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IVD	See external label	2°C-8°C	Σ=96 tests	REF Cat # 1301-11

Rubella IgG

REF **1301-11**

Test	Rubella IgG ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Sandwich ELISA: Antibody coated plate
Detection Range	Qualitative: Positive & Negative Control
Sample	5ul
Specificity	100 %
Sensitivity	100 %
Total Time	~ 75 min
Shelf Life	12 months

** Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account.*

NAME AND INTENDED USE

The ELISA, Rubella IgG 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 non-immune 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 TMB Chromogenic substrate is added. 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 calibrator and controls.

MATERIALS PROVIDED

1.	Microwell strips: Rubella antigen coated wells.	(12 x 8 wells)
2.	Enzyme conjugate: Red color solution.	1 vial (12 ml)
3.	Negative Calibrator: 0 IU/ml. Natural Cap.	1 vial (150 µl)
4.	Cut-off Calibrator: 15 IU/ml. Yellow Cap. Rubella G Index = 1.0	1 vial (150 µl)
5.	Positive Calibrator: 30 IU/ml. Red Cap.	1 vial (150 µl)
6.	Positive Calibrator: 100 IU/ml. Green Cap.	1 vial (150 µl)
7.	Negative Control: Range stated on label. Blue Cap.	1 vial (150 µl)
8.	Positive Control: Range stated on label. Brown Cap.	1 vial (150 µl)
9.	Washing concentrate 10x:	1 bottle (100 ml)

10. Sample diluent: Blue color solution.	1 vial (22 ml)
11. TMB Chromogenic Substrate: Amber bottle.	1 vial (12 ml)
12. Stop solution: 2 N HCl.	1 vial (12 ml)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 oC.
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 oC 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 20x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25 oC) and gently mix.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5µl of the test samples, negative control, positive control, and calibrators to 200µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrators, 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 30 minutes at room temperature.

4. Remove liquid from all wells. 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. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of stop solution to stop reaction.
   Make sure there are no air bubbles in each well before reading
9. Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

1. Calculate the mean of duplicate calibrator value xc.
2. Calculate the mean of duplicate positive control, negative control and patient samples.
3. Calculate the Rubella Index of each determination by dividing the mean values of each sample by calibrator mean value, xc.

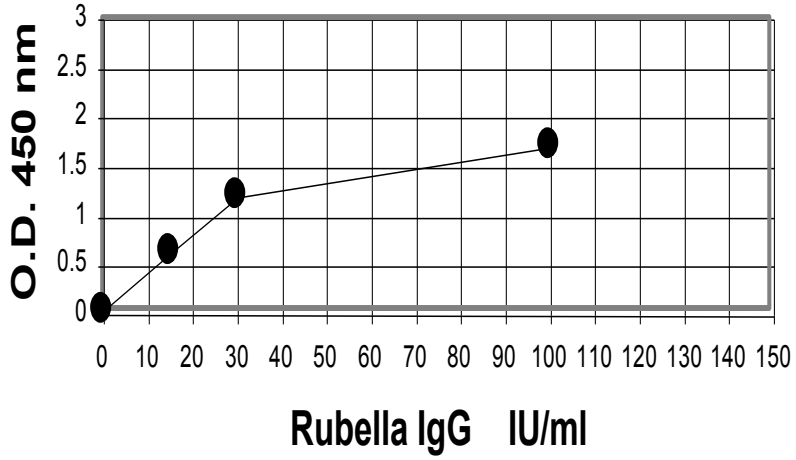
Example of typical results:

Cut-off Calibrator Rubella G Index = 1.0

Calibrator O.D. = 0.650, 0.634 xc = 0.642
Patient sample O.D. = 1.201, 1.253 xs = 1.227
Rubella G Index = 1.227 / 0.642 = 1.91

QUANTITATIVE ESTIMATION OF RUBELLA IgG

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



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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 0.150.
2. If the O.D. value of the 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 labels.

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
DIAGNOSTIC	N	71 (D)	0	0 (B)	71
AUTOMATION	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%

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%

REFERENCES Summary of Assay Procedure

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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