Sure-Vue® Rubella

Sure-Vue® Rubella is a rapid latex particle agglutination test for the qualitative and quantitative determination of rubella virus antibodies in serum. The test aids in the diagnosis of recent or active rubella infection and determination of immune status.

Summary

Rubella virus, the etiological agent of German measles, generally causes a mild viral disease which sometimes resembles common measles, but with none of the serious consequences often seen in young measles patients. When contracted in the first trimester of pregnancy, however, rubella may infect the fetus through the placenta causing deafness, cataracts, microcephaly and/or cardiac abnormalities in addition to hepatosplenomegaly, icterus, thrombocytopenic purpura, anemia and low birth weight. These multiple abnormalities are commonly referred to as a congenital rubella syndrome. Other consequences of rubella infection during pregnancy may include spontaneous abortion, miscarriage and stillbirth.[1-4]

The availability of an attenuated rubella virus vaccine has greatly reduced the natural incidence of rubella infection. Nevertheless, it is recommended that all women of childbearing age be tested for the presence of rubella antibodies to assure that nonimmune individuals are detected and subsequently vaccinated.

Patient immune status to the rubella virus has been determined for many years using various serological methods. One widely accepted method is the hemagglutination inhibition test (HAI).[4,5] Latex agglutination, in comparison with HAI, is quicker and easier to perform.[6]

The sensitivity of the **Sure-Vue® Rubella** kit is 1-2 IU/mL when performed with undiluted serum. This is greater than the sensitivity of the HAI at a 1:8 dilution. The sensitivity of the **Sure-Vue® Rubella** kit is 10-20 IU/mL when run with serum diluted 1:10. This is approximately equal to the sensitivity of the HAI at 1:8.

Principle

The **Sure-Vue® Rubella** reagent is a suspension of polystyrene latex particles of uniform size coated with soluble rubella virus antigen from disrupted virus. Latex particles allow visual observation of the antigen-antibody reaction. When a serum containing rubella virus antibodies is mixed with the latex reagent, the uniform appearance of the latex suspension will convert to a visible agglutination.

Reagents

a) **Latex reagent:**
Suspension of polystyrene latex particles coated with soluble rubella virus antigen from disrupted virus in a buffer.
Contains sodium azide 0.1%.

b) **High positive control:**
Human serum diluted to a titer of 1:160.
Contains sodium azide 0.1%.

c) **Low positive control:**
Human serum diluted to a titer of 1:10.
Contains sodium azide 0.1%.

d) **Negative control:**
Diluted non-immune human serum.
Contains sodium azide 0.1%.

e) **Dilution buffer:**
Phosphate buffered saline pH 7.2, containing bovine serum albumin.
Contains sodium azide 0.1%.

Precautions

Sure-Vue® Rubella is intended for IN VITRO diagnostic use.
The reagents and controls 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 throughly after disposing of fluids containing sodium azide.
The vaccine virus strain used in the preparation of **Sure-Vue® Rubella** latex reagent has been previously disrupted. Bioassay procedures demonstrate that disrupted virus is inactivated. However it is recommended that users follow the same safety regulations in effect for the handling of other types of potentially infectious material.
Each donor unit used in the preparation of the controls of this kit was tested by an FDA approved method for the presence of HIV1/2 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 serum samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual.[7]

Storage and handling of reagents

Reagents and controls will remain stable through the expiration date shown on the label when stored between 2 and 8°C. Do not freeze. The reagents can be damaged, or the latex sensitivity can be altered, by improper storage and/or handling. Testing with the positive and negative controls will permit detection of reagent deterioration.
To maintain optimal sensitivity, the **Sure-Vue® Rubella** kit should be removed from the refrigerator approximately 5 minutes prior to use. Warm the latex reagent by rolling vial between hands. Replace in 2-8°C immediately after use. When resuspending latex, do not shake. Invert the vial several times until the latex reagent is uniformly suspended without visible clumping. When stored, a slight sedimentation may occur with latex reagent and should be considered normal. The reagents should be discarded after the expiration date.
Although the reagents and controls contain preservatives, they remain sensitive to contamination. Handle with the necessary precautions. Discard latex reagent or controls if they become contaminated.
The reagent dropper dispenses drops of 15 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 100 tests, Cat. No. 23 038004.
Contains: 1 x 1.6 mL reagent, 1 x 1 mL high positive control, 1 x 1 mL low positive control, 1 x 1 mL negative control, 1 x 35 mL diluent buffer, 120 plastic stirrers and 8 disposable slides with 15 sections each.

Kit 500 tests, Cat. No. 23 038005.
Contains: 5 x 1.6 mL reagent, 2 x 1 mL high positive control, 2 x 1 mL low positive control, 2 x 1 mL negative control, 2 x 35 mL diluent buffer, 600 plastic stirrers and 40 disposable slides with 15 sections each.

Material required but not provided

- Rotator.
- Automatic pipettes.
- Timer.

Sample collection

Use fresh serum collected by centrifuging clotted blood.
If the test cannot be carried out on the same day, the serum must 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).
It is not necessary to inactivate the serum.
As in all serological tests, hemolytic or contaminated serum must not be used.
Do not use plasma.

For diagnosis of rubella infection, paired sera (acute and convalescent) should be obtained. The acute sera should be collected as soon after rash onset as possible or at the time of exposure and the convalescent sera should be obtained 10-21 days after the onset of rash or at least 30 days after exposure if no clinical symptoms appear. Acute and convalescent sera should be tested simultaneously for antibodies to rubella using the quantitative procedure.
For qualitative antibody assay a single sample is sufficient.[1]

Procedure

QUALITATIVE TEST

It is the opinion of the Centers for Disease Control Immunization Practices Advisory Committee that any detectable antibody level should be considered presumptive evidence of immunity.[1] Therefore, this kit can be used to screen undiluted serum. Using undiluted serum the kit sensitivity will be 1-2 IU/ml (more sensitive than HAI at 1:8). Also this kit can be used with serum diluted 1:10. Using serum diluted 1:10 will decrease kit sensitivity to 10-20 IU/ml (equivalent to HAI at 1:8). Each individual laboratory should determine which procedure it will use to determine immune status of patients.

- Remove reagents from refrigeration approximately 5 minutes prior to use, warm the latex reagent to room temperature by rolling vial between hands.
- Label test card for each sample and control to be tested.
- Invert the latex vial several times to disperse and suspend the latex particles. Vigorous shaking should be avoided.
- Before performing a series of determinations check the latex reagent with the low positive and negative controls included in the kit.

  When testing undiluted specimens, the low positive control and negative control should be used undiluted by following the procedure outlined in steps 1a-5 below. The low positive control should show agglutination different from the uniform appearance of the negative control. If no agglutination takes place the test should be repeated, and if there is no positive reaction the kit should be discarded.

  When testing specimens at a 1:10 dilution, the low positive control should be used diluted 1:10 by following the procedure outlined in steps 1b-5 below. The negative control needs not be diluted for testing. The low positive control should show agglutination different from the uniform appearance of the negative control. If no agglutination takes place, the test should be repeated with the 1:5 dilution of the low positive control prepared as in section 1b. If there is positive reaction, continue testing specimens as the low positive control is formulated to produce agglutination at a titer of 1:10 ± one dilution. If there is no positive reaction the kit should be discarded.

1a. **For undiluted specimens.**
Place 25 µl of the sample (or a drop of control) onto one of the circles of the qualitative disposable slide.

1b. **For a 1:10 specimen dilution.**
- Prepare a 1:5 dilution of the sample (or low positive control) on the qualitative disposable slide by pipetting 100 µl of the dilution buffer and 25 µl of the sample (or a drop of control) into the square section of the slide and mix several times with the same pipette (the negative control needs not be diluted before testing).
- Place 25 µl of the dilution buffer on the circle beside the square section.
- Transfer 25 µl of the 1:5 dilution from the square section into the dilution buffer and mix several times with the same pipette. Discard 25 µl from the circle.

2. Using a new plastic stirrer for each circle, spread the sample over the entire surface of the circle.
3. Dispense one drop of the latex reagent onto each circle containing the sample.
4. Rotate the slide for 8 minutes on a rotatory shaker set at 100 rpm. To prevent drying, place a moistened humidifying cover over the card prior to rotation.
5. Immediately following the 8 minutes rotation, read for the presence or absence of agglutination.

Interpretation of the results

The presence of any visible agglutination, significantly different from the negative control, indicates the presence of antibodies against rubella virus in the serum sample. This indicates previous exposure to the rubella virus. A qualitative test performed on a single serum sample can be used to estimate the immune status of the individual.
When a negative result is obtained on undiluted serum, the sample should be retested at 1:10, as occasionally a decrease in the degree of agglutination has been reported with high titered specimens.[8,9] High titered specimens, when tested undiluted, may cause the migration of agglutinated particles to the periphery of the circle.
When the **Sure-Vue® Rubella** assay is initially performed on samples which have been diluted 1:10, the sensitivity obtained is approximately equal to that obtained with the HAI test at 1:8. The data collected will correlate with that obtained using hemagglutination inhibition assays. This protocol will fail to detect low levels of antibodies found in samples that are positive undiluted.[10]

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.

QUANTITATIVE TEST

- Remove reagents from refrigeration approximately 5 minutes prior to use, warm the latex reagent to room temperature by rolling vial between hands.
- Label test card appropriately for each control and sample to be tested.
- Invert the latex vial several times to disperse and suspend the latex particles. Vigorous shaking should be avoided.
- The high positive and low positive controls should be treated as if they were samples by following steps 1-7 outlined below. Substitute one drop of control for the 25 µL of patient specimen.
- The negative control should be tested undiluted.

1. Prepare a 1:5 dilution of the sample (or control) on a square section of the quantitative disposable slide by pipetting 100 µL of the dilution buffer and 25 µL of the sample (or a drop of control) and mix several times with the same pipette.
2. Place 25 µL of the dilution buffer on the circles marked 1:10 to 1:160 of the quantitative slide.
3. Transfer 25 µL of the 1:5 dilution from the square section to the circle marked 1:5.
4. Using the same pipette, transfer 25 µL of the 1:5 dilution from the square section directly into the buffer in circle marked 1:10 and mix several times with the same pipette. The serum in this circle is now a 1:10 dilution.
5. With the same pipette, transfer 25 µL of the 1:10 dilution into the buffer in circle marked 1:20, and mix.
6. Repeat step 5 in succesion through circle marked 1:160.
7. Discard 25 µL from circle marked 1:160.

	Undiluted	1:5	1:10	1:20	1:40	1:80	1:160
PATIENT SAMPLE *	NA	P	P	P	P	N	N
HIGH POSITIVE	NA	P	P	P	P	P	P
LOW POSITIVE CONTROL	NA	P	P	N	N	N	N
NEGATIVE CONTROL	N	NA	NA	NA	NA	NA	NA

NOT APPLICABLE = NA NEGATIVE = N POSITIVE = P

* This patient sample has an antibody titer of 1:40

8. Using a new plastic stirrer for each sample and control, spread the serum or control dilutions over the entire surface of the circle starting at the highest dilution. Using the same stirrer proceed to the next lower dilution and spread the serum dilution in a similar way. Repeat this procedure until the contents of all circles are spread.
9. Dispense one drop of the latex reagent onto each of the different circles containing the serum dilutions.
10. Rotate the slide for 8 minutes on a rotatory shaker set at 100 rpm. To prevent drying, place a moistened humidifying cover over the card prior to rotation.
11. Immediately following the 8 minute rotation, read for the presence or absence of agglutination.

Interpretation of the results

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

The high positive control should show agglutination at a titer of 1:160 or greater. The low positive control should show agglutination at a titer of 1:10 ± one dilution. The negative control should show no agglutination.

When the quantitative test is performed with an acute and convalescent serum from the same patient, a four-fold or greater rise in antibody titer or seroconversion is indicative of a primary or recent rubella infection. Also a seroconversion may be seen after a vaccination procedure. Some persons previously exposed to rubella may demonstrate a rise in antibody titer. This is thought to represent reinfection and these patients rarely develop symptoms.[11]

Limitations of the procedure

- Test results obtained with **Sure-Vue® Rubella** must be evaluated by the physician in light of the clinical symptoms shown by the patient.
- **Sure-Vue® Rubella** has been tested for the detection of rubella antibodies in serum. Performance with plasma has not been established.
- To verify that the procedure works properly the use of positive and negative controls is recommended.
- Acute and convalescent sera must be tested simultaneously. The absence of a four-fold titer rise does not exclude the possibility of exposure and infection.

Expected values

A positive qualitative test on either undiluted samples (≥1-2 IU/mL) or samples diluted 1:10 (≥10-20 IU/mL and equivalent to the HAI test at 1:8) indicates previous infection with rubella virus. Each individual laboratory must determine the antibody level which it considers clinical protection against future rubella infection. A true negative result (no prozone) using undiluted samples indicates the absence of antibodies to the rubella virus (<1-2 IU/mL). A negative result using samples diluted 1:10 indicates that antibodies to rubella virus are absent or at a level <10-20 IU/mL.
The diagnosis of primary or recent rubella infection is made by comparing antibody titers in paired sera. The timing of sample collection in paired sera is critical. The first sample (acute sera) should be collected as soon after rash onset as possible or at the time of exposure, while the second sample (convalescent sera) should be obtained 10-21 days after the onset of rash or, at least 30 days after exposure, if no clinical symptoms appear. Acute and convalescent phase sera should be quantitatively analyzed simultaneously for antibodies to rubella along with positive and negative controls. A four-fold or greater titer rise between acute and convalescent sera is indicative of a primary or recent rubella infection. In unresolved cases testing for the presence of rubella IgM is recommended as an additional indicator of infection.[1,6]

Performance characteristics

QUALITATIVE TEST:

1. A clinical study of **Sure-Vue® Rubella** was conducted at the University of Connecticut Health Center. A total of 282 sera were assayed with **Sure-Vue® Rubella** using a 1:10 dilution, and the results compared to HAI assay (see Table I).

TABLE I
Sure-Vue® Rubella 1:10 vs. HAI assay

		Sure-Vue® Rubella (1:10)	
		POS	NEG
HAI	POS	204	1
(1:8)	NEG	3	74

HAI= One day old chick red blood cells; $MnCl_2$ absorption.
Sensitivity = 204/205 = 99.5%
Specificity = 74/77 = 96.1%

2. 162 of the 282 sera were also assayed with **Sure-Vue® Rubella** using the undiluted procedure and the results compared to these obtained with HAI (see Table II).

TABLE II
Sure-Vue® Rubella 1:10 and 1:1 vs. HAI assay

		Sure-Vue® Rubella (1:10)		**Sure-Vue® Rubella (1:1)**	
		POS	NEG	POS	NEG
HAI	POS	110	0	110	0
(1:8)	NEG	3	49	12	40

Sensitivity = 110/110 = 100% 110/110 = 100%
Specificity = 49/52 = 94% 40/52 = 77%

3. When the results obtained using **Sure-Vue® Rubella** on a 1:10 dilution of these same 162 clinical specimens were compared to those obtained using another commercially available latex rubella test (also at 1:10 dilution), a sensitivity and specificity of 100% was obtained.
4. A separate clinical study was conducted at Saint Francis Hospital Medical Center in Connecticut on 143 clinical serum samples comparing the results obtained with **Sure-Vue® Rubella** (using a 1:10 serum dilution) to those obtained with HAI.

Combining these two studies, a total of 425 sera were tested with the following results (see Table III).

TABLE III
Sure-Vue® Rubella 1:10 vs. HAI assay.
OVERALL (UCHC AND SFHMC)

		Sure-Vue® Rubella (1:10)	
		POS	NEG
HAI	POS	308	2
(1:8)	NEG	11*	104

Sensitivity = 308/310 = 99.4%
Specificity = 104/115 = 90.4%
True specificity* = 104/112 = 92.9%

* Upon retesting by EIA protocol:
3 confirmed positive
7 confirmed negative
1 QNS for confirmation

QUANTITATIVE TEST:

Clinical studies performed at two medical centers on a total of 100 sera compared titers obtained with **Sure-Vue® Rubella** to those obtained using HAI. 75% of sera were within ≤ 1 dilution interval, 96% of sera were within ≤ 2 dilution intervals and 100% of sera were within ≤ 3 dilution intervals.
Day to day reproducibility studies were conducted at two different medical centers. A panel of 6 sera with titers ranging from negative to 1:160 by HAI were assayed with one lot of **Sure-Vue® Rubella** using the quantitative procedure during three consecutive days. Results were 100% reproducible within one dilution.
Sure-Vue® Rubella results were studied using paired sera from 10 naturally occurring infections. All 10 serum pairs showed a four-fold or greater rise in titer. Four additional patients who received rubella vaccine were studied. Seroconversion was detected in all pairs of sera.

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