UNIVERSITY OF PENNSYLVANIA HEALTH SYSTEM

To: Penn Medicine Physicians and Staff

Date: 6/7/2022

From: Malek Kamoun, MD, PhD
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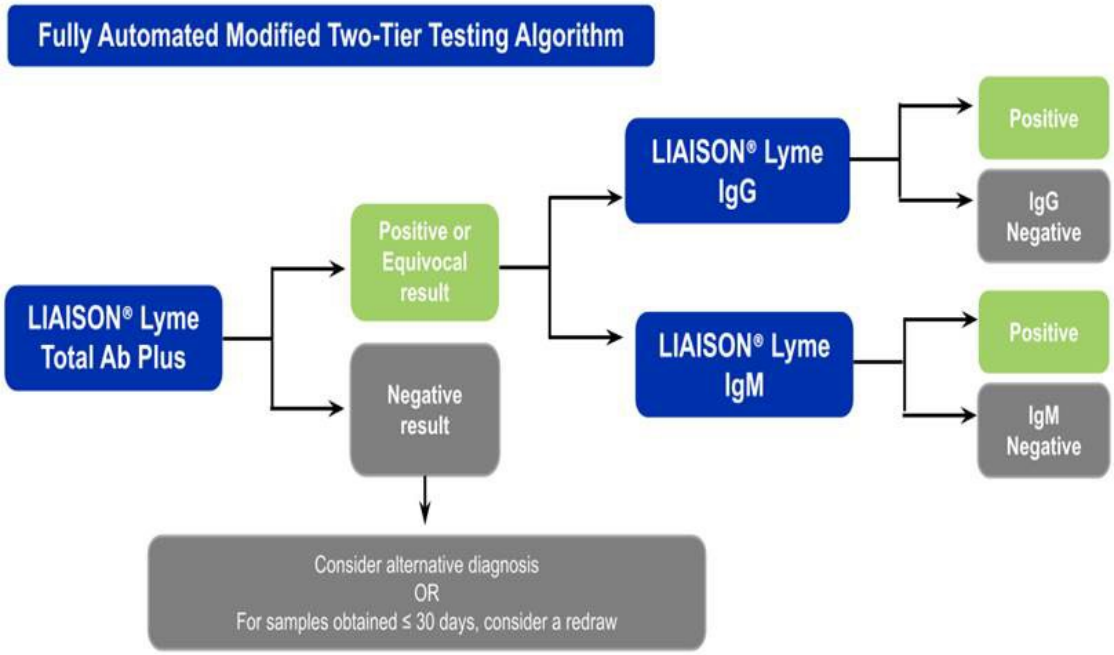
Re: Modified Two-Tiered Testing (MTTT) algorithm for the Diagnosis of Lyme Disease

Beginning on 6/9/2022, Penn Medicine Pathology and Laboratory Medicine will perform Lyme Confirmatory testing in-house, instead of sending specimens to ARUP Laboratories. The screen and confirmatory assays will follow a modified two-tier testing (MTTT) approach that was approved by the FDA in 2019 and has been endorsed by the CDC. The MTTT approach has specificity that is comparable to standard two-tiered testing that used an immunoassay as a screen and a Western blot as a confirmatory assay. Importantly, the MTTT algorithm provides increased sensitivity for detection of early infection. In addition, as the MTTT does not include a Western blot, it eliminates subjective interpretation of results associated with the Western blot.

The screening assay will still be performed on the DiaSorin Liaison XL Chemiluminescent analyzer using an IgG/IgM total assay that uses specific recombinant antigens obtained in *E. coli*. The screening assays feature a solid phase coated with OspC, the outer surface protein from *B. afzelii,* and VlsE, a variable major protein-like sequence E lipoprotein from *B. burgdorferi* and *B. garinii*. These proteins are major targets of the immune response, with OspC serving as the immunodominant antigen of the IgM response during early-stage infection and VlsE producing strong antibody responses 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 and contains the immunodominant epitope. It is therefore suitable for serodiagnosis of *B. burgdorferi.*

All presumptive positive and indeterminate results will be confirmed using two separate chemiluminescence-based assays. The assay for IgM antibodies contains recombinant antigens OspC from *B. afzelii* and recombinant VlsE from *B. burgdorferi*. The assay for IgG antibodies contains recombinant antigen VlsE and a peptide for the C6 region of VlsE antigen, both from *B. burgdorferi*. The IgG Western blot will be discontinued.

The following algorithm will be used in the MTTT:



Lyme Total IgM/IgG Screening assay by Chemiluminescence interpretive data:

< 0.90 IV	Negative - Antibody to *B. burgdorferi* not detected.
>/= 0.90 to <1.10 IV	Equivocal - Testing guidelines recommend that all equivocal samples be tested further following the modified two-tier test methodologies.
>/= 1.10 IV	Positive - Presumptive presence of antibody to *B. burgdorferi* detected. Testing guidelines recommend that all positive samples be tested further following the modified two-tier test methodologies.

Lyme IgG Confirmatory assay by Chemiluminescence interpretive data:

< 0.90 IV	Negative - Antibody to *B. burgdorferi* not detected.
>/= 0.90 to <1.10 IV	Equivocal - Repeat testing in 10-14 days may be helpful.
>/= 1.10 IV	Positive - Presence of IgG antibody to *B. burgdorferi* detected.

Lyme IgM Confirmatory assay by Chemiluminescence interpretive data:

< 0.90 IV	Negative - Antibody to *B. burgdorferi* not detected.
>/= 0.90 to <1.10 IV	Equivocal - Repeat testing in 10-14 days may be helpful.
>/= 1.10 IV	Positive - Presence of IgM antibody to *B. burgdorferi* detected.

All Lyme CSF will continue to have a serum sample screened before being sent to ARUP. Only CSF that have a positive or indeterminate serum result will be sent to ARUP for testing.

0099483 Borrelia burgdorferi Antibodies, Total by ELISA, CSF

Interpretive information for the CSF assay will be as follows:

0.99 LIV or less	Negative - Antibody to *B. burgdorferi* not detected.
1.00-1.20 LIV	Equivocal - Repeat testing in 10-14 days may be helpful.
1.21 LIV or greater	Positive - Probable presence of antibody to *B. burgdorferi* detected.

The order codes for the screening assay and tube type will not change. Positive or equivocal results will automatically reflex the confirmatory tests.

For clinical questions related to this change, please contact the Immunology Resident at 215-980-9871. For operational questions related to this change, please contact Joyce Gonzalez at Joyce.Gonzalez@pennmedicine.upenn.edu or 215-662-6023.