CE

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IVD	See external label	2°C-8°C	Σ=96 tests	REF Cat # 1408Z

Measles IgG ELISA

Cat # 1408Z

Test	Measles IgG ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	Indirect ELISA : Antigen Coated Plate
Detection Range	Qualitative: Positive, Weak Positive, Negative Control
Sample	5µL
Specificity	100%
Sensitivity	100%
Total Time	~90 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, Measles IgG is intended for use in evaluating a patient's serologic status to Measles infection. It is also used to evaluate paired sera for the presence of a significant increase in specific IgG as indicative of a recent or current Measles infection.

SUMMARY AND EXPLANATION OF THE TEST

Since the introduction of a measles virus vaccine, the U.S. has mounted an effective immunization program which has essentially eliminated measles as a major childhood disease. However, as a result of vaccine failure or the failure to be vaccinated, a recent and persistent shift in the susceptible population towards young adults has been recorded (1). In the case of measles, severity of illness and mortality rates are highest among adults (2). Thus, serology has become increasingly important as a tool for determining the immune status of the young adult population entering college or the military. In addition, the linkage between measles infection and premature delivery or spontaneous abortion supports screening pregnant mothers for susceptibility (3).

Although measles has been recognized as a disease for over two thousand years, a description of its epidemiology first appeared in a paper by Panum in 1849. In his study of an epidemic in the Faroe Islands, Panum observed that measles had an incubation period of two weeks and was contagious but that lifelong immunity followed primary infection (4). Over 100 years later, in 1963, the first live measles vaccine was licensed in the United States. Vaccine development was made possible by Enders and Peebles' discovery, in 1954, that the virus could be successfully grown in an *in vitro* cell culture system (5). The success of the vaccine program is evident by the precipitous drop in the annual incidence.

Classified as a paramyxovirus, measles produces a highly contagious respiratory infection. The disease is spread during the prodromal phase through direct contact with respiratory secretions in the form of droplets (3). Ironically, because of the low incidence of measles, younger physicians often diagnose the illness late in infection after the patient has exposed others. This has resulted in small isolated miniepidemics among the susceptible population.

Several diseases in addition to Measles have been associated but not causally linked to measles virus. This list includes subacute sclerosing panencephalitis (SSPE) (6), systemic lupus erythematosus (SLE) (7) and multiple sclerosis (MS) (8). Patients with SSPE, a chronic degenerative neurologic disease, have documented high levels of antibody to measles virus. However, for SLE and MS there is less pronounced but statistically significant elevation in antibody levels. The significance or role of measles virus infection in these disease states is unknown at the present time.

Since the presence of circulating IgG antibody to measles virus is indicative of previous infection or vaccination, screening the young adult population about to enter college or the
military, pregnant women, and other individuals at risk, for seropositivity, is a valuable tool for determining their immune status.

The immune response to infection or vaccination with measles virus is rapid and characteristic. Measles specific IgM and IgG begin to appear in the circulation simultaneously. The IgM response is in 1 to 3 months, while IgG response is sustained, resulting in life-long immunity.

The DIAGNOSTIC AUTOMATION microwell ELISA Measles IgG kit provides all the necessary reagents for the determination of measles IgG antibody with excellent sensitivity, specificity and reproducibility.

PRINCIPLE OF THE TEST

Purified Measles antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the Measles IgG 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 IgG 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: Measles antigen coated wells	(12X8 wells)
2. Sample Diluent: Blue color solution	1 bottle (22mL)
3. Calibrator: Factor Value stated on the label. Red Cap	1 vial (150µL)
4. Negative Control: Range stated on label. Natural Cap	1 vial (150µL)
5. Positive Control: Range Stated on label. Green Cap	1 vial (150 µL)
6. Washing Concentrate 10X White Cap	1 bottle (100 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)

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.

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 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 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 30 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.

CALCULATION OF RESULTS

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

For Example
If the Factor (f) value on the label = 0.4
This factor (f) is a variable value. It could be 0.35 or 0.5 etc. printed on label of calibrator.

Obtained Calibrator O.D = 1.100
Cut-off O.D. = 1.100 X 0.4 = 0.44 (By definition IgG Index =1)

Patient Samples O.D> = 0.580
IgG Index = 0.580 / 0.44 = 1.32 (Positive Result)

Patient Sample O.D. = 0.320
IgG 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 IgG Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: IgG Index of 0.90 or less are seronegative for IgG antibody.

Equivocal: IgG Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive: IgG Index of 1.00 or greater.

LIMITATIONS OF THE PROCEDURE

1. A single serum sample cannot be used to determine recent infection.
2. A serum specimen taken in an early stage during acute phase of infection may contain low levels of IgG antibody and render an IgG Index result negative. In such cases, it is recommended that an IgM assay be performed, or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion. An increase of index value of IgG greater than 30% is considered a significant change in antibody. This identifies those person who presumed to be experiencing recent or current espisodes of measles infection.
3. 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.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity:

Sensitivity, specificity and accuracy were evaluated using a commercial available ELISA kit on 59 specimens. The correlation results are summarized in the following table:

		Reference ELISA			
		N		P	Total
DIAGNOSTIC	N	2 (D)		0 (B)	2
AUTOMATION	P	0 (C)		57 (A)	57
ELISA	Total	2		57	59

Sensitivity = A / (A+B)= 57 / (57+ 0) = 100 %
Specificity = D / (C+D) = 2 / (0 + 2) = 100 %
Accuracy (Overall agreement) = (A+D) / (A+B+C+D) = 59 / 59 = 100 %

144 random samples are tested on the DIAGNOSTIC AUTOMATION microwell ELISA: There are 18 samples (12%) with IgG index lower than

1. It is 88% of total samples with positivity of measles IgG.

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:

N = 8	Negative	Low positive	Positive
Intra-assay	10.9%	10.5%	8.9%
Inter-assay	12.3%	11.1%	10.5%

Cross-reactivity:

A study was performed to determine the cross-reactivity of the test to the following antibodies:

1. Positive IgG of EBV, Mumps and Chlamydia Trachomatis.

2. Positive IgG and IgM of Rubella, Toxo, CMV, HSV 1, and HSV 2.
3. Positive IgM of RF.
4. Positive IgG of ANA, anti- ds DNA.

All positive samples tested give negative results.

REFERENCES

1. **J.A. Frank, et al.** 1985. Major Impediments to Measles Elimination: The Modern Epidemiology of an Ancient Disease. *American Journal of Diseases of Children.* 139:881-888.
2. **Measles Surveillance Report No. 11.** 1977-1981. Atlanta.
3. **Kempe, C. H. and V. A. Fulginite.** 1965. The Pathogenisis of Measles Virus Infection. *Arch-Gesamte Virusforsch.* 16: 103-128.
4. **Panum, P.** 1989. Observations Made During the Epidemic of Measles on the Faroe Islands in the Year 1846. *Med. Classics.* 3: 829-886.
5. **Enders, J. R. and T. C. Peebles.** 1954. Propagation in Tissue Cultures of Cytopathogenic Agents from Patients with Measles. *Proc. Soc. Exp. Biol. Med.* 86: 277-286.
6. **Connolly, J.H., et al.** 1967. Measles-virus Antibody and Antigen in Subacute Sclerosing Panencephalitis. *Lancet.* 1:542-546.
7. **Tannnenbaum, M., et. al.** Electron Microscopic Virus-like Material in Systematic Lupus Erythematosus: With Preliminary Immunologic Observations on Presence of Measles Antigen. *J. Urol.* 105:615-619.
8. **Adams, J. M. and D. T. Imagawa.** 1962. Measles Antibodies in Multiples Sclerosis. *Proc. Soc. Exp. Biol. Med.* 111: 562-566.

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