

RUBELLA IgG ENZYME IMMUNOASSAY TEST KIT

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ENZYME IMMUNOASSAY FOR THE DETECTION OF IgG ANTIBODIES TO RUBELLA VIRUS IN HUMAN SERUM

FOR INVESTIGATIONAL USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

Rubella IgG Enzyme Immunoassay

SUMMARY OF ASSAY PROCEDURE

1. Sample dilution 1:40		
5 µl / 200 µl		
2. Three incubations at 37°C		
Diluted Sample 100 µl 30 min.	Enzyme Conjugate 100 µl 30 min.	TMB Reagent (One-Step) 100 µl 15 min.
3. Stop with 100 µl of acid. Read O.D. at 450 nm		

INTENDED USE

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

INTRODUCTION

Rubella is a herpes virus. Generally rubella is considered a mild adolescent disease. However, a maternal infection can 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 the 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 non-immune can be educated on infection and differentiate 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 the wells, and the Rubella IgG-specific antibody, if present, binds to the antigen during incubation.

After washing the wells to remove unbound sample, antibody to human IgG conjugated with horseradish peroxidase (HRP) is added and incubated at 37°C for 30 minutes. Unbound conjugate is removed by a subsequent washing step. A solution of TMB Reagent is then added to the microwells. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrators and controls.

REAGENTS

Materials provided with the kit:

- Microtiter Wells: Rubella antigen-coated wells (12x8 wells)
- Enzyme Conjugate Reagent (red color); Red cap. 1 vial (12 ml)
- Sample Diluent (green color): 1 bottle (22 ml)
- Negative Calibrator: 0 IU/ml. Natural cap. (150 µL/vial)
- Cut-off Calibrator: 15 IU/ml. Yellow cap. (150 µL/vial)
- Positive Calibrator: 30 IU/ml. Red cap. (150 µL/vial)
- Positive Calibrator: 100 IU/ml. Green cap. (150 µL/vial)
- Negative Control: Range stated on label. Blue cap. (150 µL/vial)
- Positive Control: Range stated on label. Purple cap. (150 µL/vial)
- Wash Buffer Concentrate (20x): 1 bottle (50 ml)
- TMB Reagent (One-Step): 1 vial (11 ml)
- Stop Solution (1N HCl): Natural cap. 1 vial (11 ml)

STORAGE OF TEST KITS AND INSTRUMENTATION

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
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.

WARNING 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, 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 from 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 PREPARATION

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2-8°C for up to 7 days or frozen for up to 6 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 sera for antibody simultaneously.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (20-25°C) before use.
2. Dilute 1 volume of Wash Buffer (20x) with 19 volumes of distilled water. For example, dilute 50 ml of Wash Buffer (20x) into distilled water to prepare 1000 ml of wash buffer (1x). Wash Buffer is stable for 1 month at 2-8°C. Mix well before use.

ASSAY PROCEDURE

1. Place the desired number of coated wells into the holder.
2. Prepare 1:40 dilution of test samples, negative control, positive control, and calibrator by adding 5 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 absorbent solution in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well.
4. Incubate at 37°C for 30 minutes.
5. At the end of incubation period, remove liquid from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
6. Dispense 100 µl of Enzyme Conjugate to each well. Mix gently for 10 seconds.
7. Incubate at 37°C for 30 minutes.
8. Remove Enzyme Conjugate from all wells. Rinse and flick the microtiter wells 5 times with diluted Wash Buffer (1x).
9. Dispense 100 µl of TMB Reagent into each well. Mix gently for 10 seconds.
10. Incubate at 37°C for 15 minutes.
11. Add 100 µl of Stop Solution (1N HCl) to stop reaction.
12. Mix gently for 30 seconds. *It is important to make sure that all the blue color changes to yellow color completely.*

Note: Make sure there are no air bubbles in each well before reading.

13. Read O.D. at 450 nm within 15 minutes with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate cut-off calibrator (15 IU/ml) value x_c .
2. Calculate the mean of duplicate positive control (x_p), negative control (x_n) and patient samples (x_s).
3. Calculate the Rubella IgG Index of each determination by dividing the mean values of each sample (x) by calibrator mean value, x_c .

Example of typical results: Note: The O.D. values are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data.

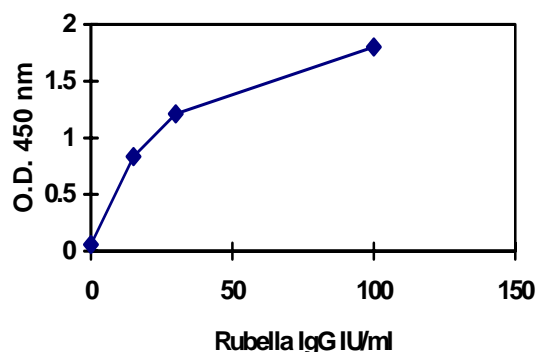
Cut-off Calibrator	Rubella IgG Index = 1.0
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1. Calibrator O.D. = 0.855, 0.816 $x_c = 0.835$
2. Negative Control O.D. = 0.199, 0.194 $x_n = 0.197$
Rubella IgG Index = $x_n / x_c = 0.197/0.835 = 0.23$
3. Positive Control O.D. = 1.570, 1.558 $x_p = 1.564$
Rubella IgG Index = $x_p / x_c = 1.564/0.835 = 1.87$
4. Patient Sample O.D. = 2.941, 2.884 $x_s = 2.913$
Rubella IgG Index = $x_s / x_c = 2.913/0.835 = 3.49$

QUANTITATIVE DETERMINATION OF RUBELLA IgG

For a quantitative determination of anti-Rubella IgG levels of positive specimens in IU/ml, OD of cut-off and positive calibrators are plotted on the Y-axis of a graph versus their corresponding anti-Rubella IgG concentrations of 0, 15, 30, and 100 IU/ml on the X-axis. The estimates of levels in patient sera are read off the graph using their individual OD values. For example:

Rubella IgG (IU/ml)	A ₄₅₀
0	0.061
15	0.914
30	1.327
100	1.843



Note: The standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

QUALITY CONTROL

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

1. The O.D. value of the reagent blank against air from a microwell reader should be less than 0.250.
2. If the O.D. value of the Cut-off Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The Rubella G Index or IU/ml unit for Negative and Positive Control should be in the range stated on the Certificate of Analysis Controls.

INTERPRETATION

- Negative: Rubella IgG Index less than 0.90 is negative for IgG antibody to Rubella virus. (<15 IU/ml)
- Equivocal: Rubella IgG Index between 0.91-0.99 is equivocal. Sample should be retested.
- Positive: Rubella IgG Index of 1.00 or greater, or IU value greater than 15 is seropositive. It indicates prior exposure to the rubella virus (>15 IU/ml).

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

PERFORMANCE CHARACTERISTICS

I. Specificity and Sensitivity:

A total of 185 patient samples were used to evaluate specificity and sensitivity of the test. The Rubella IgG ELISA test results were compared to the results of a commercial ELISA kit:

		Reference Rubella IgG ELISA			
		N	E	P	Total
Rubella IgG ELISA	N	40(D)	17	11(B)	68
	E	0	1	2	3
	P	0(C)	1	113(A)	114
Total		40	19	126	185

$$\text{Sensitivity} = A / (A+B) = 113 / 124 = 91.1\%$$

$$\text{Specificity} = D / (C+D) = 40 / 40 = 100.0\%$$

$$\text{Accuracy} = (A+D) / (A+B+C+D) = 153 / 164 = 93.3\%$$

II. Precision:

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of five different serum samples in one assay. Within-assay variability is shown below:

Sample	1	2	3	4	5
# Repts.	24	24	24	24	24
Mean (IU/ml)	95	62	36	16	3
S.D. (IU/ml)	4	4	3	1	0.2
C.V. (%)	4.0	6.9	8.8	6.8	7.1

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of five different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Sample	1	2	3	4	5
# Repts.	20	20	20	20	20
Mean (IU/ml)	97	61	42	16	3
S.D. (IU/ml)	3	2	3	0.5	0.3
C.V. (%)	3.3	3.3	6.9	3.2	9.4

REFERENCES

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MYM Laboratory & Medical Supply, Inc.
 8684 Ave. de la Fuente Ste. 14
 San Diego CA. 92154
 Tel. (619) 710-0126 Fax. (619) 710-0297
 www.mymSupply.com
 email: mail@mymSupply.com