Comparison of 2 Sets of Immunoassays Used in Modified 2-Tiered Testing Algorithms for the Diagnosis of Lyme Disease

Article:
Michael E Walsh, Daniel J Sietsma, Isabella W Martin, Lynn A Brunelle.
Comparison of 2 Sets of Immunoassays Used in Modified 2-Tiered Testing Algorithms for the Diagnosis of Lyme Disease.
J Appl Lab Med 2025; 10(2): 370–9. https://doi.org/10.1093/jalm/jfae127

Guests: Dr. Mike Walsh completed a fellowship in clinical chemistry at Dartmouth Hitchcock Medical Center, where the study was conducted and is currently at Eisenhower Army Medical Center. Dr. Lynn Brunelle is the Director of Southern Region Laboratory Services and the Assistant Director of Clinical Chemistry Services for Dartmouth Hitchcock Medical Center.

Randye Kaye: Hello and welcome to this edition of *JALM* Talk from *The Journal of Applied Laboratory Medicine*, a publication of the Association for Diagnostics & Laboratory Medicine. I'm your host, Randye Kaye.

Lyme disease is the most common vector-borne disease in the United States with an estimated 476,000 diagnoses annually. Its clinical presentation is often nonspecific, including symptoms such as skin rash, joint stiffness, and fever. If untreated, Lyme disease can progress to more severe conditions such as meningitis or carditis, making laboratory testing crucial for diagnosis.

Historically, Lyme disease testing has involved a two-tiered algorithm, starting with an enzyme immunoassay or immunofluorescence assay, followed by a confirmatory western immunoblot assay. In 2019, the CDC endorsed a new modified two-tiered testing algorithm referred to as MTTT, which includes an initial immunoassay followed by a second immunoassay targeting a different antigen. MTTTs offer improved sensitivity and are suitable for laboratory automation. However, there is a lack of studies evaluating the performance of these newly available MTTT immunoassays.

The March 2025 issue of *JALM* features an article comparing two manufacturers MTTT algorithm assays for Lyme disease testing. The study highlights the potential impact of assay choice on treatment decisions and underscores the importance of assay selection to guide patient care and treatment in Lyme disease.

Today, we're joined by two of the article's authors, Drs. Mike Walsh and Lynn Brunelle. Dr. Walsh completed a fellowship in clinical chemistry at Dartmouth Hitchcock Medical Center, where the study was conducted. He is currently the Chief of Research and Training in the Department of Clinical Investigation at Eisenhower Army Medical Center. Dr. Brunelle is the Director of Southern Region Laboratory

Services and the Assistant Director of Clinical Chemistry Services for Dartmouth Hitchcock Medical Center.

Welcome Drs. Walsh and Brunelle. Let's start with you, Dr. Walsh. Lyme disease is the most common tick-borne disease in the United States, but what are some of the challenges in diagnosing Lyme disease?

Mike Walsh: Hi Randye, thanks for having us. It can be difficult to diagnose Lyme disease as there are challenges beginning with the transmission of the disease all the way to laboratory testing. Lyme is a bacterial infection that's carried by ticks and it's really the small nymphs that spread the disease. So, people don't always notice when they've been bit and then when the patient actually has a disease. Most of the symptoms are not specific. They may have fever, joint pain, and muscle aches, but that's similar to the flu or arthritis or chronic fatigue syndrome.

And there's also this bullseye rash that's associated with Lyme disease called erythema migrans. But that doesn't always appear, and when it does, it doesn't always have that classic bullseye pattern. Lab testing for Lyme disease also has a host of issues. The bacteria that cause Lyme disease can't be cultured with routine culture methods and direct detection with molecular testing has very limited uses. So, that leaves serology, which has its own set of challenges.

With Lyme disease, it can take several weeks to develop antibodies, so if you test too early, that can result in a false negative. The assays used to detect Lyme also have cross reactivity to other infectious diseases. Then you can have false positives. Because of all this complexity, algorithms were developed that combine different types of test methods in order to improve the sensitivity and specificity. The final diagnosis is going to rely on a combination of the patient's history, their symptoms and lab results, and the judgment of the healthcare provider.

Randye Kaye: Wow, those are quite a lot of challenges. So, Dr. Brunelle, can you describe for us the advantages and disadvantages of using a modified two-tiered testing algorithm versus the standard two-tiered testing algorithm to diagnose Lyme disease?

Lynn Brunelle: Both the standard two-tiered testing algorithm and the modified two-tiered testing algorithm share similar specificities. Both are very effective at detecting Lyme disease in the later stages of the infection. Where the differentiation between the modified and the standard two-tiered testing algorithms are most apparent, occurs in their detection of the early stages of the disease. The modified two-tiered testing algorithm, through the exclusive use of

immunoassays, removes the subjectivity that can occur with western blot interpretation in the standard testing algorithm.

Additionally, with the ability to vary the type of antigens that are used in both the first-tier immunoassays and the second-tier immunoassays, improved sensitivity has been demonstrated with the modified two-tiered testing algorithm in the detection of early Lyme disease. With regard to disadvantages here, in the modified two-tiered testing algorithm, immunoassays cannot be used to monitor response over time. So, for example, if a patient becomes re-infected, the response of an immunoassay remains binary. It's either negative or it's positive.

However, the western blot offers the advantage of allowing one to monitor IgG expansion and change over time via the number and identity of bands present. So, for example, if a patient initially has six bands present and suffers a new infection and then upon retesting demonstrates say, nine IgG bands, this would be highly suggestive of a new infection.

Randye Kaye: All right, thank you. Back to you Dr. Walsh. Can you tell us more about the method comparison study that was described in the *JALM* article?

Mike Walsh: Sure thing. In our study, we compared the performance of two sets of serological assays used in modified two-tiered testing algorithms. One set of assays was from Zeus Scientific and the other was from Diasorin. In our lab, we were interested in moving to the Diasorin platform because it's more automated, but these assays are pretty new and not much is known about them. Our samples came from 120 patients, 95 of whom tested positive for Lyme disease by the Zeus assays. These samples were collected in the northeast, where Lyme disease is endemic. We retrospectively collected patient history and demographic information. About a third of the patients had a previous diagnosis of Lyme disease. Three quarters had some symptoms consistent with Lyme disease like fatigue or joint pain. But only 3% reported erythema migrans and none of the patients had symptoms of a disseminated Lyme disease like carditis.

The samples were initially tested with the Zeus assays and then we ran them on the Diasorin platform. Both sets of assays test for total Lyme disease and then reflect the separate IgG and IgM assays if the total is positive. We first compared each type of assay for total IgG/IgM. All of the negative samples and 64% of the positive samples had the same results on both platforms. For IgG, only 66% of the negative samples agreed, while 91% of the positive samples agreed. IgM was the opposite, with higher agreement between negative samples and lower agreement with positive samples.

The other thing we looked at was overall test interpretation. This is important because you can test positive for total IgG/IgM but negative for the reflex tests. And the interpretation there is that the total IgG/IgM was a false positive. You don't have Lyme disease. And in this analysis, overall test interpretation was concordant for 58% of patients who had tested positive.

Randye Kaye: Well, were you surprised by the low level of agreement that you observed between the two manufacturers' assays, Dr. Brunelle?

Lynn Brunelle: Not really. When you actually start looking at the different antigens and the different species of *Borrelia* that was utilized by the two manufacturers, that could certainly account for some of the observed differences. For example, the first-tier initial immunoassays from each manufacturer both utilize an early response marker and a late response marker of infection. So, for example, both Zeus and Diasorin assays utilize the variable membrane protein-like sequence expressed, or VlsE protein, a late response marker of infection, as one of their antigens.

However, Diasorin uses the OspC protein, or outer surface protein, an early response marker, which is required for the transmission of the spirochete from the tick into its human host. Zeus opted to use a synthetic peptide, which is derived from that OspC protein. So, while similar, slightly different, Zeus exclusively uses antigens derived from *Borrelia burgdorferi*, while Diasorin uses antigens derived from three different species of the *Borrelia* genome group, two of which are exclusively found in Europe and Asia, not in North America.

When we look at the second-tier immunoassays, Zeus utilized whole antigen extracts from both of their IgM and IgG assays, while Diasorin opted for using a combination of different species of *Borrelia* as well as different combinations of antigens.

So, for example, Diasorin uses that OspC antigen, but from *Borrelia afzelii*, which is found primarily in Asia, as well as the VlsE antigen from *Borrelia burgdorferi* for their IgM assay, and then opted to use the VlsE and the C6 peptide, which is also derived from VlsE or their IgG assay. And both of these were from *Borrelia burgdorferi*.

So, it's quite a differentiation between the two different manufacturers, not only different antigens, but different species of *Borrelia*. And so, it's not surprising that we observe different results between the two manufacturers' assays. Some authors have also suggested that the use of different

cutoffs chosen by the manufacturers behind the scenes may also be driving some of the differences that we have observed in our study.

Randye Kaye: All right, thank you. Your study indicates that treatment decisions were significantly influenced by IgM positivity. So, Dr. Walsh, what are the potential clinical implications of these results as you consider using one test versus the other?

Mike Walsh: Yeah, this was a significant finding because we looked at the rate of antibiotic administration in patients with different test results, and overall, IgM was the primary determinant in who received treatment. Seventy-nine percent of patients who received treatment had tested positive for IgM, and the agreement between Zeus and Diasorin IgM assays was only 75% for patients who tested positive. We also found that about 20% of the patients who tested positive for Lyme disease and received treatment actually tested negative by the Diasorin assays. So, presumably these patients would not have received treatment if we had been testing with Diasorin.

So, we did some math, and that suggested that testing by Diasorin would lead to a 25% reduction in antibiotic administration in our patient population. This is a major concern, but we're not sure at this point what exactly we should be concerned about. It's possible that we have been overprescribing antibiotics for years while we've been testing with the Zeus assays, but we may be under-prescribing patients with Lyme disease now that we're using the Diasorin platform. We need to do a follow-up study to see if there's been a change in adverse events related to the lower antibiotic administration since we started testing with Diasorin.

One important note is that about 30% of these patients had been symptomatic for more than 30 days, and based on CDC guidelines, the IgM test there is invalid. It's possible that our providers have been over interpreting these IgM results as reflecting a case of early acute Lyme disease, when in fact the infection was active months ago.

Randye Kaye: Thank you. So, challenges continue, but you are looking for solutions. Finally, Dr. Brunelle, do you have any closing thoughts you'd like to leave the audience with?

Lynn Brunelle: Well, certainly, we do feel that the modified two-tiered testing algorithm offers improved sensitivity. It's definitely much more practical for the laboratory from both an automation and an ease of testing perspective. However, we do feel that our data demonstrates we still have a lot to learn about the clinical drivers of such a complex disease as Lyme disease. It presents with such nonspecific findings. And as Mike just

noted, we're not really clear on how our clinicians are interpreting our results as well as the disease itself. So, such distinct differences in assay performances that we've just demonstrated clearly highlight the potential for downstream effects not only on treatment decisions by our providers, but also on the potential for some downstream effects on patient care.

Randye Kaye: All right. Well, thank you so much for this information and thank you for joining me today.

Lynn Brunelle: Thank you, Randye. It's been a pleasure.

Mike Walsh: Thank you.

Randye Kaye: That was Drs. Mike Walsh and Lynn Brunelle describing the *JALM* article "Comparison of 2 Sets of Immunoassays Used in Modified 2-Tiered Testing Algorithms for the Diagnosis of Lyme Disease." Thanks for tuning in to this episode of *JALM* Talk. See you next time. And don't forget to submit something for us to talk about.