Beaumont

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Measles IgG - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

The objective of this procedure is to serve as instructional guide, to be utilized when performing the Measles IgG assay in-conjunction with the DiaSorin Liaison XL operators manual.

II. PRINCIPLE:

The method for qualitative determination of specific IgG to Measles virus is an indirect chemiluminescence immunoassay (CLIA). The principal components of the test are magnetic particles (solid phase) coated with recombinant antigen and a conjugate of mouse monoclonal antibody to human IgG linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, Measles virus antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with Measles virus IgG that is already bound to the solid phase.

III. SUMMARY AND EXPLANATION OF THE TEST:

The LIAISON® Measles IgG assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer family for the qualitative determination of IgG antibodies to Measles virus in human serum. It is intended to be used as an aid in the determination of serological status to Measles virus.

IV. CLINICAL SIGNIFICANCE:

A. Measles is an acute viral illness caused by a morbillivirus of the paramyxovirus family and is one of the most easily transmitted diseases. Transmission is primarily by large droplet spread or direct contact with nasal or throat secretions from an infected person (1). After infection, Measles virus invades the respiratory epithelium of the nasopharynx and spreads to the regional lymph nodes. After two to three days of replication in these sites, primary viremia widens the infection to the reticulo-endothelial system. Following further replication, secondary viremia occurs five to seven days after infection and lasts four to seven days. During this viremia, infection and further virus replication may occur in skin, conjunctivae, respiratory tract and other organs, including spleen, thymus, lung, liver, and kidney. Viremia peaks 11-14 days after infection, and then declines rapidly over a few days (2).

B. Prior to vaccine availability, Measles was mostly a disease of childhood, but Measles vaccination programs (part of Measles, Mumps, Rubella, Varicella [MMRV] vaccination) have had a marked effect on the incidence of the disease and the complications associated with it. After prolonged periods of high vaccine coverage in developed countries, Measles transmission now occurs mainly in people that have never been vaccinated and in older children who did not seroconvert following vaccination. Measles outbreaks can still occur in countries with high immunization coverage. Such outbreaks demonstrate an immunity gap in the population involved (1, 3).

C. Clinically, the diagnosis of Measles is supported if Koplik's spots are detected and if the rash progresses from the head to the trunk and out to the extremities. The non-specific nature of the prodromal signs and the existence of mild cases, however, make clinical signs unreliable as the sole diagnostic criteria of measles disease. As disease prevalence falls, many medical practitioners are inexperienced in recognizing measles, increasing the need for laboratory serological method of distinguishing Measles from other clinically similar diseases (4).

D. Both IgM and IgG antibodies are synthesized during the primary immune response and can be detected in the serum within a few days of rash onset. IgM antibody levels peak after about seven to ten days and then decline rapidly, being rarely detectable after six to eight weeks. IgM is generally not detected in an immune individual following re-exposure to Measles virus (5). Re-exposure to the Measles virus induces a strong anamnestic immune response with a rapid boosting of IgG antibodies, which prevents clinical disease (6).

V. ASSAY PERFORMANCE CHARACTERISTICS:

A. Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., hemolysis, effects of sample treatment), or cross-reactive antibodies. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by hemolysis (at 1000 mg/dL hemoglobin), lipemia (at 3000 mg/dL triglycerides), icterus (at 20 mg/dL bilirubin), albuminemia (at 5.0 g/dL albumin).

B. Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than real. However, a well-optimized two-step method excludes grossly underestimated results, because the analytical signals remain consistently high (saturation curve). Analysis of saturation effect was evaluated by testing three high-titered samples positive for Measles virus IgG. All samples resulted in high concentration values as expected, indicating no sample misclassification.

C. A total of 550 prospectively collected samples were tested. They represent 500 samples from subjects sent to U.S. laboratories for measles testing plus 50 samples from children ages 0-8 years sent to European Laboratories. The testing was performed at DiaSorin. All samples were tested with the LIAISON® Measles IgG assay and an U.S. commercially available test kit. The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

Subjects Sent to the U.S. Laboratories for Measles Testing (n = 500)

LIAISON® Measles IgG	Expected Results			Total
	Negative	Equivocal	Positive	

Negative (< 13.5 AU/mL)	11	1	22	34
Equivocal (13.5-16.5 AU/mL)	0	0	4	4
Positive (≥ 16.5 AU/mL)	2	0	460	462
Total	13	1	486	500

Condition	Percent Agreement	Exact 95% Confidence Interval
Positives Negatives	94.7% (460/486) 84.6% (11/13)	92.3 – 96.5% 54.5 – 98.1%

Pediatric Subjects Sent to the U.S. Laboratories for Measles Testing (n = 50)

LIAISON® Measles IgG	Expected Results			Total
	Negative	Equivocal	Positive	
Negative (< 13.5 AU/mL)	3	1	7	11
Equivocal (13.5-16.5 AU/mL)	0	0	0	0
Positive (≥ 16.5 AU/mL)	0	0	39	39
Total	3	1	46	50

Condition	Percent Agreement	Exact 95% Confidence Interval
Positives Negatives	84.8% (39/46) 100.0% (3/3)	71.1 – 93.7% 29.2 – 100.0%

VI. STORAGE AND STABILITY OF BIO-RAD CONTROLS:

Upon receipt, the BioRad VIROTROL® MuMZ and VIROCLEAR® MuMZ controls must be stored at 2-8°C. When controls are stored sealed, they are stable at 2-8°C up to the expiration date on the vial. The controls should not be used past the expiration date indicated on the vial labels. Once opened, controls are stable for 60 days when properly stored at 2-8°C between uses. Discard controls after 30 days of use at the bench, due to repeated exposures to ambient temperature. Avoid bacterial contamination of controls. Allow controls to reach room temperature prior to use. Return controls to the refrigerator immediately after each use.

VII. SPECIMEN AND COLLECTION AND HANDLING:

This assay can only test human serum samples. Blood should be collected aseptically by venipuncture, allowed to clot, and the serum separated from the clot as soon as possible. Grossly hemolyzed, icteric or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested. Do not heat-inactivate sera. Check for and remove air bubbles before assaying. If the assay is performed within nine days of sample collection, the samples may be maintained at 2-8°C; otherwise they should be dispensed in aliquots and stored frozen (-20°C or below). If samples are stored frozen, mix thawed samples well before testing. Samples should not be repeatedly frozen and thawed. Self-defrosting freezers are not recommended for sample storage. The minimum volume required is 170 mcL per specimen (20 mcL specimen + 150 mcL dead volume).

VIII. REAGENTS:

Magnetic particles (2.5 mL)	Magnetic particles coated with recombinant Measles virus nucleoprotein (obtained in baculovirus), Bovine serum albumin (BSA), phosphate buffered saline (PBS) buffer < 0.1% sodium azide.
Calibrator 1 (0.55 mL)	Human serum/defibrinated plasma containing low IgG levels to Measles virus, BSA, phosphate buffer, EDTA, detergents, 0.2% ProClin® 300, an inert yellow dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Calibrator 2 (0.55 mL)	Human serum/defibrinated plasma containing high IgG levels to Measles virus, BSA, phosphate buffer, EDTA, detergents, 0.2% ProClin® 300, an inert blue dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Specimen Diluent (2 x 27 mL)	Casein, BSA, phosphate buffer, EDTA, detergents, preservatives, an inert blue dye.
Conjugate (28 mL)	Mouse monoclonal antibodies to human IgG conjugated to an isoluminol derivative, BSA, phosphate buffer, 0.2% ProClin® 300, preservatives, an inert yellow dye.
Number of tests	100 tests per reagent integral

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the Reagent Integral.

A. **Materials Required but not Provided**
   - LIAISON® XL Cuvettes (code X0016)
   - LIAISON® XL Starter Kit (code 319200)
   - LIAISON® XL Disposable Tips (code X0015)
   - LIAISON® Wash/System Liquid (code 319100)
   - LIAISON® XL Waste Bags (code X0025)
   - BioRad VIROTROL® MuMZ (code 00119)
   - BioRad VIROCLEAR® MuMZ (Code 00133)

B. **Reagent Integral Storage and Stability**
   Upon receipt, the reagent integral must be stored in an upright position to facilitate re-suspension of magnetic particles. See Reagent Integral Preparation for re-suspension instructions. When the reagent integral is stored sealed, the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The reagent integral must not be used past the expiration date indicated on the kit and reagent integral labels. After removing the seals, the reagent integral is stable for eight weeks when stored at 2-8°C in a refrigerator or on board the LIAISON® XL Analyzer. Undue exposure to light should be avoided. Upon opening record on the reagent integral the open date and expiration date.

C. **Reagent Integral Preparation**
   Before removing the seals from the containers, gently and carefully shake the reagent integral side-to-side. Avoid formation of foam. Remove the seal from each container of the reagent integral and turn the thumb wheel at the bottom of the magnetic particle container back and forth until the suspension turns

brown. This procedure initiates re-suspension of magnetic particles. Insert the reagent integral into the solid-state magnetic device in the instrument. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Turn thumb wheel to re-suspend the magnetic particles. Then, place the reagent integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 15 minutes before use. The analyzer automatically stirs and completely re-suspends the magnetic particles. Follow the analyzer Operator's Manual to load the specimens and start the run.

IX. CALIBRATION:

A. Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve. Each calibration solution allows four calibrations to be performed.

B. The analyzer should be calibrated in triplicate whenever one of the following conditions occurs:

   1. A new lot of reagent integral or of Starter Kit is used.
   2. The previous calibration was performed more than four weeks before.
   3. The analyzer has been serviced.
   4. The values of the recommended BioRad controls lie outside the expected ranges.

C. Refer to the analyzer operator's manual or LIAISON® Quick Guide for calibration instructions. LIAISON® XL calibrator values are stored in the Radio Frequency Identification Transponder (RFID Tag).

X. QUALITY CONTROL:

Quality control is performed once per day of use. The BioRad VIROTROL® MuMZ positive and VIROCLEAR® MuMZ negative controls are intended to monitor for substantial reagent failure. The positive control does not ensure precision at the assay cutoff. If control results lie within the expected ranges, the test is valid. If the control results lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed, and controls and samples must be retested.

The minimum volume required is 420 mcL per control (20 mcL specimen + 400 mcL dead volume).

XI. PROCEDURAL STEPS:

A. Strict adherence to the relevant analyzer operator's manual ensures proper assay performance. Each test parameter is identified via information encoded in the reagent integral RFID Tag. In the event the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact the local DiaSorin technical support for instruction.

B. The analyzer operations are as follows:

   1. Dispense calibrators, controls or specimens into the cuvette.
   2. Dispense specimen diluent.
   3. Dispense magnetic particles.
   4. Incubate.
   5. Wash with Wash/System liquid.
   6. Dispense conjugate into the cuvette.
   7. Incubate.

8. Wash with Wash/System liquid.
9. Add the Starter Kit and measure the light emitted.

C. Procedural details for the test may be viewed directly from the analyzer's assay definition displays.

XII. CALCULATIONS AND INTERPRETATIONS:

A. The Analyzer automatically calculates Measles Virus IgG antibody concentrations expressed as arbitrary units per mL (AU/mL) and grades the results. For details, refer to the Analyzer Operator Manual.

B. The assay cutoff was determined as follows: Based on available clinical and laboratory data, the samples were classified as expected positive or negative for Measles Virus IgG and evaluated with the LIAISON® Measles IgG assay. A positive cutoff of 15.0 AU/mL with an equivocal zone from 13.5 to 16.5 AU/mL was determined to provide the best sensitivity and specificity for the tested clinical samples.

Warning – If a sample result displays "invalid RLU" and an exclamation mark (!) flag, the result obtained lies below the assay signal range. The sample must be retested. If the sample result upon retest still displays "invalid RLU", call DiaSorin Technical Support.

C. **Sample results should be interpreted as follows:**

AU/mL Value	Result	Interpretation
Below 13.5	Negative	Absence of detectable Measles virus IgG antibodies. A negative result generally indicates that the patient has not been infected and is susceptible to Measles. If the subject has no history of Measles, has not been previously vaccinated and exposure to measles virus is suspected despite a negative finding, a second sample should be collected and tested one to two weeks later.
Between 13.5 and 16.4	Equivocal	Equivocal samples should be retested in duplicate. If the result remains equivocal after repeat testing, a second sample should be collected no less than one to two weeks later.
Equal to or above 16.5	Positive	Presence of detectable Measles virus IgG antibodies. A positive result generally indicates exposure to measles virus or previous vaccination.

Note: The magnitude of the measure result is not indicative of the amount of antibody present.

The results of this assay are not by themselves diagnostic. Diagnosis of infectious diseases should not be established on the basis of a single test result, but should be determined in conjunction with previous infection history, clinical findings and other diagnostic procedures as well as in association with medical judgment.

XIII. LIMITATIONS:

A. The test should be performed on serum only. The use of whole blood or plasma specimens has not been established. Grossly hemolyzed, icteric or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested. Do not heat inactivate sera. Check for and remove air bubbles before assaying.

B. Assay performance characteristics have not been established when the LIAISON® Measles IgG assay is

used in conjunction with other manufacturers' assays for detection of specific measles virus serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.

C. Potential assay interference due to circulating antibodies against Human Ehrlichiosis (HGE) and Tick Borne Relapsing Fever (TBRF) has been found. Interpret results from these patients with caution.

D. Single components of the Reagent Integral should not be removed from the Integral.

E. This kit must not be used after the expiry date printed on the package label.

F. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.

G. Bacterial contamination or heat inactivation of the specimens may affect the test results.

H. False negative results may occur if samples are collected early in the disease.

I. Test results are reported qualitatively. However, diagnosis of a disease should be established based on the patient's anamnesis, in conjunction with clinical findings and in association with medical judgment. Any therapeutic decision must also be taken on a case-by-case basis.

J. Specimens from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain human anti-mouse antibodies (HAMA). Such specimens may interfere in a monoclonal antibody-based immunoassay and their results should be evaluated with care.

XIV. WARNINGS:

A. For *in vitro* diagnostic use.

1. The human blood source material used to produce the components provided in this kit derives from donations found to be non-reactive for HBsAg, antibodies to HCV, HIV-1 and HIV-2 (AIDS) when tested by an Food and Drug Administration (FDA)-approved method and found to be non-reactive for Syphilis when tested by a serological test. Because no test method can offer complete assurance that laboratory specimens are pathogen-free, specimens should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control-National Institutes of Health (CDC-NIH) manual. Biosafety in Microbiological and Biomedical Laboratories, 5th Edition, February 2007, and Clinical & Laboratory Standards Institute (CLSI) Approved Guideline M29-A3, Protection of Laboratory Workers from Occupational Acquired Infections (7-9).
2. Some reagents contain sodium azide as a preservative. Because sodium azide may form explosive lead or copper azide in plumbing, it is recommended that drains be thoroughly flushed with water after disposal of solutions containing sodium azide.
3. Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
4. Do not pipette solutions by mouth.
5. Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands thoroughly at the end of each assay.
6. Avoid splashing or forming an aerosol. Any reagent spills should be washed with a 5% sodium hypochlorite solution and disposed of as though potentially infectious.
7. All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the

regulations of each country. Disposable materials must be incinerated: liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an *overkill* approach (USP 24, 2000, p. 2143). A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.

B. The LIAISON® XL Analyzer should be cleaned and decontaminated on a routine basis. See the Operator's Manual for the procedures.

C. Strict adherence to the instructions is necessary to obtain reliable results.

XV. REFERENCES:

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Attachments

No Attachments

Approval Signatures

Step Description	Approver	Date
	Peter Millward: Chief, Pathology Service Line	10/13/2020
Policy and Forms Steering Committee Approval (if needed)	Gail Juleff: Project Mgr Policy	10/13/2020
Policy and Forms Steering Committee Approval (if needed)	Craig Keiper: Medical Technologist Lead	9/25/2020
	Gabriel Maine: Dir, Human Leukocyte Antigen	9/25/2020
	Leah Fontana: Mgr Laboratory	9/22/2020
	Craig Keiper: Medical Technologist Lead	9/21/2020

Applicability

Royal Oak

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