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Lyme Disease Neurological Implications: II. Diagnostic Methodology

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Abstract

Lyme disease is a complex, often difficult disease to diagnose and treat effectively. Typical symptoms may vary depending on the specific type of borrelia. Lyme and other tick-borne illnesses remain poorly understood so that current surveillance guidelines are not very effective. Further, validated direct testing methods which can be applied across all stages of the disease are lacking. Still further, serologic testing, often held as a gold standard, has significant performance limitations. In this Article, I will insist on the importance of differentiating the underlying bacterial strain, and critically analyze the several issues hampering current diagnostic methodology. I will discuss the four basic principles for diagnosis and will apply them to the cases of localized (or early) Lyme, early disseminated Lyme, and late disseminated Lyme. I will describe and discuss the several diagnostic tests employed, especially for Lyme patients with neurological symptoms, and the critical importance of differentiating from the numerous confounding diseases. I will conclude with a brief review of the various clinical practice guidelines issued by governmental and professional organizations that are often used for the diagnosis of Lyme and other tick-borne diseases.

Abbreviations

AB: Antibodies;ACA: Acrodermatitis Chronica Atrophicans; AD: Alzheimer's Disease; ALS: Amyotrophic Lateral Sclerosis; ASTPHD: (U.S.) Association of State and Territorial Public Health Laboratory Directors; BBB: Blood-brain barrier; (Bbss): Borrelia burgdorferi sensu stricto; BP: Bell's Palsy; CAS: Coronary Artery Syndrome; CDC&P: (U.S.) Center for Disease Control & Prevention; CFS: Chronic Fatigue Syndrome; CJD: Creutzfeldt-Jakob disease; CLD: Chronic LD; CLD-U: Untreated CLD; CLD-PT: Previously treated CLD; CMV: Cytomegalovirus; CNS: Central Nervous System; CPG: Clinical practice

guidelines; CSF: Cerebrospinal Fluid; CSTE: (U.S.) Council of State and Territorial Epidemiologists; DHHS: (U.S.) Department of Health & Human Services; EBV: Epstein-Barr Virus; EIA: Enzyme ImmunoAssay; FDA: (U.S.) Food & Drug Administration (FDA); EKG: Electrocardiogram; ELISA: Enzyme-Linked Immuno-Sorbent Assay; EM: Erythema Migrans; FP: Facial Palsy; HHV: Human Herpes Virus; HSV: Herpes Simplex Virus; IDSA: Infectious Diseases Society of America; IFA: ImmunoFluorescence Assay; IFAT: Indirect Fluorescent Antibody Test; Ig(G, M): Immunoglobulin (G, M); ILADS: International Lyme and Associated Diseases Society; JIRA: Juvenile Idiopathic Rheumatoid Arthritis; LD: Lyme Disease; LDFP: Lyme Disease Facial Palsy; LLM: Lyme Lymphocytic Meningitis; LPC: Lymphocytic PleoCytosis; LR: Lyme Radiculopathy; MS: Multiple Sclerosis; NAM: (U.S.) National Academy of Sciences; NCCLS: (U.S.) National Committee for Clinical Laboratory Standards; NGC: National Guidelines Clearinghouse; NGS: Next Generation Sequencing; NIH: (U.S.) National Institutes of Health; NLM: (U.S.) National Library of Medicine; OSPA: Outer Specific Protein A; PCR: Polymerase Chain Reaction; PD: Parkinson's Disease; PHS: (U.S.) Public Health Service; PLDS: Post-treatment Lyme Disease Syndrome; PML: PolyMorphonuclear Leukocytes; PNS: Peripheral Nervous System; SPECT: Single-Photon Emission Computed Tomography; WB: Western immunoblot; WIBA: Western ImmunoBlot Assay.

Keywords

Alzheimer's disease; Chronic Lyme disease; Differential Lyme diagnosis; Early disseminated Lyme disease; Localized Lyme disease; Lyme clinical practice guidelines; Lyme diagnostic tests; Lyme neurology; Memory impairment; Multiple sclerosis; Neurotransmitters disruptions; Parkinson's disease; Post-traumatic Lyme disease syndrome.

Introduction

In Article I in this series, I presented Lyme disease (LD) as a complex, often difficult disease to diagnose and treat effectively. Typical symptoms, which may appear in whole or in part, include fever, headache, fatigue, and a characteristic skin rash called erythema migrans (EM). They may vary depending on the specific type of borrelia. The European specify borrelia garinii is more often associated with neurological manifestations which, if left untreated, can spread to the nervous system. Many of the associated infections can be a precursor to neurodegenerative diseases.

Lyme and other tick-borne illnesses remain poorly understood so that current surveillance guidelines are not very effective. Further, validated direct testing methods which can be applied across all stages of the disease are lacking. Still further, serologic testing, often held as a gold standard, has significant performance limitations.

In this Article, I will describe and discuss the several diagnostic tests employed including ELISA, Western blot, dark-field microscopy, Ispot, polymerase chain reaction, genomic testing, neurologic tests, and next generation sequencing. This latter test, in particular, is the most accurate of all and may allow greater sensitivity than the other tests. Lyme patients with neurological symptoms are often misdiagnosed with one or more neurological diseases including multiple sclerosis, rheumatoid arthritis, fibromyalgia, chronic fatigue syndrome, lupus, Crohn's disease, HIV or other autoimmune and neurodegenerative diseases.

On the importance of differentiating the underlying bacterial strain

Testing and monitoring LD is a complicated multi-step process that is still evolving as we learn more about the disease and new diagnostic tools are devised. It is important to have the proper tests, understand how they

work, and determine the best possible pathway for modifying treatment to attain long-term health. In this process, establishing a comprehensive list of infections associated with LD may, in fact, help patients receive a proper diagnosis in order to administer the much needed comprehensive treatments they need.

The spirochetal agent that causes LD is the *Borrelia* genus of bacteria, with *Borrelia burgdorferi sensu lato* (Bbsl) being the broad categorization of bacteria related to the condition. Specific strains of *Borrelia* bacteria within this category are prevalent in different geographical locations.

Borrelia burgdorferi sensu stricto (Bbss) is held responsible for all cases of LD in North America and some cases in Europe. The *Borrelia afzelii* and *Borrelia bavariensis* spirochetes are found in Europe along with *Borrelia garinii*, which is also prevalent in Asia.

There are several other strains which have an association with LD although infectious cases are rare and unconfirmed for most of these. Hybridization has been found in *Borrelia* bacteria and a large volume of research has already accumulated, documenting the presence and prevalence of new variations of the genus in varying locations around the world.

The importance of the particular bacterial strain that is responsible for the infection is only just being revealed as researchers are observing patterns of LD symptoms associated with each type of Borrelia bacteria. Knowing the prevalent strains of spirochete in an area can also allow the population to be on the lookout for specific symptoms in order to catch LD in its localized early (acute) stage rather than as it disseminates and becomes harder to treat.

Early LD symptoms can be easily overlooked and those who are aware of the risk of LD in their communities are usually more likely to seek early medical attention after observing more subtle symptoms of infection.

Issues with current diagnostic methodology

The diagnosis of LD is based primarily on objective signs of a known exposure, clinical findings, and supportive serologic (blood) testing. The problem in current testing for LD is the high likelihood of receiving a false negative, which occurs when a test produces results indicating that a disease is not present when, in reality, it is.

Use of old and outdated tests

Western blot and ELISA (Enzyme-Linked Immuno-Sorbent Assay) are the standard testing methods used to diagnose LD, but these older tests may have a high chance of producing false negatives because of the method they employ to produce their results. Not only old and outdated, they can be highly flawed. Unfortunately, they represent the only option for most doctors to test for LD.

The tests look for the presence of certain antibodies (AB) to produce a positive result when searching for LD. However, LD patients are often immune-compromised and their bodies may not be producing the ABs necessary to conclusively identify the existence of LD. In addition, these tests do not quantify their results so patients and doctors cannot identify the number of copies each infection type present has produced and, therefore, are unable to provide conclusive data for therapeutic removal of the infection.

LD patients are often caught in a vicious cycle of immune depression that began with the initial infection. It is important to restate that when bitten by a tick, together with LD, co-infections that the tick may carry or enable are also transferred with Borrelia burgdorferi (the main bacterial spirochete known for causing LD). Once Borrelia and these co-infections enter the patient's body, they release a multitude of endotoxins,

neurotoxins, and biotoxins. Among many other negative actions, the toxins confuse the immune system and cause it to potentially attack its own cells, resulting in autoimmune-like symptoms and chronic inflammation throughout the brain and body. The confusion caused by toxins, and other depressors of the immune system, makes the patient's body more susceptible to opportunistic secondary fungal, viral, parasitic, and other bacterial infections, further impairing the patient's immune system.

With the immune system severely impaired by Lyme and these other infections, the traditional tests may not be accurate for diagnosis. To make matters worse, the initial infection, co-infections, biotoxins, endotoxins, mycotoxins, neurotoxins, autoimmune attacks, and opportunistic secondary infections... can produce a multitude of symptoms making it difficult to recognize that Lyme may be a possibility.

It is the combination of these multiple infections with other complications that contribute to the patients' debilitating set of physical and neurological symptoms I described earlier in Article I. They all combine to make a very confusing and frustrating experience for the patient and the treating doctor alike.

Difficulties in correctly identifying the infectious load

Only in-depth, up-to-par laboratory testing can identify the above several infections. Correctly identifying and vigorously treating all of them is the key to producing lasting results. However, as previously indicated, blood tests are often negative in the early stages of the disease. Further, testing of individual ticks is not typically useful as it is their combined interacting effects (not their individual effects) that are relevant.

The common tests (Western Blot and ELISA) used to confirm a Lyme diagnosis can report incorrect results 50% of the time (see below). Indeed, erroneous test results have been widely reported in both early and late stages of the disease. They can be caused by several factors such as AB cross-reactions from other infections including Epstein-Barre virus (EBV), cytomegalovirus (CMV), and herpes simplex virus (HSV).

To complicate matters, Lyme patients with neurological symptoms are often misdiagnosed with one or more of the other neurological diseases discussed or, even worse, their symptoms are dismissed altogether. The struggle to find the right diagnosis is extremely draining for patients who are already facing depression, brain fog, memory loss, tremors, and other crippling symptoms.

[Note: An established and well-known clinical facility claims to have developed an early version of a "proprietary PCR (polymerase chain reaction) diagnosis protocol", which is still in the research and developmental stage, allowing its physicians to not only accurately diagnose LD, but be able to monitor the several infection and co-infection levels throughout treatment.

However, being proprietary, it is difficult to gauge that claim pending disclosure, peer-review, independent confirmation, and acceptance of that protocol. Further, PCR is itself outdated and inferior to genomic testing. See below more detailed comments on PCR.]

The four basic principles of the diagnosis

The four basic principles of the diagnosis are:

• **History of possible exposure to infected ticks;**

• **Signs and symptoms observed;**

• **Objective physical findings** (such as erythema migrans (EM) rash, facial palsy, arthritis, etc.); and possibly

• **Supportive laboratory tests.**

In the diagnosis process, it is useful to distinguish the following three instances: (1) localized (or early) disease, (2) early-disseminated disease, and (3) late-
• A total body skin examination for EM rashes and

• Be inquired if there was a rash in the past 1–2 months.

Presence of an EM rash and recent tick exposure (i.e., being outdoors in a likely tick habitat where Lyme is common, within 30 days of the appearance of the rash) are sufficient for Lyme diagnosis; no laboratory confirmation is needed or recommended.

Unfortunately, most people who get infected do not remember a tick or a bite, and the EM rash need not look like the typical bull's eye (actually, in the U.S., most EM rashes do not) or be accompanied by any other symptoms. In the U.S., Lyme is most common in the New England and Mid-Atlantic states and parts of Wisconsin and Minnesota, but it is expanding into other areas. Several bordering areas of Canada also carry high Lyme risk.

disseminated disease.

Case of localized (or early) LD

People with symptoms of early LD should have:

In the absence of an EM rash or a history of tick exposure, Lyme diagnosis depends on laboratory confirmation. The bacteria that cause LD are difficult to observe directly in body tissues. They are also difficult and too time-consuming to grow in the laboratory.

The most widely used tests look instead for the presence of ABs against those bacteria in the blood. However, a positive AB test result does not by itself prove active infection.It can, however, confirm an infection that is suspected because of symptoms, objective findings, and a history of tick exposure in a person. Because as many as 5%-20% of the normal population have ABs against Lyme, people without history and symptoms suggestive of LD should not be tested for Lyme ABs because a positive result would likely be false, possibly causing unnecessary treatment. This is summarized in Table 1 below:

Principle	Test	Advantages	Shortcomings
Recent history and total body skin exam for rash	**Evidence of exposure, rash, and symptoms**	**o Sufficient for diagnosis o No laboratory tests required**	**Only for early LD**
	Lack of exposure or rash	**Laboratory tests required for AB presence**	**o Positive AB test does not prove active infection o Can confirm suspected infection because of symptoms and objective findings Note: 5%-20% of the normal population have AB against Lyme**

Key: AB= Antibody; LD=Lyme disease.

Table 1: Diagnostic methodology: Localized (or early) disease

For those patients who do get diagnosed correctly, the standard-of-care involves a simple course of antibiotics.

People who suspect they may have LD should seek out specialty laboratories for a more accurate diagnosis. One such specialty laboratory, IgeneX, looks at samples of the patient's blood under a microscope. The inspection allows them to visualize and identify so-called “bands” of the spirochetes involved with LD. IGeneX also runs a separate co-infection panel to help diagnose the other infections that can be present with LD.

It is important to mention that this test is only a starting point. There is a number of other specialized laboratory tests that must be run to aid in a proper and complete diagnosis. These are necessary to create a full medical blueprint for LD and all the co-infections that can be present. The multitude of symptoms associated with LD makes it hard to diagnose it based on symptoms alone. But, with proper diagnosis and treatments tailored to all the co-infections, patients can typically rid themselves of most of the symptoms that are associated with LD and receive more long-term.

In some cases, when history and signs and symptoms are strongly suggestive of early-disseminated LD, empiric treatment may be started and re-evaluated as laboratory test results become available.The (U.S.) Center for Disease Control & Prevention (CDC&P) has recommended the following two-tiered protocol whose reliability remains controversial. Tests for ABs in the blood are ELISA and the Western blot, the former being the most widely used method for Lyme diagnosis:

• A sensitive first test, either an enzyme-linked immunosorbent assay (ELISA) or an indirect fluorescent antibody test (IFAT), followed by

• The more specific Western immunoblot (WB) test to corroborate equivocal or positive results obtained with the first test.

High titers of either immunoglobulin G (IgG) or immunoglobulin M (IgM) ABs to Borrelia antigens indicate disease, but lower titers can be misleading because the IgM ABs may remain after the initial infection; also the IgG ABs may remain for years (see Table 2 where I also mention additional tests). Patients who do not have access to DNA-sequencing may experience difficulties getting an accurate diagnosis for their LD under the standard form of testing.

Although the standard-of-care for correctly diagnosed LD patients is a simple course of antibiotics, that treatment may only be effective in some cases. Thus, there are many patients who will continue to experience ongoing resistance to antibiotics and continued symptoms (what was termed post-treatment Lyme disease syndrome, PLDS).

However, PLDS does not define the full breadth of infections the patients may have, regardless of whether they have gone through standard-of-care treatment for Lyme borrelia or not. In fact, the Lyme borrelia may not always be present in a chronic Lyme disease (CLD) patient as the term refers to a wide range of tick-borne plus other secondary infections and complications that the patient may be dealing with.

This infective load and the complications associated with it vary greatly from patient to patient so that a more personalized treatment approach would often lead to an improved patient outcome.

In particular, those patients that present with neurological Lyme need specialized care that is critical for their improvement. (Note that the CDC&P does not recommend either of the following tests: urine antigen tests; PCR tests on urine; immunofluorescent staining for cell-wall-deficient forms of Borrrelia burgdorferi; and lymphocyte transformation tests: see below.)

Principle	Test	Advantages	Shortcomings
Titers of IgG or IgM Schedule: 2-4 weeks: IgM 4-6 weeks: IgG 6-8 weeks: IgM > 8 weeks: only IgM	**ELISA (**test for AB and color changes)	o High titers indicate disease but lower titers can be misleading because IgG AB may remain for years o IgM AB may remain after the initial infection o Positive IgM and negative IgG indicate infection	o Initial sensitivity ~ 70% o Immune system not sufficiently producing AB to allow for proper diagnosis o Better for locating infection, not monitoring overall health o Often produce false-negative results in immuno-compromised patients and patients on steroids or on suppressive medication for autoimmune disease o Valid only after 30 days o Less useful after AB treatment o Antiquated and outdated for definitively diagnosing and quantifying Lyme *borreliosis* o Fails to detect and accurately quantify the presence of any present co-infection(s)
	Western blot (if ELISA not specific enough)	o Detects specific amino-acid sequences in proteins o Identifies the protein and determines the correct treatment or AB	o Initial sensitivity ~ 94%-96% o Antiquated and outdated for definitively diagnosing and quantifying Lyme *borreliosis* o Fails to detect and accurately quantify any present co-infection(s)
Microscopy	**Dark-field microscopy**	o Probably one of the best tests o Visualizes the spirochete when it emerges from hiding	o Spirochete is only seen in ~ 40% of cases o Out of the host (*borrelia*) changes shape and hides intracellularly immediately o Method is not 100% accurate
Cytokine IFN-g	**Ispot**	Secreted by patient's T-cells	o Better specificity than the Western blot
OspA antigens	**OspA** (uses nanotrap particles for detection)	o Antigens shed live bacteria in urine o Promising	o In development
Polymerase chain reaction (PCR)	**PCR**	o Detects the genetic material (DNA) of the LD spirochete o Much faster than laboratory culture o May be considered when intrathecal AB-producing test results are suspected of being falsely negative	o Susceptible to false positive results o Often shows false negative results o Recommended only in special cases (for example, Lyme arthritis)
Genomics	**DNA, RNA sequencing**	o More useful than current	o More data and validation

(a "gold standard" on the horizon)	(from patient's blood, urine, mucus, stools)	tests (including PCR) o Provides conclusive and quantifiable data on total infectious load o Leads to better diagnosis and treatment while providing a more accurate way to track the progress of treatment o Accurately tracks treatment progression o More appropriate personalized treatment o Allows selection of best treatment drugs discarding non-drug targets	are still needed
Neurologic	***Neuroborreliosis*** (Lumbar puncture and CSF analysis of pleocytosis and intrathecal AB production)	o In Europe o In the U.S.: Confirms a diagnosis of *neuroborreliosis* if positive. Does not exclude *neuroborreliosis* if negative	o U.S. guidelines consider CSF analysis optional when symptoms are confined to the PNS (for example, facial palsy without overt meningitis symptoms)
Cardiologic	**Carditis**	Uses EKG	Not done because of associated risk

(History, signs, and symptoms are strongly suggestive)

Key: AB: Antibodies; CNS: Central Nervous System; CSF: Cerebrospinal Fluid; EKG: Electrocardiogram; ELISA: Enzyme-Linked Immuno-Sorbent Assay; Ig: Immuno-globulin; LD: Lyme Disease; PCR: Polymerase Chain Reaction; PNS: Peripheral Nervous System.

Table 2: Diagnostic methodology: Early-disseminated disease

Remarks on the diagnostic tests utilized

Some brief remarks on the tests in Table 2 are provided below:

ELISA

This standardized type of laboratory blood test is the most common. It is considered to be the correct way to diagnose LD. ELISA is a type of wet-lab test that uses ABs and color changes to identify a substance, in this case, LD. What we are looking for in this instance are antigens (a cell's identifying feature, like a microscopic caller ID) from the Borrelia bacteria. When laboratories find these antigens, they attach a specific AB (a blood protein used to identify bacteria and viruses) which combines with the Borrelia antigen. Next, the enzyme's substrate (the surface on which a cell feeds itself) is added, producing a detectable signal, usually a change in color. This helps the laboratory define which foreign bodies are in the bloodstream. These standardized tests rely on the body's immune status and its production of ABs to detect the disease. However, because LD is so evasive, often times the immune system is not producing or not producing sufficient numbers of these ABs to allow for proper diagnosis. Also, as already said earlier, they can often produce false-negative results.

ELISA is a sensitive test (initial sensitivity about 70%) that is performed first. It it is positive or equivocal, then, the more specific Western blot (94%-96%

specificity for people with clinical symptoms of early LD) is run. When an EM rash first appears, because the immune system takes some time to produce ABs in quantity, ABs usually cannot yet be detected; therefore, AB confirmation at that time has no diagnostic value and is not recommended. Up to 30 days after suspected Lyme infection onset, infection can be confirmed by detection of IgM or IgG ABs. Even though the CDC&P recommends ELISA as the first line of testing, it can provide a false negative in patients with weakened immunity or on medications such as steroids. It is not uncommon for LD patients to be originally diagnosed with an autoimmune disease or fibromyalgia and, therefore, be given suppressive medication to manage symptoms instead of truly treating the disease.

The following testing schedule is usually applied:

At 2–4 weeks: IgM ABs can first be detected but they usually collapse 4-6 months after infection. Immunoglobulin M is the first AB to respond to initial antigen exposure. It is larger than IgG and the biggest AB in the human circulatory system. When EM is detected in a LD patient, IgM identifies it but only during the first four weeks of a bite, if the rash is even present. It is important to note that only 25%-30% of Lyme patients remember or experience a rash. If IgM is not used to detect Lyme, it can lead to a misdiagnosis of LD. Additional tests may be more helpful, such as IgG. However, usually, by the time patients come to recognize they have the LD complex, they are beyond IgM testing.

At 4–6 weeks: IgG ABs can next be detected and can remain detectable for years. Immunoglobulin G is an AB isotype, making up 75% of immunoglobulins (proteins that work as ABs) that are present in the bloodstream. Because IgG is so plentiful, it is the main factor in controlling infection. Its levels indicate a patient's immune status to particular pathogens (microorganisms that can cause disease). However, once positive, this is not a good tool to monitor progression or improvement. The test can stay positive, which does not provide clear clinical information in the monitoring of the patient. This is really more of an initial diagnostic tool for patients that show signs and symptoms of CLD.

At 6–8 weeks: Both IgM and IgG peak. The overall sensitivity is only 64%, although this rises to 100% in the subset of people with disseminated symptoms, such as arthritis.

After 8 weeks: It is recommended that only IgM ABs be considered.

Note that the combination of a positive IgM and a negative IgG test result suggests an early infection, especially if confirmed several weeks later by a positive IgG test result.

After antibiotic treatment, AB tests become less useful. People treated with antibiotics when they have an EM rash often subsequently test negative for Lyme ABs, whether treatment was successful or instead Lyme goes on to cause further complications. People treated later usually test positive before and after treatment, regardless of treatment success or failure. This suggests that better diagnostic tests are needed.

The overall rate of false positives is low, only about 1%-3%, in comparison to a false-negative rate of up to 36% in the early stages of infection using the two-tiered testing.

Western blot

The problem with using IgG, IgM and ELISA is that they are better for locating infection, not monitoring overall health. The Western blot is a widely accepted analytical technique that detects specific amino-acid sequences in proteins. There are hundreds of thousands of different proteins, but once the protein is identified, one can determine the correct treatment or AB.

Dark-field microscopy

The dark-field microscopy test with silver nitrate stain is probably the best test, though the spirochete is only seen in about 40% of cases. Unfortunately, it is beset by two problems. First, when taken out of the host during a blood test. the borrelia changes shape and immediately hides intracellularly. Second, the method is not always 100% accurate. However, improvements of this method are being worked on by creating a medium (serum) in which Borrelia can live up to eight weeks so that, upon retesting the blood, the spirochete reemerges from hiding.

Ispot Lyme

This test has a better specificity than the Western blot test when testing for *Borrelia*. It measures the cytokine IFN-g secreted by the patient's T cells.

Other forms of laboratory testing for LD are available, some of which have not been adequately validated. Outer specific protein A (OspA) antigens shed by live Borrelia bacteria into urine is a promising technique being studied. For their detection, the use of nanotrap particles is being looked at.

Polymerase chain reaction (PCR)

PCR tests have also been developed to detect the genetic material (DNA) of the LD spirochete. Whereas serologic studies only test for ABs of *Borrelia*, culture or PCR is the current means for detecting the presence of the organism. PCR has the advantage of being much faster than laboratory culture. However, PCR tests are susceptible to false positive results, e.g. by detection of debris of dead *Borrelia* cells or specimen contamination. Even when properly performed, PCR often shows false negative results because few Borrelia cells can be found in blood and CSF during infection. Hence, PCR tests are recommended only in special cases, e.g. diagnosis of Lyme arthritis, because it is a highly sensitive way of detecting *OspA* DNA in synovial fluid. Although sensitivity of PCR in CSF is low, its use may be considered when intrathecal AB production test results are suspected of being falsely negative, e.g. in very early (< 6 weeks) *neuroborreliosis* or in immunosuppressed people.

While PCR can detect bacterial DNA in some patients, unfortunately, this is also not helpful as a test of whether the antibiotics have killed all the bacteria. Studies have shown that DNA fragments from dead bacteria can be detected for many months after treatment. The studies have also shown that the remaining DNA fragments are not infectious. Positive PCR test results are analogous to a crime scene: just because a robbery occurred and the robber left his/her DNA, it does not mean that the robber is still in the house. Similarly, just because DNA fragments from an infection remain, it does not mean the bacteria are alive or viable.

Genomic testing: A more accurate test on the horizon?

Nothing can describe the frustration and powerlessness of being sick and in pain with no clear reason why. LD is a debilitating and painful disease that all too often goes misdiagnosed or produces false negatives on the traditional ELISA and Western blot tests. However, there is hope on the horizon through the use of modern genomic technology. This approach is more useful than current tests, including the PCR technique. Through this novel approach, the presence of LD and other specified co-infections can be more conclusively identified and quantified. This may greatly help in prescribing the appropriate personalized treatment and accurately track its progress for each patient. This new test may also aid in selecting the best drugs to target each organism in a patient's CLD.

Genomic testing is based on genomic information from

the patient's blood, urine, mucus, and stool to derive important DNA and RNA information without having to synthetically amplify the genes for detection as required by the PCR method. Not only allowing for a better diagnosis, it also paves the way for a personalized, comprehensive treatment plan and quantifies the amount of infections present. At this time, more data and validation are still needed to bring this test to patients, but we hope it may become the "gold standard" for testing and treating tick-borne infections as well as a powerful tool in helping patients with CLD. Cross-referencing the patient's test with known data on the DNA and RNA sequences of diseases will reveal the existence of LD and its co-infections, and more effectively aid with developing a treatment plan.

By testing for the genes of infectious organisms and quantifying the data, several advantages will accrue including: (1) better accuracy in telling if the patient has LD and plasmids, (2) better ability to fight LD, (3) better quality testing, (4) better information on quantity, (5) better selection of drug, and (6) discarding of non-drug targets for personalized treatment. It does not rely on detecting antibodies that may or may not be present. It helps determine a treatment plan that is best suited to attack the infections specific to the patient. It also lymphocytic pleocytosis (LPC). In LPC, the densities of lymphocytes (infection-fighting cells) and protein in the cerebrospinal fluid (CSF) typically rise to characteristically abnormal levels, while glucose levels remain normal. Additionally, the immune system produces antibodies against Lyme inside the intrathecal space, which contains the CSF. Demonstration by lumbar puncture, CSF analysis of pleocytosis, and intrathecal antibody production are required for definite diagnosis of *neuroborreliosis* in Europe - (except in cases of peripheral neuropathy associated with *acrodermatitis chronica atrophicans* (ACA) - which is usually caused by Borrelia *afzelli* and confirmed by blood AB tests.

On the other hand, in North America, neuroborreliosis allows tracking improvement of the patient's condition. Further, quantifiable data give hard evidence of the existence of CLD aiding in spreading awareness, hopefully producing a better future for those who suffer from this debilitating disease.

Next generation sequencing

Whereas PCR testing can only detect predetermined large strands of DNA, next generation sequencing (NGS) is a newer technology that is capable of sequencing millions of small strands of DNA from a single blood sample. The test sensitivity would thus be potentially larger. A clinical trial currently conducted at Stony Brooks University, New York, will investigate the capability of NGS to detect *Borrelia burgdorferi* DNA in the blood of pediatric patients with LD at all suspected phases or stages of the disease. The test will begin before or up to 24 hours after the first dose of antibiotics is administered.

Neurologic tests of neuroborreliosis cases

There is a distinction between Europe and North America. In Europe, neuroborreliosis is usually caused by *Borrelia garinii* and almost always involves
is caused by *Borrelia burgdorferi*. It may not be accompanied by the same CSF signs. A negative diagnosis of central nervous system (CNS) *neuroborreliosis* does not exclude *neuroborreliosis*. American guidelines consider CSF analysis optional when symptoms appear to be confined to the peripheral nervous system (PNS), e.g. facial palsy without overt meningitis symptoms. Those patients that present with neurological Lyme need specialized care that is critical to their improvement.

Cardiologic tests of Lyme carditis

In Lyme carditis, electrocardiograms (EKG) are used to evidence heart conduction abnormalities while

echocardiography may show myocardial dysfunction. Biopsy and confirmation of Borrelia cells in myocardial tissue may be used in specific cases but are usually not done because of the risks of the procedure.

Single-photon emission computed tomography (SPECT)

SPECT images show numerous areas where an insufficient amount of blood is being delivered to the cortex and subcortical white matter. They can also identify abnormalities in the brain of a person affected with this disease. However, SPECT images are known to be nonspecific because they show a heterogeneous pattern in the imaging. The abnormalities seen in these images are very similar to those seen in people with cerebral vacuities and Creutzfeldt-Jakob disease (CJD), which makes them questionable.

An overall summary of the diagnostic methodology for late-disseminated LD is provided in Table 3.

Principles	Test	Advantages	Shortcomings
Blood tests	Positive AB	Can exclude LD as possible cause of observed symptoms	Misses diagnosis of: o Chronic fatigue syndrome o Crohn's disease o Fibromyalgia o HIV o Lupus o Multiple sclerosis o Rheumatoid arthritis o Other autoimmune and neurodegenerative diseases
	Brain pathogens: o Cytomegalovirus o Herpes o Other pathogens		
Encephalitis	o Familial o Autoimmune		

Key: AB: antibodies; LD: Lyme disease.

Table 3: Diagnostic methodology: Late-disseminated disease

Differentiating from confounding diseases

Community clinics misdiagnose 23%-28% of EM rashes and 83% of other objective manifestations of early LD. EM rashes are often misdiagnosed as spider webs, cellulitis, or shingles. Many misdiagnoses are credited to the widespread misconception that EM rashes should look like a “bull's eye”. Actually, the key distinguishing features of the EM rash are not its anatomical appearance but its characteristic mechanical features: (1) the speed and extent to which it expands, respectively up to 2–3 cm/day and a diameter of at least 5 cm, and in 50% of cases more than 16 cm; and (2) The rash expands away from the center, which may or may not look different or be separated by a ring-like clearing from the rest of the rash:

Spider webs

Compared to EM rashes, spider bites are more common in the limbs, tend to be more painful and itchy or become swollen, and some may even cause necrosis (sinking dark blue patch of dead skin).

Cellulitis

Cellulitis most commonly develops around a wound or ulcer, is rarely circular, and is more likely to become swollen and tender. EM rashes often appear at tissue folds (armpit, groin, abdomen, back of knee) and other sites that are unusual for cellulitis.

Shingles

Like Lyme, shingles often begins with headache, fever, and fatigue, which are followed by pain or numbness. most common type of one-sided facial palsy (about 70% cases of facial palsy in areas where LD is common. Compared to LDFP, BP much less frequently affects both sides of the face. Even though LDFP and BP have similar symptoms and evolve similarly if untreated, corticosteroid treatment is beneficial for BP while being detrimental for LDFP.

The likelihood of LDFP should be based on recent history of exposure to a likely tick habitat during warmer months, EM rash, viral-like symptoms (headache, fever, and/or palsy in both sides of the face). If it is more than minimal, empiric therapy with antibiotics should be initiated, without corticosteroids, and reevaluated upon completion of laboratory tests for LD.

Lyme lymphocytic meningitis (LLM)

likely tick habitats in the last 3 months, possibly followed by a rash or viral-like symptoms and current headache, other symptoms of lymphocytic meningitis, or FP would lead to suspicion of LD and recommendation of serological and lumbar puncture tests for confirmation. LR affecting the trunk can be

However, unlike Lyme, in shingles, these symptoms are usually followed by the appearance of rashes composed of multiple small blisters along a nerve's dermatome. Shingles can also be confirmed by quick laboratory tests.

Lyme disease facial palsy (LDFP)

Facial palsy caused by Lyme disease (LDFP) is often misdiagnosed as Bell's palsy (BP). Although BP is the of cases), LDFP can account for only about 25% of Unlike viral meningitis, LLM tends to not cause fever, last longer, and recur. It is also characterized by its possible co-occurrence with EM rash, facial palsy (FP), or partial vision obstruction and having much lower percentage of polymorphonuclear leukocytes (PML) in CSF.

Lyme radiculopathy (LR)

Affecting the limbs, LR is often misdiagnosed as a radiculopathy caused by nerve root compression, such as sciatica. Most LR cases are compressive and resolve with conservative treatment (e.g., rest) within 4–6 weeks. Nonetheless, guidelines for managing LR recommend first evaluating risks of other possible causes that, although less frequent, require immediate diagnosis and treatment, including infections such as LD and shingles. A history of outdoor activities in misdiagnosed as a myriad of other conditions such as diverticulitis and coronary artery syndrome (CAS).

Against the EM rash, many of the several confounding diseases are summarized in Table 4.

Disease	Appearance/ Characteristics	Confounding factor
***Erythema migrans* rash**	o Not always a "bull's eye" o Diameter 5cm (in 50% of cases: up to 16cm) o Expands 2-3cm/day	May be present in other diseases
Spider web	o More common in the limbs o May cause necrosis	Painful, itchy, swollen
Cellulitis	Rarely circular	o Develops around a wound or ulcer o Appears at tissue folds that are rare for cellulitis
Shingles	Rashes composed of multiple small blisters along a nerve's dermatome	Begins with headache, fever, fatigue followed by pain or numbness
Facial palsy (often diagnosed as Bells' palsy)	Affects both face sides	Comparison of Bell's palsy to LDFP: o Similar symptoms o Similar evolution if untreated o Corticosteroids beneficial for Bell, not so much for LDFP
Lyme lymphocytic meningitis	o Different from viral meningitis o No fever, lasts longer, recurs o Possibly co-occurring with EM rash, facial palsy, or partial vision obstruction o Much lower percentage of people in CSF	Viral meningitis
Lyme radiculopathy	o Affects both limbs o Compressive o Resolves within 4-6 weeks with conservative treatment (rest)	o Ordinary radiculopathy caused by nerve root compression (e.g., sciatica) o Diverticulitis o Coronary artery syndrome

Key: CAS: Coronary artery syndrome; CSF: Cerebrospinal fluid; LDFP: Lyme disease facial palsy; LLM: Lyme lymphocytic meningitis; PML: Polymorphonuclear leukocytes.

Table 4: Differentiating Lyme from confounding diseases

Clinical practice guidelines

Clinical practice guidelines (CPG) are often used as reference by physicians for LD diagnosis and treatment of other tick-borne diseases. Several CPGs have been issued by governmental and professional organizations. The only CPG posted on the National Guidelines Clearinghouse (NGC), under the auspices of the (U.S.) antibiotics in the treatment of persistent LD symptoms.

Because of their importance, I briefly review below the available practice guidelines.

Department of Health & Human Services (DHHS), are those adhering to newly revised (U.S.) National Academy of Medicine (NAM), formerly the Institute of Medicine (IOM), standards for guidelines: the International Lyme & Associated Diseases Society (ILADS) Lyme Guidelines, which address the usefulness of antibiotic prophylaxis for tick bite, the effectiveness of EM treatment, and the role of

The Infectious Diseases Society of America guidelines (IDSA)

The IDSA has updated its 2019 clinical practice guidelines for clinical infectious diseases in general.

These are among high-risk populations. Unfortunately, the guidelines are too general and broadly address all infectious diseases, not specifically LD. Further, they are aimed at controlling outbreaks of infectious diseases in certain populations. They consider the care of children, pregnant, and postpartum women, and non-pregnant adults, and include special considerations for patients who are severely immunocompromised such as hematopoietic stem cell and solid-organ transplant recipients.

While the target audience includes primary care clinicians, obstetricians, emergency medicine providers, hospitalists, and infectious disease specialists, the guidelines may also be useful for occupational health physicians and clinicians working in long-term care facilities.

The guidelines add new information on diagnostic testing, use of antivirals, considerations of when to use antibiotics, and when to test for antiviral resistance. They also present evidence on harm associated with routine use of corticosteroids. The process followed that used in the development of previous IDSA guidelines that included a systematic weighting of the strength of recommendations and quality of evidence based upon the (U.S.) Public Health Service (PHS) grading system for ranking recommendations in clinical guidelines.

Unfortunately, the recommendations exclusively address seasonal influenza which, although undeniably important, is a different infectious disease than LD.

The Association of State and Territorial Public Health Laboratory Directors guidelines (ASTPHD)

In 1994, the ASTPHD, the CDC&P, the (U.S.) Food & Drug Administration (FDA), the (U.S.) National conventional testing often provides false negatives when diagnosing Lyme borreliosis, resulting in a monumental failure to provide the needed critical early

Institutes of Health (NIH), the (U.S.) Council of State and Territorial Epidemiologists (CSTE), and the National Committee for Clinical Laboratory Standards (NCCLS) convened the Second National Conference on Serologic Diagnosis of Lyme Disease during which they recommended a two-test methodology using a sensitive enzyme immunoassay (EIA) or immunofluorescence assay (IFA) as a first test, followed by a western immunoblot assay (WIBA) for specimens yielding positive or equivocal results.

Regarding future tests, the report advised that *"...evaluation of new serologic assays include blind testing against a comprehensive challenge panel, and that new assays should only be recommended if their specificity, sensitivity, and precision equaled or surpassed the performance of tests used in the recommended two-test procedure".*

With support from NIH and to assist serologic test developers, CDC&P made available a comprehensive panel of sera from patients with various stages of LD and other conditions, as well as healthy persons. Thus, on 7/2/19, the FDA cleared several LD serologic assays with new indications for use based on a modified two-test methodology.

This modified methodology uses a second EIA in place of a Western immunoblot assay. Clearance by FDA of the new LD assays indicates that test performance has been evaluated and is "substantially equivalent to or better than" a legally marketed predicate test.

Unfortunately, even though updated, the basis for the FDA/CDC&P recommendations rely too much on old technology, do not account for newer technological developments, and do not espouse principles of modern integrative and personalized medicine. Still further,

detection and treatment. I have discussed above these newer technological advances and the associated tests.

The (U.S.) CDC&P original guidelines for serologic analyses

For LD

When assessing a LD patient, health care providers should consider:

• The signs & symptoms of LD;

• The likelihood that the patient has been exposed to infected black-legged ticks;

• The possibility that other illnesses may cause similar symptoms; and

• Results of laboratory tests, when indicated.

LD is a tick-borne zoonosis for which serologic testing is currently the principal means of laboratory diagnosis. The diagnosis algorithm recommended by CDC&P consists of a two-step testing process that can be done using the same blood sample pending the development of new tests as alternatives to one or both steps. If the first step is negative, no further testing is recommended. On the other hand, if it is positive or indeterminate (sometimes called "equivocal"), the second step should be performed. The overall result is positive only when the first test is positive (or equivocal) and the second test is positive (or for some tests equivocal). (Figure 1)

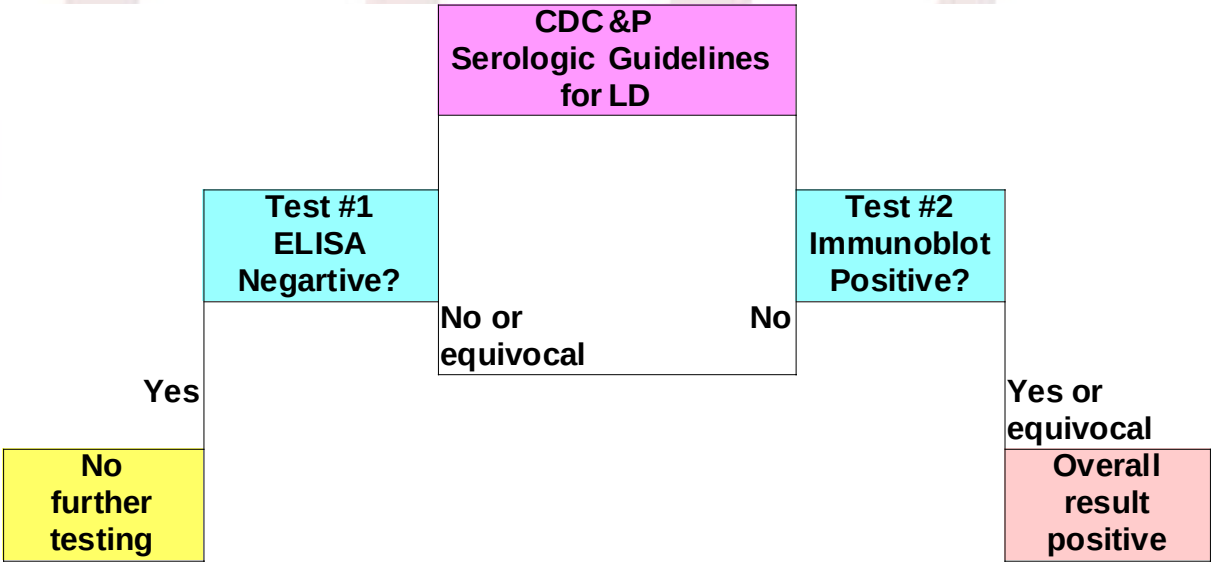

Figure 1: (U.S.) CDC&P guideline for Lyme disease serology

In so doing, key points to remember are the following:

• Most LD tests are designed to detect antibodies (ABs) made by the body in response to infection;

• ABs can take several weeks to develop, so patients may test negative if infected only recently;

• ABs normally persist in the blood for months or even years after the infection is gone; therefore, the test cannot be used to determine cure; and

• Infection with other diseases, including some tick-borne diseases, or some viral, bacterial, or autoimmune diseases, can result in false positive test results.

The above methodology was jointly recommended by the CDC&P together with the ASTPHLD, the FDA, the NIH, the CSTE, and the NCCLS at their Conference SNCSDLD held 27-29 October 1994.

Regarding serologic test performance and interpretation, the two-test approach for active disease and for previous infection using ELISA (a sensitive enzyme immunoassay) or IFA followed by a Western (immuno)blot was the algorithm of choice. Specifically:

• All specimens positive or equivocal by a sensitive ELISA or IFA should be tested by a standardized Western immunoblot. Specimens negative by a sensitive ELISA or IFA need not be tested further.

• When Western immunoblot is used during the first 4 weeks of disease onset (early LD), both immunoglobulin M (IgM) and immunoglobulin G (IgG) procedures should be performed.

• A positive IgM test result alone is not recommended for use in determining active disease in persons with illness greater than 1 month's duration because the likelihood of a false-positive test result for a current infection is high for these persons. An IgM immunoblot is considered positive if two of the following three bands are present: 24 kDa (OspC)*, 39 kDa (BmpA), and 41 kDa (Fla).

• If a patient with suspected early LD has a negative serology, serologic evidence of infection is best obtained by the testing of paired acute- and convalescent-phase serum samples. Serum samples from persons with disseminated or late-stage LD almost always have a strong IgG response to Borrellia burgdorferi antigens. An IgG immunoblot should be considered positive if five of the following 10 bands are present: 18 kDa, 21 kDa (OspC), 28 kDa, 30 kDa, 39 kDa (BmpA), 41 kDa (Fla), 45 kDa, 58 kDa (not GroEL), 66 kDa, and 93 kDa (2).

Borrelia is a terrible invader and evader in addition to being a great imitator:

For CLD

Recently, CDC&P released a case study regarding the treatment of CLD based on a few published studies involving a small number of patients. It concluded that antibiotics do not provide long-term benefits but rather gave rise to other complicating infections.

Based on these outcomes, it also inferred that patients should not be so treated but rather referred to other specialists (rheumatologists, psychiatrists, pain management specialists, and neurologists). However, while on the surface, such referrals appear sound and reasonable, they do not eliminate the root cause of the disease, which is the infections.

Unfortunately, these symptomatic treatments ultimately leave patients in a life-time of suffering and in a condition that only worsens over time. However, although providing temporary relief, IV antibiotics were never effective for numerous reasons. The important question and further case study work should help answer is why IV antibiotics alone do not work.

The updated FDA/CDC&P guidelines for serologic analyses

Very recently (16 August 2019), CDC&P has updated its recommendations for serologic diagnosis of LD. As is known, serologic testing is the principal means of laboratory diagnosis of LD. The current recommendations have been summarized in the previous section. They have now been updated following FDA clearance (on 29 July 2019) of several LD serologic assays with new indications for use, allowing for an enzyme immunoassay (EIA) rather

than a a Western immunoblot assay (IBA) as the second test in the LD testing algorithm.

For health care providers, the CDC&P recommendations are as follows. When a patient seeks care after a tick bite, topics to discuss should include:

• Tick removal (if still present), degree of engorgement, and identification;

• LD prophylaxis, as determined by the tick species and degree of engorgement; and

• Symptom watch.

Laboratory tests not recommended

Some laboratories offer LD testing using assays whose accuracy and clinical usefulness have not been adequately established. Examples of invalidated tests include:

• Capture assays for antigens in urine;

• Culture, immunofluorescence staining, or cell sorting of cell wall-deficient or cystic forms of Borrelia burgdorferi;

• Lymphocyte transformation tests;

• Quantitative CD57 lymphocyte assays;

• "Reverse Western blots";

• In-house criteria for interpretation of immunoblots;

• Measurements of antibodies in joint fluid (synovial fluid); and

• IgM or IgG tests without a previous ELISA/EIA/IFA test.

Summary and conclusions

• It is important to differentiate the actual bacterial strain as there are a number of different strains including hybridized strains that may require different treatments. Knowing the prevalent strains of spirochete in an area can allow the population to be on the lookout for specific symptoms in order to catch LD in its localized early (acute) stage rather than as it disseminates and becomes harder to treat.

• The problem in current testing for Lyme disease is the high likelihood of receiving a falsenegative, which occurs when a test produces results indicating that a disease is not present when, in reality, it is.

• ELISA and Western blot are the standard testing methods used to diagnose Lyme disease. These older tests look for the presence of certain antibodies to produce a positive result when searching for Lyme disease. However, Lyme disease patients are often immune-compromised and their bodies may not be producing the anti-bodies necessary. In addition, these tests are not quantitative and, therefore, are unable to provide conclusive data.

• When bitten by a tick with Lyme disease, co-infective agents that the tick may possess are also transferred. Once in the body, these several infections release a multitude of endotoxins, neurotoxins, and biotoxins that confuse the immune system and cause it to potentially attack its own cells resulting in autoimmune-like symptoms and chronic inflammation throughout the brain and body.

• The confusion caused by toxins, and other

factors that depress the immune system, makes the patient's body more susceptible to opportunistic secondary fungal, viral, parasitic, and other bacterial infections which work to further impair the patient's immune system.

• With the immune system severely impaired by Lyme and its co-infections, the traditional diagnostic tests may not be accurate. Worse, co-infections, biotoxins, endotoxins, mycotoxins, neurotoxins, autoimmune attacks, and opportunistic secondary infections can produce a multitude of symptoms, making it difficult to recognize Lyme.

• While most infections are tick-borne in nature, chronic Lyme disease also includes complicating primary and secondary co-infections that may also be present. It is the combination of these multiple infections with other complications that contribute to the patients' debilitating set of physical and neurological symptoms. Correctly identifying and vigorously treating all these infections is the key in producing lasting results against chronic Lyme disease.

• Diagnosis is based upon a combination of symptoms, history of tick exposure, and possibly testing for specific antibodies in the blood. The common tests (Western Blot and ELISA) used to confirm a Lyme diagnosis can report incorrect results in 50% of the time.

• To complicate matters, Lyme patients with neurological symptoms are often misdiagnosed with one or more neurological diseases.

• There are four basic principles involved in the diagnosis of chronic Lyme disease: (1) History of possible exposure to infected ticks; (2) signs and symptoms observed; (3) objective physical findings (such as erythema migrans rash, facial palsy, arthritis, etc.); and possibly (4) laboratory tests.

• The several diagnostic tests have been abundantly discussed in cases of localized (or early Lyme) disease, early-disseminated, and late-disseminated Lyme. These include: ELISA, Western blot, dark-field microscopy, Ispot, polymerase chain reaction, and genomic testing, and next generation sequencing, which may allow greater sensitivity than the polymerase chain reaction test. Other neurologic and cardiologic tests have also been discussed.

• Though controversial, certain neuroimaging tests (magnetic resonance, single-photon emission computed tomography) can provide data that are diagnostically helpful.

• There are various confounding diseases that need to be differentiated against including spider webs, cellulitis, shingles, facial palsy, Lyme lymphocytic meningitis, Lyme radiculopathy. diverticulitis, and coronary artery syndrome.

• Late-stage Lyme disease may be misdiagnosed as multiple sclerosis, rheumatoid arthritis, fibromyalgia, chronic fatigue syndrome, lupus, Crohn's disease, HIV or other autoimmune and neurodegenerative diseases.

• The Infectious Diseases Society of America has updated its 2019 clinical practice guidelines for clinical infectious diseases, in general. Unfortunately, they are too general and broadly address all infectious diseases, not specifically Lyme Disease. Further, they are aimed at controlling outbreaks of infectious diseases in certain (high-risk) populations.

• The guidelines add new information on diagnostic testing, use of antivirals, considerations of when to use antibiotics, when to test for antiviral resistance, and present evidence on harm associated with routine use of corticosteroids.

• In 1994, several U.S. governmental entities recommended a two-test methodology using a sensitive enzyme immunoassay or immunofluorescence assay as a first test, followed by a western immunoblot assay for specimens yielding positive or equivocal results.

• Unfortunately, even though updated, the basis for the FDA/CDC&P recommendations rely too much on old technology, do not account for newer technological developments (such as genomic testing), and do not espouse principles of modern integrative and personalized medicine. Still further, conventional testing often provides false negatives when diagnosing Lyme borreliosis, resulting in a monumental failure to provide the needed critical early detection and treatment.

• The CDC&P original guidelines for serologic analyses recommend that, when assessing a Lyme disease patient, health care providers should consider: the signs & symptoms of the disease, the likelihood that the patient has been exposed to infected black-legged ticks, the possibility that other illnesses may cause similar symptoms, and results of laboratory tests (when indicated).

• Most Lyme disease tests are designed to detect antibodies made by the body in response to infection. However, antibodies can take several weeks to develop so patients may test negative if infected only recently. Further, antibodies normally persist in the blood for months or even years after the infection is gone; therefore, the test cannot be used to determine cure. Still further, infection with other diseases, including some tick-borne diseases, or some viral, bacterial, or autoimmune diseases, can result in false positive test results.

• All specimens positive or equivocal by a sensitive ELISA or IFA should be tested by a standardized Western immunoblot. Specimens negative by a sensitive ELISA or IFA need not be tested further.

• When Western immunoblot is used during the first 4 weeks of disease onset, both immunoglobulin M (IgM) and immunoglobulin G (IgG) procedures should be performed.

• Very recently (16 August 2019), the CDC&P have updated its recommendations for the serologic diagnosis of Lyme disease with new indications for use, allowing for an enzyme immunoassay rather than a Western immunoblot assay as the second test in the LD testing algorithm.

• The CDC&P have also provided recommendations for health care providers when a patient seeks care after a tick bite, including: tick removal, degree of engorgement, and identification; prophylaxis, as determined by the tick species and degree of engorgement; and symptom watch.

References

1. Asch ES, Bujak DI, Weiss M, et al. (1994). “Lyme disease: An infectious and post-infectious syndrome”. J Rheumatol 21(3):454-61.
2. Aucott J, Morrison C, Munoz B, et al. (2009). “Diagnostic challenges of early Lyme disease: Lessons from a community case series”. BMC Infect Dis 9:79.
3. Bacon RM, Kugeler KJ, and Mead PS. (2008). “Surveillance for Lyme disease--United States, 1992- 2006”. MMWR Surveill Summ 57(10):1-9.
4. Barbour AG and Restrepo BI (2000). “Antigenic variation in vector-borne pathogens”. Emerg Infect Dis 6(5):449-57.
5. Bradley JF, Johnson RC, and Goodman JL (1994). “The persistence of spirochetal nucleic

acids in active Lyme arthritis". Ann Intern Med 120(6):487-9.

6. Cabello FC, Godfrey HP, and Newman SA (2007). "Hidden in plain sight: Borrelia burgdorferi and the extracellular matrix". Trends Microbiol 15(8):350-4.
7. Cairns V and Godwin J. (2005). "Post-Lyme borreliosis syndrome: A meta-analysis of reported symptoms". Int J Epidemiol 34(6):1340-5.
8. Cameron DJ (2008). "Severity of Lyme disease with persistent symptoms: Insights from a double- blind placebo-controlled clinical trial". Minerva Med 99(5):489-96.
9. Costello CM, Steere AC, Pinkerton RE, and Feder HM Jr (1989). "A prospective study of tick bites in an endemic area for Lyme disease". J Infect Dis 159(1):136-9.
10. Coyle PK and Schutzer SE. (1991). "Neurologic presentations in Lyme disease". Hosp Pract 26(11):55-66.discussion 66, 69-70 [Taylor & Francis Online].
11. Dattwyler RJ, Volkman DJ, Luft BJ, et al. (1988b). "Seronegative Lyme disease. Dissociation of specific T- and B-lymphocyte responses to Borrelia burgdorferi". N Engl J Med 319(22):1441-6.
12. Donta ST (2012). "Issues in the diagnosis and treatment of Lyme disease. Open Neurol J 6:140-5.
13. Duray PH (1989). "Clinical pathologic correlations of Lyme disease". Rev Infect Dis 11 (Suppl 6):S1487-93.
14. Duray PH, Yin SR, Ito Y, et al.(2005)."Invasion of human tissue ex vivo by Borrelia burgdorferi". J Infect Dis 191(10):1747-54.
15. Fallon BA, Levin ES, Schweitzer PJ, Hardesty D (2010). "Inflammation and central nervous system Lyme disease". Neurobiol Dis 37(3):534-41.
16. Frank C, Fix AD, Pena CA, and Strickland GT (2002). "Mapping Lyme disease incidence for diagnostic and preventive decisions", Maryland. Emerg Infect Dis 8(4):427-9.
17. Fymat AL (2017a). "Parkinson's disease and other movement disorders: a review", Journal of Current Opinions in Neurological Science 2(1):316-43.
18. Fymat AL (2017b). "Neurological disorders and the blood-brain barrier: 2. Parkinson's disease and other movement disorders", Journal of Current Opinions in Neurological Science 2(1)362-83.
19. Fymat AL (2018a). "Blood-brain barrier permeability and neurological diseases", Journal of Current Opinions in Neurological Science (Editorial).2(2):411-4.
20. Fymat AL (2018b). "Alzheimer's disease: a review", Journal of Current Opinions in Neurological Science 2(2);415-36,
21. Fymat AL (2018c). "Regulating the brain's autoimmune system: the end of all neurological disorders?" Journal of Current Opinions in Neurological Science 2(3):475-9.
22. Fymat AL (2018d). "Alzheimer's disease: prevention, delay, minimization and reversal", Journal of Clinical Research in Neurology 1(1):1-16.
23. Fymat AL (2018e). "Harnessing the immune system to treat cancers and neurodegenerative diseases", Journal of Clinical Research in Neurology 1(1):1-14.
24. Fymat AL (2018f). "Is Alzheimer's an autoimmune disease gone rogue"", Journal of Clinical Research in Neurology 2(1):1-4.
25. Fymat AL (2018g). "Dementia treatment: where do we stand?", Journal of Current Opinions in Neurological Science 3(1):1-3.
26. Fymat AL (2018h). "On dementia and other cognitive disorders", Journal of Clinical Research in Neurology 1(2):1-14.
27. Fymat AL (2018i). "Is Alzheimer's a runaway autoimmune Disease? and how to cure it?",

Newsletter European Union Academy of Sciences Annual Report (2018).

28. Fymat AL (2019a). "Is Alzheimer's a runaway autoimmune disease? and how to cure it?" Proceedings of the European Union Academy of Sciences, Newsletter, pages 379-83.
29. Fymat AL (2019b). "Dementia: a review", Journal of Clinical Psychiatry and Neuroscience 1(3):27-34.
30. Fymat AL (2019c). "The pathogenic brain", Journal of Current Opinions in Neurological Science 3(2);669-71.
31. Fymat AL (2019d). "On the pathogenic hypothesis of neurodegenerative diseases", Journal of Clinical Research in Neurology 2(1):1-7.
32. Fymat AL (2019e). "Dementia with Lewy bodies: a review", Journal of Current Opinions in Neurological Science 4(1);15-32.
33. Fymat AL (2019f). "Our two interacting brains: etiologic modulations of neurodegenerative and gastroenteric diseases". Journal of Current Opinions in Neurological Science 4(2):50-4.
34. Fymat AL (2019g). "What do we know about Lewy body dementias?" Journal of Psychiatry and Psychotherapy (Editorial) 2(1)-013:1-4. doi:10.31579/JPP.2019/018.
35. Fymat AL (2019h). "Viruses in the brain...? Any connections to Parkinson's and other neurodegenerative diseases?" Proceedings of the European Union Academy of Sciences, 2019 Newsletter.
36. Fymat AL (2019i). "Alzhei … Who? demystifying the disease and what you can do about it", Tellwell Talent Publishers, pp 235, 2019. ISBN: 978-0-2288-2420-6 (Hardcover); 978-0-2288-2419-0 (Paperback).
37. Fymat AL (2020a). "Recent research developments in Parkinson's disease", Current Opinions in Neurological Science 5(1):12-30.
38. Fymat AL (2020b). "The role of radiological imaging in neurodegenerative disorders", Journal of Radiology and Imaging Science 1(1):1-14.
39. Fymat AL (2020c). "Parkin...ss...oo...nn: Elucidating the disease and what you can do about it", Tellwell Talent Publishers, 2020.
40. Fymat AL (2020d). "Lyme disease: The dreadful invader, evader, and imitator… and what you can do about it", Tellwell Talent Publishers, pp 278.
41. Fymat AL (2020e). "Dementia: Fending off the menacing disease... and what you can do about it", Tellwell Talent Publishers, pp 488.
42. Fymat AL (2021a). "Infection-mediated heart disease – Case of Lyme Carditis", EC Journal of Emergency Medicine and Critical Care 5(1):37-38.
43. Fymat AL (2021b). "On potentially reversible forms of dementia", Journal of Current Opinions in Neurological Science 6(1):101-8.
44. Fymat AL (2021c). "Dementia – Eliminating its potentially reversible forms", Proc. European Union Academy of Sciences. Pages 270-277.
45. Fymat AL (2021d). "The human brain: Wonders and disorders", Tellwell Talent Publishers, pp 500.
46. Fymat AL (2022a). "Alzheimer's disease: A path to a cure", Journal of Neurology and Psychology Research 3(1):1-15.
47. Fymat AL (2022b). "Alzheimer's disease: A path to a cure", Current Opinions in Neurological Science, 3(1):1-16.
48. Fymat AL (2022c). "Epilepsy: The electrical storm in the brain", Tellwell Talent Publishers, pp 412.
49. Fymat AL (2023a). "Epilepsy: Surgical and non-surgical management and treatment", Current Opinions in Neurological Science 8(1);1-26.
50. Fymat AL (2023b). "Multiple system atrophy: Symptoms management and treatment", Journal of Neurology and Psychology

Research 4(1):1-37.

51. Fymat AL (2023c). “Multiple sclerosis: I. Symptomatology and etiology”, Journal of Neurology and Psychology Research 4(2):1-46.
52. Fymat AL (2023d). “Multiple sclerosis: II. Diagnosis and symptoms management”, Journal of Neurology and Psychology Research 4(2):1-21.
53. Fymat AL (2023e). “Multiple sclerosis: III. Treatment and prognosis”, Journal of Neurology and Psychology Research 4(2):1-46.
54. Fymat AL (2023f). “Tourette's syndrome: I. Symptomatology and etiology”, Journal of Neurology and Psychology Research 5(1):1-34.
55. Fymat AL (2023g). “Tourette's syndrome: II. Diagnosis and symptoms management”, Journal of Neurology and Psychology Research 5(1):1-27.
56. Fymat AL (2023h). “Tourette's syndrome: III. Treatment and prognosis”, Journal of Neurology and Psychology Research“ 5(1):1-39.
57. Fymat AL (2023i). “Tourette's syndrome: IV. Research and latest updates”, Journal of Neurology and Psychology Research 5(1):1-12.
58. Fymat AL (2023j). "Pathogens in the brain and neurodegenerative diseases", Journal of Neurology and Psychology Research 5(1):1-14.
59. Fymat AL (2023k). “Lyme disease neurological implications - I. Symptomatology and etiology". Neurology and Psychology Research Journal 5(2):1-24.
60. Fymat AL (2023l). “Multiple sclerosis: The progressive demyelinating autoimmune disease”, Tellwell Talent Publishers, pp 504.
61. Fymat AL (2023m). “Multiple system atrophy: The chronic, progressive, neurodegenerative synucleopathic disease”, Tellwell Talent Publishers, pp. 302.
62. Fymat AL (2023n). “Tourette: The self-under-siege neurodevelopmental and neuropsychiatric motor syndrome”, Tellwell Talent Publishers, pp. 466.
63. Hartiala P, Hytonen J, Suhonen J, et al. 2008). “Borrelia burgdorferi inhibits human neutrophil functions”. Microbes Infect 10(1):60-8.
64. Haupl T, Hahn G, Rittig M, et al. (1993). “Persistence of Borrelia burgdorferi in ligamentous tissue from a patient with chronic Lyme borreliosis”. Arthritis Rheum 36(11):1621-6.
65. Hayes E. (2003). “Lyme disease”. Clin Evid (10):887-99.
66. Hodzic E, Feng S, Freet KJ, and Barthold SW (2003). “Borrelia burgdorferi population dynamics and prototype gene expression during infection of immunocompetent and immunodeficient mice”. Infect Immun 71(9):5042-55.
67. Institute of Medicine, (US) Committee on Lyme Disease and Other Tick-Borne Diseases (2011): “The state of the science”. In: “Critical needs and gaps in understanding prevention, amelioration, and resolution of Lyme and other tick-borne diseases: the short-term and long-term outcomes: workshop report”. National Academies Press; Washington, DC, USA.
68. Johnson L, Aylward A, and Stricker RB (2011). “Healthcare access and burden of care for patients with Lyme disease: a large United States survey”. Health Policy 102(1):64-71.
69. Johnson L, Wilcox S, Mankoff J, and Stricker RB (2014). “Severity of chronic Lyme disease compared to other chronic conditions: A quality of life survey”. Peer J 2:e322.
70. Johnson RC, Kodner CB, Jurkovich PJ, and Collins JJ (1990). “Comparative in vitro and in vivo susceptibilities of the Lyme disease

spirochete Borrelia burgdorferi to cefuroxime and other antimicrobial agents". Antimicrob Agents Chemother 34(11):2133-6.

71. Klempner MS, Noring R, and Rogers RA (1993). "Invasion of human skin fibroblasts by the Lyme disease spirochete, Borrelia burgdorferi". J Infect Dis 167(5):1074-81.
72. Kraiczy P, Skerka C, Kirschfink M, et al. (2002). "Immune evasion of Borrelia burgdorferi: Insufficient killing of the pathogens by complement and antibody". Int J Med Microbiol 291(Suppl 33):141-6.
73. Krause PJ, Telford SR 3rd, Spielman A, et al. (1996). "Concurrent Lyme disease and babesiosis: Evidence for increased severity and duration of illness". JAMA 275(21):1657-60.
74. Lawrence C, Lipton RB, Lowy FD, and Coyle PK (2012). "Seronegative chronic relapsing neuroborreliosis". Eur Neurol 35(2):113-17.
75. Liang FT, Jacobs MB, Bowers LC, and Philipp MT (2002). "An immune evasion mechanism for spirochetal persistence in Lyme borreliosis". J Exp Med 195(4):415-22.
76. Livengood JA and Gilmore RD Jr. (2006). "Invasion of human neuronal and glial cells by an infectious strain of Borrelia burgdorferi". Microbes Infect 8(14-15):2832-40.
77. Lo R, Menzies DJ, Archer H, and Cohen TJ. (2003). "Complete heart block due to Lyme carditis". J Invasive Cardiol 15(6):367-9.
78. Logar M, Ruzic-Sabljic E, Maraspin V, et al. (2004). "Comparison of erythema migrans caused by Borrelia afzelii and Borrelia garinii." Infection 32(1):15-9.
79. Logigian EL, Kaplan RF, and Steere AC. (1990). "Chronic neurologic manifestations of Lyme disease". N Engl J Med 323(21):1438-44. Antimicrob Agents Chemother 39(3):661-7.
80. Maloney EL. (2011). "The management of Ixodes scapularis bites in the upper Midwest". WMJ 110(2):78-81.
81. Maraspin V, Lotric-Furlan S, Cimperman J, et al.(1999). "Erythema migrans in the immunocompromised host". Wien Klin Wochenschr 111(22-23):923-32.
82. Marques A, Telford SR 3rd, Turk SP, et al. (2014). "Xenodiagnosis to detect Borrelia burgdorferi infection: A first in human study". Clin Infect Dis 58(7):937-45.
83. Mursic VP, Wanner G, Reinhardt S, et al. (1996). "Formation and cultivation of Borrelia burgdorferispheroplast-L-form variants". Infection 24(3):218-26.
84. Nocton JJ, Dressler F, Rutledge BJ, et al. (1994). "Detection of Borrelia burgdorferi DNA by polymerase chain reaction in synovial fluid from patients with Lyme arthritis". N Engl J Med 330(4):229-34.
85. Oksi J, Marjamaki M, Nikoskelainen J, and Viljanen MK (1999). "Borrelia burgdorferi detected by culture and PCR in clinical relapse of disseminated Lyme borreliosis". Ann Med 31(3):225-32.
86. Rahn DW (1991). "Lyme disease: clinical manifestations, diagnosis, and treatment. Semin Arthritis Rheum 20(4):201-18.
87. Reported cases of Lyme disease by year, United States, 1995-2009. Available from: www.cdc.gov/lyme/stats/chartstables/casesbyyear.html.
88. Sapi E, Bastian SL, Mpoy CM, et al. (2012). "Characterization of biofilm formation by Borrelia burgdorferi in vitro". PLoS One 7(10): e48277.
89. Sartakova ML, Dobrikova EY, Terekhova DA, et al. (2003). "Novel antibiotic-resistance markers in pGK12-derived vectors for Borrelia burgdorferi". Gene 303:131-7.
90. Schaefer C, Chandran A, Hufstader M, et al. (2011). "The comparative burden of mild, moderate and severe fibromyalgia: results from a cross-sectional survey in the United States". Health Qual Life Outcomes 9(1):71.

91. Schwan TG and Piesman J (2000). "Temporal changes in outer surface proteins A and C of the Lyme disease-associated spirochete, Borrelia burgdorferi, during the chain of infection in ticks and mice". J Clin Microbiol 38(1):382-8.
92. Sperling J, Middelveen M, Klein D, and Sperling F (2013). "Evolving perspectives on Lyme borreliosis in Canada". Open Neurol J 6:94-103.
93. Stanek G and Reiter M (2011). "The expanding Lyme borrelia complex: Clinical significance of genomic species?" Clin Microbiol Infect 17(4):487-93.
94. Steere AC, Bartenhagen NH, Craft JE, et al. (1983b). "The early clinical manifestations of Lyme disease". Ann Intern Med 99(1):76-82.
95. Stricker RB, Johnson L (2007). "Lyme disease: A turning point". Expert Rev Anti Infect Ther 5(5):759-62 [Taylor & Francis Online].
96. Swanson SJ, Neitzel D, Reed KD, and Belongia EA (2006). "Coinfections acquired from ixodes ticks". Clin Microbiol Rev 19(4):708-27.
97. Szczepanski A and Benach JL (1991). "Lyme borreliosis: Host responses to Borrelia burgdorferi". Microbiol Rev 55(1):21-34.
98. Tang S, Calkins H, and Petri M. (2004). "Neurally mediated hypotension in systemic lupus erythematosus patients with fibromyalgia". Rheumatology 43(5):609-14.
99. Thaisetthawatkul P and Logigian EL (2002). "Peripheral nervous system manifestations of Lyme borreliosis". J Clin Neuromuscul Dis 3(4):165-71.
100. Vazquez M, Sparrow SS, andShapiro ED. (2003). "Long-term neuropsychologic and health outcomes of children with facial nerve palsy attributable to Lyme disease". Pediatrics 112(2):e93-7.
101. Weder B, Wiedersheim P, Matter L, et al. (1987). "Chronic progressive neurological involvement in Borrelia burgdorferi infection". J Neurol 234(1):40-3.
102. Wormser GP, Dattwyler RJ, Shapiro ED, et al. (2006). "The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis and babesiosis: Clinical practice guidelines by the Infectious Diseases Society of America". Clin Infect Dis 43(9):1089-134.