TABLE 1: *Clinical Sample Testing Arrangement*

Site	Fingerstick 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 Consult Mononucleosis Cassette Test and the corresponding serum/plasma samples were tested with a commercially available immunochromatographic heterophile antibody assay (Predicate) kit. When a fingerstick blood sample was tested with Consult Mononucleosis Cassette Test, 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 using Consult Mononucleosis Cassette Test. Consult Mononucleosis Cassette Test 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 Consult Mononucleosis Cassette Test 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). Consult Mononucleosis Cassette Test demonstrated a relative specificity of 98.8% (479/485) and a relative sensitivity of >99.9% (91/91). The results obtained with the Consult Mononucleosis Cassette Test correlated well to the results obtained with the commercially available immunochromatographic heterophile antibody assay test.

TABLE 2: *Total Specimens*

		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 (Fingerstick and Venous)*

		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*

		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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INTENDED USE

Consult Mononucleosis Cassette 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. Consult Mononucleosis Cassette Test 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.[2,8]

PRINCIPLE

Consult Mononucleosis Cassette 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 fingerstick or whole blood, 25 µL of blood is 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 the 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.

MATERIALS PROVIDED

- 25 Consult Mononucleosis Cassette 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.
- 1 Developer Solution: Phosphate saline buffer containing 0.1% Sodium Azide as preservative.
- 1 Negative Control: Diluted Human Serum containing 0.1% Sodium Azide as a preservative. For periodic use as external control material.
- 1 Positive Control: Diluted Human Serum containing IM heterophile antibodies and 0.1% Sodium Azide as a preservative. For periodic use as external control material.
- 25 (10 µL) (black line) sample transfer pipettes for use with serum/plasma.
- 25 (25 µL) (red line) sample transfer pipettes for use with whole blood.
- 1 Procedure card
- 1 Instructional insert

Materials 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.
- All patient samples should be handled as if they are capable of transmitting disease. Observe established precautions against microbiological hazards throughout all procedures and follow the standard procedures for proper disposal of potentially infectious specimens.
- Human blood and its products are potentially infectious; handle with appropriate precautions.
- For *in vitro* diagnostic use. Do not use after expiration date.
- 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 only in accordance with instructions supplied with the kit.

STORAGE AND STABILITY

Consult Mononucleosis Cassette Test kit should be stored at 2° - 30°C (36° - 86°F). Test Devices must remain in their sealed pouches until use. 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 and thaw whole blood; hemolyzed blood cannot be used in this test.

b). Fingerstick Blood:
For fingerstick blood, prick the finger and discard the first drop. Wipe the finger and collect blood from the second drop in the sample transfer pipette up to the red fill line (25µL). 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 or red cells as soon as possible to avoid hemolysis. When possible, clear, non-hemolyzed 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.

Specimen Storage - 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.

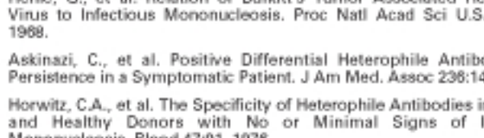
PROCEDURE

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 Consult mononucleosis Cassette Test devices, reagents and specimens to equilibrate to room temperature before testing.
- The Consult mononucleosis Cassette 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 fingerstick 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 Consult mononucleosis Cassette Test device.
- Use accepted microbiological practices for proper disposal of potentially infectious test materials and disinfection of contaminated equipment.
- After testing, dispose of Consult mononucleosis Cassette Test devices, sample

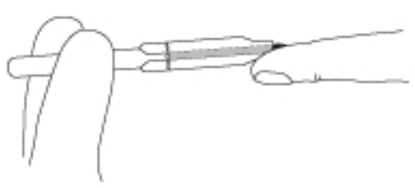
DIRECTIONS FOR USE OF SAMPLE TRANSFER PIPETTE

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.

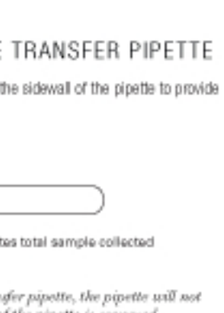
Caution: Filling is automatic: Do not squeeze the sample transfer pipette while filling. Avoid air bubbles.



STEP 1
Hold the sample transfer pipette horizontally and touch the tip of the pipette to the sample. 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.

For whole blood samples, use the 25 µL (red line) sample transfer pipette.

For serum/plasma samples, use the 10 µL (black line) sample transfer pipette. Follow the directions for sampling using the sample transfer pipette.

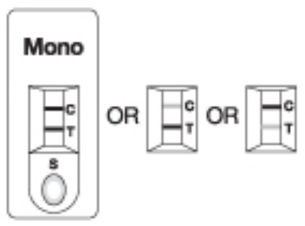
STEP 3
Add 2-3 drops of Developer Solution into the lower area of the Sample Well (S).

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.



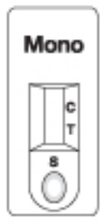
NOTE: A positive test result may be read as soon as a distinct pink-purple colored band appears at the Test position (T) and at the Control position (C). Any shade of pink-purple colored horizontal band at the Test position (T) should be reported as a positive result. The intensity of the colored band 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).



Consult mononucleosis Cassette 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 Consult mononucleosis Cassette 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 Consult mononucleosis Cassette Test 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. The included positive and negative controls can be run in place of serum or plasma according to the test procedure for this purpose.

Use the 10µL (black fill line) transfer pipette when testing external controls and serum/plasma proficiency survey samples.

If the controls do not perform as expected or the colored control band does not appear at the Control position (C), contact Technical Service at (877) 866-9335.

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. [2]
- 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 gondi[13] In primary infections of adults with clinically atypical diseases, EBV-specific laboratory diagnosis may also be helpful.
- Consult mononucleosis Cassette Test for serum and plasma is classified as moderately complex under the CLIA '88 regulations. Consult mononucleosis Cassette Test for the whole blood test is classified as waived under 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.[2]
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.[13,7] In some of these patients, serological evidence for a diagnosis of cytomegalovirus infection, toxoplasmosis, or viral hepatitis, as well as others, have been found.[22,23]

PERFORMANCE CHARACTERISTICS

SPECIFICITY

The following potentially interfering substances do not interfere with infectious mononucleosis heterophile antibody determinations in Consult mononucleosis Cassette Test 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 Consult mononucleosis Cassette Test 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 fingerstick 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).