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IVD	See external label	2°C-8°C	Σ=96 tests	REF Cat # 1300-11

Rubella IgA

Cat # 1300-11

Test	Rubella IgA ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Sandwich ELISA: Antibody coated plate
Detection Range	Qualitative: Positive & Negative Control
Sample	5ul
Specificity	99.20 %
Sensitivity	100 %
Total Time	~ 75 min
Shelf Life	12-14 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 Diagnostic Automation ELISA, Rubella IgA is intended for use in the detection of IgA 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. The Rubella antibody status of pregnant women are commonly determined at the patient's first prenatal visit. Those without antibodies are monitored through early pregnancy for seroconversion.

The antibodies present to Rubella may be of IgA, IgM and IgG. The physiological function of IgA and its clinical implication is still unclear. The DIAGNOSTIC AUTOMATION ELISA Rubella IgA is an accurate and sensitive method to detect Rubella antibody IgA isotype.

PRINCIPLE OF THE TEST

Purified rubella antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the rubella IgA 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 IgA 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: purified *Rubella* antigen coated wells. (12 x 8 wells)
2. Absorbent Solution: Black Cap. 1 vial (22 ml)
3. Washing Concentrate 10x: White Cap. 1 bottle (100 ml)
4. TMB Chromogenic Substrate: Amber bottle. 1 vial (12 ml)
5. Enzyme Conjugate: Red color solution. 1 vial (12 ml)
6. Cut-off Calibrator: Yellow Cap. *Rubella* A Index = 1 1 vial (150 µl)
7. Negative Control: Range stated on label. Natural Cap. 1 vial (150 µl)
8. Positive Control: Range stated on label. Red Cap. 1 vial (150 µl)
9. Stop Solution: 2 N HCl. 1 vial (12 ml)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 ^{o}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 ^{o}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 ^{o}C) and gently mix.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5 µl of the test samples, negative control, positive control, and calibrator to 200 µl of the absorbent solution. 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. 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 2 N HCl 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 x_c.
2. Calculate the mean of duplicate positive control, negative control and patient samples.
3. Calculate the Rubella A Index of each determination by dividing the mean values of each sample by calibrator mean value, x_c.

Example of typical results:

Cut–off Calibrator Rubella A Index = 1.0

Calibrator O.D. = 0.350, 0.375 $x_c = 0.363$

Negative control O.D. = 0.162, 0.175 $x_n = 0.169$

Rubella A Index = 0.169 / 0.363 = 0.46

Positive control O.D. = 1.086, 1.097 $x_p = 1.092$

Rubella A Index = 1.092 / 0.363 = 3.0

Patient sample O.D. = 1.119, 1.203 $x_s = 1.161$

Rubella A Index = 1.161 / 0.363 = 3.20

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 O.250.
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 A Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: Rubella A Index less than 0.90 are negative for IgA antibody to rubella virus.

Equivocal: Rubella A Index between 0.91-0.99 is equivocal. Sample should be retested.

Positive: Rubella A Index of 1.00 or greater are positive for IgA antibody to rubella virus. It is indicative of acute rubella infection in a time of zero to three months before the blood samples were obtained.

LIMITATIONS OF THE PROCEDURE

1. To prevent false negative 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.

EXPECTED VALUES

49 serum specimens from random, asymptomatic blood donors were tested with DIAGNOSTIC AUTOMATION ELISA Rubella IgA. Of the 49 specimens, 32 were found to be positive (65.3 %) and 17 were found to be negative (34.7 %). Of these 32 IgA positive specimens 30 were found to be IgG positive. Prevalence may vary depending on a variety of factors such as geographical location, age, socioeconomic status, race, type of test employed, specimen collection and handling procedures, clinical and epidemiological history.

PERFORMANCE CHARACTERISTICS

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	7.6%	6.2%	4.8%
Inter-assay	11.6%	8.6%	6.8%

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.

Date Adopted	Reference No.
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