED

- Specimen collection tubes:
 - EDTA, heparin or citrate for whole blood procedure.
- Finger lancets for fingerstick blood procedure.
- Timing device.

STORAGE AND STABILITY

The Clearview Mono-plus II Test kit is to be stored either at room temperature or refrigerated at 2°-30°C (36°-86°F). The test devices should remain in the sealed pouch until use or until the stated expiration date printed on the kit box.

PRECAUTIONS

- For *in vitro* diagnostic use only.
- For professional and laboratory use only.
- Do not use test kit beyond expiration date.
- Do not reuse test.
- Discard the test device if package is ripped, torn or device itself is damaged.
- Do not mix reagents and controls from different lots.
- Do not use whole blood which has been stored for more than three days.
- Grossly hemolyzed samples should not be used.
- Heat treated sera may cause erroneous results.
- The assay performance has not been established for anticoagulants other than those which have been evaluated.
- All patient samples should be treated as if capable of transmitting disease.

⊗ Xn Developer Buffer and Controls contain sodium azide. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of these solutions, always flush with copious amounts of water to prevent azide buildup.

Warning: Potential Biohazardous Material

Each donor unit of human plasma or serum used in the preparation of the Positive and Negative Controls were tested by FDA-approved methods for the presence of anti-HIV-1/HIV-2, HBsAg and anti-HCV, and found to be negative. However, caution should be used when handling and disposing of these items at biosafety level 2, as recommended in the Center for Disease Control/National Institutes of Health Manual, Biosafety in Microbiological and Biomedical Laboratories, 1984.

SPECIMEN COLLECTION AND HANDLING

Fingerstick

1. Clean the area to be lanced with an alcohol swab.
2. Squeeze the end of the fingertip and pierce with a sterile lancet.
3. Wipe away the first drop of blood with sterile gauze or cotton.
4. Allow the second drop of blood to flow directly into the sample well. Alternatively, the disposable transfer pipette provided can be used to obtain about 40 µl (two full drops) of fresh blood.

Whole Blood

1. Collect whole blood into a purple, blue or green top collection tube with anticoagulant (containing EDTA, citrate or heparin, respectively) by venipuncture.
2. The whole blood may be used for testing immediately or stored at 2-8°C (36°-46°F) up to three days.

TEST PROCEDURE

Test device, Developer Buffer, patient's samples, and controls should be brought to room temperature (20 to 30°C) prior to testing. Do not open pouches until ready to perform the assay to avoid condensation of moisture on the membrane.

1. Remove the test device from its foil pouch when ready to perform the test. Label the device with patient or control identification.
2. Add the specimen to the sample well.

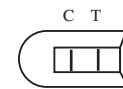
Fingerstick blood: Allow one drop of blood to flow directly into the sample well. Alternatively, use the provided transfer pipette to collect enough sample (more than 40 µl) of blood. Hold the pipette in a vertical position and dispense two full drops (about 40 µl) of fresh blood into the sample well.

Whole blood sample in collection tube: Mix well before using. Draw sample into the provided transfer pipette. Hold in a vertical position and dispense two full drops (about 40 µl) into the sample well.

3. Immediately add 3 or 4 drops of Developer Buffer into the sample well.

4. Start to count the time after addition of the Developer Buffer. Pink-red lines will begin to appear. Depending on the concentration of heterophile antibodies present, positive results may be observed within 3 minutes. However, to confirm a negative result, the complete reaction time of 5 minutes is required. Do not interpret results after 8 minutes.

INTERPRETATION OF RESULTS



POSITIVE

Two colored lines appear. One in the control region (C) and one colored line in the test region (T). A positive result indicates that there is IM heterophile antibodies in the patient sample. When testing with strong positive samples, the intensity of the control line may be lighter than expected. It is not recommended to compare the intensity of the lines.



NEGATIVE

Only one colored line appears in the control line region. No apparent faint colored line on the test line region (T). A negative result indicates that there is no IM heterophile antibodies in the patient sample or that the concentration is below the detection level.



INVALID

Absence of a colored line in the control region. The test should be voided since an improper test procedure may have been performed or deterioration of reagents may have occurred. Repeat the test with a new test device. If the problem persists, call Technical Service at (800) 257-9525.



QUALITY CONTROL

Internal Quality Control

The Clearview Mono-plus II Test has a built-in procedural control included in the test. The appearance of a pink to red line in the control region (C) assures the correct test procedure was followed, indicating sufficient volume of fluid was used and that capillary flow occurred. At the end of 5 minutes, formation of the control line verifies the sample has flowed through the test region (T) and that the test is complete. The test is invalid if the control line does not appear.

A clear background in the result window is considered an internal negative control. However, when whole blood samples are tested, the background may appear slightly reddish due to the low level hemolysis of some red blood cells. This is acceptable as long as it does not interfere with the reading of the test. The test is invalid if the background fails to clear and obscures the reading of the test result.

External Quality Control

Good laboratory practice recommends the use of external controls to assure functionality of reagents and proper performance. Positive and Negative Controls are supplied in the kit. These controls are human serum or plasma based and are tested like a patient sample.

When testing with the controls, add one drop (about 20 µl) of Positive or Negative Control in the sample well using the provided transfer pipette by holding it in a vertical position. Immediately add 3 or 4 drops of Developer Buffer. A positive signal is indicated by two lines, one in the test region (T) and one in the control region (C). A negative signal is indicated by only one line in the control region (C). Observe results at 5 minutes and do not interpret after 8 minutes.

If the controls do not perform as expected, do not interpret the test results. Repeat the test or contact Technical Service.

It is recommended that both a Positive and Negative Control be tested with every new kit. However, each laboratory should follow their state and local quality control requirements.

LIMITATIONS

This test kit is to be used for the qualitative detection of IgM antibodies to IM heterophile antigen. A positive result suggests the presence of IgM antibodies to heterophile antigen.

This test kit should be used for symptomatic individuals suspected of having IM. Diagnosis of IM should be made by confirmation with other clinical findings.

A negative result does not rule out the possibility of IM because the antibodies to heterophile antigen may be absent or may not be present in sufficient quantity to be detected.

EXPECTED VALUES

During the acute phase of IM, heterophile antibodies are detectable in 80-85% of patients. Heterophile antibodies are detectable during first month of illness and decrease rapidly after week four (ref.6).

PERFORMANCE CHARACTERISTICS

A. Accuracy

The accuracy of the Clearview Mono-plus II Test was evaluated in comparison to a commercially available qualitative color immuno-chromatographic assay for the detection of IM IgM heterophile antibodies in human serum, plasma or whole blood. Four hundred six (406) human serum, plasma and whole blood samples (288 serum, 103 plasma and 15 whole blood) were used in the comparison study. The results are summarized in Table 1.

Table 1. Summary of Comparison

	Clearview Positive	Clearview Negative	Total
Commercially Available Kit Positive	146	2	148
Commercially Available Kit Negative	3	255	258
Total	149	257	406

The results indicated that the Clearview Mono-plus II Test, demonstrated a Sensitivity of 98.6% (146/148), Specificity of 98.8% (255/258), and a total agreement of 98.8% (401/406).

B. Precision Site Study

The precision of the Clearview Mono-plus II Test has been evaluated at ABI and at two other sites, including a physician's office and an independent clinical laboratory.

Out of twenty-seven (27) positive samples with different levels, all twenty-seven (27) results were positive. Out of thirty-three (33) negative samples, all thirty-three (33) results were negative. The results obtained from all site studies demonstrated 100% agreement with the expected result. In addition, one positive and one negative control were tested each day. Results showed 100% agreement for control specimens.

C. Sensitivity:

Research standard MRC 66/235 from NIBSC was purchased to calibrate the test kit sensitivity (ref.7). Research standard MRC 66/235 was reconstituted in 1 ml of distilled water, and sequentially diluted to defined concentrations in a human infectious mononucleosis negative plasma. Diluted standards were then used for testing with Clearview Mono-plus II Test devices and three different commercially available devices. The results are shown in Table 2. The sensitivity of Clearview Mono-plus II Test is 12.5 unit/ml and is comparable to other commercially available devices.

Table 2. Test Results of NIBSC Mono Standard Serial Dilution in Human Plasma

Unit/ml	Dilution	Clearview Mono	Commercial Test 1	Commercial Test 2	Commercial Test 3
100	no	+	+	+	+
50	1:2	+	+	+	+
25	1:4	+	-	+	+
12.5	1:8	+	-	-	+
6.25	1:16	-	-	-	-

D. Interference:

Clearview Mono-plus II Test was tested for possible interference from visibly hemolyzed, lipemic and icteric samples. Lipemic samples with known levels of triglycerides were checked by both the Clearview Mono-plus II Test and a commercially available Mono Test for their heterophile antibody status. Human hemoglobin, bilirubin or albumin was spiked into samples with heterophile antibodies and tested against unspiked samples. No significant interference was observed. The results are shown in Table 3a and 3b. Testing was performed in duplicate.

It can be concluded that triglycerides up to 2,370 mg/dl, hemoglobin up to 10 mg/ml, bilirubin up to 0.06 mg/ml and albumin up to 100 mg/ml do not interfere with the performance of Clearview Mono-plus II Test.

Table 3a. Lipemic Sample Test

Sample ID	Triglycerides (mg/dl)	Cholesterol (mg/dl)	Clearview Mono	Commercial Test Kit
SD2192-9	1930	280	-	-
SD2192-10	2370	380	-	-
SD2192-11	1240	170	-	-
SD2192-17	1190	200	-	-
SD2192-20	890	190	-	-

Table 3b. Spiked Sample Test

Spike in Substances	Conc. (mg/ml)	Sample ID	Control	Spiked Sample
Hemoglobin	10	Strong Positive	++	++
		Weak Positive	+	+
		Negative	-	-
	1	Strong Positive	++	++
		Weak Positive	+	+
		Negative	-	-
Bilirubin	0.06	Strong Positive	++	++
		Weak Positive	+	+
		Negative	-	-
Albumin	100	Strong Positive	++	++
		Weak Positive	+	+
		Negative	-	-

E. Site Studies

The simplicity of using the Clearview Mono-plus II Test was evaluated at three different sites. At each site twenty (20) people, most of them non-professional with an average of a high school education, were asked to read the instructions and test three (3) blind labeled samples. The results from these studies are summarized in Table 4. Out of one hundred eighty (180) samples, only one (1) strong positive sample was interpreted as weak positive. The field results demonstrate that the Clearview Mono-plus II Test is easy to perform and can be used to obtain a visual qualitative detection of IM antibodies.

Table 4. Clearview Mono Field Test Result Summary

Sample	Number	Field Results			
		Strong Positive	Weak Positive	Negative	Invalid
Strong Positive	60	59	1	0	0
Weak Positive	60	0	60	0	0
Negative	60	0	0	60	0
Total	180	59	61	60	0

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