2. Precision

Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.82	0.08	4.39
2	16	1.31	0.07	5.34
3	16	0.26	0.02	7.69

Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.73	0.11	6.35
2	10	1.12	0.09	8.04
3	10	0.25	0.03	12.00

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CALBIOTECH
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Measles (Rubeola) IgG ELISA

Catalog No.: MS018G (96 Tests)

INTENDED USE

The Calbiotech Measles IgG ELISA Kit is intended for the detection of IgG antibody to Measles in human serum or plasma.

SUMMARY AND EXPLANATION

Measles is an acute, highly contagious viral disease. Although measles is usually considered a childhood disease, it can be contracted at any age. Measles is spread by direct contact with nasal or throat secretions of infected people or, less frequently, by airborne transmission. Measles symptoms generally appear in two stages. In the first stage, the individual may have a runny nose, cough and a slight fever. The second stage begins on the third to seventh day and consists of high fever and red blotchy rash lasting four to seven days. The rash usually begins on the face and then spreads over the entire body. Symptoms usually appear in 10-12 days, although they may occur between 8-13 days after exposure. The presence of IgG antibody to measles virus is indicative of previous exposure or vaccination. In individuals with acute measles, a significant increase in measles IgG antibody level is indicative of recent infection. IgM antibodies to measles virus are often detectable with onset of the rash and typically persist for 4 weeks. At least 80% of patients will be positive for measles IgM at 6 days and 100% at 16 days after onset of symptoms.

PRINCIPLE OF THE TEST

Diluted patient serum is added to wells coated with purified antigen. IgG 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 IgG specific antibody in the sample.

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Measles antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: 1 Vial (ready to use)	1ml
4. Positive Control: 1 vial (ready to use)	1ml
5. Negative Control: 1 vial (ready to use)	1ml
6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

MATERIALS NOT PROVIDED

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

STORAGE AND STABILITY

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.

WARNINGS AND 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. This kit is USA FDA exempt product.
3. 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.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
6. 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.

SPECIMEN COLLECTION AND HANDLING

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.

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 (18-26°C).

ASSAY PROCEDURE

Bring all specimens and kit reagents to room temperature (18-26°C) and gently mix.

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

CALCULATION OF 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

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 be greater than 1.2.

INTERPRETATION

The following is intended as a guide to interpretation of Measles IgG 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 Measles IgG by ELISA
0.9-1.1	Borderline positive. Follow-up testing is recommended if clinically indicated.
>1.1	detectable antibody to Measles IgG 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 patient's history, physical findings and other diagnostic procedures.
2. Lipemic or hemolyzed samples may cause erroneous results.

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Specificity
   327 human sera were tested by this Measles IgG ELISA and a reference ELISA method. 284 sera were positive and 33 were negative by both methods (97% agreement). The results are summarized below:

		Measles IgG ELISA		
		+	–	Total
Reference ELISA kit	+	284	4	288
	–	6	33	39
	Total	290	37	327