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Infectious Mononucleosis (IM) Latex Test

INTENDED USE

The PULSE IM LATEX TEST (IM TEST) is intended for the qualitative screening and semi-quantitative determination of heterophile antibodies in serum as an aid in the diagnosis of infectious mononucleosis. This product is “For Laboratory Use Only”.

SUMMARY AND PRINCIPLES

IM is a viral disease caused by Epstein-Barr virus that affects the reticuloendothelial system and has a broad spectrum of clinical symptoms. The patients usually develop transient IgM heterophile antibodies, have an abnormal white cell picture, and abnormal liver function.

Paul and Bunnell[1] first reported that patient serum with IM contained heterophile antibodies which agglutinated sheep erythrocytes, reacting with an antigen which apparently is not responsible for heterophile antibody production. This test lacked specificity because the natural Forssman antibody found in serum from some individuals with no history of IM agglutinates unmodified sheep or horse erythrocytes[2]. In 1937, Davidsohn[3] employed a differential absorption procedure which removed the Forssman antibody but made the test time consuming and cumbersome to perform. Attempts to find a suitable alternative were made. Studies by Bailey and Raffel[4] discovered that bovine erythrocytes were more sensitive than sheep or horse erythrocytes for detecting IM heterophile antibodies. Since that time, antigens which have been extracted from bovine red cell membranes have been used in various enzyme immunoassays which are both highly sensitive and specific for heterophile antibodies associated with IM[5].

The PULSE IM TEST reagent contains polystyrene latex particles coated with antigenic extract of bovine erythrocyte membranes, which will bind with the heterophile antibody associated with IM. This binding is evident by rapid visible agglutination of the latex.

MATERIALS SUPPLIED

IM Latex:	Latex particles coated with extract of bovine erythrocytes membranes in buffer and < 0.1% sodium azide.
IM Positive Control:	Human serum that produces a positive reaction with the IM Latex Reagent and < 0.1% sodium azide.
IM Negative Control:	Human serum that produces a negative reaction with the IM Latex Reagent and < 0.1% sodium azide.

Disposable pipettes and test slides.

Additional Items Required:
Physiological saline, serological pipettes, test tubes, mechanical rotator (optional), vortex mixer (optional), and timing device.

STORAGE & STABILITY

When not in use, store reagents and controls at 2 - 8°C. DO NOT FREEZE. Prior to use, allow reagents and controls to warm up to room temperature. Expiration date is specified on the kit label and on each vial. Biological indication of product instability is evidenced by inappropriate reaction of the latex reagent with the corresponding positive and negative control sera.

PRECAUTIONS

This product is for In Vitro Diagnostic Use Only.
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 (Hepatitis B Surface Antigen) 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. The preservative (sodium azide) may react with metal plumbing to form explosive metal oxides. In disposal, flush with a large volume of water to prevent metal azide build up.

SPECIMEN COLLECTION

Fresh serum is preferred. However, serum can be stored up to 7 days at 2-8°C or up to 3 months at – 20°C. Samples with presence of fibrin should be centrifuged. The test sera and controls should not be heat activated. DO NOT use highly hemolyzed or lipemic samples.

PROCEDURE:

A. Method I (Qualitative)

1. Bring all test reagents and serum specimens to room temperature.
2. Mix the IM Latex reagent thoroughly or on a vortex mixer before using.
3. Positive and negative controls should be included with each series of tests.
4. Place 50µL (1 drop) of the sample and one drop of positive and negative controls onto separate circles on the test card. Use a new pipette each time. Add 1 drop of latex reagent next to the samples and controls on the test card.
5. Using the sealed end of the stirrer, mix the drops and spread over the entire surface of the circle. Use different stirrers for each sample.
6. Gently tilt and rotate the test card by hand for 2 minutes. Alternatively, place the test card on a mechanical rotator at 80-100 r.p.m. for 2 minutes.
7. Observe for macroscopic clumping under direct light and compare sample results to the controls.

B. Method II (Semi-Quantitative)

1. For each test serum to be titrated, set up at least 6 test tubes (12 x 75 mm) and label 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, etc.
2. To each tube add 0.2 ml of physiological saline.
3. To Tube No. 1 add 0.2 ml of undiluted test serum (1:2). Mix and transfer 0.2ml of Tube No. 1 ml to Tube No. 2. This serial dilution will provide the dilutions range from 1:2 to 1:64. If required, additional serum dilutions can be added.
4. Repeat steps 3 to 7 as given in Method I (Qualitative).

RESULTS

Qualitative
Positive Result: Agglutination
Negative Result: Smooth milky suspension

Semi-Quantitative
The titer of IM heterophile antibody is the reciprocal of the highest dilution which exhibits a positive reaction. The actual titer of the antibody cannot relate to the stage or severity of the disease[6,7]. 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.

QUALITY CONTROL

Positive and Negative controls should be included in each test series. The Positive control should produce visible agglutination; and Negative control should produce no agglutination.

LIMITATIONS OF THE PROCEDURE

1. The results of this test SHOULD NOT be used as a single diagnostic tool to make a clinical diagnosis. Instead, the test results must be evaluated together with other clinical findings and observed symptoms to aid in the final diagnosis.
2. False positive results could appear if the test is read later than two minutes.
3. Apparent false positive reactions have been associated with sera from patients with other diseases such as rheumatoid arthritis, certain respiratory infections, leukemia, Burkitt's lymphoma and serum sickness[8-12].
4. False positive results may result in geographical areas where "horse serum' is used a as prophylactic measure (Vaccination).
5. Although most patients develop heterophile antibodies within 3 weeks after the onset of symptoms, occasional patients may take several months to develop detectable levels.
6. If the IM TEST is negative in the presence of strong evidence of suggesting IM, or because of delayed IM heterophile antibody response, repeat testing on samples obtained at intervals of several days will likely reveal heterophile agglutinin.
7. Some patients with hematological and clinical evidence of IM remain persistently negative[12-14].
8. 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.
9. The sensitivity of the test may be reduced at low temperatures.

INTERFERENCES

Hemoglobin (10 g/L), bilirubin (20mg/dL), lipemia (10g/L) and rheumatoid factors (300IU/ml), do not interfere. Other substances may interfere[15].

PERFORMANCE CHARACTERISTICS

1. Serum and plasma specimens from 285 individuals which had been submitted to clinical laboratories by physicians for IM testing were examined. The PULSE IM TEST and another commercial Red Cell IM Test Kit were used to evaluate the specimens. One Hundred Thirty-Two (132) specimens were found to be positive by both assays. The remaining 153 specimens gave negative results using both products. These data indicate that both sensitivity and specificity of the PULSE IM TEST are 100%.
2. No prozone effect was detected up to 1/256 sample titer.