PERFORMANCE CHARACTERISTICS

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%. 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 Quantitative Method (100 determinations). No determinations gave more than a 2-fold difference from the mean titer for a sample.

BIBLIOGRAPHY

1. Paul, J.R. and W.W. Bunnell, Am.J. Med.Sci., 183, 90, 1932.
2. Beer, P.J. Clin.Invest., 15, 591, 1936.
3. Davidsohn, I., Am. J.A.M.A., 108, 289, 1937.
4. Bailey, G.H., and Raffel, S., Hemolytic Antibodies for Sheep and Ox Erythrocytes in Infectious Mononucleosis, J. Clin.Invest., 14, 228-244, 1935.
5. Fletcher, M.A., and B.J. Woolfolk, Immunochemical Studies of Infectious Mononucleosis: Isolation and Characterization of Heterophile Antigens from Hemoglobin-Free Stroma, J. Immunol., volume 107.
6. Lee, C.L., I. Davidsohn and R. Slaby, Am. J, Clin. Path., 49, 3-11, 1968.
7. Davidsohn, I. and C.L. Lee, Med.Clin.N.Am., 46, 225, 1962.
8. Baehner, R.L. and S.E., Shuler, Clin. Pediat., 6, 393, 1967.
9. Horwitz, C.A., H. Polesky, T. Stillman, P.C.J. Ward, G. Henle and W. Henle, Brit.Med.J., 1, 591, 1973.
10. Bender, C.E., Ann.Intern.Med., 49, 652, 1958.
11. Carpenter, G., J. Kahler and E.B. Reiley, Am. J. Med. Sci., 220, 195, 1950.
12. Henle, G., W. Henle and V. Diehl, Proc. Natl. Acad. Sci., 220, 195, 1950.
13. Davidsohn, I., J. Immunol., 16, 259, 1929.
14. Penman, H.C., J. Clin. Path., 21, 50, 1968.
15. Henle, W. and G. Henle, New Engl. J. Med., 288, 263, 1973.
16. Andiman, W.A., Antibody Responses to Epstein-Barr Virus in "Manual of Clinical Immunology", editors, Rose, N.R., H.Freidman and J.L. Hahey, 3 ed., Am. Soc.Microbiol., Washington, D.C., 1985.

PULSE SCIENTIFIC INC.

Infectious Mononucleosis Latex Test

INTENDED USE

The PULSE IM LATEX TEST (PULSE IM TEST) is intended to be used 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

Paul and Bunnell[1] were the first to report that serum from a patient with 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[3] employed a differential absorption procedure which removed the Forssman antibody but retained the heterophile agglutination characteristic of IM. 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 were made by Bailey and Raffel[4] and they 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 walls 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 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 IM binds to the corresponding antigenic determinants on the glycoprotein coated latex. This binding is evident by rapid agglutination of the latex. As a result of the purification of the bovine red cell glycoprotein, the coated latex particles are not agglutinated by Forssman or Serum Sickness antibodies at levels normally encountered in the U.S. population. Thus, no differential absorption is required.

MATERIALS SUPPLIED

IM Latex: Contains suspension of polystyrene latex particles coated with partially purified glycoprotein from bovine red

cell in a stabilization buffer, and less than 0.1% sodium azide preservative.

IM Positive Control: Human serum known to have a positive reaction with the IM Latex Reagent and contains less than 0.1% sodium azide as preservative.

IM Negative Control: Human serum known to have a negative reaction with the IM Latex Reagent and contains less than 0.1% sodium azide as preservative.

Disposable pipettes and test slides.

Additional Items Required:
Physiological saline, serological pipettes, 12 x 75 mm test tubes and timing device.

STORAGE & STABILITY
When not in use, store reagents and controls at 2 - 8 degrees Celsius. 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 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
The test should be performed on serum. The test sera and controls should not be heat inactivated. Fresh specimens (less than 24 hours) should be used in performing the test. If testing is delayed, specimens should be refrigerated (or frozen where applicable). Bacterial contamination may cause false positive agglutination.

PROCEDURE:

A. Method I (Qualitative)
1. Bring all test reagents and serum specimens to room temperature.
2. Gently shake the IM latex vial to disperse and suspend latex particles.
3. Positive and negative controls should be tested with each series of test.
4. Using the disposable pipette provided, place one drop of test serum onto a circle on the slide. Use a separate disposable pipette for each test serum. Important: The Pulse IM Latex Reagent must be agitated well for about 10 seconds prior to using on each day's testing. Do not use a vortex mixer. Deliver one drop of IM Latex to each circle that contains specimens on the slide. Spread the resulting mixture by using the paddle end of the pipette. Do not use the same paddle end to mix each test serum or control as this will cause cross-contamination.
5. Gently tilt and rotate slide by hand for three (3) minutes.
6. Observe for macroscopic clumping under direct light.
7. Compare the reaction of the test serum to the IM positive and negative control sera.

B. Method II (Semi-Quantitative)
1. For each test serum to be titrated, set up a 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.
4. Serially make two-fold dilutions by mixing contents of Tube No. 1 with pipette and transferring 0.2 ml to Tube No. 2. Repeat serial transfers for each tube. For the tubes, the dilutions range from 1:2 to 1:64. If required, additional serum dilutions can be added.
5. 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 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.

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. This test is designed to be performed by hand rotation. The use of a mechanical rotator could yield false positive/negative results.
3. Incubation of the test for longer than the recommended time or microbial contamination may cause false positive reactions.
4. 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[9-13].
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 PULSE IM TEST is negative in the presence of strong evidence of suggesting a diagnosis of IM, repeat testing on samples obtained at intervals of several days will generally reveal development of the heterophile agglutinin.
7. Some patients with hematological and clinical evidence of IM remain persistently negative[12,14,15].
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.