No-blot testing charts new course for Lyme Dx

Anne Paxton

January 2020—"You can eliminate blots altogether!" may sound like a pitch for a cleaning product. But it's a line from a webpage of Zeus Scientific touting the new algorithm for Lyme disease testing that ditches Western immunoblot in favor of a two-enzyme immunoassay test sequence.

The new algorithm, which the CDC recommended last year, is the first approved alternative to the standard algorithm in 25 years.

"The big picture is, it's a change," says Carol A. Rauch, MD, PhD, adjunct associate professor of pathology, microbiology, and immunology at Vanderbilt University School of Medicine. "And by doing it differently, the components of possibly unintended Western blot testing and unintended interpretation of bands as positive results when they are actually negative, and people reacting to bands at all, may be minimized. I'd like it to be headed in a healthier direction so that we can get everybody the right diagnosis."

The update in the CDC's guidelines is significant for a variety of reasons, says John A. Branda, MD, associate director of the microbiology laboratory at Massachusetts General Hospital. "The modified algorithm may bring only an incremental improvement in Lyme disease diagnosis, but it's an important change."

Since 1994, the strategy of testing for Lyme disease has been to use EIA and a confirmatory Western immunoblot to achieve high sensitivity and specificity. But this standard algorithm has weaknesses that include insensitivity during the first weeks of Lyme disease and a confirmatory test with its own limitations. Lyme is also one of the rare diseases over which there is a running public controversy about how the diagnostic test should be interpreted.

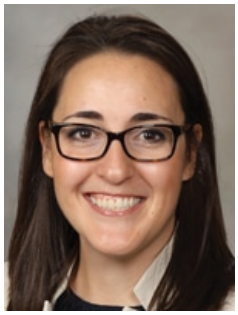
The CDC's recommendation followed the FDA's clearance of Zeus' existing Lyme disease ELISAs for use in the modified two-test method. With improved EIA design and development, studies have shown, a single multiplex or a two-tiered strategy involving two different EIAs performs as well as or better than the traditional algorithm.

By eliminating the need for the confirmatory Western immunoblot test, experts in Lyme disease diagnostics suggest, the modified algorithm is likely to speed test results and lead to fewer reference lab tests. It may also lower costs, reduce false-negative results in recently infected patients, and improve time to treatment. And there are hopes that the modified algorithm could reduce misinformation among the public about Lyme disease diagnosis.

Four Zeus ELISAs already approved for Lyme disease testing but typically used only for the first tier of the standard algorithm are now CDC endorsed for use in both tiers of the modified algorithm: Zeus ELISA *Borrelia* VlsE1/pepC10 IgG/IgM Test System, Zeus ELISA *Borrelia burgdorferi* IgG/IgM Test System, Zeus ELISA *Borrelia burgdorferi* IgM Test System, and Zeus ELISA *Borrelia burgdorferi* IgG Test System.

Under the standard algorithm, a reactive result from one of those tests was reflexed to a Western blot test for confirmation. The CDC has now okayed conducting a second ELISA—or in some cases two more ELISA tests—instead, explains Elitza S. Theel, PhD, director of the infectious diseases serology laboratory at Mayo Clinic in Rochester, Minn.

"Zeus has put forth two different versions of the modified algorithm using these ELISAs, which may appear to be more complicated," Dr. Theel says. "Regardless of which modified algorithm version you choose to use, however, Zeus indicates to start with the VlsE1/pepC10 IgG/IgM ELISA as the first-tier test and then, if that's reactive—positive or equivocal—you can do one of two things."



‘In my opinion, it’s more helpful to have that differentiation for second-tier test results.’ **— Elitza Theel, PhD**

“In the first version, the second-tier assay is the Zeus whole-cell antigen IgM/IgG ELISA, which doesn’t differentiate between which class of antibody is present. Alternatively, the other version of the modified algorithm involves performing two separate ELISAs for second-tier testing. These two ELISAs are also based on whole-cell antigen material, but they test for IgM and IgG separately, so you would be able to tell which class of antibody you’re detecting.”

For diagnosis, it’s important to know whether the antibodies are IgG or IgM, she says. “The current CDC guidelines state that if the patient has had more than 30 days of symptoms at the time of testing, only the IgG component should be considered. On the other hand, if the patient has had less than 30 days of symptoms, you’d want to look at that IgM antibody result as well. In my opinion, it’s more helpful to have that differentiation for second-tier test results. Are we just detecting IgG or just IgM, or is it both? With the total IgM/IgG ELISA, you can’t answer that question.”

Knowing the antibody class would not necessarily have treatment implications. “It would help guide clinicians on whether the patient has an acute or recent infection, or if just IgG-class antibodies are present, it might be more suggestive of a past infection given that IgG-class antibodies to Lyme disease can persist for months or years.” In such a case, “the clinician has to interpret the results in context with the patient’s presentation and duration of symptoms at the time of testing.”

Testing for antibodies to *Borrelia burgdorferi*, the causative agent of Lyme disease, is typically performed on routinely collected serum samples, or testing can be added on to already collected samples. But the standard algorithm has multiple weaknesses, Dr. Theel says. “First, its sensitivity in the early stage of Lyme disease is pretty poor, reported as about 40 to 60 percent, depending on the study. The immunoblot test can also be challenging for some laboratories to implement. And interpretation of the banding patterns, by the lab, clinicians, and increasingly also by patients, can be confusing.”

The modified algorithm has been winning support in part because the EIA method provides increased sensitivity during the early stage of disease. “Depending on the study and the combination of ELISAs used, sensitivity increases from 10 to maybe 20 percent for modified algorithms versus the standard algorithm.” In later stages of infection, when the patient might have Lyme-associated arthritis or arthralgia, the test sensitivity is about the same whether the standard or modified algorithm is used, Dr. Theel says.

The Western immunoblot has limitations as well. Originally—and some labs may still do it this way—the test is performed manually and the presence or absence of bands is interpreted visually. “That comes with quite a bit of subjectivity and variability in interpretation. Other labs, including many reference labs, have moved to processing these blots using an automated system, and band presence is determined by taking advantage of scanning software to provide more objective and standardized results.”

“While this has somewhat improved the subjectivity associated with interpretation of blot results, there are still challenges associated with blot testing, including false-positive results, particularly with IgM blots. Also, from a lab perspective, we have to maintain competency in blot testing, and for smaller hospital labs that send their blot testing to commercial labs, the turnaround time to results is significantly longer.”

The three most common causes of false-positive blot test results are syphilis, infectious mononucleosis, and multiple sclerosis, Dr. Theel notes. Fortunately, the typical treatment for Lyme, a course of doxycycline, doesn’t subject patients who are being treated unnecessarily to too many side effects. “But the bigger problem is that you are missing the true diagnosis and unnecessarily administering antibiotics.”

Large numbers of providers order the test for multiple reasons, she says. “Lyme symptoms are fairly nonspecific: fever, fatigue, headache, myalgia, arthralgia. So if the patient presents with those symptoms, in a Lyme disease-endemic area in the right season, chances are they’re going to get Lyme disease testing.”

Lyme disease’s prominence in the media enters into the test-ordering decision too, Dr. Theel says. “Frequently, we see patients who come in and specifically request testing for Lyme disease, so there’s some pressure on providers to perform the testing even though the patient may not meet the criteria or epidemiologic factors for Lyme disease. This can lead to overtesting for Lyme disease, which in low-risk individuals equates to a higher risk for false-positive results.”

People sometimes misunderstand Lyme disease and how it can be diagnosed and treated. “There have been a lot of good public health campaigns to make people more aware,” says Laura Gillim-Ross, PhD, discipline director for infectious disease immunology at LabCorp and a director in the Department of Science and Technology. “What I try to stress when I talk to providers is that the laboratory test is just a tool to aid in the diagnosis and should be considered in conjunction with clinical presentation and epidemiological factors.”

LabCorp and other labs cannot police whether a patient has symptoms, she notes. “We test what we receive. And we—and I’m sure all labs—receive a fair number of samples from patients who do not have travel history or known tick bite or anything else that would connect them with Lyme. Clinicians at times may test for numerous infectious diseases, especially if they aren’t savvy enough about the testing’s weaknesses.”

In Dr. Gillim-Ross’ view, the modified algorithm should make interpretation easier, “as long as the sample is being submitted on a patient who meets the recommended testing criteria.”

Two-tier serologic testing has an edge over the standard algorithm for Lyme testing because of performance and practical advantages, Dr. Branda says. “What made it feasible, and what allowed CDC to endorse this test, was that if you use two different EIAs sequentially or concurrently, you can achieve specificity equal to that of conventional two-tiered testing with ELISAs followed by Western immunoblots.”

“In very early infections, the sensitivity of the modified approach is significantly better than with the conventional testing because ELISAs tend to be more sensitive.”

The main practical advantage, Dr. Branda says, is that ELISA tests are much more approachable and feasible for typical clinical laboratories than Western blots. “You can do two ELISAs, which are objectively interpreted, tend to have very high throughput, can easily be automated, and, for all those reasons, tend to have short turnaround time.” It’s also generally less costly to perform two ELISAs than it is to perform an ELISA followed by an IgM/IgG Western blot.

For straightforward cases of suspected Lyme handled by non-Lyme disease experts, he says, “a lot of the information that is lost by doing the modified approach may not have been well used in the first place and may even have sometimes been misinterpreted.”

That is, “one does lose some information by replacing Western blotting with another ELISA because Western blotting not only gives us detailed information about the presence of an antibody response, and whether it is a predominantly IgM or IgG antibody response, but also tells us something about the maturity and breadth of the

antibody response."

But the modified two-tier testing has the potential to simplify interpretation in the vast majority of cases, Dr. Branda says. "On the flip side, if the case is difficult and challenging and more information is needed than can be acquired through ELISA, then having only the modified two-tier testing will make interpretation more difficult in a way. In those cases one would still want to pursue Western blotting to get as much information as possible."

Dr. Gillim-Ross and Dr. Theel agree that Western immunoblot might still have a place in Lyme testing. "One of the benefits of blots, particularly for the IgG, is that you can look at the expansion of the immune response over time," Dr. Theel says. "This antibody expansion may help providers differentiate between a new infection versus a past infection with lingering low-level antibodies. In addition, based on which bands are present on your IgG immunoblot, savvy providers can tell whether this is a recent infection or a past infection. So I think it will be helpful in more difficult or challenging cases."

A switch away from Western immunoblot has turnaround time and staffing implications, Dr. Gillim-Ross says. "If you were to do them side by side, the two-EIA method would be completed more quickly than the traditional algorithm including Western immunoblot. But because the immunoblot is manual, smaller labs may not perform testing every day. If a sample is tested Monday and is positive, it might not have the Western blot done until Thursday."

If there's a demand in a hospital or it's something of interest for its patient population, with the modified algorithm the laboratory will have the opportunity to complete the full testing algorithm on site, with no delays, Dr. Gillim-Ross says. "That's one positive. We have an opportunity to have faster results and utilize the same instruments to complete all steps of the algorithm."

Dr. Branda

"But I still caution that this is a change in required tests or the sequence of tests that is meant to improve the confidence in the final result. That final result is still only as good as the clinical presentation and the risks of the patient. So the issue with Lyme testing where we have a fair amount of false-positives in populations who have no reason to have Lyme disease is still going to exist with this algorithm."

Dr. Branda thinks only a small proportion of laboratories will continue with the Western immunoblot. "As this modified two-tier testing becomes adopted, it's likely that more and more laboratories will do two ELISAs on site, and they may want to maintain the ability to obtain a Western blot through a reference lab. But it would be on a much smaller scale than previously."

A recent paper by the CDC's tick-borne disease group reviewed Lyme disease testing at seven large commercial laboratories. "They found that 86 percent of the tests being sent for Lyme disease were for patients with a skin rash," Dr. Branda says. "Those aren't the kind of complex cases I'm talking about, where one would get the most from having the detailed antibody information that Western blot provides. Using that study for a touchstone, I would say it would be a small minority of cases where you would want to revert to Western blot."

It wouldn't be complicated for labs that do one ELISA to add another assay because they can often be done on the same platform, Dr. Branda notes. "Any laboratory that has an open platform for an automated ELISA instrument could perform all the relevant Zeus assays, and other manufacturers will follow suit, no doubt."

"So there will be more options eventually. But if you do assays on the Luminex or on the DiaSorin Liaison or

something similar, you may need to wait for those manufacturers to come out with a second complementary assay to be used in modified two-tier testing, or you may need to use two different assays from two different manufacturers on two different platforms. And you would need to do your own validation if the assays haven't been FDA cleared for use in a modified two-tiered testing protocol."

A key benefit of the modified algorithm is that it increases the chances of earlier treatment. "And right now," Dr. Branda says, "because serologic testing is insensitive in very early infection, we rely on physicians to recognize potential early Lyme disease and treat it empirically without necessarily having laboratory confirmation."

"That works pretty well, but the recognition of early Lyme disease is not always as straightforward as we like to think. So if a patient has a classical erythema migrans rash, for example, with a target or a bull's-eye appearance, then most clinicians recognize that as likely to be Lyme, especially if the patient had appropriate exposure risks, and they would treat it empirically and not rely on laboratory tests."

But there are likely to be other types of cases too, Dr. Branda explains. "For example, the patient has a skin lesion that is not a classical bull's-eye lesion or they have no skin rash that's observable but they have some of the constitutional symptoms we associate with early Lyme disease. In those cases, they still may receive empiric treatment, but they may not. If they don't, then those are the kinds of cases that potentially are more likely to be confirmed with this more sensitive modified approach."

Dr. Theel predicts another important outcome from use of the modified algorithm. "Clinicians will be able to interpret the results more easily, because they don't have to look at the banding patterns and then determine, 'Is this positive? Is this negative?'"

Most labs performing immunoblots provide a qualitative positive or negative result plus the bands that were detected, Dr. Theel notes. But the current algorithm often creates interpretive confusion. "If the result is negative but they still see bands, questions may come up from the clinician and the patient. For IgG, for example, you need at least five bands present to call it positive. But you'll hear: 'You called my blot negative but I still see these three bands here.'" Bands would be absent from the modified version, so patients may be less alarmed, she says.

'At a higher level I think we all need to focus on getting people the right diagnosis.' — **Carol Rauch, MD, PhD**

Dr. Gillim-Ross agrees: "Patients are going to get a quicker result, many of them are seeing it online, and the results are much easier to understand. It's typically a positive or a negative or a reactive or not reactive, versus a Western blot, which includes every band and whether it was present and a final interpretation." The modified algorithm results will be easier for clinicians to interpret as well.

Dr. Rauch of Vanderbilt hopes the modified algorithm will put more focus on following the right testing and interpreting it the right way—"aligning everyone to what I would call 'the due north' of the CDC guidelines on

testing," she says.

Right now, for a variety of reasons, nonapproved-algorithm testing is being done in the industry as a whole, Dr. Rauch says. "Some advocacy groups have pushed patients to demand that they get a Western blot and demand that they know their bands—whatever is reactive on the Western blot. It tends to lead to more testing, follow-up testing, potential therapy, and all that."

"So if we could just pull back a little on those unintended things that may have been triggered by an initial event that was not following the guidelines strictly, I'd like to see the improved accuracy, utility, and effectiveness of the testing as a result of this," Dr. Rauch says.

While those groups are well intentioned in trying to help people who are struggling to find a diagnosis, "At a higher level I think we all need to focus on getting people the right diagnosis. Sometimes, when someone has access to a Western blot alone or they're following the recommendations of a non-CDC-aligned organization," they may end up having therapies that are not appropriate, says Dr. Rauch, who is working with Dr. Theel to oversee the CAP's Lyme Survey. Both are members of the CAP Microbiology Committee.

Through whatever means, people can push to have the Western blot, and there are laboratories that don't follow the guidelines, Dr. Rauch says. So sometimes the Western blot is performed when not indicated. "If they have access to a laboratory that will perform a Western blot only, they'll report some bands and further may not use the highest, most endorsed criteria for how to interpret band patterns. But if the Western blot is divorced from the original enzyme immunoassay results, which are step one of the algorithm, we end up with things not being put together as a package. That isn't exactly how the rhythm of testing is supposed to work. Then in some cases, patients are being treated for bands rather than a true positive result that is at the end of the approved algorithm sequence."

Interpretation of Lyme disease testing has always been a challenge, she says, so the CDC and the Association of Public Health Laboratories as well as a number of reference laboratories have formed a Lyme disease serology working group. The group is assembling a document, similar to those that have been done for HIV and syphilis, to outline how to interpret Lyme disease testing results.

Says Dr. Theel, "We hope to provide recommended, standardized language for laboratories to use in reporting Lyme disease serologic results, and I think this will be very helpful as laboratories roll out the modified two-tiered algorithm." The organizations plan to endorse and issue that document by midyear.

Anne Paxton is a writer and attorney in Seattle.

Direct testing for Lyme disease

Direct testing of the *Borrelia burgdorferi* bacterium is not a realistic option for most laboratories, but promising research is underway on a test that does not require detection of an antibody response. As Dr. Branda says, "The modified algorithm is not a game-changer because if patients present very early, they may not have a detectable antibody response yet, no matter what method you apply. The new algorithm doesn't entirely solve that problem. It improves it but doesn't solve it, because the sensitivity in early disease, though better than it was with conventional two-tier testing, is still suboptimal since it still relies on development of an antibody response."

The modified algorithm also doesn't solve the problem of differentiation between active or recent infection versus past or remote infection. Dr. Branda believes that problem can be solved only by direct detection methods. That's one reason why direct testing is a hot topic in the research community, he says. "I don't think there have been any breakthroughs quite yet, but there are a lot of different approaches being taken."

One obstacle is that there is no FDA-cleared direct test for the agent of Lyme disease. Everything that's available is a homebrew test, he says. "Mainly what's available are PCR assays offered by commercial labs. Often we don't know much about the performance characteristics of commercially available PCR assays, but we can say that,

regardless of the PCR method, a blood PCR is poorly sensitive. It has not worked well on readily available sample types like blood or cerebrospinal fluid, regardless of the stage of Lyme disease or manifestation of suspected Lyme disease."

The place where PCR has worked better would be in a patient with a skin rash that's suspected to be erythema migrans, Dr. Branda says. "Biopsying the skin and then applying the PCR to the skin has worked with reasonably high sensitivity. However, it's very impractical because of the need to do a biopsy, which is not usually done in a typical primary care office."

In addition to PCR, researchers are working to develop antigen-based detection assays for early Lyme disease, though sensitivity is still not as high as serologic testing, Dr. Theel notes. "But other methods are being worked on, such as evaluation of the metabolic profile of patients with early Lyme disease versus non-infected patients. And development of interferon-gamma release assays for Lyme disease looks promising as well."

"The challenge," says Dr. Gillim-Ross, "would be that you could do direct testing potentially at point of care, but then, based on the current algorithms, you would need a second test." In diagnosing HIV, by way of comparison, "we do have HIV primary care tests. Typically those then need to be confirmed by a laboratory-based test. But I certainly think there's a big opportunity for a point-of-care device to detect antigen and/or antibody for Lyme disease just as we do for many other infections." —*Anne Paxton*