Cross-reactivity

Samples negative to IM heterophile antibodies by another technique and positive to antibodies of other viral infections were tested with **Sure-Vue® Color Mono** in order to determine possibility of cross reactivity. Samples positive to IgM CMV (10 samples), IgM Toxoplasmosis (13 samples) and Rheumatoid Factor (11 samples) did not show any reactivity to IM heterophile antibodies.

Reproducibility

Reproducibility studies were conducted using an in-house IM heterophile antibody calibrator diluted from 1:1 to 1:32 in saline solution and tested by three different operators on 5 consecutive days twice a day following the semiquantitative procedure. The kit controls (negative and positive) were also tested following the qualitative procedure. Accepting only one dilution of difference between days, times and operators, the results indicated that the **Sure-Vue® Color Mono** semiquantitative and qualitative tests gave 100% reproducibility.

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Sure-Vue® Color Mono

Sure-Vue® Color-Mono is a simple color-enhanced slide test for the qualitative and semiquantitative detection of infectious mononucleosis heterophile antibodies in serum or EDTA plasma.
Sure-Vue® Color Mono aids in the diagnosis of infectious mononucleosis.

Summary

Infectious mononucleosis (IM) is an acute infectious disease of viral etiology. In 1968, the etiologic agent of infectious mononucleosis was described.[1] It was called the Epstein-Barr virus (EBV), a member of the herpes virus group.
In 1932, Paul and Bunnell[2] reported that serum samples from patients with IM have heterophile antibodies to sheep erythrocytes. Also were described agglutinins to red blood cells from other mammals. The proteins responsible for this agglutination are glycoproteins from red cell membranes called Paul-Bunnell antigen by several authors. Heterophile antibodies to sheep erythrocytes, which are different from those present during IM, may also be detected in sera from normal people, from individuals who have received injections of serum, and others.[2-4]
The diagnosis made on clinical history and symptomatology alone is difficult. Numerous cases in which IM has been misidentified with other non-related viral and bacterial diseases have been cited.[4] For this reason, hematologic and serologic tests are very helpful in diagnosis. Traditionally the IM heterophile antibodies have been distinguished from other heterophile antibodies by a "differential" absorption test[4,5] with bovine red blood cells and guinea pig kidney tissue. In 1968, Lee *et al* have shown that horse erythrocytes contribute to higher sensitivity and specificity than do sheep erythrocytes in the IM heterophile antibodies detection.[6,7] Now, the use of chemical treated horse erythrocytes provides a simple method with improved sensitivity for the specific detection of IM heterophile antibodies.

Principle

The **Sure-Vue® Color Mono** reagent is a suspension of specially treated horse red blood cells. Added coloration of the suspension facilitates the recognition of positive and negative reactions.
The serum or plasma being tested is mixed on a test slide with the reagent. The appearance of dark agglutinates against a blue-green background indicates the presence of IM. If no heterophile antibodies are present, the horse cells remain unagglutinated against a green-brown background.

Reagents

a) **Red cells reagent:**
Color-enhanced treated horse red blood cells suspension.
Contains sodium azide 0.1%.

b) **Positive control:**
Diluted positive human serum.
Contains sodium azide 0.1%.

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

Precautions

Sure-Vue® Color Mono is intended for IN VITRO diagnostic use.
The reagents contain sodium azide as a preservative. Azides may react with metal plumbing, forming explosive components. Upon disposal, please flush abundantly with water.
All human source material used in the preparation of reagents has been tested by an FDA approved method for the presence of HIV 1/2 and HCV antibodies, as well as for HBsAg and found to be negative.

WARNING: POTENTIALLY BIOHAZARDOUS MATERIAL.
Because no test method can offer complete assurance of the absence of infectious agents, the reagents should be handled carefully.[8]
Do not exchange components of different kits.
Dispose all used materials in a suitable biohazardous waste container.

Storage

The reagents will remain stable through the expiration date, shown on the label, if stored between 2 and 8°C. Do not freeze.
When stored the reagents containing red cells show a complete sedimentation. Once shaken an uniform suspension should be obtained.

Available packaging

- Kit 24 tests, Cat. No. 23 038016.
  Contains: 1 x 0.7 mL reagent, 1 x 1 mL positive control, 1 x 1 mL negative control, and 9 disposable slides with 3 circles each.

- Kit 50 tests, Cat. No. 23 038017.
  Contains: 1 x 1.4 mL reagent, 1 x 1 mL positive control, 1 x 1 mL negative control, and 18 disposable slides with 3 circles each.

Material required but not provided

- Normal saline (0.9% NaCl, only for semiquantitative test).
- Automatic pipettes.
- Rotator.
- Stirrers.
- Timer.

Sample collection

Use fresh serum or plasma (EDTA). Samples may be stored at 2-8°C for up to 72 hours. For longer periods, samples should be frozen (-20°C).

Procedure

Quality control: Before performing a set of determinations, it is recommended to test the reagent with each of the two controls included in the kit, following the steps outlined in the QUALITATIVE TEST section below. The reaction between the positive control and the reagent should show a clear agglutination, different from the uniform appearance of the negative control. **If these results are not obtained, do not use the kit.**

To assure proper delivery the reagent dropper must be held vertically and a single drop allowed to fall.

QUALITATIVE TEST

- Allow the reagents to reach room temperature (20 to 30°C).
- Place 50 µL of the sample (or a drop of control) onto one circle of the slide.
- Shake the reagent vial and add one drop of reagent next to the drop of sample.
- Mix both drops with a stirrer covering the whole surface of the circle.
- Rotate the slide on a rotary shaker set at 80-100 rpm or manually rock the slide slowly and gently for ONE MINUTE. Then allow it to remain UNDISTURBED on a flat surface for an additional one minute.
- WITHOUT DISTURBING THE SLIDE, examine immediately for agglutination and record the results.

Interpretation of results

Dark agglutination against a blue-green background constitutes a positive result and indicates the presence of IM heterophile antibodies. No agglutination of the cells against a green-brown background constitutes a negative result.

POSITIVE REACTIONS:

3+ Large clumping with clear blue-green background.
2+ Moderate clumping with blue-green background.
1+ Small clumping with green-brown background.

NEGATIVE REACTIONS:

Roughness or not visible clumping with green-brown background.

SEMIQUANTITATIVE TEST

There is no correlation between severity of illness and the level of IM heterophile antibodies, but sequential titration may provide helpful information to the clinician in following the course of the disease.

Make two-fold serial dilutions of the sample in normal saline, starting at the 1:2 dilution (see the descriptive diagram below for the procedure):

- Place 50 µL of normal saline onto each one of the circles 1 through 6 (use two disposable slides).
- Using an automatic pipette, place 50 µL of the sample (or a drop of control) into the drop of normal saline on circle 1.
- Using the same pipette aspirate and expel several times the mixture on circle 1. Take 50 µL of the mixture made on circle 1 and transfer it to circle 2.
- Repeat the aforementioned operations to obtain a thorough mixing of reagents, through circle 6, thereafter discarding 50 µL.

Circle	1	2	3	4	5	6
Saline µL	50	50	50	50	50	50
Sample µL	50	-	-	-	-	-
Mix and transfer µL		50	50	50	50	50	50 →
Dilution	1:2	1:4	1:8	1:16	1:32	1:64

- Test each dilution as described in the QUALITATIVE TEST section.

Interpretation of results

The IM heterophile antibodies titer is the highest dilution showing a positive result.

Limitations of the procedure

- The results of the **Sure-Vue® Color Mono** assay should be interpreted in light of the clinical, hematological and serological information of the patient.
- Occasionally detectable levels of heterophile antibodies are late in developing in patients symptomatic for IM. If symptoms persist it is recommended to repeat the assay in several days. Some patients may remain persistently negative, especially children and adolescent. It has been reported that only 80 to 90% of adults and less than 50% of young children develop IM heterophile antibodies.[9-11]
- Detectable levels of heterophile antibodies may persist for months, and more rarely for years, in some individuals.[5,9]
- The time limits prescribed in the procedure must be strictly observed.
- Assay performance characteristics have not been established for matrices other than serum and EDTA plasma.
- Disturbing the slide when interpreting the results may cause agglutination to be dispersed.

Expected values

Different studies[12] of the presence of IM heterophile antibodies in blood donors show that the incidence of the disease ranges from 0.9 to 1.7%. As presence of IM heterophile antibodies indicates a relatively recent infection, these results suggest that the true incidence of the disease is higher than the number of diagnosed cases. In a study performed with **Sure-Vue® Color Mono** using 99 blood bank sera from a Center of Barcelona, the incidence of positive results was 3%.

Performance characteristics

The sensitivity of **Sure-Vue® Color Mono** was qualitatively tested using 48 samples presumptively positive for IM heterophile antibodies and compared to a commercially available horse red cell slide test. The sensitivity of **Sure-Vue® Color Mono** relative to the red cell slide test was 97.9% (95% Confidence Interval = 88.7 - 99.9%).
The specificity of **Sure-Vue® Color Mono** was qualitatively tested using 200 randomly selected serum patient samples presumptively negative for IM heterophile antibodies. The specificity of **Sure-Vue® Color Mono** relative to the horse red cell slide test was 95.8% (95% Confidence Interval = 91.9 - 98.2%).