

ACCUTEX INFECTIOUS MONONUCLEOSIS LATEX TEST

For serologic detection of heterophile antibodies associated with infectious mononucleosis.

QUALITATIVE AND SEMIQUANTITATIVE

SUMMARY

Paul and Bunnell were the first to report that serum from a patient with infectious mononucleosis (IM) contained heterophile antibodies which agglutinated sheep erythrocytes. These heterophile antibodies react with an antigen which apparently is not responsible for their production. However, it was soon discovered that the test lacked specificity because the naturally occurring Forssman antibody found in serum from some individuals who apparently have not had recent infectious mononucleosis agglutinates unmodified sheep or horse erythrocytes. ⁽²⁾ In 1937, Davidsohn employed a differential absorption procedure which removed the Forssman antibody yet retained the heterophile agglutination characteristic of IM. ⁽³⁾ The Davidsohn modification added specificity but made the test time-consuming and cumbersome to perform. Therefore, the Davidsohn test has been relegated to the role of a reference method for diagnosis of IM. Attempts to find a suitable alternative include enzyme immunoassays, latex fixation and chemically treated horse erythrocytes in a 1-step agglutination procedure. When properly selected and treated, horse RBC have been shown to be sensitive and specific for the serological detection of the heterophile antibodies associated with infectious mononucleosis (95% positive in the course of serologically proven IM and 80% positive by the first week) ^(2,4,5,6,16)

PRINCIPLE

The Accutex Infectious Mononucleosis Latex Test provides a suspension of polystyrene latex particles which have been coated with partially purified glycoprotein from bovine red blood cells. The heterophile antibody associated with infectious mononucleosis binds to the corresponding antigenic determinants on the glycoprotein coated latex. This binding is evident by rapid agglutination of the latex. Due to the purification of the bovine red cell, the glycoprotein coated latex is not agglutinated by Forssman or serum sickness antibodies at levels normally encountered in the U.S. population; therefore, no differential absorption is required.

STABILITY AND STORAGE

Indication of deterioration: lack of clear agglutination with the Positive Control Serum, agglutination with the Negative Control Serum or extreme turbidity in either control serum. The reagents in this kit are stable until their expiration date when stored as directed. However, as with most reagents, they can be damaged by improper handling, especially temperature extremes. Use of the Positive and Negative Control Sera provided will permit detection of reagent deterioration.

Store reagents at 2-8°C when not in use. All other kit components may be stored at room temperature if desired. Do not freeze reagents.

Discard any unused control serum when the IM Latex Reagent is depleted.

SPECIMEN COLLECTION AND HANDLING

Use fresh serum or plasma free from contamination. Collect blood in a clean, dry tube and allow to clot at room temperature for at least 10 minutes before removing serum. If not tested immediately, specimens may be stored at 2-8°C for maximum of 72 hours. If longer storage is required, the sample may be frozen and tested at a later time. Repeated freezing and thawing should be avoided.

Specimens must be clear and free of particulate matter before testing. If necessary, centrifuge to clarify specimens before testing. Contaminated or grossly hemolyzed specimens should not be used.

PROCEDURE

REAGENTS AND MATERIALS PROVIDED

1. IM Latex Reagent: a suspension of polystyrene latex particles coated with partially purified glycoprotein from bovine red cells.
2. IM Positive Control Serum: a human serum pool known to have a positive reaction with the Accutex IM Latex Reagent.
3. IM Negative Control Serum: a human serum pool known to have a negative reaction with the Accutex IM Latex Reagent.

All 3 reagents contain 0.1% sodium azide as a preservative.

4. Test Slide
5. 50 disposable dispensing-spreading pipets

MATERIALS REQUIRED BUT NOT PROVIDED

1. Timer
2. Mechanical slide rotator (optional)
3. Physiologic saline (0.85% or 0.9% sodium chloride)
4. Serological pipets or safety pipetting device with disposable tips.
5. Disposable test tubes (12 x 75 mm, 10 x 75 mm or 13 x 100 mm)
6. Test tube rack

Warning: Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. If discarded into sink, flush with a large volume of water to prevent azide buildup.

Warning: Human sourced material. Treat as potentially infectious. Each donor unit used in the preparation of this product has been tested by an FDA-approved method and found non-reactive for the presence of HBsAg and antibody to HIV virus. Because no known test method can offer complete assurance that Hepatitis B virus, HIV virus or other infectious agents are absent, all human blood based products should be handled in accordance with good laboratory practices using appropriate precautions as detailed in Centers for Disease Control/National Institute of Health Manual "Biosafety in Microbiological and Biomedical Laboratories," 1984.

PROCEDURE OUTLINE

A. QUALITATIVE TEST

1. Bring reagents and specimens to room temperature before use.
2. Place one drop (50 ul) of the IM Positive Control on the first field of the reaction slide. Place one drop (50 ul) of the IM Negative Control on the second. The remaining fields are used for test specimens. Using pipets provided, place one drop of the specimens on successive fields. Retain the Pipet/Stir Sticks for mixing step.
3. Gently resuspend the IM Latex Reagent and add one drop to each test field. Use Pipet/Stir Stick to spread reaction mixture over entire test field.
4. Rotate the slide for 3 minutes and read immediately under direct light.

B. SEMIQUANTITATIVE TEST

1. Bring reagents and specimens to room temperature before use.
2. Using physiologic saline, dilute the specimens 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 or as needed.
3. Place one drop (50 ul) of each dilution on successive fields of the reaction slide.
4. Gently resuspend the IM Latex Reagent and add one drop to each test field. Use Pipet/Stir Stick to spread reaction mixture over entire test field.
5. Rotate the slide for 3 minutes and read immediately under direct light.

QUALITY CONTROL

IM Positive and Negative Control Sera should be included in each test series. The Positive Control Serum should produce clear agglutination; the Negative Control Serum should produce no agglutination.

RESULTS

A. QUALITATIVE TEST:

Agglutination (positive reaction) indicates the presence of the heterophile antibody associated with infectious mononucleosis. The lack of agglutination (negative reaction) indicates the absence of the heterophile agglutinin associated with infectious mononucleosis.

B. SEMIQUANTITATIVE TEST

The titer of IM heterophile antibody is the reciprocal of the highest dilution which exhibits a positive reaction. The actual titer of the antibody has not been related to the stage or severity of the disease.^(7,8) However, an increase in IM heterophile agglutination titer may be clinically significant in the early stages of the disease and may assist in the diagnosis of IM.

LIMITATIONS OF TEST PROCEDURES

Although the Accutex IM Latex Reagent is highly sensitive and specific, a diagnosis of infectious mononucleosis should not be made on the basis of a positive test result without the support of patient history and hematological or other clinical evidence. Similarly, a negative test result cannot completely rule out infectious mononucleosis. Incubation of the test for longer than the recommended time or microbial contamination may cause false positive reactions.

Apparent false positive reactions have been associated with sera from patients with other diseases such as infections, leukemia, Burkitt's lymphoma and serum sickness.^(9,10,11,12,13)

Although most patients develop heterophile antibodies within 3 weeks of the onset of symptoms, occasional patients may take several months to develop detectable levels. If the Accutex IM Latex Test is negative in the presence of strong evidence suggesting a diagnosis of infectious mononucleosis, repeat testing on samples obtained at intervals of several days will generally reveal development of the heterophile agglutinin. Some patients with hematological and clinical evidence of infectious mononucleosis remain persistently negative.^(12,14,15)

A single heterophile antibody titer cannot be interpreted as an indication of the stage or severity of the disease.^(7,8) However, titrations on sequential samples may be useful in following the course of the disease in an individual patient.

SPECIFIC PERFORMANCE CHARACTERISTICS

Serum and plasma specimens from two hundred eighty-five (285) individuals which had been submitted to clinical laboratories by physicians for IM testing were examined. The Accutex IM Latex Test and a commercial RBC kit were used to evaluate the specimens. One hundred thirty-two (132) specimens were found positive using both assays. The remaining one hundred fifty-three (153) specimens gave negative results using both assays. This data indicates that both sensitivity and specificity of the Accutex IM Latex Test are 100%.

In a study on precision, a panel of 10 serum samples with IM heterophile antibody titers from 1 to 256 were tested 10 consecutive days by the Semi-Quantitative Test method (100 determinations). No determinations gave more than a 2-fold difference from the mean titer for a sample.

REFERENCES

1. Paul, J.R. and W.W. Bunnell. 1932. Am. J. Med. Sci. 90:1932.
2. Beer, P. 1936. J. Clin. Invest. 15:591.
3. Davidsohn, I. 1937. J.A.M.A. 108:289.
4. Hoff, G. and S. Bauer. 1965. 194:351.
5. Wilkinson, P.C. and D.S. Carmichael. 1964. J. Lab. Clin. Med. 64:529.
6. Lee, C.L., I. Davidsohn and R. Slaby. 1968. AM. J. Clin. Path. 49:3.
7. Davidsohn, I. and C.L. Lee. 1962. Med. Clin. N. Am. 46:225.
8. Baehner, R.L. and S.E. Shuler. 1967. Clin. Pediat. 6:393.
9. Horwitz, C.A., H. Polesky, T. Stillman, P.C.J. Ward, G. Henle and W. Henle. 1973. Brit. Med. J. 1:591.
10. Bender, C.E. 1958. Ann. Intern. Med. 49:852.
11. Carpenter, G., J. Kahler and E.B. Reilly. 1950. AM. J. Med. Sci. 220:195.
12. Henle, G., W. Henle and V. Diehl. 1968. Proc. Natl. Acad. Sci. 59:94.
13. Davidsohn, I. 1929. J. Immunol. 16:259.
14. Penman, H.C. 1968. J. Clin. Path. 21:50.
15. Henle, W. and G. Henle. 1973. New Engl. J. Med. 288:263.
16. Andiman, W.A. 1985. Antibody Responses to Epstein-Barr Virus. in Rose, N.R., H. Friedman and J.L. Fahey (ed.) Manual of Clinical Immunology. Third edition. Amer. Soc. Microbiol. Washington, DC