Table 8

Sample	Expected result	Laboratory 1			Laboratory 2			Laboratory 3		
		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
R08	Negative	N	N	N	N	N	N	N	N	N
9215	Weak positive	2+	1+	1+	2+	2+	2+	1+	1+	1+
0827 (R2)	Strong positive	3+	3+	3+	3+	3+	3+	3+	3-4+	3-4+
9061 (R12)	Moderate positive	2+	2+	2+	2+	2+	2+	1-2+	1+	1+
61084K	Positive	3+	2+	3+	3+	3+	3+	3+	3+	2-3+

These data indicate that the day-to-day, batch-to-batch, and person-to-person reproducibility of the **Sure-Vue® Select Rubella** with various rubella samples is good. Also, the results from different laboratories are comparable with the panel of rubella samples.

D. Precision around the cutoff value:

To assess the precision of the **Sure-Vue® Select Rubella** to detect rubella specific antibodies at or near the cutoff value for the sensitivity of the latex reagent (approximately 10 IU/mL) five samples with antibody concentrations ranging from approximately 5.0 to 20 IU/mL were tested in triplicate. Test results were interpreted independently by two technologists. A summary of the test results is presented in Table 9.

Table 9

Sample No.	Approximate concentration (IU/mL)*	Test result		
793	10	1+	1+	1+
9061	10-20	1+	1+	1+
F13151	10-20	1+	1+	1+
CDC Rubella Standard	10	1+	1+	1+
CDC Rubella Standard	5	N	N	N

* The concentration of antibodies (IU/mL) was determined using the semi-quantitative procedures of two latex agglutination tests.

References

1. Center for Disease Control. Recommendation of the Immunization Practices Advisory Commitee (ACIP). Morbidity and Mortality Weekly Report 33: 301-318, 1984.
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4. Biosafety in Microbiological and Biomedical Laboratories. CDC/NIH manual, 5th Edition, 2007.

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Sure-Vue® Select Rubella

The Sure-Vue® Select Rubella detects both IgG and IgM anti-rubella virus antibodies in clinical specimens. When used qualitatively, the test determines the immune status of patients. When used semi-quantitatively with paired sera, the test aids in the diagnosis of recent or current infections.

Summary

Due to the potential complications caused by a rubella virus infection during pregnancy, it is recommended that all women of child-bearing age be tested for immunity to rubella virus. According to CDC and NCCLS guidelines, if a serum sample contains at least 10 IU/mL of rubella-specific antibody, the donor is considered immune. Individuals with less than 10 IU/mL of specific antibody should be vaccinated with an attenuated strain of rubella virus.[1]

The immune status of an individual typically is determined by testing an appropriately collected specimen using hemagglutination inhibition (HAI), enzyme immunoassay (EIA), or latex agglutination (LA).[2,3] The **Sure-Vue® Select Rubella** is a rapid latex agglutination test in which latex particles are coated with rubella virus antigens. When the latex reagent is mixed with a sample containing rubella specific antibodies at a concentration of approximately 10 IU/mL or greater, agglutination of latex particles will be observed. If the amount of specific antibodies is insufficient or in the absence of specific antibodies, agglutination of latex particles will not be observed.

Principle

The latex reagent is prepared by coating latex particles with rubella viral antigens. When the latex reagent is mixed with specimens containing a sufficient quantity of rubella-specific antibodies, a detectable agglutination of latex particles occurs.

Reagents

REF. 3018-4011

a) **Latex reagent:** 1 x 1.6 mL.
Latex particles coated with rubella virus (Strain HPV-77, ≥ 90% purity) antigen and suspended in a buffer containing bovine serum albumin and 0.1% sodium azide.

b) **High positive control:** 1 x 1.5 mL.
Human serum with a titer of 240 ± 80 IU/mL as determined by comparison with the 2nd WHO Rubella Reference Standard. Contains 0.1% sodium azide.

c) **Low positive control:** 1 x 1.5 mL.
Human serum with a titer of 30 ± 10 IU/mL as determined by comparison with the 2nd WHO Rubella Reference Standard. Contains 0.1% sodium azide.

d) **Negative control:** 1 x 1.5 mL.
Non-immune human serum. Contains 0.1% sodium azide.

e) **Diluent:** 1 x 15 mL.
Phosphate buffered saline containing bovine serum albumin and 0.1% sodium azide.

f) **Disposable slides:** 13 units with 8 sections each.

Precautions

Sure-Vue® Select Rubella is intended for IN VITRO diagnostic use.
The rubella virus used to prepare the latex reagent is disrupted prior to use. The results of bioassay procedures indicate that the disrupted virus is inactivated. However, it is recommended that users follow safe laboratory practices when handling reagents and serum controls provided with each test kit. Sera used in the preparation of controls were tested with FDA approved reagents and were found to be negative for HBsAg and antibodies to HIV and hepatitis C virus. Since no test method can offer complete assurance that HIV, hepatitis B virus, hepatitis C virus and other infectious agents are absent, controls included in the **Sure-Vue® Select Rubella** kit should be handled as recommended for any potentially infectious human serum or blood specimen (Refer to the Centers for Disease Control/National Institutes of Health manual for specific instructions).[4]

The latex reagent and serum controls contain sodium azide as a preservative. Lead and copper in laboratory plumbing can react with sodium azide to produce lead and copper azides which can explode on percussion. After disposing of reagents containing sodium azide, drains should be flushed thoroughly with water.
Do not use beyond the expiration date. Do not mix reagents from different kit lot numbers.

Storage

Reagents should be stored at 2 - 8°C when not in use. If stored at 2 - 8°C, reagents will perform as expected through the expiration date shown on the label.

Exposure to adverse storage temperatures may affect the performance of reagents and controls. The latex reagent should not be frozen or stored at temperatures greater than 25°C.

For optimal performance, allow test kit reagents to warm to room temperature (18 - 30°C) before testing controls and clinical specimens. During storage, latex particles in the latex reagent will settle out. Before using, gently shake the bottle containing the latex reagent to resuspend latex particles. Inspect the bottle for visible signs of latex clumping.

The latex reagent, serum controls and the diluent should be handled with the necessary precautions to prevent microbial contamination. Discard reagents and controls that become contaminated.

Materials required but not provided

- Rotator (optional, flat bed, 100 rpm).
- Timer.
- Calibrated pipettes, 25 µL and 100 µL.

Specimen collection

A venous blood sample should be collected in a tube which does not contain an anticoagulant. Allow blood to clot completely. Clotted blood should be centrifuged. Serum may be stored at 2 - 8°C for up to 48 hours after collection. For periods longer than 48 hours, serum must be stored frozen at ≤ -20°C.

Hemolyzed or contaminated specimens may interfere with the performance of the test. Hemolyzed and contaminated specimens should be discarded. **NOTE: Do not use heat inactivated samples. When performing the qualitative procedure, do not dilute specimens.**

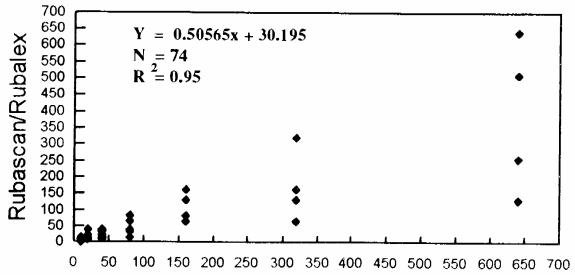
For diagnosis of current or recent rubella infection, obtain paired sera (acute and convalescent). The acute specimen should be collected as soon as possible after the onset of a rash or at the time of exposure. The convalescent specimen should be obtained from 10-21 days following observation of a rash or at least 30 days after exposure in the absence of clinical symptoms associated with a rubella infection. Acute and convalescent specimens should be tested simultaneously for antibodies to rubella virus using the semi-quantitative procedure. For the qualitative antibody test, a single specimen is sufficient.

Quality control

Each **Sure-Vue® Select Rubella** kit contains a high positive control, a low positive control and a negative control. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff. Controls should be tested with each test run. A test run may be defined as a period of up to 24 hours. Positive controls must give the expected agglutination pattern when mixed manually or with a mechanical rotator for 5 minutes. When mixed with the negative control (manually or with a mechanical rotator for 5 minutes), the latex reagent should not exhibit agglutination. If the test performance with the controls is not as expected, the test run is invalid and the results with clinical specimens should not be reported. Additional controls may be tested in accordance with the guidelines of local, state, and/or federal agencies or accrediting organizations.

Positive controls may be used to monitor the performance of test kit reagents for the duration of the shelf life assigned to the reagents by the manufacturer. Controls should be tested as described for a patient sample. Results should be read as described in the "Interpretation" section of this package insert. When the semi-quantitative procedure is performed, the endpoint for each positive control should be within one dilution step of the titer listed on the Test Control Data Card provided with the test kit. If the dilution obtained differs more than one dilution step from the expected value for each positive control, testing should be repeated to verify endpoints. If the reagents do not perform as expected, it is recommended that you discontinue using the reagents to test clinical samples and immediately contact Technical Services.

Figure 1



Sure-Vue® Select Rubella

REPRODUCIBILITY

A. Person-to-person:

A panel comprised of one non-reactive and four reactive samples was tested by at least two technologists at each of the clinical sites. Each technologist tested each sample in triplicate. A summary of the results are shown in Table 6.

Table 6

Sample	Expected result	Laboratory 1		Laboratory 2		Laboratory 3	
		Tech 1	Tech 2	Tech 1	Tech 2	Tech 1	Tech 2
R08	Negative	N	N	N	N	N	N
9215	Weak positive	±	N	2+	2+	1+	1+
0827 (R2)	Strong positive	3+	3+	3+	3+	2+	3+
9061 (R12)	Moderate positive	2+	1+	2+	2+	1+	2+
61084K	Positive	1+	2+	3+	3+	2+	3+

B. Lot-to-Lot:

At each clinical site, a technologist evaluated the performance of a test kit from each of three production lots of **Sure-Vue® Select Rubella** kits. A panel comprised of one non-reactive and four reactive samples was tested in triplicate. A summary of the results are shown in Table 7.

Table 7

Sample	Expected result	Laboratory 1			Laboratory 2			Laboratory 3		
		Lot # 2402-1	Lot # 2402-2	Lot # 2402-3	Lot # 2402-1	Lot # 2402-2	Lot # 2402-3	Lot # 2402-1	Lot # 2402-2	Lot # 2402-3
R08	Negative	N	N	N	N	N	N	N	N	N
9215	Weak positive	N	2+	1+	2+	2+	2+	1+	1+	1+
0827 (R2)	Strong positive	3+	3+	3+	3+	3+	3+	2+	3+	4+
9061 (R12)	Moderate positive	2+	3+	3+	2+	2+	2+	1+	1+	1+
61084K	Positive	3+	2+	2+	3+	3+	3+	3+	3+	3+

C. Day-to-Day:

At each clinical site, test kits were evaluated on three consecutive working days. A panel comprised of one non-reactive and four reactive samples was tested in triplicate. A summary of the results are shown in Table 8.

Performance characteristics

QUALITATIVE TEST

Table 4. Comparison of **Sure-Vue® Select Rubella** with two commercially available latex agglutination tests.

		Reference latex test	
		Reactive	Non-reactive
Sure-Vue® Select Rubella	Reactive	650	2*
	Non-reactive	0	145

* Two samples non-reactive in a reference latex agglutination test were reactive in both an alternative latex test and the **Sure-Vue® Select Rubella**

Relative sensitivity: 100% (95% CI: 99.4% - 100%)
Relative specificity: 98.6% (95% CI: 95.2% - 99.8%)

Performance of the **Sure-Vue® Select Rubella** with the CDC Rubella Reference Panel:

The following information is from a serum panel obtained from the CDC and tested in an external laboratory. These results are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC. The panel consists of 82% positive and 18% negative samples. The **Sure-Vue® Select Rubella** demonstrated 100% agreement with the CDC results. Of the results obtained, there was 100% agreement with the positive specimens and 100% agreement with the negative specimens.

Table 5. Comparison of **Sure-Vue® Select Rubella** and a commercial latex agglutination test with a rubella seroconversion panel.

Rubella seroconversion panel
Comparison of the performance of Sure-Vue® Select Rubella and a reference latex test

Sample No.	EIA result* (No. pos/No. tested)		**Sure-Vue® Select Rubella Latex** Lot No.			Reference latex test
	IgG	IgM	2402-1	2402-2	2403-2	
PTR901-01	0/5	0/5	N	N	N	N
PTR901-02	0/5	0/5	N	N	N	N
PTR901-03	0/5	0/5	N	N	N	N
PTR901-04	0/5	0/5	N	N	N	N
PTR901-05	0/5	0/5	N	N	N	N
PTR901-06	2/5	3/5	**±	**±	**±	**±
PTR901-07	5/5	5/5	2+	3+	2+	2+
PTR901-08	5/5	5/5	2+	1+	1+	1+
PTR901-09	5/5	3/5	3+	3+	3+	3+

* EIA data are presented in the table as the number of positive tests versus the number of tests run. The information presented in the table is a summary of the results of testing the seroconversion panel with Rubella IgG and IgM enzyme immunoassays obtained from five manufacturers. Data were obtained from Boston Biomedica, Inc., the supplier of the seroconversion panel.

** Since specimen PTR901-06 contained aggregates prior to testing, the test results should be interpreted with caution.

These data indicate that the performance of the **Sure-Vue® Select Rubella** is similar to both rubella enzyme immunoassays (IgG and IgM) and a commercial rubella latex test.

SEMIQUANTITATIVE TEST

A total of 74 samples were diluted and tested using **Sure-Vue® Select Rubella** and two other latex agglutination tests. A review of the data indicate that the performance of the **Sure-Vue® Select Rubella** is equivalent to two other latex agglutination tests.

Procedure

Procedural note

Both qualitative and semi-quantitative procedures are provided. The sensitivity of the **Sure-Vue® Select Rubella** is approximately 10 IU/mL as determined by comparison with the 2nd WHO Rubella Reference Standard. Rubella antibody levels less than 10 IU/mL may not provide sufficient protection against a rubella infection. According to the Centers for Disease Control and Prevention (CDC), Immunization Practices Advisory Panel (ACIP), and the National Committee for Clinical Laboratory Standards (NCCLS), antibody levels greater than 10 IU/mL (>1:8 HAI titer) indicate immunity against rubella infection.

QUALITATIVE TEST

The high and low positive controls and the negative control should be tested as described for patient samples.

1. Remove the test kit from the refrigerator and allow the reagents to warm to room temperature (18 - 30°C) before testing specimens.

2. Dispense one drop (approximately 25 µL) of the control into the appropriate circle. Spread the control over the entire surface area of the circle using the flattened end of a mixing pipette.

3. Using separate mixing pipettes, dispense one drop (approximately 25 µL) of the patient sample into the appropriate circle. Spread the sample over the entire surface area of the circle using the flattened end of the mixing pipette.

4. Mix the latex reagent by shaking the vial gently for approximately 10 seconds. Dispense one drop (approximately 15 µL) into each circle containing control or sample.

5. Manually rotate the test card for 5 minutes and examine each circle for agglutination. For mechanical rotation, place the test card on a flatbed rotator at 100 rpm for 5 minutes and examine each circle visually for agglutination.

SEMIQUANTITATIVE TEST

The high positive control should be diluted and tested as described for patient samples. The negative control should be tested undiluted.

1. Remove the test kit from the refrigerator and allow the reagents to warm to room temperature (18 - 30°C) before testing specimens.

2. Using a calibrated pipette, dispense 25 µL of the diluent into circles marked 2 to 8.

3. Dispense two drops of the high positive control into the circle marked 1, or using a calibrated pipette, dispense approximately 50 µL of the specimen. DO NOT CREATE BUBBLES.

4. Using a calibrated pipette, transfer 25 µL from the circle marked 1 to the circle marked 2. Mix by aspirating and dispensing the mixture several times using the same mixing pipette. DO NOT CREATE BUBBLES. This is a 1:2 dilution.

5. Repeat step 4 in succession through the circle marked 8. NOTE: Discard 25 µL from the circle marked 8.

6. Using the flattened end of separate mixing pipettes, spread specimens or control dilutions over the entire surface of the circle starting with the highest dilution. Using the same pipette, proceed to the next lower dilution and spread the specimen dilution in a similar way. Repeat the procedure until contents of all circles are spread.

7. Mix the latex reagent by shaking the vial gently for approximately 10 seconds. Dispense 1 drop (approximately 15 µL) into each circle containing control or sample.

8. Manually rotate the test card for 5 minutes and examine each circle for agglutination. For mechanical rotation, place the test card on a flatbed rotator at 100 rpm for 5 minutes and examine each circle visually for agglutination.

Interpretation of results

QUALITATIVE TEST

POSITIVE REACTIONS:

3+/4+ Large clumping with clear background
2+ Moderate clumping with slightly opaque fluid in background
1+ Small clumping with opaque fluid in background

A person is presumed to be immune if his or her sample gives visible agglutination that is different from the appearance of the latex reagent with the negative control.

NEGATIVE REACTIONS:

No visible agglutination with a uniform suspension.
A person is presumed to be non-immune if agglutination is not observed.

Test results should be read promptly after the five minute incubation to avoid an erroneous result. Studies suggest that antibody levels less than 10 IU/mL may be insufficient to protect against a rubella virus infection. The sensitivity of the **Sure-Vue® Select Rubella** is approximately 10 IU/mL (as determined by comparison with the 2nd WHO Rubella Reference) when the test procedure is performed as described in the package insert. Samples with a concentration of up to 3200 IU/mL of rubella antibodies did not cause a prozone effect in the **Sure-Vue® Select Rubella**. Samples containing aggregates should be centrifuged before testing. If aggregates are observed after centrifugation, collect and process a second specimen.

SEMIQUANTITATIVE TEST

Titers of the high and low positive controls should fall within the expected ranges. Agglutination should not be visible in the negative control circle.

The titer of each sample, expressed in IU/mL, corresponds with the reciprocal of the highest sample dilution that still presents visible agglutination multiplied by the sensitivity of the latex reagent (approximately 10 IU/mL).

Example:

Latex reagent sensitivity:	Approximately 10 IU/mL
Highest sample dilution:	1:8
Approximate concentration:	80 IU/mL (8 x 10 IU/mL)

Acute and convalescent samples must be tested simultaneously. An increase in the antibody titer of four-fold or greater when testing convalescent and acute sera indicates a recent infection with rubella virus. Less than a four-fold increase in titer between acute and convalescent specimens may be observed when using a test that detects both IgG and IgM antibodies. This situation would be anticipated when the acute specimen contains predominantly IgM and the convalescent specimen contains predominantly IgG. Seroconversion may indicate either a primary infection or a response to vaccination. Some individuals may experience a reinfection without developing symptoms. Absence of a detectable difference in titers between acute and convalescent samples does not exclude a recent exposure to rubella virus.

Limitations of the procedure

1. Test results must be evaluated by health care professionals in conjunction with clinical symptoms presented by the patient.
2. Performance of the test with plasma has not been validated.
3. Test results should be read promptly after the 5 minute incubation to avoid erroneous results.
4. The performance of test kit reagents should be evaluated at least once each day the test kit is used. Positive and negative controls provided with the test kit should be used for the Quality Control testing.
5. The **Sure-Vue® Select Rubella** detects both IgG and IgM.
6. Results obtained from testing immunocompromised patients must be interpreted with caution.
7. The performance characteristics of this device have not been established in neonates or cord blood.
8. Due to the high prevalence of rubella IgG antibodies in individuals residing in the U.S., studies to assess the potential cross-reactivity with other viral pathogens were not performed.
9. In general, latex agglutination tests are less sensitive for the detection of IgM than IgG; therefore, falsely negative results may be observed.

10. The performance of the **Sure-Vue® Select Rubella** with specimens other than serum has not been established.
11. Specimens with obvious microbial contamination should not be tested.
12. The semi-quantitative protocol should be used with properly paired specimens to determine recent infection. Care must be used in the timing of sample collection. If the first (acute phase) sample is taken too late or the second (convalescent phase) sample is taken too soon, the seroconversion or four-fold rise in titer characteristic of recent infection may not be seen. The acute phase specimen should be collected as nearly as possible to the time of exposure and no later than three days after the onset of rash. The convalescent phase specimen should be taken 7-21 days after the onset of rash or at least 30 days after exposure. The absence of a four-fold rise in the titer does not necessarily rule out the possibility of exposure and infection. A subclinical infection may not produce clinical symptoms.

Expected values

The results of testing the WHO Reference Standard at various concentrations using the **Sure-Vue® Select Rubella** are shown in Table 1. The performance of the **Sure-Vue® Select Rubella** with samples which contain concentrations of rubella antibodies at or near the cutoff value of 10 IU/mL are shown in Table 9. These data indicate the sensitivity of the **Sure-Vue® Select Rubella** is approximately 10 IU/mL.

Table 1. Performance of the **Sure-Vue® Select Rubella** with the 2nd WHO Rubella Reference Standard

WHO Rubella Reference (IU/mL)	Test result (Lot No. 2402-1)		
30	2+	2+	2+
15	1+	1+	1+
10	1+	1+	1+
5	N	N	N
1	N	N	N

The distribution of semi-quantitative results was not ascertained for the samples included in the evaluation of the **Sure-Vue® Select Rubella**. The distribution of rubella antibodies in the populations tested are shown in Tables 2 and 3. The estimated percent positives in the populations evaluated by Laboratory 1 (a Community Hospital) and Laboratory 2 (a University Hospital) was approximately 90%.

A. Laboratory 1 (Community Hospital)

Table 2

Patient type	**Sure-Vue® Select Rubella**		Reference latex test	
	Reactive	Non-reactive	Reactive	Non-reactive
Pre-Natal	199*	39	198	40
Pre-Marital	6	1	6	1
Employee Health	10	3	10	3
Out Patient	1	0	1	0

* One specimen which was non-reactive in the reference latex agglutination test was reactive in an alternative latex test and the **Sure-Vue® Select Rubella**.

B. Laboratory 2 (University Hospital)

Table 3

Patient type	**Sure-Vue® Select Rubella**		Reference latex test	
	Reactive	Non-reactive	Reactive	Non-reactive
Obstetrics	45*	8	44	9
Family Practice	15	15	15	15
Student/Employee Health	166	31	166	31

* One specimen which was non-reactive in the reference latex agglutination test was reactive in an alternative latex test and the **Sure-Vue® Select Rubella**.