

LABORATORY PROCEDURE

BBL™ MonoSlide™ Test

One-Minute Color-enhanced* Differential Slide Test
for Infectious Mononucleosis, using serum or plasma.

I. INTENDED USE

The **BBL™ MonoSlide™** Mononucleosis Test is a rapid, differential test for the serological detection of IgM class heterophile antibodies associated with infectious mononucleosis.

II. SUMMARY AND EXPLANATION

Paul and Bunnell¹ were first to suggest the use of hemagglutination of sheep erythrocytes as a method of detecting heterophile antibodies associated with infectious mononucleosis. Other antibodies such as Forssman, which occasionally appeared in sera and caused hemagglutination, were found by Davidsohn^{2,3} to be absorbed out with guinea pig kidney antigen, while the antibody of infectious mononucleosis remained reactive. Species of erythrocytes other than sheep, particularly the horse erythrocytes, have been shown by Beer^{4,6} and others to react similarly to sheep erythrocytes, but with greater sensitivity.

Heterophile antibodies of infectious mononucleosis may be present as early as the fourth day to indicate a positive diagnosis, and practically always by the twenty-first day of illness persisting as long as several months. Infectious mononucleosis has been reported to be associated with the Epstein-Barr Virus.^{8,9} Positive heterophile tests have been reported with hepatitis, rubella, leukemia,¹⁰ rheumatoid arthritis, Burkitt's lymphoma,¹¹ and other pathological conditions.^{12,13} Since false positive and false negative results occur with all known tests,¹⁴ results should be correlated with clinical and hematological findings.

III. PRINCIPLES OF THE PROCEDURE

This test utilizes a disposable card, guinea pig kidney antigen for absorption, and specially treated horse erythrocytes (color-enhanced) to increase specificity, sensitivity and enhance readability. No special equipment is required to read the **BBL™ MonoSlide™** Mononucleosis Test.

*When used on serum or plasma specimens.

IV. REAGENTS

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|-------------------|------------------------|--|
| Reagent A, | BBL™ MonoSlide™ | Guinea pig kidney antigen, with 0.1 % sodium azide (preservative). |
| Reagent B, | BBL™ MonoSlide™ | Preserved horse erythrocytes, with 0.1 % sodium azide (preservative). |
| Control +, | BBL™ MonoSlide™ | Positive control, human serum, with 0.1 % sodium azide (preservative). |
| Control -, | BBL™ MonoSlide™ | Negative control, human serum, with 0.1 % sodium azide (preservative). |

Precautions: For *in vitro* Diagnostic Use.

Reagents: Do not use beyond the expiration date. Upon removal from the refrigerator, allow reagents to warm to room temperature before use. Do **NOT** mix reagents from different kit lot numbers.

Controls: Do not use the kit if positive and negative controls do not yield appropriate results.

The serum controls are derived from human blood tested by an FDA (U.S. Food and Drug Administration)-approved method for the presence of the antibody to HIV (human immunodeficiency virus) and HBsAg (hepatitis B surface antigen) and found to be nonreactive.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL. Because no test method can offer complete assurance that HIV, hepatitis B virus, hepatitis C virus or other infectious agents are absent, SPECIMENS AND THESE REAGENTS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE. The FDA recommends such material be handled at a Biosafety Level 2. BSL2 is referenced in the Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) manual, *Biosafety in Microbiological and Biomedical Laboratories*.

Reagents contain sodium azide which may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Test Cards: Cards must be flat for proper reactions. If necessary, flatten cards by bowing back in a direction opposite to that of the curl. Care should be taken not to finger-mark the test areas, since this may result in an oily deposit and improper test results. Use each card once and discard. Store cards in the original package in a dry area at room temperature.

Pipettes and Dropper Rod Assemblies: Hold vertically when dispensing free-falling drops of reagents and sample; otherwise, test accuracy may be compromised due to

imprecise volume delivery. Before dispensing reagents, clear the dropper rod channel by squeezing the dropper bulb.

Storage of Reagents: Refrigerate at 2 to 8° C. DO NOT FREEZE. Prolonged day-to-day room temperature storage may compromise reagent stability; therefore, reagents should be recapped and returned to refrigeration when not in use.

V. SPECIMEN COLLECTION AND PREPARATION

Serum/Plasma: Serum or plasma may be used with the **MonoSlide** Mononucleosis Test, provided whole blood is collected by an acceptable medical technique. Avoid using grossly hemolyzed sera. Serum should be clear and free of bacterial contamination.

Specimens can be stored at 2 to 8°C for 24 h. For prolonged storage, specimens should be frozen (-20°C). It is not necessary to inactivate sera with heat before use. Plasma with EDTA or Acid Citrate Dextrose (ACD) as an anticoagulant can be used.

No special preparation of the patient is required prior to specimen collection.

VI. PROCEDURES

Review “Precautions” and “Specimen Collection and Preparation” prior to performing procedures. The testing area, reagents, test specimens and test components should be at room temperature when used.

Materials Provided:

No. 240901
(60 tests)

| | | |
|--|---|--------|
| Reagent A, | MonoSlide™ Guinea Pig Kidney Antigen | 3.0 mL |
| Reagent B, | MonoSlide Preserved Horse Erythrocytes | 3.0 mL |
| Control +, | MonoSlide Positive Control (human serum) | 1.0 mL |
| Control -, | MonoSlide Negative Control (human serum) | 1.0 mL |
| Test cards, and test disposables and accessories. | | 20 |

Materials Required But Not Provided: Timer, test tubes (13 x 75 mm), 0.85% sodium chloride solution, transfer pipettes for dilutions.

Also required are the necessary laboratory equipment used for preparation, storage and handling of serologic specimens.

Qualitative Test: (Serum and Plasma):

Remove the caps on **Reagents A** and **B** and replace with the dropper rod assemblies provided. Determine the number of test circles required (one circle for each patient sample or control to be tested). The test card may be separated at the perforation line to remove and save extra test circles for future testing.

Pipettes - To use, squeeze bulb of pipette and insert into sample. Release pressure to draw up the sample. Dispense free-falling drops by gently squeezing the pipette while holding vertically over the test card. Use a separate pipette for each sample. When handling pipettes, avoid touching the tip.

1. Gently shake **Reagent A** to resuspend antigen. Add one drop to the left side of the test circle on the card.
2. Invert **Reagent B** several times to mix thoroughly. Add one drop to the right side of the test circle.
3. Using a pipette, add one drop of patient serum or plasma to **Reagent A** on the left side of the test circle.
4. Invert the pipette and use the paddle end to THOROUGHLY mix (10 to 15 circular strokes) **Reagent A** (clear liquid) and sample (patient or control). Gradually mix this solution into **Reagent B** (reddish-brown liquid), covering entire test circle.
5. Rock the card by hand SLOWLY and GENTLY for one min (approximately 13-16 rocks per minute).
6. IMMEDIATELY read results macroscopically. Reading with direct light source is not necessary for test interpretations.

Quantitative Test (Serum and Plasma):

Titration-

1. Make serial dilutions of serum or plasma being tested using 0.85% sodium chloride, starting with a 1:2 dilution and continuing with 1:4, 1:8, 1:16, etc.
2. Test the diluted samples following the procedure outlined under "Qualitative Test."

VII. READING RESULTS (Serum/Plasma)

1. A positive infectious mononucleosis reaction will have dark clumps against a blue-green background, distributed uniformly throughout the test circle.

2. A negative reaction will have no agglutination, but may have fine granularity against a brown/tan background. Peripheral color development associated with fine granularity should be interpreted as negative; i.e., a faint blue-green color halo on the periphery of the test circle should not be interpreted as positive.

Quantitative Test: The highest dilution in which agglutination occurs is the end point.

The **MonoSlide™** Mononucleosis Test has been adjusted to provide a positive test approximating the sensitivity of a guinea pig kidney absorbed Davidsohn sheep cell titer of 1:28 to 1:56. Therefore, multiplying the reciprocal of the highest dilution in which agglutination occurs (end point) by 28 can approximate a quantitative result. While no correlation has been found between the severity of illness and the heterophile titer,⁷ it may be of interest to the clinician in following the course of the disease.

VIII. QUALITY CONTROL

The **MonoSlide™ Controls** + and - should be used to check the reagents upon receipt of the kit. This is suggested for two reasons: 1) to ensure proper kit performance, and 2) to familiarize the user with positive and negative test result interpretations. When performing positive and negative control sera, follow instructions under "Procedures, Qualitative Test." Under Step 3, add one drop of each control to a separate test circle in place of patient serum.

IX. LIMITATIONS OF THE PROCEDURES

Diagnosis of infectious mononucleosis should be based upon the results of all clinical and laboratory findings.

Some segments of the population do not produce detectable heterophile antibody; e.g., approximately 50% of children under 4 years of age and 10% of adolescents.^{15,16}

Detectable levels of heterophile antibody may persist for months, and more rarely for years, in some individuals.^{16,17}

X. PERFORMANCE CHARACTERISTICS

One hundred thirty-four serum specimens were evaluated for heterophile antibody by the **BBL™ MonoSlide™** test and a leading latex agglutination test. Both tests yielded the same results for ninety-nine positives and thirty negative sera. The five remaining sera were negative by the **BBL™ MonoSlide™** test and positive by the latex test. These sera were all negative by further tests using the Davidsohn Differential Tube procedure.¹⁸

XI. AVAILABILITY

| Cat. No. | Description |
|----------|--|
| 240901 | BBL™ MonoSlide™ Test, 60 test kit, qualitative/quantitative |

XII. REFERENCES

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18. Data on file at BD Diagnostic Systems.

XIII. TECHNICAL INFORMATION

In the United States, telephone BD Diagnostic Systems Technical Services, toll free (800) 638-8663

Approved by: _____

Date Effective: _____

Supervisor: _____ Date: _____

Director: _____ Date: _____

Reviewed:

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