Diagnostic Automation/Cortez Diagnostics, Inc.

IMMUNO DIAGNOSTICS

CE

AccuDiag™ Rubella IgM ELISA Kit

REF 1302-P3

IVD See External Label 2°C 8°C 96 Tests

Rubella IgM ELISA	
Principle	Indirect ELISA
Detection	Qualitative
Sample	10 µL serum/plasma
Incubation Time	50 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. Rubella IgM ELISA Kit is intended for the detection of IgM antibody to Rubella in human serum or plasma.

SIGNIFICANCE AND SUMMARY

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.

ASSAY PRINCIPLE

Diluted patient serum (serum diluent contains sorbent to remove Rheumatoid Factor and human IgG interference) is added to wells coated with purified antigen. IgM 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 IgM specific antibody in the sample.

SPECIMEN COLLECTION & 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.

REAGENTS

Materials provided with the kit

1. Microwell coated with Rubella antigen
2. Sample Diluent: 1 bottle (Ready to use)
3. Calibrator: 1 Vial (ready to use)
4. Positive Control: 1 Vial (ready to use)
5. Negative Control: 1 vial (ready to use)
6. Enzyme conjugate: 1 bottle (ready to use)
7. TMB Substrate: 1 bottle (ready to use)
8. Stop Solution: 1 bottle (ready to use)
9. Wash concentrate 20X: 1 bottle

Materials required but not provided

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (20-25°C) and gently mix.

1. Place the desired number of coated strips into the holder.

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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 200µl of sample diluent. Mix well.
3. Dispense 100 µl of diluted sera, calibrator and controls into the appropriate wells.
4. For the reagent blank, dispense 100µl 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.
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer.
6. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
8. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer.
9. Blot on absorbance paper or paper towel Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
10. Add 100 µL of stop solution.
11. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

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

INTERPRETATION

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

Antibody Index Interpretation
<0.9 No detectable antibody to Rubella IgM by ELISA.
0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1 Detectable antibody to Rubella IgM by ELISA.

QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:
1. The O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab Index for Positive control should fall within the range specified on the COA/label.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity

142 patient sera were tested by Rubella IgM ELISA and a reference ELISA method. 15 sera were positive and 126 were negative by both methods (99% agreement). The results are summarized below:

Reference ELISA Kit	Rubella IgM ELISA		
	+	-	Total
+	15	1	16
-	0	126	126
Total	15	127	142

2. Precision

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.43	0.074	5.17
2	16	0.92	0.57	6.20
3	16	0.11	0.006	5.83

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.45	0.143	9.86
2	10	0.95	0.112	11.78
3	10	0.10	0.012	12.00

LIMITATIONS OF THE ASSAY

1. To enhance sensitivity and specificity of this IgM test provided sample diluent has been formulated to block IgG and Rheumatoid Factor (RF) interferences. Turbidity could be seen after diluting serum with sample diluent. This turbidity is due to the blocking of serum IgG and has shown no interference with test results. It can be removed by centrifugation.
2. In specimens with high RF and high autoimmune antibodies, the possibility of eliminating the interferences cannot be ruled out entirely.
3. 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.
4. Lipemic or hemolyzed samples may cause erroneous results.

STORAGE CONDITIONS

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.

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.

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

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