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Rubella IgG ELISA Kit

10/18

(Catalog # E4666-100, 96 assays; Store at 2-8^{0}C)

I. Introduction:

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 exanthema, 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.

II. Application:

Detection of IgG antibody to Rubella

III. Sample Type:

Human serum or plasma

IV. Kit Contents:

Components	E4666-100	Part No.
Microplate	12 strips x 8 wells	E4666-100-1
Sample Diluent	22 ml	E4666-100-2
Calibrator	1 ml	E4666-100-3
Positive Control	1 ml	E4666-100-4
Negative Control	1 ml	E4666-100-5
Enzyme conjugate	12 ml	E4666-100-6
TMB Substrate	12 ml	E4666-100-7
Stop Solution	12 ml	E4666-100-8
Wash Buffer (20X)	25 ml	E4666-100-9

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VI. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{o}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash Buffer:** 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 (18-26°C).

VII. Warning & Precautions:

- 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."
- 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.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- 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.

VIII. Sample Preparation and Storage:

Collect blood specimens & separate the serum immediately. Specimens may be stored refrigerated at (2-8°C) for 7 days. Store frozen at (-20°C) for up to six month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well.

IX. Assay Protocol:

Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
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 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 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.
4. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature.
8. Add 100 µl of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

X. Calculation

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. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF). 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

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.

INTERPRETATION
The following is intended as a guide to interpretation of IgG antibody 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 IgG antibody by ELISA.
0.9 - 1.1	Borderline positive. Follow-up testing is recommend if clinically indicated.
> 1.1	Detectable antibody to IgG antibody by ELISA.

LIMITATIONS OF THE TEST
1. The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patients history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

Sensitivity and Specificity
47 patient sera were tested by this M. pneumoniae IgG ELISA and a reference ELISA method. 109 sera were positive and 31 were negative by both methods (95% agreement). The results are summarized below:

		M. pneumoniae IgG ELISA		
		+	–	Total
Reference ELISA Kit	+	109	4	113
	–	3	31	34
	Total	112	35	147

Precision
Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.77	0.08	4.5
2	16	0.97	0.06	6.2
3	16	0.15	0.01	6.6

Inter Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.54	0.13	8.4
2	10	0.85	0.07	8.2
3	10	0.18	0.02	12.7

XI. RELATED PRODUCTS:

- Gentamicin (serum/urine) ELISA Kit (K4315)
- Tetracyclines ELISA Kit (E4273)
- Rubella IgM ELISA Kit (E4665)
- Mycoplasma DNA Kit (K1416)
- Mycoplasma Arginine Deiminase (ADI), Recombinant Protein (P1278)

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