Subject/Title: ASI RUBELLA IgG EIA TEST		Doc#: 800096AG
Effective Date: 09/06/17	Supersedes Revision/Date: 02/18/16	Revision: 09/06/17
Prepared by: ASI Thomas	QA Approval by:	Copy/Dept.:

FOR IN VITRO DIAGNOSTIC USE

1.0 INTENDED USE: For the qualitative, semiquantitative and quantitative detection of human IgG antibodies to rubella virus in human serum by enzyme immunoassay, to aid in the assessment of the patient's immunological response to rubella, and as a qualitative screening test to determine the immune status of individuals, including women of childbearing age. These reagents have not received FDA clearance for use in testing blood or plasma donors.

2.0 SUMMARY AND EXPLANATION OF THE TEST:

The most serious manifestation of acute rubella infection is congenital rubella syndrome, as a result of infection during pregnancy, particularly during the first trimester. To prevent congenital rubella syndrome, the screening and immunization of susceptible women of childbearing age is essential. The detection of IgG antibodies to rubella virus is indicative of previous infection and presumptive immunity[1,2], therefore, screening by serological methods will establish the immune status of individuals with regard to their resistance or susceptibility to rubella infection.

The usual means of diagnosis of active or recent rubella infection is also serological. This can be accomplished either by the demonstration of a four- fold or greater rise in virus-specific IgG antibody in acute / convalescent serum pairs, or by the detection of virus-specific IgM antibody in a single, early serum specimen.

The traditional serologic test for evaluating rubella antibody, hemagglutination-inhibition, has been replaced by the enzyme-linked immunosorbent assay (ELISA)[3]. The Rubella IgG EIA test is an ELISA which utilizes a microwell format. Test results are obtained after one and one-half hours incubation time. They are objective and normalized as Index values, or as International Units (IU/ml), which are traceable to the WHO International Reference Preparation of Anti-Rubella Serum[4].

3.0 PRINCIPLE OF THE PROCEDURE:

Diluted samples are incubated in antigen-coated wells. Rubella antibodies (if present) are immobilized in the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme labeled antibodies to human IgG) is added and incubated. If IgG antibodies to rubella are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

4.0 REAGENTS

4.1	Coated Wells	Coated wells with sonicated rubella antigen (strain HPV-77, produced in Vero cells, and purified by proprietary centrifugation steps). 12 eight-well strips.
4.2	Well support	One
4.3	Diluent*	25 ml (pink color). Diluent for specimens. Phosphate-buffered saline with a protein stabilizer.
4.4	Rubella IgG Calibrator 1*	0.3 ml. Human serum. Strongly reactive for rubella IgG antibodies. Index or IU/ml values shown on vial label.
4.5	Rubella IgG Calibrator 2*	0.3 ml. Human serum. Moderately reactive for rubella IgG antibodies. Index or IU/ml values shown on vial label.
4.6	Rubella IgG High Positive Control*	0.3 ml. Human serum. Reactive for rubella antibodies. Index or IU/ml values shown on vial label.
4.7	Rubella IgG Low Positive Control*	0.3 ml. Human serum. Weakly reactive for rubella antibodies. Index or IU/ml values shown on vial label.
4.8	Rubella Negative Control*	0.3 ml. Human serum. Non-reactive for rubella antibodies.
4.9	Rubella IgG Conjugate	12 ml (green color). Goat anti-human IgG labeled with alkaline phosphatase (calf).
4.10	Substrate	12 ml. p-nitrophenyl phosphate
	Note: The substrate may develop a slight yellow color during storage. One hundred microliters of substrate should yield an absorbance value less than 0.35, when read in a microwell against air or water.	
4.11	Wash Concentrate*	30 ml. Tris-buffered saline with Tween 20, pH 8.0. Prepare Wash Solution by adding the contents of the Wash bottle to 1 liter, of distilled or deionized water.
4.12	Stop Reagent	12 ml. Trisodium Phosphate 0.5 M.

* Contains 0.1% sodium azide

Store these reagents at 2-8°C. up to the expiry date indicated on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

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5.0 WARNINGS AND PRECAUTIONS

5.1 For *in vitro* diagnostic use.

5.2 Test samples, Calibrator(s), Controls and the materials that contact them, should be handled as potential biohazards. The calibrators and controls have been found to be negative for HIV, hepatitis B surface antigen and HCV antibodies by licensed tests. However, because no method can offer complete assurance that HIV, hepatitis B virus, HCV or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories", 1993, or latest edition.

5.3 The concentrations of anti-rubella IgG in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

5.4 Avoid contact with open skin.

5.5 Never pipet by mouth.

5.6 Certain of the test reagents contain sodium azide. Azides are reported to react with lead and copper in plumbing to form compounds that may detonate on percussion. When disposing of solutions containing sodium azide, flush drains with large volumes of water to minimize the build-up of metal-azide compounds.

5.7 R 21/22: Harmful in contact with skin and if swallowed.

5.8 S24/25 - 36/37/39: Avoid contact with skin and eyes. Wear suitable protective clothing, gloves and eye/face protection. For further information, refer to product SDS.

5.9 Do not interchange reagents from different reagent lots, except for Diluent, Wash solution, Substrate and Stop solution.

5.10 Do not use reagents beyond their stated expiration date.

5.11 Incubation times recommended in the Test Procedure section should be adhered to.

5.11 Unused Coated Wells should be kept in their resealable bag with desiccant, and stored in the refrigerator.

5.12 This product should be used by qualified personnel.

5.13 Do not smoke, eat, drink, or apply cosmetics in areas where plasma/serum samples are handled.

6.0 HANDLING AND PROCEDURAL NOTES

6.1 In order to obtain reliable and consistent results, the instructions in the package insert must be strictly followed. Do not modify the handling and storage conditions for reagents or samples.

6.2 Do not use past the expiration date indicated on the kit.

7.0 STORAGE INSTRUCTIONS

Store all reagents at 2 to 8°C in an upright position when not in use. Do not freeze reagents.

8.0 INDICATIONS OF DETERIORATION

8.1 Turbidity or precipitation in controls is indicative of deterioration and the component should not be used.

8.2 Bacterial contamination of reagents or specimens may cause false positive results.

9.0 SPECIMEN COLLECTION AND STORAGE

9.1 Sera should be separated from clotted blood.

9.2 If specimens are not tested within 8 hours, they should be stored at 2 to 8°C. for up to 48 hours. Beyond 48 hours specimens should be stored at -20°C. or below.

9.3 Multiple freeze-thaw cycles should be avoided.

9.4 Samples containing visible particulate matter should be clarified by centrifugation; and hemolyzed, icteric or grossly contaminated samples should not be used.

9.5 Samples should not be heat-inactivated before testing.

10.0 PERFORMANCE OF TEST

Materials Provided:

96 Tests

Coated Wells	12 eight-well strips
Well Support	1
Rubella IgG Calibrator 1	0.3 ml
Rubella IgG Calibrator 2	0.3 ml
Diluent	25 ml
Rubella IgG High Positive Control	0.3 ml
Rubella IgG Low Positive Control	0.3 ml
Rubella Negative Control	0.3 ml
Rubella IgG Conjugate	12 ml
Substrate	12 ml
Wash Concentrate	30 ml
Stop Reagent	12 ml

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Additional Materials Required:

1. Microplate washer
2. Pipettors for dispensing 4, 100 and 200 µl
3. Timer
4. 1 or 2 liter container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm. Dual wave length readers are recommended.

11.0 TEST PROCEDURE

Preparation for the Assay

11.1 Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use. The test procedure follows:

11.2 Prepare 1:51 dilutions of test samples, Calibrator(s), Positive and Negative Controls, in the Diluent. For example: add 4 µl of sample to 200 µl of Diluent in a dilution well or tube, and mix well.

Note: *For qualitative assays, a single Calibrator may be used (RUBELLA IgG Calibrator 2); for semiquantitative or quantitative assays, use Rubella IgG Calibrator 1 and Rubella IgG Calibrator 2..*

12.0 ASAY PROTOCOL

12.1 Place an appropriate number of Coated Wells from Rubella IgG Plate in the Well Support.
Note*: For combination testing (multiple assays per plate), the strips should be assembled on a white background with good lighting. Be sure to note the placement of each strip and the corresponding color.*

12.2 Transfer 100 µl of each diluted Calibrator, Control and patient sample to the wells.
Note*: Include one well which contains 100 µl of Diluent only. This will serve as the reagent blank and will ultimately be used to zero the photometer before reading the test results.*

12.3 Incubate the wells at room temperature (20 to 25° C) for 30 ± 5 minutes.

12.4 Wash wells four times with at least 250 µl/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.

12.5 Place 100 µl of Rubella IgG Conjugate into each well.

12.6 Incubate the wells at room temperature for 30 ± 5 minutes.

12.7 Wash wells four times with at least 250 µl/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.

12.8 Place 100 µl of Substrate into each well.

12.9 Incubate at room temperature for 30 ± 5 minutes.

12.10 Place 100 µl of Stop solution into each well.

12.11 Read and record the absorbance of the contents of each well at 405 nm against the reagent blank.
Note: *Adjust the photometer to zero absorbance at 405 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.*

13.0 QUALITY CONTROL

Quality control requirements must be performed in accordance with applicable local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control Procedures. Controls and calibrators should be included. If they do not yield the expected response, the assay should be considered invalid and the assay repeated. If the repeat assay does not elicit the expected results for the controls and calibrators, discontinue use of the kit and contact ASI Technical Support at 800-654-0146.

14.0 INTERPRETATION OF RESULTS –

Calculation of Results

Qualitative results may be calculated using a single calibrator. For semi-quantitative and quantitative results, use a calibration curve consisting of two or more calibrators.

Single Calibrator (Rubella IgG Calibrator 2)
Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Rubella IgG Calibrator 2 Index}}{\text{Rubella IgG Calibrator 2 Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

If the Calibrator is run in duplicate, use the average absorbance value to calculate results.

Calibration Curve

Alternatively, test results may be calculated from a three-point curve comprised of: Rubella IgG Calibrator 1 (high-point), Rubella IgG Calibrator 2 (mid- point) and the reagent blank (zero / origin), using a point-to-point curve fit.
The upper range of the curve may be expanded by adding additional points. For example: the concentration of Rubella IgG Calibrator 1 may be increased 1.5-fold, and 2-fold, by adding 6 µl and 8 µl of Rubella IgG Calibrator 1 to 200 µl of the Diluent, and transferring 100 µl of each dilution to coated wells. The Index, or IU/ml values, assigned to these points, should be 1.5 and 2 times respectively, the value shown on the Rubella IgG Calibrator 1 label. The extent to which the upper range of the standard curve may be expanded, will be limited by the Calibrator being used.

Test Validation Criteria

1. The Calibrator(s), Positive and Negative Controls must be included in each test run.
2. The absorbance value of Rubella IgG Calibrator 1 must be at least 0.6, when read against the reagent blank.
3. The absorbance value of the reagent blank should be less than 0.35.
4. The Rubella IgG Negative Control must have an Index value less than 0.9, or an IU/ml value less than 9. This control is used to validate the assay below the cutoff of the assay.
5. The Positive Controls must have Index values, or IU/ml values, within the ranges printed on the labels. The Rubella IgG Low Positive Control is used to validate the assay in the low end of the dynamic range of the assay, and the Rubella IgG High Positive Control is used to validate the upper range of the assay. When performing qualitative tests, users may supply alternative positive controls if they wish.
6. To validate the upper range of the assay when performing the semiquantitative and quantitative procedures, the Rubella IgG High Positive Control may be run at higher concentrations. For example, the Rubella IgG High Positive Control may be assayed at 1.5-fold and 2-fold concentrations by adding 6 µl and 8 µl of the Rubella IgG High Positive Control, to 200 µl aliquots of the Diluent, and transferring 100 µl of each of these dilutions to coated wells. The expected value ranges for these concentrated controls would be 1.5 times and 2 times respectively, the expected value ranges printed on the Rubella IgG High Positive Control label. The assay results for these controls must fall within the corrected ranges. Optionally, users may supply alternative positive controls if they wish.
   If any of these criteria are not met, the test is invalid and should be repeated.
7. The Negative and Positive Controls are intended to monitor for substantial reagent failure. The Positive Controls will not ensure precision at the assay cutoff. Users may wish to establish an in-house control having a quantitative value determined by replicate testing, at or near the cutoff of the assay, to monitor the precision of the assay cutoff. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to NCCLS document C24-A, Internal Quality Control Testing: Principles and Definitions.

Interpretation of Results

Index Value	IU/ml	Interpretation
<0.9	<9	Negative for rubella IgG, presumed non-immune to rubella infection.
≥0.9<1.0	≥9<10	Equivocal
≥1.0	≥10	Positive for rubella IgG, presumed immune to rubella infection.

The Rubella IgG EIA cut-off value has been set at 10 IU/ml based on the WHO International Reference Preparation of Anti-Rubella Serum, 1970, in accord with the CLSI Guideline for the Detection and Quantitation of Rubella IgG Antibody[5].
When equivocal results are obtained, another specimen should be obtained ten to fourteen days later, and tested in parallel with the initial specimen. If the second specimen is also equivocal, the patient is negative for primary or recent infection, and equivocal for antibody status. If the second sample is positive, the patient can be considered to have a primary infection. The conversion of an individual patient's serum from negative to positive for antibodies to the infectious agent in question, is defined as seroconversion, and indicates active or recent infection.

To determine a significant difference between acute/convalescent serum pairs, both specimens should be assayed concurrently. Dose response experiments performed at Laboratory C, Miami, FL, have shown that a 60 to 90 percent increase in the Rubella IgG EIA Index or IU/ml value, corresponds to a two-fold increase in the rubella IgG antibody level; and a 120 to 180 percent increase in Rubella IgG EIA Index or IU/ml value, corresponds to a four-fold increase in the rubella IgG antibody level.

Specimens which yield absorbance values above the range of the test set calibrator(s), may be reported as greater than the Index or IU/ml value of the uppermost point of the calibration curve. Alternatively, such specimens may be pre-diluted in the Diluent and reassayed. The resulting Index or IU/ml value must be multiplied by the dilution factor for reporting. *Example: If the specimen has been pre-diluted 1:5 before testing, the resulting Index value should be multiplied by 5.*

Values obtained with different manufacturer's assay methods may not be used interchangeably. The magnitude of the reported IgG level cannot be correlated to an endpoint titer. When the assay is used qualitatively, the magnitude of results above the cut-off is not an indicator of total antibody present.

15.0 LIMITATIONS OF THE PROCEDURE

The results obtained with the Rubella IgG EIA test serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves. A single positive result only indicates previous immunologic exposure; the level of antibody response or class of antibody response may not be used to determine active infection or disease stage.

Paired specimens should be collected during the acute and convalescent stages of infection, and tested concurrently to detect significant antibody increases. The acute phase sample should be collected early in the infection, preferably within 7 days of the onset of symptoms, and the convalescent phase sample one to two weeks after the first sample, but not earlier than 10 days after the onset of symptoms[4]. The semiquantitative procedure should be used when testing paired sera only. Serum specimens obtained during the acute phase of infection may be negative by serological tests.

Timing of specimen collection for paired sera may be critical. In some patients, antibody titers may rise to significant levels and fall to lower or undetectable levels within a month. Other patients may not develop significant antibody levels. Culture results, serology and antigen detection methods should all be appropriately used along with clinical findings for diagnosis.

The assay performance characteristics have not been established for matrices other than serum.

If the assay is used with cord blood as the specimen source, positive results should be interpreted with caution. The presence of IgG antibodies to rubella in cord blood may be the result of passive transfer of maternal antibody to the fetus. A negative result however, may be helpful in ruling out infection. Performance characteristics have not been determined with neonatal or cord blood.

Titration experiments (please see Figure 2) have shown that the upper limit of linearity for Rubella IgG EIA IU/ml values is approximately 40.

16.0 EXPECTED VALUES

Since the program for rubella immunization was initiated in the United States, epidemiological studies have shown that 85 to 90 percent of adults have antibodies to rubella virus[5]. Serum samples obtained randomly from 214 normal South Florida blood donors were assayed at Laboratory C, Miami, FL, using the Rubella IgG EIA test. One hundred and eighty-eight samples (88 %) were positive for IgG antibodies to rubella, nineteen (9 %) were negative, and seven (3 %) were equivocal. The positive samples yielded Index values between 1.0 and 8.7; and IU/ml values between 10 and 86.8. The mean Index and IU/ml values were 4.4 and 44 respectively. The incidence of these values is shown in Table 1.

Table 1. Results of tests of 214 Specimens (100% frozen), from Normal South Florida Donors, Performed at Laboratory C, Miami, FL, Using the Rubella IgG EIA Test. Seventeen Percent of the Specimens Tested were Obtained from Women of Childbearing Age.

IU/ml Value Ranges	Index Value Ranges		Specimens
< 10	< 1	27 {3}	12.6 %
≥ 10 to < 30	≥ 1 to < 3	44 {14}	20.6 %
≥ 30	≥ 3	143 {19}	66.8 %

{ } Number of female donors of childbearing age.

One hundred and sixty-four women of childbearing age (18 to 45 years) were identified in the clinical studies. They ranged in age from 18 to 45, with a mean age of 31. Of these, 126 (76.8 %) were positive, 9 (5.5 %) were equivocal, and 29 (17.7 %) were negative, when tested by the Rubella IgG EIA test. The incidence of the values obtained for these women is shown in Table 2.

Table 2. Results of tests of 164 Specimens, from Women of Childbearing Age (18-45), Performed at Laboratory A (Atlanta, GA), Laboratory B (Miami, FL) and at Laboratory C (Miami, FL) Using the Rubella IgG EIA Test.

IU/ml Value Ranges	Index Value Ranges		Specimens
< 10	< 1	38	23.1 %
≥ 10 to <30	≥ 1 to < 3	48	29.3 %
≥ 30	≥ 3	78	47.6 %

17.0 PERFORMANCE CHARACTERISTICS

Comparative Testing

Rubella IgG EIA test results correlate well with results of other serological tests. Sera from normal blood donors were assayed for the presence of rubella IgG antibodies, using the Rubella IgG EIA test and three other commercial tests, at two independent laboratories (Lab A, Atlanta, GA, and Lab B, Miami, FL), and at Laboratory C (Miami, FL). These results are shown below in Tables 3, 4, 5 and 6, respectively.

Table 3. Results of Tests of 201 Specimens (89% frozen and 11% fresh), from North and South Carolina, Alabama, Georgia and Florida, Performed at Laboratory A (Atlanta, GA), Using the Rubella IgG EIA Test and Another Commercial Test. Fourteen Percent of the Specimens Tested were Obtained from Women of Childbearing Age.

Comparative Test #3	Rubella IgG EIA Positive	Equivocal	Negative		95 % C.I.
Positive	143 {25}	3 {2}	2 {1}	Relative sensitivity	94.0 to 100**
Equivocal	0	0	0		
Negative	0	0	53	Relative specificity	93.3 to 100**
				Overall Agreement*	97.6 to 100***

*Excluding equivocal results

**Calculated by the Exact Method.

***Calculated by the Normal Method (6).

{ } Number of female donors of childbearing age.

Table 4. Results of tests of 200 Specimens (62% frozen and 38% fresh), Performed at Laboratory B (Miami, FL), Using the Rubella IgG EIA Test and Another Commercial Test. Forty-nine Percent of the Specimens Tested were Obtained from Women of Childbearing Age.

Comparative Test #2	Rubella IgG EIA Positive	Equivocal	Negative		95 % C.I.
Positive	126 {68}	8 {4}	2 {1}	Relative sensitivity*	94.5 to 99.8**
Negative	0	2 {2}	62 {25}	Relative specificity*	94.2 to 100**
				Overall Agreement*	97.5 to 100***

* Excluding equivocal results
** Calculated by the Exact Method.
*** Calculated by the Normal Method (6).
{ } Number of female donors of childbearing age.

Table 5. Results of tests of 214 Random Specimens (100% frozen), from South Florida, Performed at Laboratory C (Miami, FL), Using the Rubella IgG EIA Test and Another Commercial Test. Fourteen Percent of the Specimens Tested were Obtained from Women of Childbearing Age.

Comparative Test #1	Rubella IgG EIA Positive	Equivocal	Negative		95 % C.I.
Positive	188 {33}	3 {1}	10 {2}	Relative sensitivity	90.9 to 97.6**
Negative	0	0	13	Relative specificity	75.3 to 100**
				Overall Agreement*	92.4 to 98.1***

* Excluding equivocal results
** Calculated by the Exact Method.
*** Calculated by the Normal Method (6).
{ } Number of female donors of childbearing age.

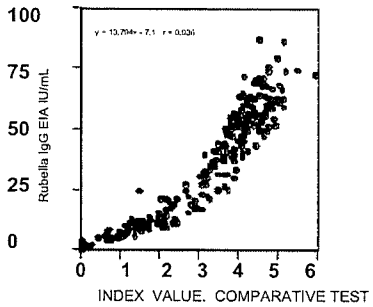
Table 6. Results of tests of 46 Selected Negative and Weakly Positive Specimens (100% frozen), from South Florida, Georgia and Delaware, Performed at Laboratory C (Miami, FL), Using the Rubella IgG EIA Test and Another Commercial Test.

Comparative Test #1	Rubella IgG EIA Positive	Equivocal	Negative		95 % C.I.
Positive	16	1	1	Relative sensitivity	71.3 to 99.8**
Negative	0	0	28	Relative specificity	87.7 to 100**
				Overall Agreement*	93.5 to 98.1***

* Excluding equivocal results
** Calculated by the Exact Method
*** Calculated by the Normal Method (6).

The data obtained at Lab C and tabulated in Tables 5 & 6, has been plotted below in Figure 1.

Figure 1. Results of Tests of 260 Serum Specimens Performed at Lab C (Miami, FL), Using the Rubella IgG EIA Test and Another Commercial Test.



Calibration, Quantitative Procedure

To demonstrate the accuracy of the quantitative procedure, dilutions of the WHO standard, were assayed versus the Rubella IgG EIA calibration curve. The results are shown in Table 7.

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Table 7. Results Obtained When Triplicate Dilutions of the WHO Anti-Rubella Serum, 2nd International Standard Preparation, Were Assayed in Parallel with the Rubella IgG EIA Calibration Curve (Calibrator 1, Calibrator 2 and the Blank).

RUBELLA IgG EIA CALIBRATOR	ASSIGNED IU/ml VALUE	ABSORBANCE* 405 nm
BLANK	0	0.113
CALIBRATOR 2	12	0.838
CALIBRATOR 1	45	1.705

DILUTION OF WHO STANDARD	EXPECTED IU/ml VALUE	ABSORBANCE 405 nm	RECOVERED IU/ml VALUE	MEAN IU/ml	STD. DEV. IU/ml	95 % C.I. IU/ml
1:25	40	1.589	40.5			
1:25	40	1.392	33.1	37.9	4.2	30.9 to 44.9
1:25	40	1.577	40.1			
1:50	20	1.083	21.3			
1:50	20	1.028	19.2	20.9	1.5	18.3 to 23.5
1:50	20	1.107	22.2			
1:100	10	0.692	9.5			
1:100	10	0.675	9.3	9.8	0.6	8.7 to 10.9
1:100	10	0.747	10.5			

* Mean of duplicate results.

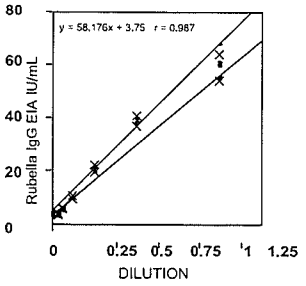
Cutoff Verification

Rubella IgG EIA utilizes the CDC Low-titer Anti-Rubella Human Reference Serum, CDC Biological Standard, to verify the Rubella IgG EIA assay cutoff. This standard, which contains 21.0 IU/ml of Rubella IgG antibody, is diluted 1:2 in the test set diluent, and assayed during the quality control testing of each production lot of Rubella IgG EIA. The expected value for the 1:2 dilution of the CDC standard is 10 to 15 IU/ml, which is in agreement with the CDC immunity cutoff reference level. For example: the Rubella IgG EIA lot which was used to obtain the majority of the data presented in the Performance Characteristics section of this package insert, gave a value of 11.2 IU/ml for the 1:2 of the CDC standard, during quality control testing.

Titration curve

Several strongly positive serum specimens were serially diluted (two-fold) in triplicate, and assayed by the Rubella IgG EIA test. Typical results are shown in Figure 2.

Figure 2. Titration Curve for a Strongly Positive Specimen.



The triplicate data for each dilution are shown as points, the 95 % confidence limits for each set of triplicate data are indicated by (x's), and the 95 % confidence limits for the slopes and y-intercepts are represented by straight lines. The formula for the linear regression for the triplicate data is shown in Figure 2.

Specificity

The Rubella IgG EIA test is specific for IgG antibodies directed against rubella, and does not cross-react with a variety of other viruses or nuclear antigens. Of fifty-nine specimens which were unreactive in the Rubella IgG EIA test, 11 were shown to contain moderate to high levels of IgG antibody directed against cytomegalovirus, 14 against herpes simplex virus, 33 against varicella zoster virus, 35 against Epstein-Barr virus, 2 against type A influenza, 10 against measles, 2 against parvovirus B-19, and 8 against the nuclear antigens. The IgG antibodies directed against cytomegalovirus, herpes simplex virus, varicella zoster virus and Epstein-Barr virus were detected using commercially available enzyme immunoassays. IgG antibodies to type A influenza, measles and parvovirus B-19, were detected by complement fixation methods; and IgG antibodies against the nuclear antigens were detected by immunofluorescence.

Precision

Eight serum specimens (3 negative and 5 positive) and the Rubella IgG EIA Low Positive and Negative Controls, were assayed in triplicate, on three separate occasions. The precision experiments were performed manually at two independent laboratories (Lab A and Lab B), and at Laboratory C. These results are shown below.

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Table 8. Results of Intra-assay and Interassay Precision Tests Performed at Lab A. Values were calculated from the Rubella IgG EIA Index & IU/ml values.

	INTRA-ASSAY						INTERASSAY					
SAMPLE	MEAN INDEX	S.D.	C. V. %	MEAN IU/ml	S.D	C.V. %	MEAN INDEX	S.D.	C.V. %	MEAN IU/ml	S.D.	C.V. %
Pos.	1.2	0.070	6.0	14.3	2.234	15.6	1.4	0.211	14.7	19.9	4.429	22.2
Neg.	0.2	0.008	NA	0.5	0.058	NA	0.2	0.024	NA	0.5	0.194	NA
1	0.2	0.002	NA	1.2	0.000	NA	0.2	0.012	NA	1.0	0.217	NA
2	0.2	0.008	NA	1.0	0.115	NA	0.2	0.008	NA	0.7	0.194	NA
3	1.4	0.066	4.7	17.8	1.845	10.4	1.4	0.102	7.4	17.0	2.934	17.3
4	0.4	0.009	NA	3.4	0.115	NA	0.4	0.018	NA	3.4	0.164	NA
5	1.2	0.095	7.7	13.3	2.173	16.4	1.3	0.071	5.6	14.0	1.816	13.0
6	1.1	0.055	5.2	10.3	0.252	2.5	1.1	0.055	5.2	10.5	0.572	5.5
7	1.4	0.049	3.6	16.4	1.350	8.3	1.4	0.121	8.3	19.1	3.563	18.7
8	1.8	0.188	10.7	27.2	5.140	18.9	2.0	0.203	10.4	33.6	6.233	18.5

Table 9. Results of Intra-assay and Interassay Precision Tests Performed at Lab B. Values were calculated from the Rubella IgG EIA Index & IU/ml values.

	INTRA-ASSAY						INTERASSAY					
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN IU/ml	S.D	C.V. %	MEAN INDEX	S.D.	C.V. %	MEAN IU/ml	S.D.	C.V. %
Pos.	1.7	0.031	1.9	23.3	0.751	3.2	1.6	0.053	3.3	22.8	1.848	8.1
Neg.	0	0.009	NA	0.1	0.058	NA	0	0.011	NA	0.1	0.173	NA
1	0.1	0.005	NA	0.7	0.058	NA	0.1	0.037	NA	0.6	0.406	NA
2	0.1	0.007	NA	0.5	0.058	NA	0	0.013	NA	0.4	0.176	NA
3	1.4	0.027	1.9	16.5	0.611	3.7	1.4	0.087	6.1	17.8	1.979	11.1
4	0.4	0.008	NA	4.1	0.115	NA	0.4	0.047	NA	4.1	0.502	NA
5	1.4	0.097	6.7	17.8	2.329	13.1	1.5	0.096	6.6	18.7	2.243	12.0
6	1.3	0.066	5.1	13.9	1.582	11.4	1.3	0.064	5.0	13.6	1.344	9.9
7	1.8	0.159	9.0	25.6	3.790	14.8	1.8	0.112	6.3	26.6	2.558	9.6
8	2.1	0.065	3.0	34.5	1.587	4.6	2.2	0.076	3.5	37.3	2.939	7.9

Table 10. Results of Intra-assay & Interassay Precision Tests Performed at Lab C. Values calculated from the Rubella IgG EIA Index & IU/ml values.

	INTRA-ASSAY			INTRA-ASSAY			INTRA-ASSAY			INTRA-ASSAY		
SAMPLE	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	S.D.	C.V. %	MEAN INDEX	S.D.	C.V. %	MEAN IU/ml	S.D.	C.V. %
Pos.	1.8	0.108	6.1	25.4	2.542	10.0	1.7	0.109	6.4	24.2	2.652	11.0
Neg.	0.1	0.024	NA	0.3	0.252	NA	0.1	0.024	NA	0.3	0.252	NA
1	0.1	0.006	NA	0.4	0.058	NA	0.1	0.006	NA	0.3	0.071	NA
2	0.1	0.018	NA	0.3	0.208	NA	0.1	0.016	NA	0.3	0.181	NA
3	1.5	0.135	8.8	19.7	3.139	16.0	1.5	0.135	8.9	19.8	3.400	17.2
4	0.5	0.016	NA	4.9	0.153	NA	0.5	0.039	NA	4.7	0.409	NA
5	1.4	0.025	1.7	17.2	0.577	3.4	1.4	0.105	7.5	17.0	2.717	16.0
6	1.3	0.072	5.4	14.9	1.701	11.4	1.3	0.117	9.0	14.6	2.919	19.9
7	1.9	0.160	8.3	29.0	3.781	13.0	1.9	0.178	9.5	28.6	4.658	16.3
8	2.3	0.180	7.7	38.4	4.215	11.0	2.3	0.106	7.1	37.9	4.194	11.1

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Table 11. Interlaboratory Precision. Tests Were Performed at Lab A, Lab B and Lab C. Values were calculated from the Rubella IgG EIA Index & IU/ml values.

	INDEXES			IU/ml		
SAMPLE	MEAN	S.D	C.V. %	MEAN	S.D.	C.V. %
Pos.	1.6	0.177	11.2	22.3	2.164	9.7
Neg.	0.1	0.076	NA	0.3	0.208	NA
1	0.1	0.074	NA	0.6	0.340	NA
2	0.1	0.069	NA	0.5	0.213	NA
3	1.4	0.121	8.4	18.2	1.450	8.0
4	0.5	0.056	NA	4.1	0.656	NA
5	1.4	0.122	8.9	16.6	2.366	14.3
6	1.2	0.131	10.8	12.9	2.173	16.8
7	1.7	0.231	13.6	24.8	5.025	20.3
8	2.1	0.200	9.4	36.3	2.324	6.4

Table 12. Results of Lot-to-Lot Precision Tests Performed at Lab C. Values were calculated from the Rubella IgG EIA Index values.

	Lot L0305			Lot L0405			Lot L1005A					
	INTERASSAY			INTERASSAY			INTERASSAY			LOT-TO-LOT		
SAMPLE	MEAN	S.D.	C.V. %	MEAN	S.D.	C.V. %	MEAN	S.D.	C.V. %	MEAN	S.D.	C.V. %
Pos.	1.7	0.109	6.4	1.6	0.147	9.1	1.6	0.135	8.2	1.7	0.132	8.0
Neg.	0.1	0.024	NA	0.1	0.003	NA	0.3	0.021	NA	0.2	0.119	NA
1	0.1	0.006	NA	0.1	0.005	NA	0.3	0.033	NA	0.2	0.124	NA
2	0.1	0.016	NA	0.1	0.004	NA	0.3	0.022	NA	0.2	0.115	NA
3	1.5	0.135	8.9	1.4	0.124	9.0	1.4	0.125	9.1	1.4	0.141	9.9
4	0.5	0.039	NA	0.4	0.039	NA	0.5	0.044	NA	0.5	0.059	NA
5	1.4	0.105	7.5	1.3	0.097	7.4	1.3	0.104	8.3	1.3	0.117	8.9
6	1.3	0.117	9.0	1.3	0.153	12.1	1.2	0.151	12.4	1.3	0.141	11.2
7	1.9	0.178	9.5	1.8	0.156	8.9	1.6	0.139	8.7	1.7	0.190	10.9
8	2.3	0.160	7.1	2.1	0.177	8.3	2.0	0.138	6.8	2.1	0.184	8.6

Table 13. Results of Lot-to-Lot Precision Tests Performed at Lab C. Values were calculated from the Rubella IgG EIA IU/ml values.

	Lot L0305			Lot L0405			Lot L1005A					
	INTERASSAY			INTERASSAY			INTERASSAY			LOT-TO-LOT		
SAMPLE	MEAN	S.D.	C.V. %	MEAN	S.D.	C.V. %	MEAN	S.D.	C.V. %	MEAN	S.D.	C.V. %
Pos.	24.2	2.652	11.0	21.8	3.627	16.7	25.7	3.635	14.1	23.9	1.988	8.3
Neg.	0.3	0.252	NA	0	0.053	NA	2.8	0.200	NA	1.1	1.544	NA
1	0.3	0.071	NA	0.2	0.112	NA	3.1	0.350	NA	1.2	1.621	NA
2	0.3	0.181	NA	0.1	0.100	NA	2.8	0.224	NA	1.1	1.508	NA
3	19.8	3.400	17.2	16.1	2.808	17.4	18.8	3.605	19.2	18.2	1.891	10.4
4	4.7	0.409	NA	3.7	0.443	NA	4.4	0.436	NA	4.3	0.539	NA
5	17.0	2.717	16.0	14.8	2.232	15.1	15.2	2.998	19.7	15.7	1.193	7.6
6	14.6	2.919	19.9	13.7	3.317	24.3	14.2	4.195	29.5	14.2	0.485	3.4
7	28.6	4.658	16.3	24.8	3.942	15.9	24.7	4.121	16.7	26.0	2.217	8.5
8	37.9	4.194	11.1	34.4	4.575	13.3	35.7	3.255	9.1	36.0	1.772	4.9

CDC Panel Results

The following information was obtained with the Centers for Disease Control and Prevention (CDC) serum panel for rubella serology assays, which was tested at Laboratory C by the Rubella IgG EIA test. 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 by the CDC.

The panel consists of 82% positive and 18% negative samples. The Rubella IgG EIA test demonstrated 100% total agreement with the CDC results. Of the results obtained by Lab C, there was 100% agreement with the positive specimens, and 100% agreement with the negative specimens.

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