Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Rubella IgM ELISA

REF 1302-11

IVD See external Label 2°C 8°C Σ 96 Tests

Test	Rubella IgM ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Sandwich ELISA: Antibody coated plate
Detection Range	Qualitative: Positive, Negative Control
Sample	5 µL
Total Time	~ 75 min.
Shelf Life	12 Months from the manufacturing date
Specificity	100%
Sensitivity	100%

INTENDED USE

The Diagnostic Automation, Inc. Rubella IgM is intended for use in the detection of IgM antibody to the rubella virus.

SUMMARY AND EXPLANATION

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 abnormalities. 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.

TEST PRINCIPLE

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 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 IgM specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

SPECIMEN COLLECTION AND PREPARATION

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.

MATERIALS AND COMPONENTS

Materials provided with the test kits

1. Microwell Strips: Purified Rubella antigen coated well	(12 X 8 wells)
2. Absorbent Solution	1 vial (22mL)
3. Calibrator: Factor value (f) stated on label	1 vial (150µL)
4. Negative control: Range Stated on label	1 vial (150µL)
5. Positive control: Range stated on label	1 vial (150µL)
6. Washing Concentrate (H) 20x	1 bottle (50 mL)
7. Enzyme Conjugate: Red color solution	1 vial (12 mL)
8. TMB Chromogenic substrate: Amber Bottle	1 vial (12 mL)
9. Stop solution	1 vial (12 mL)

REAGENT PREPARATION

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 °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 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.

RESULTS

1. To obtain Cut off OD value: Multiply the OD of Calibrator by Factor (f) printed on label of Calibrator.
2. Calculate the Rubella IgM index of each determination by dividing the OD values of each sample by obtained OD value of Cut off.

For Example:

If Factor (f) value on label = 0.4

Diagnostic Automation/ Cortez Diagnostics, Inc.
21250 Califa St, Suite 102 and 116, Woodland Hills, CA 91367 USA Phone: 818-591-3030, Fax : 818-591-8383
Email: onestep@rapidtest.com Website: www.rapidtest.com

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

Obtained Calibrator O.D. = 1.100
Cut-off O.D. = 1.100 x 0.4 = 0.44 (By definition Rubella IgM Index = 1)

Patient sample O.D. = 0.580
Rubella IgM Index = 0.580 / 0.44 = 1.32 (Positive result)

Patient sample O.D. = 0.320
Rubella IgM Index = 0.320 / 0.44 = 0.73 (Negative result)

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 M Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: Rubella M Index less than 0.90 are negative for IgM antibody to rubella virus.

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

Positive: Rubella M Index of 1.00 or greater are positive for IgM 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.

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	8.5%	7.4 %	5.2%
Inter-assay	9.6%	8.2%	6.5%

LIMITATIONS OF 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, in 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.

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.

STORAGE

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.

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.
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Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

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