GRUPO MEXLAB®

Chemiluminescence Immunoassay

Rubella IgG

Catalog No. CL10226 (96 tests)

SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:40 = 5 µL / 200 µL		
2	Diluted samples, controls & calibrators	100 µL	30 minutes
3	Washing buffer (3 times)	350 µL	
4	Enzyme conjugate	100 µL	30 minutes
5	Washing buffer (3 times)	350 µL	
6	Substrate A and Substrate B mixture	100 µL	5 minutes
7	Read with Luminometer in 5~30 minutes		

NAME AND INTENDED USE

Rubella IgG Chemiluminescence ELISA is intended for use in evaluating a patient's serologic status to rubella virus infection. It is also used to evaluate paired sera for the presence of a significant increase in specific IgG as indicative of a recent or current rubella virus infection.

SUMMARY AND EXPLANATION OF THE TEST

Rubella is a herpes virus. Generally rubella is considered a mild adolescence disease. However a maternal infection could be transmitted through the placenta to the fetus, causing congenital rubella. Congenital rubella may result in chronic cardiac disease, growth retardation, hepatosplenomegaly, malformations and other severe anomalies. Children born asymptomatic may develop these abnormalities later in life.

To reduce risk of such severe complications, accurate serological methods must be performed to determine the serologic status of childbearing aged women. The presence of rubella specific IgG in the bloodstream attests immunity to rubella. A woman tested to be nonimmune can be educated on the availability of vaccination. An increase in rubella IgG denotes an acute infection and differentiates rubella from other exanthematous diseases. Expecting women with current rubella infection should be counseled on the consequences of congenital infection.

PRINCIPLE OF THE TEST

Purified rubella antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the rubella IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and substrate A & substrate B mixture is added. The light generated (RLU) is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell luminometer compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1.Microwell strips: Rubella antigen coated wells.(12 x 8 wells)

2.Sample diluent: Blue color solution. 1 vial (22 mL)
3.Washing concentrate 10x. 1 bottle (100 mL)

4.Enzyme conjugate: Red color solution. 1 vial (12 mL)

5.Substrate A: H2O2 in buffer. Natural bottle. 1 vial (6 mL)

6.Substrate B: Luminol in buffer. Amber bottle. 1 vial (6 mL)

7.Negative Calibrator: 0 IU/mL. Natural Cap. 1 vial (150 µL)

8.Cut-off Calibrator: 15 IU/mL.Yellow Cap. 1 vial (150µL) Rubella G Index = 1.0

9.Positive Calibrator: 30 IU/mL. Red Cap. 1 vial (150µL)

10.Positive Calibrator: 100 IU/mL. Green Cap. 1 vial (150µL)

11.Negative control: Range on label. Blue Cap. 1 vial (150µL)

12.Positive control: Range on label. Brown Cap. 1 vial (150µL)

STORAGE AND STABILITY

1.Store the kit at 2 - 8 ˚C.

2.Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.

3.The reagents are stable until expiration of the kit.

4.Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1.Potential biohazardous materials:

The calibrator and controls contain human source components which have been tested and found nonreactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984

2.Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.

3.The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.

4.This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

SPECIMEN COLLECTION AND HANDLING

1.Collect blood specimens and separate the serum.

2.Specimens may be refrigerated at 2 - 8 ˚C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

3.If rubella is suspected clinically, a blood specimen should be taken within three days after onset of a rash and a second specimen taken at least two weeks later. Test both serums for antibody simultaneously.

PREPARATION FOR ASSAY

1.Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to a final volume of 1 liter.

2.Bring all specimens and kit reagents to room temperature (20-25 ˚C) and gently mix.

ASSAY PROCEDURE

1.Prepare 1:40 dilutions by adding 5 µL of the samples, negative control, positive control, and calibrators to 200 µL of sample diluent. Mix well.

2.Place the desired number of coated strips into the holder.

3.Dispense 100 µL of diluted sera, calibrators, and controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.

4.Remove liquid from all wells and repeat washing three times with washing buffer.

5.Dispense 100 µL of enzyme conjugate to each well and incubate for 30 minutes at room temperature.

6.Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.

7.Mix equal volume of Substrate A & Substrate B, then dispense 100 µL of this mixture to each well.

8.Read RLU with a microwell luminometer within 5~30 minutes.

CALCULATION OF RESULTS

Determination of Index values

1.Calculate the mean of duplicate RLU values (B).

2.Calculate the Rubella G Index of each determination by dividing the mean values of each sample (B) by Cut-off calibrator mean value (C).

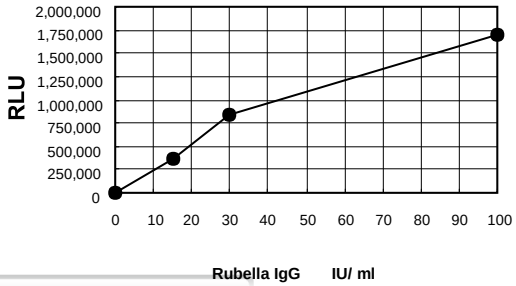
Example 1:

Sample	Well No	RLU (A)	Mean RLU(B)	INDEX B/C
Cut-off Calibrator	A1 B1	360888 354484	357686 (C)	1
Positive Calibrator	C1 D1	822955 842450	832703	2.3
Negative Control	E1 F1	10197 10777	10487	0.03
Positive Control	G1 H1	1208500 1192400	1200450	3.36
Patient Sample	A2 B2	1670150 1709850	1690000	4.7

QUANTITATIVE ESTIMATION OF RUBELLA IgG

For a quantitative determination of anti-Rubella IgG levels of specimens in IU/ml unit, RLU of calibrators are plotted on Y-axis in graph versus their corresponding anti-Rubella IgG concentration 0, 15, 30, and 100 IU/ml on X-axis. The estimates of levels in patient sera are read off the point to point curve using their individual RLU values.

Example 2:



QUALITY CONTROL

1.In order for the assay results to be considered valid the controls should be within the ranges indicated on the labels.

2.The RLU values vary with the different luminometer used.

3.Each laboratory should assay controls at levels in low, normal and elevated ranges for monitoring assay performance. Quality control trends should be maintained to monitor batch to batch consistency.

INTERPRETATION

Negative: Rubella G Index of 0.90 or less are seronegative for IgG antibody to Rubella virus. (< 13 IU/mL)

Equivocal: Rubella G Index of 0.91 - 0.99 are equivocal. Sample should be retested.

Positive: Rubella Index of 1.00 or greater, or IU value greater than 15 are seropositive. It indicates prior exposure to the rubella virus. (> 15 IU/mL)

Significant change in antibody level:
The ratio between the Rubella G Index of convalescent sample and that of pre-vaccination sample should be greater than 1.5 to be suggestive of a significant rise in antibody level.

LIMITATIONS OF THE PROCEDURE

1.A single serum sample cannot be used to determine recent infection.

2.A serum specimen taken in an early stage during acute phase of infection may contain low levels of IgG antibody and render a Rubella G Index result negative.

3.As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity:

Sensitivity, specificity and accuracy were evaluated using a commercial available ELISA kit on 117 specimens. The correlation results are summarized in the following table:

		Reference ELISA			
		N	E	P	Total
Rubella IgG	N	71 (D)	0	0 (B)	71
Chemiluminescence	E	0	3	0	4
ELISA	P	0(C)	1	41(A)	42
	Total	71	4	41	117

Sensitivity = A / (A+B)= 41 / (41+0) = 100%
Specificity = D / (D+C)= 71 / (71+0) = 100% Accuracy
= (A+D) / (A+B+C+D)
= 112 / 112 = 100%

Parallelism / Recovery Study:
In dilution experiments, sera with high IgG antibody concentration were diluted with Sample Diluent and assayed with the test kit. For positive IgG sample Index 2.5 and below, percentage of recovery results are between 110% to 90%. For Index IgG higher than 2.5, a further dilution have to make in order to obtain a better accuracy of quantitative determination.

Precision:
The precision of the assay was evaluated by testing three different sera eight replicates on 3 days. The intra-assay and inter-assay C.V. are summarized below:

	Negative	Low positive	Positive
Intra-assay	9.1%	8.5%	6.4%
Inter-assay	10.5%	8.9%	7.5%

Cross-reactivity:
A study was performed to determine the cross-reactivity of the test with samples which tested for positive IgG. The results indicate an absence of cross-reactivity of CLIA Rubella IgG with positive RF, ANA, VZV, Measles, Chlamydia trachomatis, HSV 1, HSV 2, Toxo, CMV and H. pylori.

REFERENCES

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