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| **Total Lyme (*Borrelia burgdorferi*)**  |
| **Purpose** | This procedure provides instructions for performing TOTAL LYME (*BORRELIA BURGDORFERI*) on the DiaSorin LIAISON XL®. The LIAISON® Lyme Total Antibody Plus assay (also known as Total Lyme) uses chemiluminescent immunoassay (CLIA) technology for the qualitative determination of lgG and IgM antibodies to Borrelia burgdorferi in human serum and plasma samples. This assay is intended for use on samples from patients with signs and symptoms that are consistent with Lyme disease. Positive or equivocal results should be supplemented by testing with a standardized western blot procedure. Positive supplemental results provide evidence of exposure to B. burgdorferi and can be used to support a clinical diagnosis of Lyme disease. Negative results by LIAISON® Lyme Total Antibody Plus assay should not be used to exclude Lyme disease. The test has to be performed on the LIAISON® XL Analyzer. |
| **Policy Statements** | This procedure applies to all laboratory technical staff responsible for performing Total Lyme (*Borrelia burgdorferi*) testing on the DiaSorin LIAISON XL®. |
| **Principle** | The method for qualitative determination of IgG and IgM antibodies to *Borrelia burgdorferi* is an indirect chemiluminescence immunoassay (CLIA). All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the Analyzer. The principal components of the test are magnetic particles (solid phase) coated with recombinant *Borrelia* antigens and a conjugate reagent containing two mouse monoclonal antibodies (anti-human IgG and anti-human IgM) linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, antigen-specific antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the conjugates react with *Borrelia burgdorferi* IgG and IgM antibodies captured by the solid phase. Unbound material is removed with a wash cycle following incubations. 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 *Borrelia burgdorferi* antibodies present in calibrators, samples or controls.  |
| **Clinical Significance** | Lyme disease is caused by the tickborne spirochete Borrelia burgdorferi and is the most common vector-borne disease in the United States. The CDC initiated surveillance for Lyme disease in 1982, and since 1991 Lyme disease has been a nationally reportable disease. In the United States, the disease is primarily localized to states in the northeast, mid-Atlantic, upper north-central regions and northwestern California. The bacterium, Borrelia burgdorferi is the etiologic agent of Lyme borreliosis, a disease which is transmitted by different tick species of the genus Ixodes.Lyme borreliosis is a multisystemic disorder that can affect several organs, such as skin, nervous system, large joints and cardiovascular system. Even though Lyme disease spirochetes elicit a vigorous immune response, Borrelia bacteria may survive and persist in the circulation of infected patients. Similar to syphilis, Lyme borreliosis generally progresses through several different stages, from early to late infection:Stage 1 – Localized infection: After an incubation period, a slowly expanding skin lesion, erythema migraines (EM), forms at the site of the tick bite in 70-80% of the cases. General flu-like symptoms including malaise, fatigue, headache, arthralgia, myalgia and fever may accompany the skin lesion.Stage 2 – Disseminated infection: B. burgdorferi often disseminates within days to weeks after disease onset. Possible clinical manifestations include secondary skin lesions, acute lymphocytic meningitis and musculoskeletal pain in joints, tendon, muscle or bone.Stage 3 – Persistent infection: After weeks of disseminated infection, the Lyme disease agents may still survive in localized niches and may persist up to several years. Months after onset of illness, about 60% of untreated patients with this infection experience intermittent attacks of arthritis.Differential diagnosis of Lyme disease is difficult as clinical manifestations associated with different stages of the disease are variable and diagnosis of persistent infection is challenging in that the IgM/IgG anti-Borrelia antibody response can remain positive for months or even years after antibiotic therapy. Diagnosis of Lyme disease is based upon a physician’s review of clinical symptoms, patient’s exposure to an endemic area, and laboratory test results. In ambiguous cases, there may be a greater reliance on laboratory data to confirm the diagnosis.To provide accurate diagnosis of Lyme borreliosis, the LIAISON® Lyme Total Antibody Plus assay uses specific recombinant antigens obtained in E. coli. This assay features a solid phase coated with Borrelia VlsE, variable major protein-like sequence, expressed antigens (B. burgdorferi and B. garinii) and Borrelia OspC, the outer surface protein (B. afzelii). These outer surface proteins are thought to play major roles in the immune response with OspC serving as the immunodominant antigen of the IgM response during early stage infection and VlsE producing strong antibody response at all stages of disease, including the early stage. The recombinant B. afzelii OspC shares a high degree of homology with the B. burgdorferi OspC at the C-terminus which contains the immunodominant epitope and is therefore suitable for serodiagnosis of B. burgdorferi infection. The LIAISON® Lyme Total Antibody Plus assay was validated for the presumptive detection of human IgM and IgG antibodies to B. burgdorferi by method comparison, specificity, and sensitivity studies using samples from US patient population.In 1994, the Second National Conference on Serological Diagnosis of Lyme disease recommended a two-step testing system toward standardizing laboratory serologic testing for B. burgdorferi. Because EIA and IFA methods were not sufficient to support clinical diagnosis, it was recommended that positive or equivocal results from an EIA or IFA (first step) should be further tested, or supplemented by using a standardized western blot method (second step) for detecting antibodies to B. burgdorferi. Two-step positive results provide supplemental evidence of exposure to B. burgdorferi, which could support a clinical diagnosis of Lyme disease but should not be used as a sole criterion for diagnosis. |
| **Instrument** | DiaSorin LIAISON® XLSunquest Method Code: **XL** |
| **Sunquest Test Code** | **LYMS:** Lyme Serology IgG, IgM**LYCON:** Confirmation by Western Blot to Mayo for positive LYMS |
| **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**: 500 μL (includes 300 μL for Western Blot to Mayo)**Stability**: Room Temperature up to 8 hours, 2-8 °C / 7 days, 90 days at -20 ºC or colderDo not store in self-defrosting freezer. Do not exceed 5 freeze-thaw cycles**Rejection criteria**: Unlabeled tube, gross hemolysis (>1000 mg/dL)**Preparation:**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. |

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| **Reagents** | ***Product Description*** | ***Product Code*** | ***Stability*** |
|  | LIAISON XL® Borrelia burgdorferiIntegral, (100 tests) supplied ready to use, containing magnetic particles, calibrators, diluent and conjugate. Refer to the Operating Procedure for instructions on how to prepare integrals for use and loading onto the analyzer.Each calibration solution allows 5 calibrations to be performed.  | 318330 | Store at: 2-8°. Upon receipt, the Reagent Integral must be stored in an upright position to facilitate re-suspension of magnetic particles. When the Reagent Integral is stored unopened the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The Reagent Integral should not be used past the expiration date indicated on the kit and Reagent Integral labels. After removing seals the Reagent Integral is stable for 8 weeks when returned to the kit box and stored upright at 2-8°C or stored on board the Analyzer.Unopened: Date on cartonOpened or on board: 8 weeks at 2-8° C |
|  | LIAISON XL® Serum Controls Borrelia burgdorferi(negative, positive) | 318331 | Store at: 2-8° C Unopened: Date on vialOpened: 12 weeks at 2-8° C |
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| **Risk and Safety** | Refer to safety data sheet on Children’s [StarNet](https://msdsmanagement.msdsonline.com/site-notification/?guid=a07dc954-23d8-42a9-b591-ef5763cdfd33)All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents.Specimens should be handled at the BSL 2 level recommended for any potentially infectious human serum or blood specimen.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.Some reagents contain sodium azide as a preservative. Flush drains thoroughly with water after disposal to prevent azide build up.* 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.

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.* Controls contain ProClin as a preservative, and human source material
	+ May cause an allergic skin reaction.
	+ Avoid breathing mist or spray.
	+ Contaminated work clothing should not be allowed out of the workplace.
	+ Wear protective gloves and clothing, and eye protection
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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 8 weeks.
* After servicing the LIAISON XL® Analyzer.
* If quality controls are out of acceptable range.

Verify new reagent lots before use by testing **LIAISON XL® Borrelia burgdorferi****Controls (310871):** negative and positive. If results are within the acceptable limits, the reagent lot is acceptable for use.Discrepant results must be resolved before the reagent can be used for patient testing. |
| **Analytical Measuring Range (AMR)** | LIAISON XL® Borrelia burgdorferiis 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. |
| **Reagent Integral Preparation**  | **How to prepare and load new 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 with 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. Let stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.

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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| **Quality Control** | **LIAISON XL® Borrelia burgdorferi****Controls (318831):** negative and positive are used to monitor the performance of the LIAISON XL® Borrelia burgdorferi chemiluminescent immunoassay (CLIA) for the qualitative determination of IgG/IgM antibodies to *B. burgdorferi* in human serum.* Negative control (0.8 mL x 2 vials) containing a barcode label and ProClin® 300 as a preservative
* Positive control (0.8 mL x 2 vials) containing a barcode label and ProClin® 300 as a preservative
* Controls are not lot specific and may be interchanged between integral reagent lots

The LIAISON® Lyme Total Antibody Plus Control Set is provided ready to use. Allow controls to reach room temperature and mix thoroughly by gentle inversion prior to use. Remove caps from the controls and place controls into the appropriate sample rack type with the barcode showing outward and slide rack into the patient sample area. Control identification is detected by the bar code label or may be manually programmed into the instrument. Follow the analyzer operator’s manual to start the run. Return controls to the refrigerator immediately after each use. The certificate of analysis bar codes give specific information on the lot of controls and should be read by the hand-held bar code scanner of the LIAISON® XL analyzer prior to loading the control vials on board. For details, refer to the analyzer operator's manual. **Frequency:** Run 2 levels once per day of use. **Stability:** Unopened: Store at 2-8°C. Stable until the date on vial. Do not use past the expiration date Opened: 12 weeks stored tightly capped at 2-8°C between uses. Mark vial with expiration date and initials upon opening.**Acceptable ranges:** * New lots of control should be verified that control values lie within the expected ranges provided on the certificate of analysis. A Bio-Rad Unity Real Time administrator (technical specialist or designee) must be notified several days before the new lot is used to allow for the new lot to be configured in Unity Real Time using the certificate of analysis.
* **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 **Liaison XL Operating Procedure**.**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 coated magnetic particles into the reaction module. 2. Dispense specimen diluent. 3. Dispense calibrators, controls or specimens. 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 reagents and measure the light emitted. |
| **Interpretation/****Results/Alert Values** | The Analyzer automatically calculates LIAISON XL® Borrelia burgdorferiantibody concentrations expressed as an index value and grades the results. For details, refer to the Liaison XL Operator’s Manual. The measuring range is 0.1 to 10.0.An **index of 1.0** provides the best balance of sensitivity and specificity. An **equivocal range of 0.90-1.09** was applied to the assay to account for normal measurement imprecision.Results between 0.90-1.09 (***equivocal)*** should be repeat tested. If the result is the same after repeat testing, a second sample should be collected and tested no less than one or two weeks later. If the result is not the same, retest a third time.**Equivocal and Positive results:** will automatically reflex to order a Western blot confirmatory test at Mayo Medical Laboratories when resulted in OEM. Equivocal results must be repeated prior to resulting in Sunquest.**Warning** – When a sample result displays “invalid RLU” and the exclamation mark **(!) flag**, the result obtained lies below the assay’s signal range. The sample must be retested. If the retest still displays “invalid RLU”, call DiaSorin technical support.**Note** – *The magnitude of the measured result*, *above the cutoff, is not indicative of the amount of antibody present*.The results of this assay are not by themselves diagnostic. Diagnosis of Lyme disease should not be established on the basis of a single test result, but should be supplemented by testing with a standardized Western blot assay and evaluated in conjunction with clinical findings and in association with medical judgment. |

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| **Dilutions** | Do not dilute. See result Reporting. |
| **Reference Intervals** | < 0.90 = Negative Absence of detectable *Borrelia burgdorferi* antibodies. A negative result does not exclude the possibility of *Borrelia burgdorferi* infection. If early Lyme disease is suspected, a second sample should be collected and tested two to four weeks later.0.90 to 1.09 = Equivocal The imprecision inherent in this method does not allow definitive categorization of samples that read close to the cutoff. Current testing guidelines recommend that equivocal results be tested further in a standardized Western blot assay.≥ 1.10 = Positive Presence of detectable *Borrelia burgdorferi* antibodies. Current testing guidelines recommend that all positive results be supplemented by further testing in a standardized Western blot assay.  |
| Limitations | 1. The test should be performed on **serum only** so that equivocal and positive results may be confirmed by Mayo Medical Laboratories. 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.
2. The results from this kit are not by themselves diagnostic and should be considered in association with the second step Western blot for IgG and IgM and other clinical data and patient symptoms.
3. The DiaSorin LIAISON® Lyme Total Antibody Plus assay contains antigens from Borrelia burgdorferi, Borrelia garinii and Borrelia afzelii. Results from the second-step western blots that detect only B. burgdorferi specific antigens should be interpreted with caution.
4. The assay contains antigens to *B. garinii,* which is known to infect populations in Europe and other parts of the world, but not generally detected in the U.S. patients.
5. Some patient samples may be reactive with the DiaSorin LIAISON XL® *Borrelia burgdorferi* assay, but non-reactive by the second-tier Western Blot test due to the use of different antigens in the Western Blot. Treatment of these patients for Lyme disease should be based on clinical manifestations present and patient history, including travel outside of the US.
6. The DiaSorin LIAISON XL® *Borrelia burgdorferi* assay contains antigens from *Borrelia burgdorferi* and *Borrelia garinii.* Results from the second-step Western blots that detect only *B. burgdorferi* specific antigens should be interpreted with caution.
7. Screening of the general population should not be performed. The positive predictive value depends on the likelihood of Lyme disease being present. Testing should only be performed on patients with clinical symptoms of Lyme disease or when exposure is suspected.
8. 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.
9. The cross-reactivity study was designed to evaluate 238 specimens from 24 disease states either known to contain potentially cross reactive antibodies to B. burgdorferi or from patients with diagnoses that can exhibit signs and symptoms similar to late manifestations of Lyme disease and cause false positive results. See the package insert for details.

**Interferences:** assay performance was not affected by:Hemolysis (at 1000 mg/dL hemoglobin)Lipemia (at 1500 mg/dL triglycerides)Icterus (at 40 mg/dL bilirubin)Total Protein (at 12 g/dL)Cholesterol (at 500 mg/dL)Biotin (at 3600 ng/mL) |
| **Result Reporting** | * Review, validate, and tag results and send to Sunquest. Send only the repeated equivocal result to Sunquest.
* Release results in Sunquest following LIS procedures for OEM.
* Equivocal and Positive results automatically order the confirmatory Western blot test (#9535) to Mayo when the result is accepted.
* Transfer the sample to the Send Outs department to complete the reflex testing.
* **Results <0.90 without error messages are reported as negative.** The comment “Absence of detectable *Borrelia burgdorferi* antibodies. If early Lyme disease is suspected, a second sample should be collected and tested two to four weeks later” will append.
* **Results between 0.90 – 1.09 must be repeated prior to reporting and are reported as Equivocal.** The comment “Current testing guidelines recommend that all equivocal results be supplemented by further testing in a standardized Western blot assay. The sample will be referred for confirmation by Western blot” will append. Verify the reflexive Western blot confirmatory test code # 9535 to Mayo was ordered on resulting.
* **Results 1.10 without error messages are reported as positive.**  The comment “Presence of detectable *Borrelia burgdorferi* antibodies. Current testing guidelines recommend that all positive results be supplemented by further testing in a standardized Western blot assay. The sample will be referred for confirmation by Western blot” will append. Verify the reflexive Western blot confirmatory test code # 9535 to Mayo was ordered on resulting.
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| **Alternate Methods** | * When test performance does not meet quality standards, consult the technical specialist or Medical Director, and refer testing to Mayo Medical Laboratory.
* Order test 9129, Lyme Disease Serology, and submit 0.5 mL of serum.
* If Western blot (confirmation) is required order test 9535, Lyme Disease Antibody, Western Blot, Serum, and submit 0.5 Ml serum. DO NOT REFER proficiency samples for confirmatory testing.
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| **References** | 1. LIAISON® Lyme Total Antibody Plus (318830) Directions for Use, DiaSorin, Inc., Stillwater, MN 55082, February 2020
2. LIAISON® Lyme Total Antibody Plus Controls (318831) Directions for Use, DiaSorin, Inc., Stillwater, MN 55082, February 2020
3. Jacobs & DeMott Laboratory Test Handbook, Lexi-Comp, Inc., Hudson, OH, 5th Edition, 2001
4. Mayo Medical Laboratories Test Catalogue, Lyme Disease Antibody, Western Blot, 12/2020
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| **Appendices** | Refer to LIAISON XL® *Borrelia Burgdorferi* (318330) Directions for Use for specific performance characteristics. |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Erin Bartos | December 2, 2020 | Updated for reformulation of Lyme assay |
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