

<p>CALDON BIOTECH INC. Rubella IgG ELISA</p>

Catalog No. RB025G
 (96 tests)

NAME AND INTENDED USE

The CALDON BIOTECH INC. (CBI), Rubella IgG ELISA is intended for use in the evaluation of patient's immune status or exposure to rubella virus.

SUMMARY AND EXPLANATION OF THE TEST

Rubella is usually a mild disease with infrequent complication. In unvaccinated populations, rubella is primarily a childhood disease. Where children are well-immunized, adolescent and adult infections become more evident. Rubella is spread by direct contact with nasal or throat secretions of infected individuals. Symptoms may include a rash, slight fever, joint aches, headache, discomfort, runny nose and reddened eyes. The incubation period for rubella is 12-23 days; in most cases, symptoms appear within 16-18 days. If contracted during the first trimester of pregnancy, Rubella infection can lead to congenital rubella syndrome (CRS). Infection of a pregnant woman may result in a miscarriage, stillbirth or the birth of an infant with abnormalities, which may include deafness, cataracts, heart defects, liver and spleen damage and mental retardation. CRS occurs among at least 25 percent of infants born to women who have had rubella during the first trimester of pregnancy. The presence of IgG antibody to rubella virus is indicative of vaccination or previous exposure. In individuals with acute rubella infection, four-fold or greater increase in IgG antibody level is indicative of recent infection. Rubella IgM antibodies are detected by ELISA in 100% of patients between days 11 -25 after onset of the exanthem, in 60-80% of individuals at days 15-25 after vaccination and in 90-97% of infants with congenital rubella between 2 weeks and 3 months after birth. Rubella IgM antibody often persists for 20-30 days after acute infection or vaccination.

PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind to the antibody-antigen complex, if present. Excess enzyme

conjugate is washed off and substrate is added. The plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

MATERIALS PROVIDED

- Microwell Strips: Rubella antigen coated wells (12 x 8 x 1 wells)
- Sample Diluent: 1 bottle (22 mL). Ready to use.
- Calibrator: Yellow Cap. (1.50 mL/vial). Ready to use.
- Positive Control: Red Cap. (1.50 mL/vial). Ready to use.
- Negative Control: Blue Cap. (1.50 mL/vial). Ready to use.
- Enzyme Conjugate: 1 bottle (12 mL). Ready to use.
- TMB Substrate: 1 bottle (12 mL). Ready to use.
- Stop Solution: 1N H₂SO₄; 1 bottle (12 mL). Ready to use.
- Wash Concentrate: 1 bottle (50 mL), 20X concentrate.

STORAGE AND STABILITY

- Store the kit at 2-8°C.
- Keep microwells sealed in a dry bag with desiccants.
- The reagents are stable until expiration of the kit.
- 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 non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, 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. Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of

different lots should not be mixed. 5. Control sera and sample diluent contain 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.

PREPARATION FOR ASSAY

1. Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.
2. Prepare washing buffer by adding the contents of the bottle (50 mL, 20X Wash concentrate) to 950 mL of distilled or deionized water in one-liter container. Store at room temperature.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. **Negative control, positive control and calibrator are ready to use.**
Prepare 1:21 dilution of test samples, by adding 10 µL of the sample to 200 µL of sample diluent. Mix well.
3. Dispense 100 µL of diluted sera, calibrator and controls into the appropriate wells. For the reagent blank, dispense 100 µL sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with wash buffer.
5. Dispense 100 µL of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with wash buffer.
7. Dispense 100 µL of TMB substrate solution and incubate for 10 minutes at room temperature.
8. Add 100 µL of 1N H₂SO₄ to stop reaction.
9. Read O.D. within 30 min at 450 nm using microwell reader.

CALCULATION OF RESULTS

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).

3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:

Calibrator mean OD = 0.8
 Calibrator Factor (CF) = 0.5
 Cut-off Value = 0.8 x 0.5 = 0.400
 Positive control O.D. = 1.2
 Ab Index = 1.2 / 0.4 = 3
 Patient sample O.D. = 1.6
 Ab Index = 1.6 / 0.4 = 4.0

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

- The O.D. of the Calibrator should be greater than 0.250.
- The Ab index for Negative control should be less than 0.9.
- The Ab Index for Positive control should be greater than 1.2.

INTERPRETATION

The following is intended as a guide to interpretation of CBI Rubella IgG test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

1. Antibody Index Interpretation

<0.9 No detectable antibody to Rubella IgG by ELISA.

0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.

>1.1 Indicative of vaccination, current or previous exposure to Rubella infection.

2. Converting of Ab Index to IU/mL

As an option, Ab index may be converted to IU/mL by multiplying Ab index by 9.0. IU/mL values may then be interpreted as follows:

<8.1 IU/mL No detectable IgG antibody to Rubella

8.1-9.9 IU/mL Borderline positive. Follow-up testing is recommend if clinically indicated.

> 9.9 IU/mL Indicative of vaccination, current or previous Rubella infection

NOTE: Patient values above 25 IU/mL should be reported as > 25 IU/mL" or retested after dilution. In case of dilution, final results must be multiplied by the dilution factor.

LIMITATIONS OF THE TEST

1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history,

physical findings and other diagnostic procedures.
 2. Lipemic or hemolyzed samples may cause erroneous results.

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	10	0.90	0.08	9.4
2	10	0.84	0.08	10.3
3	10	0.17	0.02	11.8

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

327 patient sera were tested by both CBI Rubella IgG ELISA and a reference ELISA method. 240 sera were positive and 74 were negative by both methods (96% agreement). The results are summarized below:

	CBI Rubella IgG			Total
	ELISA +	ELISA -	Reference ELISA Kit +	
Reference ELISA Kit +	240	2	246	4
Reference ELISA Kit -	3	74	78	0
Total	247	76	327	4

2. Precision Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation%
1	16	1.94	0.04	1.9
2	16	0.88	0.02	2.05
3	16	0.18	0.01	6.73

Inter-Assay Study

REFERENCES

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