

Will There Ever Be An Accurate Test for Lyme Disease?

A Reference Booklet For Lyme Patients

By
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Lyme Disease is a complex systemic disease that is caused by highly motile bacterium in the spirochete family. The bacteria *Borrelia burgdorferi* was first isolated from the skin of a Lyme patient with the distinctive bull's-eye rash in the early 1980s. Since that time, culturing the elusive bacterium has been difficult, frustratingly unpredictable, and miserably inconsistent.

Borrelia burgdorferi can very quickly move from the site of a tick bite into the circulatory system, where it can circulate throughout the body. This unique bacterium has a distinct and insidious method for passing through the capillary walls of blood vessels and nestling its way deep inside many organs and tissues of the human body. In animal models, the Lyme spirochete within mere hours of a tick bite, can cause a breakdown of the **blood-brain-barrier**.

It is the bacteria's ability to exit the blood stream, hide, and survive that makes Lyme disease so difficult to detect with any one test. It is the unique microbiology of the bacteria that gives it the ability to hide and survive undetected within the human body. That is why using other bacterial diseases as a model for Lyme disease is difficult and leads to misunderstandings in the medical community on how to diagnose and treat Lyme disease. Infections caused by the family of bacteria known as **Borrelia** are unique in their microbiology and cannot be dismissed with a rubber stamped one-treatment-fits-all approach.

There are five main methods of testing for Lyme disease:

1. Antibody tests (either the Elisa or Western Blot serology tests)
2. Bacterial DNA detection by polymerase-chain-reaction test (PCR)
3. Live culture
4. Antigen detection: Finding particles and proteins of the bacteria in blood, CSF, urine, or tissue samples.
5. Direct observation by microscope: This can employ the use of biopsy and stain, or centrifuged blood, spinal fluid, and urine.

By far, the most commonly used tests for Lyme disease are antibody serologies. These tests are indirect measurements of your body's response to the bacterial pathogen. Antibody serology tests are most often employed not for their accuracy, but more for their convenience, low risks, and low costs. Essentially, these tests look for your antibody response to an infection in the blood stream.

The most common of the antibody tests is the **Enzyme Linked Immune Sera Assay** test (ELISA). This test is often the first test given to Lyme patients and can only give you a very general idea of the presence or absence of anti-Lyme antibodies.

The Main Drawbacks of Using Elisa Tests for Lyme Disease:

First, each lab can have their own version of an ELISA test, using different enzyme-linked antigens that are used to trap specific anti-Lyme antibodies in the patient's blood. When an enzyme is in the presence of the correct anti-Lyme antibody, there is an enzymatic color change that occurs which is read by a machine. If the lab chooses antigens that do not reflect the same set of antibodies that the patient is producing then the test will fail to detect Lyme disease. The individual that tests negative with one lab's test may have a detectable Lyme antibodies if another lab's ELISA test is given employing a slightly different set of antigens.

Think of it like this: If you take a gold fish bowl and drop a paperclip and a penny into the bowl and then use a magnet to find the paper clip, you cannot conclude that there is no other metal in the bowl. The magnet is simply incapable of detecting the penny. The same is true if you look for the wrong antibodies in a Lyme patient.

Another problem is consistent accuracy within labs in interpreting results. his lack of consistency in laboratory accuracy was borne out in Lori Bakken's ELISA test study. This study showed that more than 50% of the time, labs could not correctly or consistently replicate identical results in identical triple-paired serum samples highly positive for Lyme antibodies. This is despite claims by labs of being 99% specific and accurate. However, what is accurate by laboratory definition has *nothing* to do with accuracy as a diagnostic test to determine the presence or absence of active infection in a patient.

- **Bakken LL, Callister SM, Wand PJ, Schell RF. Interlaboratory Comparison of Test Results for the Detection of Lyme Disease by 516 Participants in the Wisconsin State Lab of Hygiene/College of American Pathologists Proficiency Testing Program. J Clin Microbiol 1997; Vol 35, No. 3:537-543**
- **Bakken LL, Case KL, Callister SM et al. Performance of 45 Laboratories Participating in a Proficiency Testing Program for Lyme Disease Serology. JAMA 1992;268:891-895**

You see, when companies marketing their products use buzzword terms like *specific*, *sensitive*, or *accurate*, these marketing terms do **not** mean *diagnostically* accurate!

The next problem for both the Western Blot and the ELISA tests is timing. In Lyme disease, timing is everything! Timing is everything for both successful diagnosis and successful treatment.

In most infections of the human body, the body's immune system releases early and late antibody responses. However, there are some caveats to this general assumption. First, the immune system is most effective when infection is in the blood stream, or places where blood can travel to easily. This is because several white blood cells in the blood stream must interact with the pathogen and then with each other to create the proper immune response.

About four to six weeks after first detecting a harmful pathogen within the blood-stream, a white blood cell called the B-cell lymphocyte expands and becomes a plasma cell and produces immunoglobulin type M (IgM) antibody. Then, in another four weeks, if the infection is still in the blood stream, a new set of plasma cells creates IgG antibodies. Therefore, in general, the presence of IgM antibody and the absence of IgG antibody mean an early infection. (Early means the initial exposure to the bacteria was probably within eight to twelve weeks.) Unfortunately, in Lyme disease this general rule of the lone presence IgM antibody as an indicator of early infection does not apply: IgM antibody can and will appear throughout the infection.

Within mere days after an infected tick bite, the Lyme bacteria can already find its way into the brain and joints of patients. Since *Borrelia burgdorferi* is prefers other locations within the human body other than the blood, it is equipped to seek out those environs more suitable to long-term survival of the bacteria

The migration of the Lyme spirochete from the human blood stream into the tissues has the same effect as muting the patient's antibody response. This happens because the fewer bacteria that remain in the bloodstream, the less stimulation there is to evoke and provoke a B-Cell to become a plasma cell. If the initial infection never makes a strong presence in the blood stream, the host's antibody response may be muted or never initiated. In primate studies a strong antibody response was dependent on the infection-load that the animal was given. The more bacteria injected the greater the antibody titer.

The next problem with timing is this: If you give an antibody serology test too early, it will always be negative. This is simply by virtue of not allowing the immune system enough time to clone enough of the correct plasma cells to create a measurable antibody response. The immune system must manufacture exact copies of the cells that can fight the disease. In some patients it is thought that they may not have the correct B-cells to even make an effective assault against this highly variable pathogen. It

is akin to trying to fix a broken motor with the wrong tools. You do the best you can but will it be good enough?

Finally, since the infection can already be beyond the reach of the blood stream, the antibody serology tests are worthless for early-sequestered infections of the brain, joints, bladder, skin, and tendons. These are sites where the immune system has little effect. The assumption that the absence of antibodies means the absence of infection could prove disastrous if the infection has already established itself in the brain. There is evidence from looking at spinal taps on dozens of “*Early*” Lyme patients with a bull’s-eye rash that the infection can penetrate into the CNS early and without symptoms! If not treated effectively a trapped infection within the CNS is hard to detect and very hard to treat. ***Unlike other infections of the brain, Lyme does not routinely produce immune cells and antibodies in the spinal fluid.***

Further Considerations:

IgM antibodies in Lyme disease can occur at *any* point in the infection, early or late. This is most probably because of the infection seeding from one site to another via the breach of capillaries in that area. In other words, if the Lyme spirochete can escape out of the capillaries during early infection by stimulating the blood vessel cells to produce digestive enzymes, then it also reasons that the sequestered spirochetes can occasionally seed back into the circulatory system by a similar mechanism.

The ELISA test is sometimes used to test cerebrospinal fluid (CSF) in order to detect antibodies in the central nervous system (CNS). While the presence of Lyme antibodies in the CNS is highly significant, no accurate conclusion whatsoever can be made by the absence of Lyme antibodies in CSF. Short of an autopsy, an infection of the brain with *Borrelia burgdorferi* is almost impossible to rule out. The brain is a very poor producer of immune responses. This is why Lyme infections of the brain appear to be aseptic (the absence of white blood cells in the spinal fluid or brain.).

ELISA Recap:

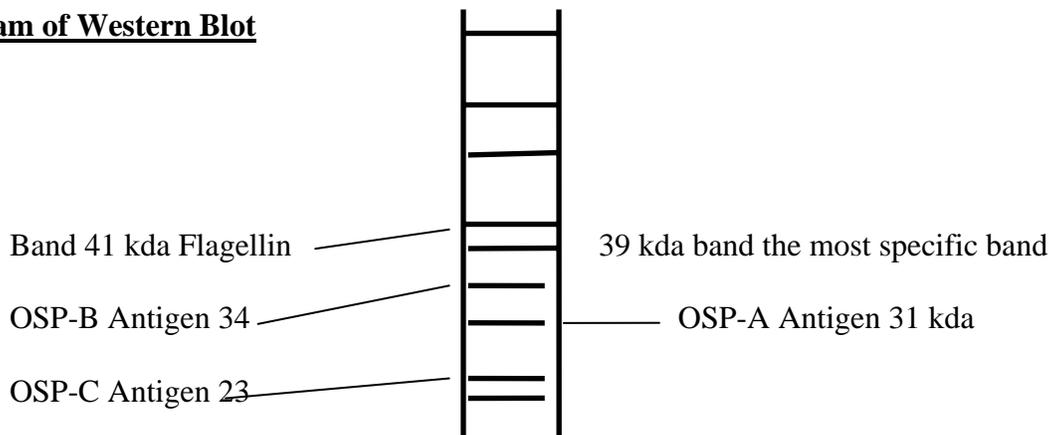
- The ELISA test is useless within the first four weeks of a tick bite.
- The ELISA may not detect late infection because the bacteria can find immune privileged sites in which to hide.
- The ELISA test is not a standardized test. The design of the test can vary greatly from lab to lab.
- The choice of antigens used in the test is derived from a laboratory strain B-31 instead of the naturally occurring wild strains. The B-31 strain is proving to be highly variable and changing. Using a high passage lab strain may be cheap and convenient, but not an accurate representation of the various strains of *Borrelia* found in nature.
- The accuracy of the test varies even on identical samples, meaning that even the labs themselves introduce a variable of inaccuracy by poor procedure, interpretation, or quality control.

Western Blot:

The Western Blot antibody test has only two *slight* advantages over the ELISA test. First, it is slightly more sensitive, probably due to the inclusion of more bacterial antigens. Second, it tells us which bacterial proteins are eliciting an antibody response in that patient. However, in the end, the Western Blot still suffers from all the same downfalls as the ELISA, with one additional disadvantage. In Dearborn, Michigan, a group of state epidemiologists decided to standardize the interpretation of the Western Blot test: ***The IgM Western Blot must have two significant bands to be positive and the IgG Western Blot must have 5 out of 10 possible bands present.*** These numbers are somewhat arbitrary, because the presence of the single band 39 kda is specific for *only Borrelia burgdorferi*, and *no* other bacteria yet discovered.

Lyme antibodies in the patient's serum at the bottom of the filter paper infuse upward and are trapped as lines where the corresponding bacterial protein is found.

Diagram of Western Blot



In essence, the conference in Dearborn, Michigan, chose to eliminate the reporting of a half dozen significant bacterial proteins from the test. Even if antibodies are found present against these significant excluded bacterial proteins the Western Blot using these new reporting criteria cannot be interpreted as a positive test.

The IgG Western Blot must show the strong presence of five out of ten pre-selected bacterial proteins, and the presence of any other highly significant bands is to be ignored. In fact, most state labs are prohibited from even reporting these other significant bands. They are allowed only to report the test as positive or negative, thus taking the ability to interpret the test away from the physicians.

How badly did the new reporting criteria bootstrap the Western Blot accuracy? Compare these results in a 66-patient study on children with physician-diagnosed bull's-eye Lyme skin rashes:

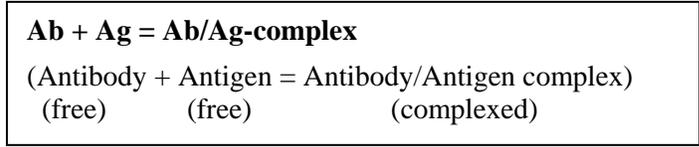
- **1995 Rheumatology Symposia Abstract # 1254 Dr. Paul Fawcett et al.** This abstract shows that, under the old criteria, all 66 pediatric patients with a history of a tick bite and bull's-eye rash who were symptomatic were accepted as positive under the old Western Blot interpretation. Under the newly proposed criteria, only 20 were now considered positive. This means 46 children, all symptomatic, would probably be denied treatment. That's a success rate of only 31%!

Sixty-Six Children with Bull's-Eye Rash Tested by Both Criteria	
Old Western Blot criteria: 100 % positive	False positives on controls: 0%
New NIH criteria: 31% positive	False Positives on controls: 0%

*** Note:** *A misconception about Western Blots and ELISA tests is that they have as many false positives as false negatives. This is not true. False positives are rare. Negative serologies despite a rash or a positive culture is routine. Remember words like sensitivity, specificity, and accuracy DO NOT MEAN DIAGNOSTICALLY ACCURATE to determine if a patient has an infection. A NEGATIVE TEST CANNOT RULE OUT AN INFECTION THAT HAS ESCAPED THE BLOODSTREAM.*

The conclusion of the researchers in this pediatric study was: ***“The proposed Western Blot Reporting Criteria are grossly inadequate, because it excluded 69% of the infected children”***

Finally, the failings of all antibody tests are that they can only capture *uncomplexed* antibody. Once an antibody finds something to latch on to in the bloodstream, it is no longer available to be detected with our current tests.



Free antibodies are detectable with the current Lyme tests, but antigen/antibody complexes are undetectable using either the ELISA or Western Blot tests. It has been suggested that complexes are the biggest weakness of both the ELISA and Western Blot tests and yet they are completely ignored when Lyme tests are given.

- *Schutzer SE, Coyle PK, Belman AL, Golightly MG, Drulle J. Sequestration of antibody to Borrelia burgdorferi in immune complexes in seronegative Lyme disease. Lancet 1990;335:312-315*

When the ELISA or Western Blot tests are positive, they are significant. However, *when they are negative, they are incapable of determining the complete absence of active infection somewhere in the body.* All it takes to validate this is a single case history of a patient who is sero-negative for Lyme disease (the absence of Lyme antibodies), but is culture positive for the bacteria. Here are a few such examples of patients found to be actively infected despite having no measurable antibodies.

- *Masters EJ, Lynxwiler P, Rawlings J. Spirochetemia after Continuous High Dose Oral Amoxicillin Therapy. Infect Dis Clin Practice 1994;3:207-208*
- *Schmidli J, Hunzicker T, Moesli P, et al. Cultivation of Bb from Joint Fluid Three Months After Treatment of Facial Palsy Due to Lyme Borreliosis. J Infect Dis 1988;158:905-906*
- *Liegner KB, Shapiro JR, Ramsey D, Halperin AJ, Hoglefe W, and Kong L. Recurrent erythema migrans despite extended antibiotic treatment with minocycline in a patient with persisting Borrelia burgdorferi infection. J. American Acad Dermatol. 1993;28:312-314*

The biggest misunderstanding physicians have about Lyme antibody tests is thinking that a high titer of antibody in a patient means that the patient is more ill than a patient with a low titer of antibodies. What these tests are really measuring is the body's natural immunity against the bacteria. A patient who is able to mount a strong antibody defense against this pathogen is far better off than a patient who makes little or no antibody. It only stands to reason that a patient with a high infection load and no antibodies is going to be more symptomatic than a patient with a high natural immunity. Unfortunately, most physicians look at higher titers and assume those patients are the most ill, but their reasoning is backwards. Low titers in a highly symptomatic Lyme patient is a very bad thing.

DNA Amplification or PCR (Polymerase Chain Reaction) Tests:

Every living cell has DNA that is unique to that species of organism. This is true from the simplest bacteria to the most complex mammal. The polymerase chain reaction test is a test that can search for a specific sequence of DNA and multiply that sequence a billion times in less than a day. It is often said that the PCR test is the most sensitive and specific diagnostic test available. However, PCR tests are not without their drawbacks.

Specificity is a big problem with today's Lyme tests. It sounds good to hear things like, "This is the most specific test on the market!" Those buzz words meant to promote and sell a test, however, should throw up a red flag to anyone who understands this family of bacteria. The family of bacteria that Lyme disease spirochetes belong to is *the borrelia* family. *Borrelia* is the same group of bacteria that cause tick-

borne-relapsing-fever. There are more than 40 different disease-causing subspecies of borrelia that are closely related to the Lyme spirochete.

Variability in the borrelia family of bacteria is not only routine; it is actually built into the genetics of the bacteria to add variations its proteins when stressed during division. This is what causes relapses of symptoms in relapsing fever. The bacteria presents one set of proteins to your immune system and then six weeks later, the bacteria adapt and present new variations of surface proteins to your immune system. In turn, your own immune system is what makes you feel sick, sweaty, feverish, and ill with each new set of antigens presented.* The Lyme bacteria uses a slightly more subtle form of this morphogenic antigen variability, but it, too, like its cousins, is capable of changing its outward appearance like a criminal changing his disguise.

Escaping the immune system is very important if you want to survive as a bacterium. *Borrelia* has several mechanisms to do this, but this mechanism of gene variability and variable proteins make both serology antibody tests and PCR tests less effective.

One could easily make a case that Lyme disease and all of its Lyme-like cousins are nothing more than variations of Relapsing-Fever. In America, we now have at least two geno-species that cause Lyme disease, which are not *Borrelia burgdorferi*. The truth is, we may have a situation with Lyme disease that is similar to relapsing fever, in that there are dozens of yet to be discovered species of borrelia that cause disease but are not *Borrelia burgdorferi*.

Would you want to be denied medical treatment, medical coverage, and your good health, all because the test you are given is so specific that it excludes all other borrelia? What we need in America and other Lyme-endemic countries is not a hundred different species-specific tests, but rather one general screening test for *Borrelia*. This is true for the antibody tests as well as the PCR DNA tests. Suddenly the buzz-word SPECIFICITY has become a dirty word. Specific also can mean exclusionary!

***NOTE:** There is no substantial evidence that a Herxheimer reaction during antibiotic treatment is due to the release of bacterial toxins. There is good evidence that a true Herxheimer reaction is the rapid synthesis of cytokines from your immune cells. This increases when internal bacterial antigens are exposed when the bacteria ruptures with treatment. This was established in Relapsing Fever patients in the late 1960s. To date the evidence of *Borrelia burgdorferi* producing significant toxins in symptomatic patients is virtually non-existent.

How a PCR test works:

The first step is to create a template or primer for the PCR test that is specific to the organism you are looking for. Second, you have to be sure that the test sample has a high probability of containing the DNA sequence for which you are searching. Another concern is inhibitory factors in the samples. Finally, you have to be sure there are no false positives from lab contamination due to DNA floating around like dust in the air.

In Lyme disease, the PCR test really has limited value. The main problem is that doctors and labs like to use blood, urine, and spinal fluid to test simply because of the ease of collection. In the case of Lyme disease, however, the borrelia spirochete does not like the blood stream, and PCR tests of fluids are frequently negative while the same PCR tests of skin biopsies in the same patient are positive. This indicates that finding a sample with the bacterium's DNA is a bit like finding a needle in a haystack.

The example I like to use to demonstrate this is the parable about the bucket and the hailstorm. If it is hailing outside, you could run around all day with a teacup, trying to catch just one hailstone. Just one hailstone in the teacup would be proof that it is hailing outside: But your chance of success is low. If, however, you used a bucket to catch the hailstones, you would have a slightly better chance to make your case. If you had an entire parking lot to catch hail you would have even a better chance!

However, remember, just because it is hailing in New Jersey doesn't mean it's hailing in New York. If the hail represents borrelia DNA, it is clear the bigger and better the sample you collect the better

the result. However, if New Jersey represents your blood and New York represents your brain, you cannot use a hailstone from New Jersey to prove whether it's hailing in New York. To make matters worse, the tests we have now are not as good as they could be, so it compares to using teacups with holes in the bottom to collect hailstones! Absence of proof is not proof of absence of infection.

It has been reported that there are inhibitory enzymes in the urine that can affect the PCR tests. Most labs using PCR tests don't address this but the truth is the whole organism rarely shows up in the urine so a PCR test like serology tests can be inconclusive if negative.

Cerebrospinal fluid has been reported PCR positive in only 7% of the cases of documented neurological Lyme disease. This indicates that the organism is highly tissue bound in the CNS and that bacterial DNA in the spinal fluid is the exception, not the rule, during infection.

The biggest problem with the PCR test in Lyme disease isn't its accuracy or sensitivity, but the squabbling over patents. Every lab wants its own patent on its own test and will often present data on their tests in a favorable way to sell the test, but is misrepresentative of the test's abilities to diagnose Lyme disease at any stage.

Most labs use the OSP-A gene as a primer for its PCR test, but, in truth, a PCR test that can use multiple primers is probably better, there are some species of Borrelia burgdorferi don't even have a gene for OSP-A. However, using multiple primers can mean paying royalties to many different labs for the use of proprietary patents. This raises costs and means cooperation between competitors.

PCR Recap:

No single sample from a patient can be diagnostic for Lyme disease using PCR as the test. Even the University of Minnesota reported a mere 18% success rate in early Lyme when skin biopsies were obtained from culture positive bull's-eye rashes.

Patent disputes often result in poor PCR tests being marketed with little or no sharing of technology between competitors. This competition often leads to over-hyped performance and over hyped accuracy claims by manufacturers.

Often the best tissues for PCR tests are tissue biopsies, which are rarely done because of costs, risk, and inconvenience. In truth, PCR tests are best used in the post-mortem exam on tissues such as the brain, bladder, and heart. As a diagnostic tool, it can be useful when positive, but tells us nothing when negative. I see few patients lining up for a brain biopsy.

- *Appel MJ, Allan S, Jacobson RH, Lauderdale TL, Chang YF et al. Experimental Lyme disease in dogs produces arthritis and persistent infection. J Infect Dis, March 1993;167(3):651-4*

Culturing *Borrelia burgdorferi*:

Culturing spirochetes can be difficult (see my article on the Lyme Alliance web site). Some spirochetes, such as *T. pallidum*, which causes syphilis, have never been successfully cultured in culture media, and are maintained only in live animal models. *Borrelia burgdorferi* is difficult to culture. The University of Minnesota, when comparing culturing of erythema migrans rashes to their early PCR test, reported culture success rates as low as 4%.

As discussed earlier, culturing blood is frustrating because the bacteria do not like the blood stream. The same is true for the spinal fluid and urine. Therefore, once again we are left with the option to biopsy at great expense and risk, or to use other, less costly, tests. Culturing is the gold standard for confirmation of an infection, and a positive culture is highly important. However, as a diagnostic tool in Lyme diagnosis, culturing has a poor success rate, and the cost and time often make it prohibitive except as a research tool.

The technique of culturing the non-classical form of the spirochete known as spheroid-forms or L-forms is controversial and has yet to be corroborated and commercially reproducible. L-forms seem to be shed by the spirochetes when they are stressed and moribund. They appear to be able to transform back

into viable spirochete indicating that the vesicles retain a full compliment of bacterial genetic material. Until the link can be established between these L-forms and disease, and the cultures can be corroborated by PCR and consistently reproducible by other labs the presence of L-forms will be debated by opponents as artifacts and aberrations.

Just as in the case of PCR and antibody tests, culturing can also be affected by the species of bacteria that is present. Some laboratory strains of Lyme disease have been known to take months to culture. Most hospital labs don't even stock culture media that is suitable for culturing *Borrelia*, and the choices for culture media are extremely limited. Until better techniques and culture medias are made available, culturing spirochetes is a techniques best used as a research tool. But the inexperience of hospital labs with spirochete culturing techniques is by far the biggest hurdle in seeing this test flourish.

Microscopic observation of the organism:

Using a microscope to find the Lyme bacteria is quite literally like looking for a needle in a haystack. The *Borrelia burgdorferi* organism is found in such low numbers in fluids and tissue that it requires hours and hours of lab time to do an adequate search of just a few millimeters of tissue. As a research tool biopsy and stain is useful, but to use microscopy to diagnose Lyme is too slow, too costly, and quite unreliable.

The methods used today have remained, for the most part, unchanged for a hundred years. It has been known since the turn of the century that spirochetes stain heavily with silver acid-loving stains. This means spirochetes normally invisible under a light microscope will stain jet black and can be seen in silver-stained tissue biopsies. (Nucleic acids in the bacteria's membrane absorb the silver-stain.)

The modern variation of spirochete microscopy is to use a fluorescent stain attached to an antibody that will latch on to the bacteria. Under a fluorescent microscope, the bacteria stand out as brightly colored spirals.

The final method is simply to take a bodily fluid, such as CSF or urine, and ultra centrifuge it, then look for spirochetes under a light microscope with a drop of acrodine orange dye.

All of these techniques are direct observation of the organism, but it is impossible to determine the species just by looking at the bacterium's gross morphology. The most useful place for this tool is in the post-mortem exam, where deep tissues can be collected, preserved in paraffin, stained, and studied months later. Any autopsy performed on a Lyme patient should include silver stain sections of paraffin-fixed brain biopsies. However, using microscopic exam to diagnose Lyme would be expensive and time consuming, and would yield poor results. (In a post-mortem exam the presence of spirochetes is significant. Spirochetes should never be found in the brain. One wonders what percentage of dementia patients would have spirochetes in the brain if we looked for them?)

Conclusion:

One of the biggest misconceptions about Lyme disease antibody tests, which has led to years of unnecessary morbidity and mortality for Lyme disease patients, is *the insistence that the absence of Lyme antibodies means the absence of active infection*. You cannot equate the absence of antibodies with the absence of infection, nor can you use antibody serologies as an endpoint in treatment studies to determine the effectiveness of any treatment regimen. It isn't just a bad idea - it is just plain bad science.

Will there ever be a Lyme test that can be used dependably to diagnose Lyme disease? I don't believe such a test is within our current technology, and until such a test exists denying patients treatment using our current tests is bad medicine.

Key words and concepts used in this article:

Systemic: *Borrelia burgdorferi* and other spirochetes in the tick-borne relapsing fever family of bacteria circulate through the blood stream to the entire body. They can find passage through blood vessel walls and invade other tissues and organs, including known target tissues such as skin, tendons, joints, heart, nerves, and brain.

Sequestered: The Lyme spirochete can find haven in areas of the body that are poorly protected by the immune system (the brain), have poor areas of blood circulation (tendons), or where the bacteria can lay metabolically inactive for lengths of time and resist antibiotic penetration (the skin and the brain).

Multi-systemic: The bacteria has affected more than one system of the body, such as: Peripheral nervous system, skin, joints and connective tissues, cardiovascular system and circulatory system, eyes, muscles, liver and spleen, and central nervous system.

Blood Brain Barrier (BBB): The network of capillaries surrounding the brain that selectively let things in and out of the brain. A healthy BBB prevents infections, white blood cells, and many medicines from entering the brain. A breakdown of the BBB is not a good thing, and occurs very early in most animal models of Lyme disease.

Antigen: A protein usually on the surface of the bacteria that stimulates the immune system to make antibodies against that protein. Antigens can also stimulate other immune responses, such as T-cell and macrophage responses.

Serologies: Using the centrifuged serum extracted from blood to look for antibodies against the Lyme bacterium.

Titer: A measurement of antibody content in the serum. Titers are usually reported as dilution series. The higher the dilution of serum where antibodies are still detectable means there are more antibodies present in that patient's serum. The cut off for reporting a positive is a bit arbitrary and varies from lab to lab. Institutions conservative on the diagnosis of Lyme have raised their cutoffs from 1:256 to 1:1024 Which is rare to see in most Lyme patients.

Infection Load: The actual number of bacteria in a host. If the bacteria remain in the bloodstream, the number of bacteria per unit of blood can be calculated, but this number is meaningless if the infection has moved beyond the bloodstream. In Lyme disease, there is no accurate way of determining true infection load. If the bacteria out pace antibody production then there is no FREE ANTIBODY only ANTIBODY COMPLEXES.

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- Preac-Mursic V, Wilske B, Schierz G, et al. Repeated Isolation of Spirochetes From the Cerebrospinal Fluid of an Antibiotic-treated Patient with Meningoradiculitis Bannwarth' Syndrome. *Eur J Clin Microbiol* 1984;3:564-565
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- Abstract # D654: J. Nowakowski, et al. Culture-Confirmed Treatment Failures of Cephalexin Therapy for Erythema Migrans. Two of six patients biopsied had culture-confirmed *Borrelia burgdorferi* infections despite up to 21 days of Cephalexin (500 mg TID) antibiotic treatment.
 - Abstract # D655: Nowakowski, et al. Culture-confirmed Infection and Reinfection with *Borrelia Burgdorferi*. A patient, despite antibiotic therapy, had a recurring Erythema Migrans rash on three separate occasions. On each occasion, it was biopsied and revealed the active presence of *Borrelia burgdorferi* on two separate occasions, indicating reinfection had occurred.
 - Abstract # D657: J. Cimperman, F. Strle, et al. Repeated Isolation of *Borrelia Burgdorferi* from the CSF of Two Patients Treated for Lyme Neuroborreliosis. Patient 1 was a twenty-year-old woman who presented with meningitis, but was seronegative for *Borrelia burgdorferi*. Subsequently, six weeks later, Bb was cultured from her CSF and she was treated with IV Rocephin, 2 grams a day for 14 days. Three months later, the symptoms returned, and Bb was once again isolated from the CSF. Patient 2 was a 51-year-old female who developed an EM rash after tick bite. Within two months, she had severe neurological symptoms, but her serology was negative. She was denied treatment until her CSF was culture positive, nine months post-tick bite. She was treated with 2 grams of Rocephin for 14 days. Two months post-antibiotic treatment, Bb was once again cultured from her CSF. In both of these cases, the patients had negative antibodies but were culture positive, suggesting that the antibody tests are not reliable predictors of neurological Lyme disease. In addition, standard treatment regimens are insufficient when infection of the CNS is established, and Bb can survive in the brain despite intravenous antibiotic treatment.
 - Abstract # D658: F. Strle et al. Reinfection with *Borrelia Burgdorferi* in Endemic Areas. Conclusion: Even despite high antibody titers, as seen in ACA patients, 7% of 2273 patients with previous Lyme disease became reinfected and presented with an EM rash and late symptoms after a recent tick bite.
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