GRUPO MEXLAB®

Chemiluminescence Immunoassay

Rubella IgM

Catalog No. CL10227 (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 IgM Chemiluminescence ELISA is intended for use in the detection of IgM antibody to the rubella virus.

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.

PRINCIPLE OF THE TEST

Purified rubella antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the rubella IgM 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 and

Substrate B are added. The light generated (RLU) is proportional to the amount of IgM 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: purified Rubella antigen coated wells. (12 x 8 wells)

2.Absorbent Solution: Black Cap. 1 vial (22 mL)

3.Calibrator: Factor value (f) stated on label. Red Cap.1 vial (150 µL)

4.Negative Control: Range stated on label. Natural Cap.1 vial (150 µL)

5.Positive Control: Range stated on label. Green Cap.1 vial (150 µL)

6.Washing Concentrate 10x. 1 bottle (100 mL)

7.Enzyme Conjugate: Red color solution.1 vial (12 mL)

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

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

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

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 absorbent solution. 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.

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.

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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.To obtain Cut off value: Multiply the RLU of Calibrator by Factor (f) printed on label of Calibrator.

2.Calculate the IgM Index of each determination by dividing the RLU values of each sample with obtained RLU value of Cut off.

NOTE: This factor (f) is a variable. It is specific for a lot manufactured and printed on label of Calibrator. **For example:**

If Factor (f) value on label = 0.40
900110 x 0.40 = 360044

Sample	RLU	Mean RLU (A)	Calculated Cut off Value(B)	INDEX A/B	Interpretation
Calibrator	897142				
f = 0.40	903078	900110	360044		
Positive Control	1449370 1450960	1450165		4.02	Positive
Negative Control	6556 6978	6767		0.02	Negative
Patient Sample 1	1712547 1800691	1756619		4.88	Positive
Patient Sample 2	128620 118540	123580		0.34	Negative

QUANTITATIVE ESTIMATION OF RUBELLA IgG

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.

	Negative	Low positive	Positive
Intra-assay	10.7%	9.4%	8.6%
Inter-assay	12.8%	10.4%	8.9%

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

Rubella IgM Index	**Interpretation**
< 0.90	Negative for IgM to Rubella
0.91 ~ 0.99	Equivocal, sample should be retested
1 ~ 2	Low positive
2 ~ 2.5	Moderate positive
> 2.5	High positive

LIMITATIONS OF THE PROCEDURE

1.To prevent false negative and false positive IgM test results caused by the presence of specific IgG and rheumatoid factor (RF) in some specimens, reagents provided in this kit has been formulated to resolve these interferences. However, specimens with extremely high

RF and high autoimmune antibodies, the possibility of these interferences cannot be ruled out entirely.

2.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

Specificity and Sensitivity:

A total of 42 patient samples were used to evaluate specificity and sensitivity of the test. Rubella IgM test results were compared to a commercial ELISA kit results:

		Reference ELISA			
		N	E	P	Total
Rubella IgM	N	26 (D)	0	0 (B)	26
Chemiluminescence	E	0	0	0	0
ELISA	P	0(C)	0	16(A)	16
	Total	26	0	16	42

Sensitivity = A / (A+B) = 16 / 16 = 100%

Specificity = D / (C+D) = 28 / 28 = 100% Accuracy

= (A+D) / (A+B+C+D) = 42 / 42 = 100%

Expected Values:

49 random samples were determined with Rubella IgM Chemiluminescence ELISA. The test results were computed as IgM Index using a chosen reference serum (cut off) as IgM index 1. None were found to be positive (0%), and 49 were found to be negative (100%). Others reported 0% positivity from a study of 182 sera. A third set of 48 random samples, the positivity was found 8.30%.

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:

REFERENCES

1.Gravell, M., P.H. Dorcett, O. Gutenson, and A.C. Ley. Detection of antibody to rubella virus by enzyme-linked immunosorbent assay. J. Infect. Dis. 136:S300, 1977.

2.Hermann, K.L. Rubella virus. Manual of Clinical Microbiology, 3rd Edition. Lennette, Balows, Hausler, Truant (ed). Chapt. 86:862, 1980.

3.Katz, S.L. Rubella (German measles). Zinssmer Microbiology, 18th Edition. Jolik, Willett, Amos (ed). Chapt. 75:1067, 1985.

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