Measles IgG
Enzyme Immunoassay

For Individual Laboratory to Complete:

Laboratory Name		
Adopted		
Reviewed		
Reviewed		
Revised		
Supercedes		

Method: **Diamedix Corp., Immunosimplicity®**
Manual or in conjunction with one of the Diamedix Automated EIA Systems such as the MAGO Plus, the DSX, or the DS2. For *In Vitro* Diagnostic Use.

Clinical Significance

Measles (Rubeola) is a highly contagious infection caused by an RNA myxovirus. The incubation period is 10 to 11 days and the infection is characterized by fever, myalgias, nonproductive cough, conjunctivitis and exanthem and enanthem (Koplik's spots). The rash of rubeola almost always begins on the face and then spreads to the trunk and extremities. Typically, the illness crests on day three of fever and the temperature falls to normal on day seven (1,2).

Prior to the introduction of vaccines, measles was an inevitable disease of childhood. However, since the introduction of the measles vaccine in 1963, the incidence of this disease has dropped dramatically and physicians have become less familiar with the disease (3). Diagnosis of the disease can become further complicated by the emergence of atypical forms of measles. These atypical forms generally occur in recipients of inactivated measles vaccine who were immunized with this vaccine in 1963-1967 and were subsequently exposed to the natural disease. The atypical form of measles may be severe and is often confused with Rocky Mountain spotted fever (1). In addition, acute measles infection can be complicated by secondary infections of the lower respiratory tract and ear. Additional complications such as encephalomyelitis occur in about 0.1% of patients (3). Measles infection during pregnancy has been associated with an increased risk of miscarriages or premature delivery (4). Persistent measles infection has also been suspected in chronic autoimmune disease. Patients with multiple sclerosis, as well as those with systemic lupus erythematosus, have consistently been found to have elevated levels of antibodies to measles virus. This does not necessarily indicate a role of the virus in the etiology of these diseases, but may reflect polyclonal activation of B cells known to occur during the courses of these diseases (3).

The presence of specific antibodies in a single serum specimen indicates past measles infection or vaccination. Demonstration of a significant

increase in antibody titers in a serum pair taken at a 7-14 day interval is the basis for diagnosis of acute infection (3).

The traditional methods of antibody detection such as hemagglutination inhibition (HI), and neutralization (Nt) have been replaced by the enzyme immunoassay (EIA) which is more sensitive, equally specific and less labor intensive (5,6).

The Diamedix Immunosimplicity Is-Measles IgG Test Kit is an EIA procedure intended for the semi-quantitative detection of antibodies to measles virus antigen. The test can be performed either manually or in conjunction with one of the Diamedix Automated EIA Systems. The results are objective and reported in ELISA units (EU/ml), standardized against in-house reference materials.

Principle of the Procedure

Diluted samples are incubated with measles virus antigen bound to the solid surface of a microtiter well. If IgG antibodies against measles are present in the samples, they will bind to the antigen forming antigen-antibody complexes. Residual sample is eliminated by aspirating and washing. Conjugate (horseradish peroxidase-labeled anti-human IgG) is added and will bind to these complexes. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm (reference 600-630 nm) and is directly proportional to the concentration of IgG antibodies to measles antigen present in the sample.

Specimen Collection

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2 to 8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples are to be frozen at -20°C. Prior to testing, bring frozen sera to room temperature slowly and mix gently, avoiding foam formation. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used.

For the diagnosis of acute Measles infection, the acute-phase specimen should be drawn as soon after onset as possible, preferably within the first 7 days. The convalescent-phase specimen should be drawn 10 or more days after the acute-phase specimen.

CAUTION: Serum samples must not be heat-inactivated prior to use.

Reagents

Antigen Wells Twelve, 8-well microwell breakapart strips,

	color-coded dark blue, coated with Measles virus antigen (partially purified extract of Vero cells infected with the Edmonston strain of measles virus).
Calibrator	One vial with blue cap containing 1.8 ml of pre-diluted human serum, highly reactive for Measles IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
Positive Control	One vial with white cap containing 1.8 ml of pre-diluted human serum, moderately reactive for Measles IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Assigned EU/ml value printed on label. Note that controls and Calibrators are produced from different serum lots.
Negative Control	One vial with black cap containing 1.8 ml of pre-diluted human serum, non-reactive for Measles IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90ppm active ingredient.
Sample A Diluent	One bottle with blue cap containing 60 ml Phosphate buffer with protein stabilizers. Contains 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Color-coded blue.
Wash S Concentrate	Two bottles with clear caps containing 50 ml of Phosphate buffered saline with Proclin™ 300, 15 ppm active ingredient. Color-coded light blue/green. Each bottle is sufficient to make 1 liter of wash solution.
Conjugate	One bottle with red cap containing 25 ml goat anti-human immunoglobulin G labeled with horseradish peroxidase. Also includes protein stabilizers and Proclin™ 300, 30 ppm active ingredient. Color-coded pink.
Substrate HRP	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethylbenzidine).
Stop M Solution	One bottle with white cap containing 30 ml of 1 N Phosphoric and 1 N Hydrochloric acids. **CAUTION:** Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Precautions section.

These reagents should be stored at 2 to 8° C.

Other Materials Required

Manual Users:

1. Wash bottle or automated microplate washer

2. Pipettors capable of dispensing appropriate volumes
3. Timer
4. One liter graduated cylinder
5. One liter wash solution reservoir
6. Deionized or distilled water
7. Absorbent toweling
8. Tubes or microwell plate for serum dilution
9. Reader capable of reading absorbance at 450nm, reference at 600 or 630 nm.

Diamedix Automated EIA System Users:

1. One liter graduated container
2. Deionized or distilled water
3. Dilution containers appropriate to system
4. Sample and Reagent tips required by system
5. Reagent containers required by system

Warnings:

1. Handle samples, Calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the standards and controls has been found negative for Hepatitis B surface antigen and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1993.
2. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.
4. Certain of the test reagents contain Proclin™ 300 as a preservative. When disposing of reagents containing Proclin™ 300, flush drains with copious amounts of water to dilute the active components below active levels.
5. Serum components contain sodium azide as preservative. Azides are reported to react with lead and copper in plumbing to form compounds that may become explosive. When disposing of solutions containing sodium azide, flush with copious amounts of water to minimize the build up of metal azide compounds.
6. Sodium azide inhibits conjugate activity. Clean pipet tips MUST be used for conjugate addition so that azide is not carried over from other reagents.
7. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.

8. The substrate contains 3,3' 5,5' Tetramethylbenzidine (TMB) which has been shown to cause possible mutagenic effects in laboratory experiments.

Calibration

This test uses an in-house reference standard (or Calibrator). This Calibrator has been prepared from a pool of sera strongly positive for the antibody under investigation. The Calibrator functions as an internal reference preparation and is assigned a unitage in ELISA units (EU) per ml. The Calibrator must be included in every test run.

These tests have been optimized to permit the use of single point calibration. This is possible because the dose response curves are sufficiently linear and pass near to, or through the origin. The linearity of the dose response has been validated by the manufacturer during quality control testing.

Patient samples which contain very high levels of antibody may produce absorbance values greater than the Calibrator absorbance. Patient sample results greater than the Calibrator value should be reported as "Greater than Calibrator value EU/ml". If numerical results are required for such samples, dilute the sample using Sample Diluent and re-assay. Several dilutions (for example 1/5, 1/10 and 1/20) of the pre-diluted sample may be re-assayed simultaneously. Select the dilution that has an absorbance reading about 50% of the absorbance reading of the Calibrator; calculate the EU/ml for this dilution and multiply by the dilution factor to obtain estimated values.

Quality Control

a) The Positive and Negative Controls must be included in each test run.
b) The absorbance of the Blank must be < 0.2.
c) The Positive Control must be within its assigned range.
d) The Negative Control must be < 15.0 EU/ml.

If any of these criteria is not met, the run is invalid and must be repeated.

Notes: The Negative and Positive Controls are intended to monitor substantial reagent failure. The controls will not control all parts of the procedure such as technical dilution of patient specimens. The Positive Controls will not ensure precision at the assay cut-off. Users may wish to establish an in-house control having a quantitative value determined by replicate testing, at or near the cut-off to monitor the precision of the assay cut-off. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Procedure

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 50 ml of Wash Concentrate(20X) to one liter of deionized or distilled H_2O.

Manual Users:
The Calibrator and Controls are provided ready to use: DO NOT DILUTE FURTHER.

1. Prepare 1:101 dilutions of the patient samples in Sample Diluent. (e.g., by addition of 2 µl sample to 200 µl Sample Diluent or 5 µl sample to 500 µl Sample Diluent)
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of Calibrator, controls and diluted patient samples to the antigen wells. Avoid formation of bubbles when transferring diluted samples.

   NOTE: *Include one well which contains 100 µl of Sample Diluent only as the reagent blank. This will ultimately be used to "zero" the photometer before reading the test results.*
3. Allow the wells to incubate at room temperature(18-30°C) for 30 ± 5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling if necessary. Wash the wells by rinsing 3 times with at least 300 µl per well of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.
9. Allow the wells to incubate uncovered at room temperature(18-30°C) for 30 ± 5 minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Read the absorbance of each well at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 60 minutes of adding Stop Solution.

Refer to the BP-96 Plate Reader Operation Manual for complete instructions on set-up and operating procedures.

Diamedix Automated EIA System Users:
If using one of Diamedix's Automated EIA Systems, please refer to the corresponding Operating Manual(s) for the test setup, procedure, and accessories/consumables needed.

Calculation of Results

Determine the EU/ml (ELISA Units/ml) for each patient specimen or control using the following formula:

$$\frac{\text{EU/ml of Calibrator}}{\text{Absorbance of Calibrator}} \times \text{Absorbance of Sample} = \text{EU/ml of sample}$$

Reference Ranges

The following is only a guide to interpretation. **Each laboratory can establish its own "normal" ranges based on populations encountered.**

Less than 15.0 EU/ml Index < 0. 75	Nonreactive (Negative) for anti-Rubeola IgG; presumed non-immune to measles virus.
Greater than/equal to 20.0 EU/ml Index > 1.0	Reactive (Positive) for anti-Rubeola IgG; presumed immune to measles virus.
15.0 to 19.9 EU/ml Index 0.75-0.99	Equivocal*

- When equivocal results are obtained, another specimen should be collected ten to fourteen days later and tested in parallel with the initial specimen. If the second sample is also equivocal, the patient is negative for primary or recent infection, and equivocal for antibody status. If the second sample shows a significant increase in antibody level, the patient may be considered to have a primary infection. The conversion of an individual patient's serum from negative to positive for antibodies to the infectious agent in question, is defined as seroconversion, and indicates active or recent infection.

Reporting Results

When the EU/ml value is reported for a single specimen the following statement should be included: "The following results were obtained with the Diamedix Immunosimplicity® Is-Measles IgG EIA test system. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present. The magnitude of the reported IgG level cannot be correlated to an endpoint titer".

When the assay is used semi-quantitatively, the following statement should be included when reporting results: "Timing of specimen collection for paired sera may be critical. In some patients, antibody titers may rise to significant levels and fall again to lower or undetectable levels

within a month. Other patients may not develop significant antibody levels. Culture results, serology and antigen detection methods should all be appropriately used along with clinical findings for diagnosis".

Paired Sera

To determine a significant difference between acute/convalescent serum pairs, both specimens should be run within the same assay. In addition, paired sera should be evaluated within the linear range of the assay. The upper limit of the linear range has been set at 100 EU/ml. In-house studies performed manually and using the Diamedix Automated EIA Systems have shown that a 2.1-fold to a 4.4-fold (mean 3.2-fold + 2 SD) increase in Index Ratio (convalescent serum Index value / acute serum Index value) corresponds to a four-fold increase in measles IgG antibody level. An Index Ratio in the range of 1.5 to 2.1 indicates an equivocal status for the paired sample Index Ratio. In this case, paired samples can be retested or additional samples collected if necessary. If paired sera controls are desired, it is recommended that a four-fold dilution of the Calibrator, or other known positive sample of Calibrator strength, is made first in sample diluent and then diluted according to assay procedures. The undilute and 4-fold diluted material will provide a simulated serum pair. The four-fold dilution Index ratio is compared against the established range (see above).

Procedure Notes

1. Do not interchange reagents from different reagent lots except for Sample Diluent, Wash Concentrate, Substrate and Stop Solution.
2. Do not use reagents beyond their expiration date.
3. Store unused reagents at 2 to 8 ^{o}C.
4. Incubations above or below the recommended temperatures or times may give erroneous results.
5. The EIA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
6. Antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
7. *(Manual Procedure Only)* The washing procedure is very important and requires special attention. (Please refer to the Procedure section)

   NOTE: *Improperly washed* wells *may* give *erroneous results.*
8. The concentration of anti-Rubeola (measles) IgG in a given specimen determined from assays from different manufacturers can vary due to differences in assay methods and reagents.

Limitations

1. The results obtained with the Is-Measles IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.

2. Assay performance characteristics have not been established for visual result determination.
3. The Diamedix Is-Measles IgG test system is linear from 20.0 EU/ml (1.0 Index Value) to 100 EU/ml.
4. The test should be performed on serum. The use of whole blood, cord blood or plasma has not been established.
5. A single positive result only indicates previous immunologic exposure; the level of antibody response or class of antibody may not be used to determine active infection or disease stage.
6. A negative result does not rule out the diagnosis of Rubeola infection. The sample may have been collected before appearance of detectable antibodies. Negative results in suspected early Rubeola infection should be repeated in 4-6 weeks.
7. A significant rise in the level of Measles IgG cannot distinguish between primary infection and reinfection with measles. Lack of a significant rise in the level of Measles IgG does not exclude the possibility of measles infection.
8. Rare heterotypic responses with rubella virus and varicella virus have been reported for measles virus (7).
9. For individuals experiencing a polyclonal response when infected with a heterotypic virus, a differential diagnosis can be made on the basis of the fact that antibody to the infecting virus type is absent or at a very low titer in the acute-phase specimen, whereas antibody to the viral heterotype is already present (8).
10. The results on serum from immunosuppressed individuals must be interpreted with caution.
11. The performance characteristics of the Diamedix Is-Measles IgG Test Kit with automated equipment other than the Diamedix Automated EIA Systems, have not been established.

References

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