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| **Measles IgG** |
| **Purpose** | This procedure provides instructions for performing Measles IgG on the DIASORIN LIAISON XL. |
| **Policy Statements** | This procedure applies to all laboratory technical staff responsible for performing Measles IgG testing on the DiaSorin Liaison XL. |
| **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. After each incubation, unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and indicates the presence or absence of IgG to measles virus in calibrators, samples or controls. |
| **Clinical Significance** | 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. 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.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.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.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. Re-exposure to the measles virus induces a strong anamnestic immune response with a rapid boosting of IgG antibodies, which prevents clinical disease. |
| **Instrument** | DiaSorin LIAISON® XLSunquest Method Code: **XL** |
| **Sunquest Test Code** | ROGM |
| **Sample** | Serum is the only acceptable specimen for this assay collected aseptically by venipuncture. Refer to specimen collection procedures.Grossly hemolyzed, lipemic or particulate samples are not recommendedMinimum volume: 200 μLStability: 2-8 °C / 9 days, 30 days at -20 ºC or colderDo not store in self-defrosting freezer.Rejection criteria: Unlabeled tube, plasma, grossly hemolyzedPreparation:Whole blood specimens should be centrifuged as soon as clotted, according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.Clarify samples having particulate matter, turbidity, lipemia, or erythrocyte debrisRemove air bubbles before testingTransfer serum to a properly labeled tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freeze-thaw cycles. Check for and remove air bubbles before assaying |
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| **Materials** | **Reagents** | **Supplies** | **Equipment** |
|  | LIAISON® Measles IgG ([REF] 318810) | polypropylene sample tubes | DiaSorin Liaison XL System |
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| **Reagent Integral Preparation** | **How to prepare and load new reagent integrals**1. Remove from refrigerated storage, maintaining upright orientation
2. Inspect integral for leakage
3. Mix magnetic particle for 30 seconds
4. Seat test integral in Xcelerator for 30 seconds
5. Gently rotate the magnetic particle vial for 30 seconds
6. Remove new integral sealing flaps slowly
7. Remove all liquid from the surfaces of the membranes to prevent cross-contamination of the reagent vials by blotting using a kim wipe folded in half lengthwise
8. Open the reagent bay on the analyzer
9. Using a smooth motion, insert the integral into an unoccupied lane in the reagent area until it rests firmly against the docking pins at the rear.

**Note:** if more than one integral of the same reagent is loaded place the newest integral to the right of the old integral. The analyzer will sample from the left integral until empty then move right. |
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|  | Reagent Integral Storage and Stability:Upon receipt, the reagent integral must be stored in an upright position to facilitate resuspension of magnetic particles.Stored sealed, the reagents are stable at 2-8°C up to the expiration date. After removing the seals, the reagent integral is stable for eight weeks when stored at 2-8°C or on board the LIAISON XL® Analyzer. Record new expiration date and tech initials on the integral.Do not freeze. The reagent integral must not be used past the expiration date indicated on the kit and reagent integral labels.Keep away from direct light. |
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| **Special Safety Precautions** | For additional information, refer to Safety Data Sheet available on Star net.Reagent integral contains: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin® 300).Avoid direct contact with potentially infected material by wearing appropriate PPE* All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each country.
* Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.
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| **Calibration** | Assay of calibrators contained in the reagent integral allows the analyzer to recalibrate the stored master curve, as indicated by Radio Frequency Identification transponder (RFID Tag) on the reagent integral label. Refer to the Operator's Manual or LIAISON XL® Quick Guide for calibration instructions.Recalibration is required:* With each new lot of reagents (reagent integral or Starter reagents).
* Every 14 days.
* After servicing the LIAISON XL® Analyzer.
* If quality controls are out of your acceptable range.

Verify new reagent lots before use by testing Liaison Measles IgG Controls. Comparable results verify the new reagent lot. Discrepant results must be resolved before the reagent can be used for patient testing. |
| **Analytical Measuring Range (AMR)** | Measles IgG is an FDA-cleared/approved in vitro diagnostic assay that reports the qualitative result based on a predefined cut-off value. Verification of AMR or the cut-off value is not required by CAP or CLIA. The AMR of Measles IgG per DiaSorin is 5.00-300.00 AU/mL (arbitrary units). |
| **Quality Control** | **LIAISON® Control Measles IgG (**[REF] **318811*** Negative control (0.7 mL x 2 vials) containing a barcode label
* Positive control (0.7 mL x 2 vials) containing a barcode label
* Allow controls to reach room temperature prior to use. Return controls to the refrigerator immediately after each use.

**Frequency:** Run 2 levels every day of use. Load the bar-coded control vials into the “T” rack on the Liaison XL.**Stability:** Unopened: When controls are stored sealed and kept upright, they are stable at 2-8°C up to the expiry date.Opened: Once opened controls are stable for eight weeks when properly stored at 2-8°C.**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* Refer to the [Westgard Rules in Chemistry procedure](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.18-westgard-rules-in-chemistry.pdf) for current Westgard rules in place for each analyte.
* **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface.
* In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| **Procedure** | Refer to the instrument Operating procedure.Strict adherence to the relevant Analyzer Operator’s Manual ensures proper assay performance. **LIAISON**® **XL Analyzer**. Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.The Analyzer operations are as follows:1. Dispense calibrators, controls or specimens into the reaction module.2. Dispense specimen diluent 3. Dispense magnetic particles.4. Incubate. 5. Wash with Wash/System liquid.6. Dispense conjugate into the reaction module.7. Incubate. 8. Wash with Wash/System liquid.9. Add the Starter Kit and measure the light emitted. |

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| **Dilutions** | Do not dilute. See result Reporting. |
| **Reference Intervals** | 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 30.0 AU/mL with an equivocal zone from 25.0 to 30.0 AU/mL was determined to provide the best sensitivity and specificity for the tested clinical samples.< 25.0 AU/mL: 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 within one to two weeks later.≥ 25.0 AU/mL and< 30.0 AU/mL: Equivocal samples should be retested. If the result remains equivocal after repeat testing, a second sample should be collected no less than one to two weeks later.≥ 30.0 AU/mL: Presence of detectable measles virus IgG antibodies. A positive result generally indicates exposure to measles virus or previous vaccination.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

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| **Warning** - If the 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.  |

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| Limitations | False negative results may occur if samples are collected early in the disease.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.Bacterial contamination or heat inactivation of the specimens may affect the test results. |
| **Result Reporting** | 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.Results <25 AU/mL without error messages are reported with the numerical result, and interpreted as Negative. Results between 25 and 29.9 AU/mL must be repeated prior to reporting. If test remains in this range after repeat, results is reported with the numerical result and interpreted as Equivocal. Results ≥30 AU/mL without error messages are reported with the numerical result, and interpreted as Positive.  |
| **Alternate Methods** | RUBES: To Mayo Medical Laboratory.  |
| **References** | 1. LIAISON® Measles IgG ([REF] 318810) Directions for Use, DiaSorin, Inc., Stillwater, MN 55082, September 2016
2. LIAISON® Control Measles IgG ([REF] 318811)QC Directions for Use, DiaSorin, Inc., Stillwater, MN 55082, September 2016
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Stephen Gripentrog, Erin Bartos | August 13, 2019 | Initial Procedure |
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