

## **RUBAscan™**

### **FOR THE DETECTION AND/OR SEMI-QUANTITATION OF ANTIBODIES TO RUBELLA VIRUS**

#### **I. INTENDED USE**

The **RUBAscan™** Card Test is a passive latex agglutination test for the qualitative detection of rubella IgG and IgM antibodies in human serum or as an aid in the determination of immune status. The **RUBAscan™** Card Test can also be used to give semi-quantitative results when serial dilutions of properly paired specimens are tested to determine recent or active infection.

#### **II. SUMMARY AND EXPLANATION**

The **RUBAscan™** Card Test is based upon the well-established principles of passive latex agglutination. Latex is sensitized according to a patented process using solubilized rubella virus antigens from disrupted virions. This latex reagent, when mixed with serum containing rubella antibodies on a card surface, will agglutinate forming visible clumps. In the absence of antibody, or if the concentration is insufficient to react, the latex will remain smooth and evenly dispersed.

In a qualitative sense, the presence of rubella antibodies is an indication of previous infection and presumptive of immunity.<sup>1-3</sup> An interpretation of the test results can be used to evaluate the immune status of the individual with regard to resistance or susceptibility to primary rubella infection. The test is configured to give qualitative results by testing specimens either undiluted or diluted 1:10 to yield two sensitivity levels for detecting rubella antibodies. The **RUBAscan™** Card Test correlates to Hemagglutination Inhibition (HAI) and approximates a 10 IU/mL sensitivity level using the 1:10 Qualitative Procedure.

The semi-quantitative test differs in procedure by having provision for a primary specimen dilution and six consecutive test dilutions. Results are determined by dilution titers, which are indicated by limiting dilutions containing sufficient antibody to agglutinate the added latex reagent. The **RUBAscan™** Card Test is a more sensitive test than HAI, but correlation does exist between it and conventional hemagglutination inhibition procedures (cf., "Expected Values" and "Performance Characteristics"). Consequently, **RUBAscan™** Card Test results can be related to a large existing body of knowledge drawn from HAI test data pertaining to the significance of antibody titers. Moreover, with properly collected specimen pairs, seroconversion of a four-fold titer rise can be used to diagnose recent or current infection.

### III. PRINCIPLES OF THE PROCEDURE

The **RUBAscan™** Card Test depends upon the reaction of antibodies in a serum with the patented latex reagent on a card surface. Results are interpreted from the presence or absence of agglutination, following rotation on a rotator. A single specimen can be used to obtain a qualitative judgment about its antibody content. Analysis of dilutions of the specimen can give semi-quantitative results.

### IV. REAGENTS

Reagent A, **RUBAscan™** Latex Antigen, coated with purified rubella virus with 0.2% sodium azide and 0.02% gentamicin (preservatives).

Reagent B, **RUBAscan™** Card Dilution Buffer, Phosphate buffered saline solution, containing bovine serum albumin, with 0.02% sodium azide (preservative).

Control ++, **RUBAscan™** High Reactive Control (human serum), with 0.1% sodium azide (preservative).

Control +, **RUBAscan™** Low Reactive Control (human serum), with 0.1% sodium azide (preservative).

Control -, **RUBAscan™** Nonreactive Control (human serum), with 0.1% sodium azide (preservative).

Precautions: For *in vitro* Diagnostic Use.

Reagents: Do not use beyond the expiration date. Upon removal from the refrigerator, allow to warm to room temperature (23-29°C) before use. Refrigerate at 2-8°C. DO NOT FREEZE. Reagents should be recapped and returned to refrigeration when not in use.

To assure proper drop delivery when dispensing **RUBAscan™** Latex Antigen (Reagent A), the dispensing bottle must be held vertically.

Reagent A has been prepared from disrupted vaccine strain virus, which has been judged to be inactivated by bioassay procedures.

The serum controls are derived from human blood tested by an FDA (U.S. Food and Drug Administration) approved method for the presence of the antibody to HIV (human immunodeficiency virus) and HBsAg (hepatitis B surface antigen) and found to be nonreactive.

**WARNING:** Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, SPECIMENS AND THESE REAGENTS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE. The FDA recommends such material be handled at a Biosafety Level 2. BSL 2 is referenced in the Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) manual, *Biosafety in Microbiological and Biomedical Laboratories*.

Warning: Reagents contain sodium azide which is very toxic by inhalation, in contact with skin, and if swallowed. Contact with acids liberates very toxic gas. After contact with skin, wash immediately with plenty of water. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

Test Cards: Cards must be flat for proper reactions. If necessary, flatten cards by bowing back in a direction opposite to that of the curl. Care should be taken not to finger-mark the test areas, since this may result in an oily deposit and improper test results. Use each card once and discard. Store cards in the original package in a dry area at room temperature.

Reading of Test Results: To help differentiate weak agglutination from no agglutination, a brief hand rotation of the card must be made following mechanical rotation. Results should be read promptly under a high intensity incandescent lamp. Fluorescent lighting is generally insufficient to distinguish minimally reactive results. The use of magnification in reading test results is not recommended.

Rotation: The recommended speed for mechanical rotation is  $100 \pm 2$  rpm, but rotation between 95 and 110 rpm does not significantly affect the results obtained. The rotator should circumscribe a circle approximately two centimeters in diameter in the horizontal plane. A moistened humidifying cover should be used to prevent drying of test specimens during rotation.

## V. SPECIMEN COLLECTION AND PREPARATION

The rationale for the manner of serum collection varies with testing objectives. Single specimens are required for qualitative antibody level determinations. In suspected clinical infections or exposure, two specimens for semi-quantitative testing should be obtained. The first should be collected within three days of the onset of rash or at the time of exposure and tested upon arrival at the laboratory. This specimen should then be stored frozen until the second specimen is collected 7-21 days after the onset of rash or at least 30 days after exposure if no

clinical symptoms occur. *Both specimens should be tested simultaneously for antibodies to rubella.* Review "Limitations of Procedure" prior to performing tests.

Whole blood is collected and the serum is separated. Specimens may be stored up to 48 hours at 2 - 8°C. Specimens should be frozen if longer storage is required.

Do not heat inactivate serum. Serum that displays excessive particulate matter, lipemia, or hemolysis could affect the test.

No special preparation of the patient is required prior to specimen collection.

## VI. PROCEDURES

Review "Precautions" and "Specimen Collection and Preparation" prior to performing procedures. The testing area, reagents, test specimens and test components should be at room temperature (23 - 29°C) when used.

Materials Provided:	Cat. #	Cat. #.	Cat #.
	261926	261701	261805
	30 Tests	100 Tests	500 Tests
Reagent A, <b>RUBAscan™</b> Latex Antigen	0.5 mL	1.6 mL	5 x 1.6 mL
Reagent B, <b>RUBAscan™</b> Card Dilution Buffer	5.0 mL	20.0 mL	5 x 20.0 mL
Control ++, <b>RUBAscan™</b> High Reactive Control (human serum)	0.5 mL	0.5 mL	5 x 0.5 mL
Control +, <b>RUBAscan™</b> Low Reactive Control (human serum)	0.5 mL	0.5 mL	5 x 0.5 mL
Control -, <b>RUBAscan™</b> Nonreactive Control (human serum)	0.5 mL	0.5 mL	5 x 0.5 mL
Test Cards, Qualitative	5	8	40
Plastic Stirrers	33	120	600

Materials Required But Not Provided: Rotator with humidifying cover, micropipettors, 25 and 100 µL delivery, centrifuge and high intensity incandescent lamp.

Procedural Notes: There are three procedure options for testing sera with the **RUBAscan™** Card Test. Two are for qualitative testing (Undiluted Qualitative Procedure and 1:10 Qualitative Procedure) and one is for semi-quantitative testing.

A qualitative procedure should be used to screen a single serum for the presence or absence of rubella antibody. The choice of which qualitative procedure is to be used should be made based upon judgements concerning the significance of low levels (< 10 IU/mL) of rubella antibody in protecting the individual from rubella infection. The Undiluted Qualitative Procedure is more sensitive than the 1:10 Qualitative procedure because low levels of rubella antibody (<10 IU/mL) may yield positive reactions.

The 1:10 Qualitative Procedure should be used when a sensitivity level correlating to a 10 IU/mL breakpoint or HAI (Hemagglutination Inhibition) is desired. The National Committee for Clinical Laboratory Standards (NCCLS) tentative document I/LA6-T states that the majority of seropositive persons are detected with a 10 IU/mL breakpoint as an indicator of immune status.<sup>20</sup> The 1:10 Qualitative Procedure, however, will fail to detect low level antibody specimens that are reactive undiluted.

The semi-quantitative procedure uses serial dilutions of the specimen to give a relative indication of how much antibody is present. With properly paired specimens, the semi-quantitative procedure can be used to determine recent or active infection.

---

#### UNDILUTED QUALITATIVE PROCEDURE

---

1. Mark the card to identify Control +, Control -, or all samples being tested.
2. With a micropipettor, place 25 µL of Control +, Control -, or patient sample onto the appropriate circle.
3. Using a new plastic stirrer for each circle, spread the serum to fill the entire circle.
4. Gently invert the dispensing bottle several times to thoroughly mix Reagent A.
5. Before uncapping the bottle, gently tap base on counter top to assure no latex reagent remains in the tip. Dispense one free-falling drop of Reagent A (approx. 15µL) onto each circle containing the serum.
6. Place the card on a rotator and rotate for 8 min under a moistened humidifying cover.

7. Immediately following mechanical rotation read the card macroscopically in the wet state under a high intensity incandescent lamp. Gently tilt the card (three or four back-and-forth motions) to help differentiate weak agglutination from no agglutination.

### **Interpretation of Undiluted Qualitative Results:**

The Control + should show agglutination, while the Control - should show no agglutination.

Report as “Antibody Present”: Specimens showing any agglutination of the RUBAscan™ Latex Antigen (Reagent A).

Occasionally strong reactivity may cause the center of the test circle to appear clear because agglutination latex has migrated to the periphery.

To follow the recommendation of NCCLS guideline I/LA6-T, an undiluted positive serum should be repeated at a 1:10 dilution before a patient result is reported (refer to 1:10 Qualitative Procedure).

Report as “No Antibody Present”: Suspension remains evenly dispersed showing no agglutination of the RUBAscan™ Latex Antigen (Reagent A).

---

### 1:10 QUALITATIVE PROCEDURE

---

This procedure will approximate a 10 IU/mL sensitivity level.

1. Mark the card to identify Control +, Control -, or all samples being tested.
2. With a micropipettor, add 100  $\mu$ L of Reagent B to the appropriate squares for each control and sample being tested.
3. With a micropipettor, add 25  $\mu$ L of Reagent B to the appropriate circles for each control and sample being tested.
4. Using the same micropipettor with a new tip, place 25  $\mu$ L of Control + directly into the buffer in the appropriate square and mix the serum and buffer by drawing up-and-down with the micropipettor 12 times. Avoid the formation of bubbles. The serum in this square is now a 1:5 dilution.
5. Using the same micropipettor and tip, transfer 25  $\mu$ L of the 1:5 dilution from the square and place directly into the buffer in the correspondingly numbered circle and mix by drawing up-and-down with the micropipettor six times.

Withdraw 25  $\mu$ L from the circle and discard. The serum in this circle is now a 1:10 dilution.

6. Repeat the procedures in step 3 (with a new tip each time) for the Control - or for each patient sample being tested.
7. Using a new plastic stirrer for each circle, spread the serum to fill the entire circle.
8. Gently invert the dispensing bottle several times to thoroughly mix Reagent A.
9. Before uncapping the bottle, gently tap base on counter top to assure no latex reagent remains in the tip. Dispense one free-falling drop of Reagent A (approx. 15 $\mu$ L) onto each circle containing the serum.
10. Place the card on a rotator and rotate for 8 min under a moistened humidifying cover.
11. Immediately following mechanical rotation, read the card macroscopically in the wet state under a high intensity incandescent lamp. Gently tilt the card (three or four back-and-forth motions) to help differentiate weak agglutination from no agglutination.

#### **Interpretation of 1:10 Qualitative Results:**

The Control + should show agglutination, while the Control - should show no agglutination.

Report as "Presumed Immune": Specimens showing any degree of agglutination of the **RUBAscan™** Latex Antigen (Reagent A).

Report as "Presumed Non-Immune": Suspension remains evenly dispersed showing no agglutination of the **RUBAscan™** Latex Antigen (Reagent A).

---

#### **SEMI-QUANTITATIVE PROCEDURE**

---

Performance of the **RUBAscan™** Test with Sera Diluted 1:5 through 1:160:

1. Mark card to identify Control ++, Control + and Control - and all samples being tested.
2. Dilution of Control (++):

- a. With micropipettor, add 100  $\mu\text{L}$  of Reagent B to appropriate square in row marked Control ++.
  - b. With a micropipettor, add 25 $\mu\text{L}$  of Reagent B to circles 1 through 6.
  - c. With a micropipettor, place 25 $\mu\text{L}$  of Control ++ directly into the buffer in the appropriate square and mix the serum and buffer by drawing up-and-down with the micropipettor 12 times. Avoid the formation of bubbles. The serum in the square is now a 1:5 dilution.
  - d. Using the same micropipettor and tip, transfer 25  $\mu\text{L}$  of the 1:5 dilution from the square, and place directly into the buffer in circle 1, and mix by drawing up-and-down with the micropipettor six times. The serum in circle 1 is now a 1:10 dilution.
  - e. Using the same micropipettor and tip, transfer 25  $\mu\text{L}$  of the 1:10 dilution directly into the buffer in circle 2, mix as before, and continue this preparation of serial two-fold dilutions through circle 6. Withdraw 25 $\mu\text{L}$  from circle 6 and discard. The resulting serum dilution in circle 1 is 1:10, in circle 2 is 1:20, in circle 3 is 1:40, in circle 4 is 1:80, in circle 5 is 1:160 and in circle 6 is 1:320.
3. Dilution of the Control (+):
- a. With micropipettor, add 100  $\mu\text{L}$  of Reagent B to appropriate square in the row marked Control +.
  - b. With a micropipettor, add 25  $\mu\text{L}$  of Reagent B to circle 2 through 4, leaving circle 1 empty.
  - c. With a micropipettor, place 25  $\mu\text{L}$  of Control + directly into the buffer in the appropriate square and mix the serum and buffer by drawing up-and-down with the micropipettor 12 times. Avoid the formation of bubbles. The serum in the square is now a 1:5 dilution.
  - d. Using the same micropipettor and tip, transfer 25  $\mu\text{L}$  of the 1:5 dilution from the square and place into circle 1.
  - e. Using the same micropipettor and tip, transfer an additional 25  $\mu\text{L}$  of the 1:5 dilution from the square, place directly into the buffer in circle 2, and mix by drawing up-and-down with the micropipettor six times. The serum in circle 2 is now a 1:10 dilution.
  - f. Using the same micropipettor and tip, transfer 25  $\mu\text{L}$  of the 1:10 dilution directly into the buffer in circle 3, mix as before, and continue this preparation of serial two-fold dilutions through circle 4.

Withdraw 25  $\mu\text{L}$  from circle 4 and discard. The resulting serum dilution in circle 1 is 1:5, in circle 2 is 1:10, in circle 3 is 1:20, in circle 4 is 1:40.

4. Control -: With a micropipettor, add 25  $\mu\text{L}$  of Control - to circle 1 in the row marked Control -.
5. Dilution of Test Samples:
  - a. Using a new micropipettor tip for each sample being tested, repeat the procedure in step 3, except continue to circle 6, 1:160 dilution.
  - b. If further dilutions are required, continue procedure described in step 3 through the next row of circles.
6. Using a new plastic stirrer for each control and test sample, start at the highest numbered circle and spread the serum dilution to fill the entire circle. Using the same stirrer in each row, proceed to the next lower circle and spread the serum dilution in a similar manner. Repeat this procedure until the contents of all circles are spread.
7. Gently invert the dispensing bottle several times to thoroughly mix Reagent A.
8. Before uncapping the bottle, gently tap base on counter top to assure no latex reagent remains in the tip. Dispense one free-falling drop of Reagent A (approx. 15  $\mu\text{L}$ ) onto each circle containing the serum dilutions.
9. Place the card on a rotator and rotate for 8 min under a moistened humidifying cover.
10. Immediately following mechanical rotation, read the card macroscopically in the wet state under a high intensity incandescent lamp. Gently tilt the card (three or four back-and-forth motions), to help differentiate weak agglutination from no agglutination.

#### **Interpretation of Semi-Quantitative Results:**

Report reactivity in terms of the highest dilution showing any agglutination of the **RUBAscan™** Latex Antigen (Reagent A). Specimens showing no agglutination at any dilution are reported as no antibody present. Occasionally strong reactivity may cause the center of the test circle to appear clear because agglutinated latex has migrated into the periphery.

Demonstration of seroconversion or a four-fold or greater rise in antibody titer on properly collected paired specimens indicates recent exposure to rubella virus.

With the **RUBA<sub>scan</sub>**<sup>™</sup> test system, seroconversion means a positive test result of 1:5 or greater after an initial nonreactive result of less than 1:5.

## VII. **CONTROLS**

The Control ++ is formulated to produce agglutination at a titer of 1:160 or greater. The Control + is formulated to produce agglutination at a titer of 1:10 ± one dilution. The Control - should show no agglutination. Titers should be reported as last dilution showing agglutination. Additional controls may be tested in accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

## VIII. **LIMITATIONS OF THE PROCEDURE**

The **RUBA<sub>scan</sub>**<sup>™</sup> Card Test has been designed to detect the presence of rubella antibody. At a single dilution the qualitative protocol will perform satisfactorily with both acute phase and convalescent phase antibodies. In cases when the presence or absence of a four-fold titer rise in paired specimens must be demonstrated, the semi-quantitative protocol is required. Results of these tests should be interpreted by a physician in light of other critical findings and diagnostic procedures.

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 phase (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 the rash or at least 30 days after exposure, if no clinical symptoms appear (possible subclinical infection). **BOTH SPECIMENS SHOULD BE TESTED SIMULTANEOUSLY.** The absence of a four-fold titer rise does not necessarily rule out the possibility of exposure and infection.

The affinity and avidity of Rubella IgG and IgM for the rubella antigen have not been determined with this assay. Some tests that detect both IgM and IgG antibodies may be less sensitive for IgM and IgG.<sup>21</sup> As with other rubella tests, results from patients in the acute stages of primary infection should be interpreted with caution.<sup>21</sup>

Pregnant women and women of child bearing age were included in the study population for the **RUBAscan™** Card Test. However, the specific performance characteristics for this subgroup of the study population were not determined.

Serum specimens with obvious microbial contamination should not be assayed with this method.

The use of plasma in the **RUBAscan™** Quantitative Card Test has not been established. Performance characteristics of this assay have only been established with human serum.

Reduction in the degree of agglutination has been reported with rare high titered specimens when **RUBAscan™** Card Test screening is performed undiluted. However, no prozone was detected in a study of 378 sera with endpoint titers up to 1:2560 (>500 IU/mL).

## IX. EXPECTED VALUES

Expected values for the **RUBAscan™** Qualitative Card Test Procedures were derived from a composite of 16 geographically dispersed **RUBAscan™** evaluations involving 2,252 fresh specimens. The specimens represented various patient populations and included specimens from hospital and Public Health laboratories.

Eighty-two percent of the specimens were rubella antibody positive and 18% were rubella anti-body negative using the qualitative 1:10 procedure. From the same specimen set, 83% were rubella antibody positive and 17% were rubella antibody negative using the undiluted qualitative procedure.

Based on data in Table 5 (“Performance Characteristics”) which demonstrate seroconversion of 74 paired samples from individuals with natural infection or vaccination, the **RUBAscan™** Card Test will reliably determine seroconversion on appropriately collected pairs of samples.

## X. PERFORMANCE CHARACTERISTICS

Qualitative Testing: The **RUBAscan™** Card Test using the 1:10 Qualitative Procedure and an ELISA assay were studied in comparison to HAI. The results are depicted in Table 1.

Table 1: Pooled Results of Clinical Studies Comparing the **RUBAscan™** 1:10 Qualitative Procedure and ELISA<sup>18, 19</sup> to HAI

<b>RUBAscan™</b> Card Test	ELISA
----------------------------	-------

		1:10			
		Pos	Neg	Pos	Neg
HAI	Pos	703	2	679	4
	Neg	2	71	24	71

Sensitivity = 99.7% (95% C.I. = 98.9 - 99.9)      99.4% (95% C.I. = 98.5 - 99.8)  
 Specificity = 97.3% (95% C.I. = 90.5 - 99.7)      77.8% (95% C.I. = 64.8 - 83.1)

Additionally the **RUBAscan™** Card Test, using the undiluted and 1:10 Qualitative Procedure, was studied in comparison to HAI. The results are depicted in Table 2.

Table 2: Pooled Results Comparing the **RUBAscan™** Undiluted and 1:10 Qualitative Card Test to HAI\*

		<b>RUBAscan™</b> Card Test		<b>RUBAscan™</b> Card Test	
		1:1		1:10	
		Pos	Neg	Pos	Neg
HAI (1:8 and 1:10)	Pos	133	0	133	0
	Neg	4	19	0	23

Sensitivity = 100.0% (95% C.I. = 97.3 - 100.0)      100.0% (95% C.I. = 97.3 - 100.0)  
 Specificity = 82.6% (95% C.I. = 61.2 - 95.5)      100.0% (95% C.I. = 85.0 - 100.0)

\*Data combined from separate studies at Becton Dickinson and Company and the University of Tennessee Medical Center, USA.

Analysis (using the undiluted [or 1:10] test protocols) of a panel of 100 sera selected to evaluate the suitability of various rubella test kits for the determination of immunity and for serodiagnosis further established the reliability of the **RUBAscan™** Card Test. Agreement with rubella-positive sera (latex card/HAI) was 98% (61/62); the agreement with all sera was 96% (96/100).<sup>5</sup>

Semi-Quantitative Testing: The **RUBAscan™** Card Test procedure was extensively compared with microdilution plate HAI tests using test combinations of trypsinized human Group O cells, fresh chick cells and stabilized chick cells with both kaolin and heparin-MnCl<sub>2</sub> absorption. Virtually all were fresh specimens representing various populations tested at several geographical sites in the United States. Public Health and hospital laboratories were included. The data from 16 such studies involving 2252 specimens clearly indicates the overall greater sensitivity of the **RUBAscan™** Card Test. The data also show correlation between the **RUBAscan™** Card Test and several HAI methods with the observed variation derived from known differences among HAI procedures.<sup>2-16</sup>

A study<sup>17</sup>, comparing research ELISA, latex agglutination, and HAI results, was conducted with paired sera from 21 persons with natural infection and 53 who had been immunized with rubella vaccines. Post exposure sera were collected 30 - 60 days after infection or vaccination. The results in Table 3 show that latex agglutination test accurately detected seroconversions in all pairs of sera.

Table 3: Evaluation of Latex Agglutination Tests for Detection Seroconversion After Rubella Immunization and Infection

Rubella Exposure	Number of Pairs of Sera	Mean Titer (Range) in Post Immunization/Infection Sera <sup>a</sup>		
		Research ELISA	RUBAscan™ <sup>b</sup> Card Test	HAI
RA27/3	33	1600 (400 - 3200)	64 (16 - 256)	64 (16 - 256)
Cendihill	11	1600 (400 - 6400)	64 (32 - 256)	128 (64 - 128)
HPV <sub>77</sub> DE <sub>5</sub>	9	800 (400 - 3200)	32 (16 - 64)	32 (16 - 64)
Rubella infection	21	3200 (400 - 12800)	128 (16 - 256)	128 (64- 512)

- a. All pre-exposed sera were negative in each test.
- b. Pre-exposed sera were negative when tested undiluted and 1:4 by latex agglutination.

CDC Evaluation Panel: The following information is from a serum panel obtained from the CDC and tested by BD Diagnostic Systems. The 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. BD Diagnostic Systems demonstrated 100% total agreement with the CDC results.

Testing (using the undiluted [or 1:10] test protocols) of a CDC rubella evaluation serum panel consisting of coded serum pairs yielded no false positive (0/36) and no false negative (0/162) results. Reproducibility (within test and between test) was 100% (Table 4).<sup>6</sup>

Table 4: Reproducibility of Semi-Quantitative RUBAscan™Card Test

Agreement of Duplicates	Technician No.1		Technician No.2	
	No.	%	No.	%
Both same titer	39	78	42	84

One dilution difference	11	22	8	16
Two dilution difference	0	0	0	0
Reproducibility	100%		100%	

The sensitivity and specificity of the **RUBAscan™** Card Test are shown in Table 5. This table is a summary of a study in which tests were performed on 100 randomly coded specimens representing 50 pairs of duplicates (supplied by CDC). The series was composed of 82 HAI positive samples (with titer range of 1:10 - 1:1280) and 18 HAI negative samples. Each laboratory assayed all 100 specimens and submitted the results to the CDC for decoding and tabulation.

Table 5: Multi-Site Study Using Coded Serum Panel

	Lab A	Lab B	Lab C
Sensitivity	100%	100%	100%
Specificity	94%	100%	100%

10 IU/ml Validation: In accordance with NCCLS document 1/LA6-T, a serum panel of 378 samples was tested by the **RUBAscan™** Card Test and by a commercial EIA method. This serum panel is heavily weighted at the detection limits of rubella antibody assays and does not reflect the distribution of rubella titers in a normal population. Of the 378 samples, 50 contained approximately 10 - 20 IU/mL, 149 contained > 20 IU/mL and 179 contained < 10 IU/mL of rubella antibody as determined by EIA. The **RUBAscan™** Card Test detected 190/199 of EIA reactive samples and 154/179 of EIA negative samples. When the 34 discordant results were tested by a second commercially available latex method, 27 agreed with the **RUBAscan™** Card Test results. All 9 discordant EIA reactive samples were also non-reactive by the second latex method. 18/25 discordant EIA non-reactive samples were also reactive by the second latex method.

In additional studies, a subset of the 378 samples were tested at 2 hospital sites (n=191). Of the 93 samples containing  $\geq 10$  IU/mL rubella antibody, the **RUBAscan™** Card Test detected 90 and 84 at sites 1 and 2 respectively. Of the 98 samples containing < 10 IU/mL rubella antibody, the **RUBAscan™** Card Test correctly identified 98/98 and 97/98 at sites 1 and 2 respectively.

A study was conducted at BD Diagnostic Systems to correlate the cutoff of the **RUBAscan™** Card Test to the CDC Reference Preparation. The CDC Anti-Rubella Human Serum Reference Preparation (labeled as 21.0 IU/mL of IgG) was diluted to yield a concentration of 10 IU/mL. This preparation was tested undiluted and serially diluted using 3 lots of **RUBAscan™** Card Test. All results were positive at the 1:10 screening dilution with endpoint titers  $1:20 \pm 1$  dilution.

A study was conducted at BD Diagnostic Systems to correlate the cutoff of the **RUBAscan™** Card Test to the WHO standard. The WHO standard (1,047 IU/ml) was diluted in rubella antibody-negative human serum to yield concentrations of 5, 7.5, 10, 15, and 25 IU/ml (based on labeled value). All samples were tested in triplicate both undiluted and diluted 1:10. The results summarized in Table 6 show the 1:10 dilution correlation to a 10 IU/ml cutoff for immunity.

Table 6: WHO Standard Study

Concentration IU/ml	Lot #1		Lot #2	
	Undiluted	1:10	Undiluted	1:10
0	N	N	N	N
5	R	N	R	N
7.5	R	N	R	N
10	R	R	R	R
15	R	R	R	R
25	R	R	R	R

R = Reactive

N = Nonreactive

## XI. AVAILABILITY

Description	Catalog #
<b>RUBAscan™</b> 30 Test Kit (Qualitative).	261926
<b>RUBAscan™</b> 100 Test Kit (Qualitative).	261701
<b>RUBAscan™</b> 500 Test Kit (Qualitative).	261805
<b>RUBAscan™</b> Semi-Quantitative Test Cards, Box of 30.	262030
<b>Macro-Vue™</b> Card Test Rotator (with humidifying cover), 100 ± 2 rpm, automatic timer, friction drive, Model 51-II (110V).	278051
<b>Macro-Vue™</b> Card Test Rotator Accessories	277979

Package, containing one 15" x 7" extension top and two humidifying covers.

## XII. REFERENCES

1. Rawls, W.E. and Chervesky, M.A.: Rubella Virus, *Man. Clin. Immun.*, Rose, N.E. and Friedman, H., Eds., Chap. 61:452-455, 1976.
2. Millian, S.J. and Wegman, D.: Rubella Serology: Applications, Limitations and Interpretations., *Amer. J. Pub. Health*, 62:170-176, 1972.
3. Centers for Disease Control Morbidity and Mortality Weekly Report, Vol. 30, No. 4, Feb. 6, 1981. Recommendation of the Advisory Committee on Immunization Practices (ACIP).
4. Ambrose, C.T. and Donner, A.: Application of the Analysis of Variance to Hemagglutination Titrations, *J. Immun. Meth.*, 3:165-210, 1973.
5. Castellano, G.A., Madden, D.L., Hazzard, G.T., Cleghorn, C.S., Vails, D.V., Ley, A.C., Tzan, N.R. and Sever, J.L.: Addendum, Evaluation of Commercially Available Diagnostic Test Kits for Rubella, *J. Infec. Dis.*, 143:583, 1981.
6. Herrmann, K.L.: Centers for Disease Control, Personal Communications, 1981.
7. Urquhart, G.E.D., Worswick, D.A.W., Grist, N.R.: *J. Clin. Pathol.*, 29:1101-1104, 1976.
8. Kilgore, J.M.: *J. Med. Virol.*, 3:231-236, 1979.
9. Brody, J.P., Binkley, J.H., Harding, S.A., *J. Clin. Microbiol.*, 10:708-711, 1979.
10. Haukenes, G.: *Acta Pathol. Microbiol. Scand.*, (B)88:85-87, 1980.
11. Roberts, P.C., Hobbs, S.J.: *J. Clin. Pathol.*, 30:1011-1014, 1977.
12. Birch, C.J., Glaun, B.P., Hunt, V., Irving, L.G., Gust, I.D.: *J. Clin. Pathol.*, 32:128-131, 1979.
13. Meurman, O.H.: *Infec. Immun.*, 19:369-372, 1978.
14. Castellano, G.A., Madden, D.L., Hazzard, G.T., Cleghorn, C.S., Vails, D.V., Ley, A.C., Tzan, N.R., Sever, J.L.: *J. Infec. Dis.*, 143:578-584, 1981.
15. U.S. Dept. of HEW: Quality Control for Immunologic Tests, June, 1979.
16. Christensen, M.L.: *Clin. Microbiol.*, Newsletter, 2, Number 11, 1980.
17. Meegan, J.M., Evans, B.K. and Horstmann, D.M.: *J. Clin. Microb.*, 16:644-649, Oct. 1982.
18. Horvath, L.M., LeBar, W.D.: *Am. Clin. Prod. Rev.* 6-4:18-19, 1987.
19. Steece, R.S., et al., *J. Clin. Path.*, 19:923-925, 1984.
20. NCCLS Document, Evaluation and Performance Criteria for Multiple Component Test Products Intended for the Detection and Quantitation of Rubella IgG Antibody 1/LA6-T, 1992.
21. NCCLS Document, Specifications for Immunological Testing for Infectious Diseases, Approved Guideline 1/LA18-A, 1994.

TECHNICAL INFORMATION: In the United States, telephone Becton Dickinson Diagnostic Systems Technical Services, toll free (800)-638-8663.

Approved by:

Supervisor

Date

Director

Date

Reviewed:

PI Rev. 5/99  
Rev. 07/02