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Lyme Diagnosis: Transition from Standard to Modified Two-Tier Testing

A diagnosis of Lyme disease can be made clinically if the characteristic erythema migrans (EM) rash is observed. However, 20-30% of patients do not present with typical EM. Since PCR cannot reliably detect *Borrelia burgdorferi* in early Lyme, the diagnosis relies on the detection of antibodies to the bacteria. However, antibodies take time to develop and can be negative when patients present early for care. In addition, all IgM assays are prone to false positive results.

Standard vs Modified two-tier testing: To increase the accuracy of serologic testing, the CDC recommends two-tier antibody testing (1). The **standard two-tier testing (STTT)** algorithm involves an initial total antibody screen by ELISA, followed by IgM and IgG detection by western blot when the initial screen is positive or equivocal. However, poor sensitivity of STTT for diagnosis of early Lyme disease has prompted the development of a **modified two-tier testing algorithm (MTTT)** using two successive ELISAs (2, 3).

Advantages of MTTT algorithm: Several studies have demonstrated a slightly higher sensitivity of MTTT in the early stages of Lyme (**Table 1**), without compromising specificity (3-6). In addition, MTTT has a shorter time to result and is more cost-effective than STTT (7). The MTTT algorithm received FDA approval in July 2019.

Table 1. Estimated Sensitivity of Standard vs Modified Lyme Test Protocols in the U.S.

Disease stage	Disease manifestation	Standard 2-tier ELISA/ immunoblot (STTT)	Modified 2-tier ELISA (MTTT)
Early localized	EM rash	30% (acute) 60% (convalescent)	35% (acute) 75% (convalescent)
Early disseminated	Multiple EM Early neuroborreliosis Lyme carditis	70-90%	85-95%
Late	Lyme arthritis Late neuroborreliosis	100%	100%

The first FDA approved MTTT utilizes *Borrelia burgdorferi* VlsE1/pepC10 IgM/IgG ELISA screen, followed by second tier ELISA with whole cell antigen for detection of IgM and IgG (Zeus Scientific, Zeus, Branchburg, NJ).

Comparison study at YNHH: Before the transition to MTTT, a comparison of MTTT with STTT was performed. All samples submitted for Lyme testing to the Clinical Immunology Laboratory in July 2021, peak Lyme season, were tested by both algorithms (8).

Of 537 samples tested, 84 (15.6%) were positive or equivocal by Zeus ELISA and reflexed to tier 2 testing for IgM and IgG testing by both ELISA (MTTT) or immunoblot (STTT). As shown in **Table 2**, **MTTT detected more active Lyme infections and had fewer false negative tier 2 results than STTT**.

Zeus ELISA MTTT provided earlier detection of IgG than immunoblot STTT, giving fewer "IgM only" positive results and more IgM and IgG dual positives. The earlier detection of IgG provides greater confidence in diagnostic accuracy due to the greater reliability of IgG seroconversion compared to IgM (9). Due to increased sensitivity for IgG, MTTT also detected more cases of past Lyme.

False positive IgM results: Our test interpretive comment for IgM positive (IgG negative) results alerts providers to the possibility of a false positive IgM result, due to primary EBV or CMV infection, other bacterial or viral infections or autoimmune diseases, and recommends follow-up testing to document seroconversion of IgG if indicated. Thus, the earlier detection of IgG by the MTTT ELISA not only provides greater confidence in result accuracy, but also reduces the need for follow-up testing.

Table 2. Comparison of MTTT and STTT test in detection of active Lyme at YNHH (n=537)

Tier 2 Result	**Positive Screen: Zeus VlsE1/pepC10 ELISA** (n=84)	
	STTT tier 2 Viramed immunoblot	**MTTT tier 2 Whole cell antigen ELISA**
IgM and IgG Negative[a]	30	21
IgM positive only[b]	27	20
IgM and IgG positive[c]	20	32
IgG positive only[d]	7	11
Total positive Tier 2[b,d, e]	54	63
Active Lyme by chart review	43/54	49/63[e]

a, False negative tier 2 results: 10/30 STTT and 5/21 MTTT IgM/IgG negative results were cases of active Lyme disease.

b, Nonspecific IgM positives due to other infections or autoimmune diseases were found in 5/27 STTT and 4/20 MTTT.

c, Most dual IgM & IgG positives were active Lyme by chart review (17/20 STTT, 31/32 MTTT)

d, Lyme arthritis (4/7 STTT, 2/11 MTTT). Two of 4 cases of Lyme arthritis were IgM & IgG positive by MTTT, but IgG-only by STTT.

e, MTTT detected more cases of past Lyme than STTT. These were true results, but not "active Lyme".

Conclusion: Our study confirmed the superior performance of Zeus MTTT over STTT, as reported by others.

Implementation of Lyme MTTT serology at YNHHS:

1. Previously STTT methods varied between system hospitals, including some testing sent to reference labs.

2. Since **October 17, 2023**, Lyme serology testing for all YNHHS hospitals has been performed at YNHH **Clinical Immunology Laboratory** using the **Zeus Borrelia modified two-tier testing (MTTT**) protocol.

3. New test name: **Lyme Antibodies w/rflx to confirm (Modified Two-Tier Testing) [LAB4977]**

4. **Test schedule and time to result in EPIC:**
   a. Lyme antibody ELISA screen performed daily. Time from collection to result: Mean 21 h (95% <44 h).
   b. Lyme IgM & IgG ELISA performed 3 times/wk. Time from collection to result: Mean 49 h (95% <4 d).

5. Despite more sensitive MTTT ELISA, some early infections may be antibody-negative by screen or IgM/IgG.

6. **Note: Repeat Lyme serology should NOT be done for test of cure**, as specific antibodies to the organism persist after effective therapy.

7. **Other Lyme testing with limited indications:**
    - For **typical early neuroborreliosis** (cranial nerve palsies or meningitis), **antibody testing of serum** is usually sufficient to document active Lyme.

    - **PCR testing of CSF is usually negative (**80-95% negative by PCR**)** and thus is not recommended unless the patient is immunosuppressed and unable to make antibody (3).

    - For **late neuroborreliosis and atypical or severe early neuroborreliosis**, the current preferred test is the **Lyme CNS infection IgG with antibody index reflex, serum and CSF [LAB11150]** to detect intrathecal production of IgG (3). Samples are sent to the Mayo Clinic Reference Laboratory. Paired Serum and CSF collected within 24 hrs are required.

    - For **late Lyme arthritis**, serology **showing strong IgG reactivity** is ~100% sensitive and is the test of choice.

    - However, PCR of synovial fluid [LAB6943] has reasonable sensitivity (>70% if tested prior to antibiotic therapy) when needed to diagnose an active *B. burgdorferi* arthritis in a patient with past Lyme who is IgG positive. Note however that PCR of synovial fluid can remain positive for months after effective therapy (3).

For questions on test methods or interpretation, contact Marie Landry, MD at marie.landry@yale.edu.
For questions on testing schedule and operations, contact Penny Smith at penny.smith@ynhh.org.

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