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| **Rubella IgG**  |
| **Purpose** | This procedure provides instructions for performing LIAISON® Rubella IgG on the DIASORIN LIAISON XL. |
| **Policy Statements** | This procedure applies to all laboratory technical staff responsible for performing LIAISON® Rubella IgG testing on the DiaSorin Liaison XL. |
| **Principle** | The LIAISON® Rubella IgG uses chemiluminescence immunoassay (CLIA) technology on the LIAISON® XL analyzer for the qualitative determination of IgG antibodies to rubella virus in human serum specimens. It is intended for use as an aid in the determination of immune status to rubella in individuals including pregnant women. The performance of this device has not been established for cord blood, neonatal samples, or for any matrices other than human serum. Likewise, performance has not been established for population(s) of immunocompromised or immunosuppressed individuals.The method for qualitative determination of specific IgG to rubella virus is an indirect chemiluminescence immunoassay (CLIA). All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the LIAISON® XL analyzer. The principal components of the test are magnetic particles (solid phase) coated with rubella antigen and a conjugate of mouse monoclonal antibody to human IgG linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, rubella virus antibodies present in the calibrators, specimens or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with rubella virus IgG already bound to the solid phase. After each incubation, the 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 is indicative of the presence of rubella virus IgG in calibrators, specimens or controls. A result of positive or negative is determined according to the CLSI I/LA6-A recommended cutoff of 10 IU/mL established with the calibrators. |
| **Clinical Significance** | Rubella is a viral exanthematous, infectious disease that typically follows a benign clinical course. Symptomatology is generally characterized by fever, with maculopapular rash accompanied by enlargement of lymph nodes. Widespread vaccination of children has greatly reduced the incidence of rubella in the general population. However, total eradication of rubella in the population is unlikely. Because many rash illnesses mimic rubella and because many rubella infections are asymptomatic, rubella can often be overlooked or misdiagnosed. By far, the greatest consequence of rubella is Congenital Rubella Syndrome (CRS), which could cause miscarriages, stillbirths, fetal abnormalities, and therapeutic abortions as a result of infection in early pregnancy, especially in the first trimester. Fetal abnormalities may be hepatosplenomegaly, psychomotor retardation, bone alterations, cardiopathies, or neuropathies. Pathological consequences to the fetus depend on the virus teratogenic potency and on when the infection is contracted during pregnancy. The consequences to the fetus are highest in cases of rubella infection during the first two months of pregnancy, but decrease progressively if infection occurs at the 4th or 5th month. The risk of severe neonatal complication points to the need for accurate methods to determine the immune status of women of childbearing age. Reinfection can occur following either naturally acquired or vaccine-induced immunity, but is much more likely to occur in the latter. Reinfection is almost always subclinical and is defined by a rise in preexisting antibody. Viremia and systemic manifestation are uncommon. The risk of fetal transmission and subsequent CRS resulting from maternal reinfection during pregnancy is low. Since the availability of the vaccine in the late 1960s, the assay of rubella IgG has been widely used to determine the immune status, to determine the need for vaccination and to evaluate vaccine efficacy and screening for rubella virus is now generally performed by immunoassay. Specific IgG antibodies generally appear several weeks post infection; reach their maximum concentration and then progressively decrease to a low level persisting for life. Rubella vaccines will induce the production of IgG antibodies similar to that observed with natural infection. However, antibody titers following vaccination may be delayed and somewhat lower than the titers observed with natural infections |
| **Instrument** | DiaSorin LIAISON® XLSunquest Method Code: **XL** |
| **Sunquest Test Code** | **RUBEG** |
| **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 recommended.Minimum volume: 200 μLStability: 2-8 °C / 3 days, 30 days at -20 ºC or colderDo not store in self-defrosting freezer.Rejection criteria: Unlabeled tube, plasma, grossly hemolyzed samplesPreparation: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 debris.Remove air bubbles before testing.Transfer 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® Rubella IgG ([REF] 310460) | 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 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.  |
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| **Special Safety Precautions** | See additional and detailed information in SDS on Children’s StarNet.5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H -isothiazol-3-one May cause an allergic skin reaction.All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents.Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands at the end of each assay. |
| **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® Rubella IgG ([REF] 310460) 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)** | Rubella 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. DiaSorin stated AMR is 0.100-33.000 Index. |
| **Quality Control** | LIAISON® Rubella IgG ([REF] 310460)Negative control (0.9 mL x 2 vials) containing a barcode labelPositive control (0.9 mL x 2 vials) containing a barcode labelAllow 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 four 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 coated 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** | < 0.90 Index: Sample is considered negative for IgG antibodies to rubella virus. A negative result presumes that immunity has not been acquired. If exposure to rubella virus is suspected despite a negative finding, a second specimen should be collected and tested one or two weeks later. Seroconversion from a negative specimen to a positive specimen is evidence of either recent infection, response to vaccination, or administration of immunoglobulins.≥ 0.90 Index and < 1.00 Index: The equivocal specimen should be re-tested. In case the result remains in this range after repeat testing, a second specimen should be collected.≥ 1.00 Index: Sample is considered positive for IgG antibodies to rubella virus.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. |
| Limitations | * Human anti-mouse antibodies (HAMA) and other heterophile antibodies may be present in patient samples. Although patient specimens are washed away prior to the addition of the mouse monoclonal (conjugate), the performance characteristics of HAMA specimens have not been established and may occasionally influence results. Carefully evaluate results from patients suspected of having such antibodies
* Cross-reactivity could not be determined for HBsAg, measles IgG, mumps IgG, anti-HCV, anti-HIV-1/2, parvovirus IgG, gammaglobulin, rheumatoid factor and Treponema pallidum due to low prevalence of rubella antibody negative/cross-reacting condition positive patients
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| **Result Reporting** | * Results 0.9 Index without error messages are reported with the numerical result, and interpreted as Negative. The comment “Samples is considered negative for IgG antibodies to rubella virus. A negative result presumes that immunity has not been acquired. If exposure to rubella virus is suspected despite a negative finding, a second sample should be tested no less than one to two weeks later” is appended.
* Results between 0.90 and 1.0 Index: Equivocal samples must be repeated. If, upon repeat, the result is still in the equivocal range, the comment “Equivocal samples have been retested. A second sample should be collected no less than one to two weeks later” is appended.
* Results ≥ 1.0 Index without error messages are reported with the numerical result, and interpreted as Positive. The comment “Sample is considered positive for IgG antibodies to rubella virus” is appended.
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| **References** | 1. LIAISON® Rubella IgG ([REF] 310460) Directions for Use, DiaSorin, Inc., Stillwater, MN 55082, January 2019
2. LIAISON® Rubella IgG Tri-Controls ([REF] 310461) Directions for Use, DiaSorin, Inc., Stillwater, MN 55082, January 2019
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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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