Performance characteristics

Evaluations comparing **Sure-Vue® Mono** to a commercially available differential hemagglutination test were performed to determine the sensitivity and specificity of the reagent. 106 positive sera and 114 negative sera were selected by a differential hemagglutination slide test for the study. Discrepancies between the results given by **Sure-Vue® Mono** and the hemagglutination test were resolved using Epstein-Barr Virus (EBV) specific serological assays.

In these assays, the titers of specific antibodies to the EBV capsid antigen (both IgG and IgM), EBV early antigen (both diffused -D- and restricted -R-) and EBV nuclear antigen were determined. The results of these assays specified whether and EBV infection was recent or acute, in which case the serum was considered positive, or if antibodies were absent or relics of an old infection, in which case the serum was considered negative. Twelve of the 100 nondiscrepant positive sera and five of the 106 nondiscrepant negative sera were also analyzed using these same EBV specific serological assays. In all cases the EBV specific assay confirmed the positivity or negativity of the samples.

Compared with hemagglutination, **Sure-Vue® Mono** was found to have a sensitivity of 94% and a specificity of 93%. Assuming that the concordant results of the EBV serology performed on nondiscrepant sera apply to all the samples tested, it can be inferred that the sensitivity of **Sure-Vue® Mono** relative to EBV specific tests is 99% and its specificity relative to the same is 93%. There was only one result negative to **Sure-Vue® Mono** and positive to EBV serology. It was determined to be a recent infection and not an acute infection due to the absence of anti-VCA IgM and nuclear antigen. For this reason this negative result cannot be interpreted as prozone effect.

In a separate study **Sure-Vue® Mono** was compared to a qualitative red horse cell slide test involving a total of 224 EDTA plasma samples. There was complete agreement in test results which included 51 positive and 173 negative samples. Overall, the results of this study indicate clearly that **Sure-Vue® Mono** is highly sensitive and specific for the diagnosis of infectious mononucleosis.

A panel of 10 positive serum samples was tested on three consecutive days using the semiquantitative test. The results of the study indicate that **Sure-Vue® Mono** has 100% precision. The error of repeated estimations was expected to be only one doubling dilution.

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Sure-Vue® Mono

Sure-Vue® Mono is a one step rapid latex particle agglutination test for the qualitative and semiquantitative determination of infectious mononucleosis heterophile antibodies in serum or plasma. Sure-Vue® Mono aids in the diagnosis of infectious mononucleosis.

Summary

Infectious mononucleosis is an acute infectious disease of viral etiology. The most frequent symptoms are fever, sore throat, tender lymphadenopathy, anorexia, malaise, headache and myalgia. Splenomegaly occurs in most patients. A macular, maculopapular or petechial rash occurs in up to 50% of the cases, but such rashes occur most commonly in patients who have been treated with ampicillin.

The complications of infectious mononucleosis include secondary bacterial pharyngitis, rupture of the spleen, autoimmune hemolytic anemia, autoimmune thrombocytopenia, myocarditis, hepatitis and central nervous system involvement with meningoencephalitis or transverse myelitis. Fatal fulminant infectious mononucleosis or acquired hypogammaglobulinemia is rarely seen.

The diagnosis made on clinical history and symptomatology alone is difficult. Numerous cases in which infectious mononucleosis has been misidentified with other non-related viral and bacterial diseases have been cited.[1] For this reason, hematologic and serologic tests are very helpful in diagnosis.

In 1932, Paul and Bunnell[2] noted that sera from patients with infectious mononucleosis have heterophile antibodies to sheep erythrocytes. Also were described agglutinins to red blood cells from other mammals.[3,4] The proteins responsible for this agglutination are glycoproteins from red cell membranes called Paul-Bunnell antigen by several authors. Studies made on these glycoproteins show that those purified from bovine red blood cells are the most sensitive to infectious mononucleosis heterophile antibodies. Heterophile antibodies to sheep erythrocytes (which are different from those present during infectious mononucleosis) may also be detected in sera from normal people, from individuals who have received injections of serum, and others.[3,5]

Traditionally the infectious mononucleosis heterophile antibodies have been distinguished from other heterophile antibodies by a "differential" absorption test[6,7] with bovine red blood cells and guinea pig kidney tissue. Now, the use of a purified Paul-Bunnell antigen attached to latex particles provides a simple method with improved sensitivity for the specific detection of heterophile antibodies associated with infectious mononucleosis.

In 1968, the etiologic agent of infectious mononucleosis was described.[8] It was called the Epstein-Barr virus (EBV), a member of the herpes virus group. Subsequently, several serologic techniques involving EBV-related antigens have been developed.

The mode of transmission of infectious mononucleosis appears to be intimate salivary contact, salivary contamination of eating and drinking vessels and airborne dissemination of EBV.[9]

Principle

The **Sure-Vue® Mono** reagent is a suspension of polystyrene latex particles of uniform size coated with highly purified Paul-Bunnell antigen from bovine red cell membranes. The degree of purity of the antigen is such that **Sure-Vue® Mono** only reacts with infectious mononucleosis heterophile antibodies. For this reason, "differential" absorptions are not necessary.

Latex particles allow visual observation of the antigen-antibody reaction. If infectious mononucleosis heterophile antibodies are present in either serum or plasma, the latex suspension changes its uniform appearance and a clear agglutination becomes evident.

Reagents

a) **Latex reagent:**
Suspension of polystyrene latex particles coated with Paul-Bunnell antigen in a buffer.
Contains sodium azide 0.1%.

b) **Positive control:**
Rabbit IgG anti-Paul-Bunnell antigen diluted in a buffer.
Contains sodium azide 0.1%.

c) **Negative control:**
Non reactive diluted human serum.
Contains sodium azide 0.1%.

Precautions

Sure-Vue™ Mono is intended for IN VITRO diagnostic use.
The reagents in this kit contain sodium azide as a preservative. Sodium azide has been reported to form lead or copper azide in laboratory plumbing which may explode on percussion. Flush drains with water thoroughly after disposing of fluids containing sodium azide.
Each donor unit used in the preparation of this material was tested by an FDA approved method for the presence of HIV 1/2 and HCV antibodies, as well as for hepatitis B surface antigen and found to be negative.
WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.
Because no test method can offer complete assurance that HIV 1/2, HCV, hepatitis B virus, or other infectious agents are absent, the controls of this kit and specimens should be handled carefully following procedures recommended for biohazardous material.[10]

Storage

The reagents will remain stable through the expiration date shown on the label if stored between 2 and 8°C. Do not freeze. The reagents can be damaged by improper handling, especially temperature extremes. Checking reagent with the positive and negative controls provided will permit detection of reagent deterioration.
The reagents should not be used after the expiration date shown on the label.
The latex reagent, once shaken, must be uniform without visible clumping. When stored a slight sedimentation may occur and should be considered normal.
Do not use reagents if they become contaminated.
The reagent dropper dispenses drops of 28 µL ± 10%. The dropper must be held perpendicular to the slide surface and a single drop allowed to fall. Do not use another dropper without previously checking the volume of the drop.

Available packaging

- Kit 50 tests, Cat. No. 23 038006.
  Contains: 1 x 1.4 mL reagent, 1 x 1 mL positive control, 1 x 1 mL negative control and 9 disposable slides with 6 sections each.
- Kit 150 tests, Cat. No. 23 038007.
  Contains: 3 x 1.4 mL reagent, 1 x 2 mL positive control, 1 x 2 mL negative control and 27 disposable slides with 6 sections each.
- Kit 1000 tests, Cat. No. 23 038008.
  Contains: 20 x 1.4 mL reagent, 2 x 2 mL positive control and 2 x 2 mL negative control and 180 disposable slides with 6 sections each.

Material required but not provided

- Normal saline (0.9% NaCl, only for semiquantitative test).
- Automatic pipettes.
- Disposable stirrers.
- Rotator.
- Timer.
- Disposable slides (not provided in Cat. No. 23 038008).

Sample collection

Serum:
Use fresh serum collected by centrifuging clotted blood. If the test cannot be carried out on the same day, it may be stored between 2 and 8°C for no longer than 8 days after collection. For longer periods the samples must be frozen (-20°C).

Plasma:
Collect blood into a tube containing anticoagulant (EDTA). Other anticoagulants should be evaluated before use. Centrifuge to separate plasma from cellular elements. Test the specimen within 24 hours of blood collection.

Do not use hemolyzed or contaminated samples.

Procedure

PREVIOUS MANIPULATIONS

Control of the latex reagent:
- Before performing a set of determinations it is advisable to check the latex reagent with each of the controls, positive and negative, included in the kit.
- Both controls should be used following the steps outlined in the QUALITATIVE TEST.
- The reaction between the positive control and the reagent should show a clear agglutination, different from the uniform appearance of the negative control. If no agglutination takes place, the test should be repeated, and the kit discarded if there is no positive reaction.

QUALITATIVE TEST

- Allow reagents and samples to reach room temperature (20 to 30°C).
- Gently shake the reagent vial to disperse and suspend the latex particles in the buffer solution. Vigorous shaking should be avoided.
- Place 50 µL of the sample onto one section of the disposable slide.
- Place one drop of reagent next to the drop of sample.
- Mix both drops with a stirrer covering the whole surface of the slide section.
- Gently rotate the slide for 3 minutes manually or on a rotary shaker set at 80-100 rpm.
- Look for the presence or absence of agglutination after the aforementioned period of time.

Interpretation of results

The presence of agglutination indicates a clinically significant concentration of infectious mononucleosis heterophile antibodies in the sample.

POSITIVE REACTIONS:

3+ Large clumping with clear background.
2+ Moderate clumping with fluid slightly opaque in background.
1+ Small clumping with opaque fluid in background.

NEGATIVE REACTIONS:

No visible clumping, uniform suspension.

SEMIQUANTITATIVE TEST

Allow reagents and samples to reach room temperature (20 to 30°C).
Preparation of the sample dilutions on slide (see descriptive diagram):

- Place 50 µL of normal saline on slide sections 2 through 6.
- Using an automatic pipette, place 50 µL of the sample onto slide section 1 and 50 µL directly into the drop of normal saline on slide section 2.
- Using the same pipette take in and release several times the mixture made on section 2 and transfer 50 µL of the mixture to section 3. Repeat in this manner serially through section 6, discarding 50 µL from section 6.

Section	1	2	3	4	5	6
Saline µL	-	50	50	50	50	50
Serum µL	50	50	-	-	-	-
Mix and transfer µL			50	50	50	50	50 →
Dilution	1:1	1:2	1:4	1:8	1:16	1:32

- Gently shake the reagent vial and add one drop of reagent to each section
- Mix both drops using a stirrer covering the whole surface of the slide section.
- Gently rotate the slide for 3 minutes manually or on a rotary shaker set at 80-100 rpm.
- Look for the presence or absence of agglutination after the aforementioned period of time.

Interpretation of the results

The approximate titer will correspond to the highest sample dilution that still presents a clearly visible agglutination (see diagram).

Limitations of the procedure

- As with all diagnostic assays, the results of the **Sure-Vue® Mono** assay should be interpreted in light of the clinical symptoms shown by the patient.
- Occasionally detectable levels of heterophile antibodies are late in developing in patients symptomatic for infectious mononucleosis. If symptoms persist it is recommended to repeat the assay in several days.
- Although titers of heterophile antibodies have little relation with the severity of infection, the semiquantitative procedure can be used to follow the evolution of the disease.

Expected values

Different studies[11,12] of presence of infectious mononucleosis heterophile antibodies in blood donors show incidence of the disease in from 0.9 to 1.7% of population. As presence of antibodies indicates a relatively recent infection, these results suggest that the true incidence of the disease is higher than the diagnosed cases.