

# Laboratory Tests

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## Three Main Categories of Lyme Disease Tests:

### 1. Indirect Tests (serum antibody tests):

ELISA; Western Blot; IFA; Borreliacidal Antibody Assay (Gunderson test); T-cell Activation Test

### 2. Direct detection tests:

PCR (DNA amplification); Lyme Urine Antigen Test (LUAT); Antigen Capture Test; culturing of skin, blood, CSF, urine, or tissue; immune complex / antigen-antibody test

### 3. Tissue Biopsy and Staining:

Silver Stain; Gold Stain; Fluorescent Tagged Monoclonal Antibody Stains; Acrodine Orange; Gram Stain; Muramidase; etc.

There is a great deal of confusion and controversy surrounding Lyme disease testing. The first problem is that most of the manufacturers of these tests want you to believe that their tests are the best. At every medical convention, I listen to sales pitch after sales pitch from sales people making their product sound infallible. Often the terminology is confusing and the customer frequently misinterprets what is really being said.

For example, a salesman may say the rate of false positive or false negative is less than one percent. This sounds like the test is more than 99% accurate. In reality, what it is saying is if you have 1000 test samples from the same known laboratory sample, then in less than ten samples will there be a result that differs significantly from the other 990.

In any of this, did you hear the words: "percent reliability" or "percent accuracy" in diagnosing Lyme disease in humans? No! People often mistake "false positive rate" for accuracy. The truth is that no Lyme disease test to date is close to 100% accurate, because each test has its own particular set of shortcomings. So, while the first problem with Lyme disease tests is in the way they are promoted, the second problem is the way the tests are primed to recognize laboratory strains of Bb, rather than wild types. Third, the Lyme spirochete can hide in the human body, and fool the immune system into thinking it isn't there. So, no antibodies are produced, resulting in negative tests. Stealth technology isn't new, it evolved millions of years ago by the first bacteria that evaded its host's defenses.

## Immune Responses

The first antibody our body makes in response to a foreign invader is usually immunoglobulin type M, abbreviated as IgM. This large antibody takes two to four weeks to be made in quantities large enough to be consistently measured. It is at its peak of production four weeks after exposure to an antigen. The IgM antibody will only stay in circulation for about six months, and then levels are usually too low to detect. If infection persists, this antibody may also persist. In general, a Lyme patient who consistently has detectable IgM levels is usually chronically ill, but its absence is not a reliable indicator of cure.

The second antibody we make after the IgM is the IgG antibody. This antibody takes four to eight weeks to form, and is gone in less than twelve months. It peaks at about six weeks. This antibody crosses the placenta, so an infected mother can pass this antibody

to her child. An IgG antibody titer in a newborn does not have to mean active infection. It does mean the mother has had exposure, and the child must be carefully monitored for signs of the disease.

Because of the difference in the two antibodies, two separate tests are available to test for their presence. Therefore, a physician must specify whether or not a patient should have an IgM or IgG Western Blot, or an IgM or IgG ELISA test.

### **IgM:**

This is the earliest of the antibodies to appear in response to an infection. It is produced in quantity. It is six times larger than the IgG antibody. Because of its size, this immunoglobulin does not cross the placenta. Since it cannot enter the fetus from the mother, any newborn that starts to make IgM antibodies against Lyme disease must be infected. However, a fetus exposed to *Borrelia burgdorferi* early in the pregnancy may never make an antibody response to the Lyme bacteria because the baby's immune system doesn't recognize it as foreign.

### **IgG:**

This antibody remains the longest and is the foot soldier of the immune system. It attacks viruses, bacteria, yeast, toxins, and transplants. The IgG antibody can kill bacteria indirectly by tagging or marking the foreign invaders for destruction by the killer cells (T-cells, macrophage). Or, it can kill the bacteria directly by evoking complement, a series of enzymes and proteins that will dissolve the intruder.

*Note: It was once thought that plasma cells could produce antibodies that could conform to any shape necessary to attack foreign intruders. If this were true, we would have almost unlimited immunity. It is now thought that each person has a finite collection of specialized lymphocytes that are able to create a finite number of antibodies. Each antibody shape is predetermined, and can be produced by only one type of lymphocyte. When the body is invaded by a foreign antigen, it will stimulate one of these cells, and only that cell will begin to clone itself. This process takes several weeks. If we lack the right cell type to do the job, we are left with a gap in our immunity. This might account for why some Lyme patients with certain tissue types have greater morbidity, while others have relatively mild symptoms.*

Dr. Alan Steere, M.D., observed that Lyme arthritis patients with tissue type HLA-DR2 and HLA-DR4 had more severe arthritis and chronic disease. Other tissue types have been associated with an increased incidence of multiple sclerosis and other neurological diseases. It might be that different patient tissue types might account for a difference in patient's symptoms to a greater degree than different strains of the bacteria.

It is known that this bacteria has an affinity for specific tissues. If you have a specific lack of immunity, this may cause the disease to manifest differently in those tissues. For example, let's say hypothetically that your heart is infected with *Borrelia burgdorferi* bacteria. Perhaps most people make an antibody that suppresses attachment of Bb to certain fibers in the heart. If you lack that antibody, the infection may continue more aggressively and manifest differently - for instance, causing an enlargement of the muscle fibers or destruction of the conduction pathways.

Instead of lacking a specific antibody, perhaps some individuals make a different kind of antibody, an antibody that not only attacks the bacteria - but may attack the heart as well! It is well known and documented that some patients produce auto-antibodies, which are antibodies that our own body produces that attack our own tissues. This is the basis of autoimmune disease. In some Lyme disease patients, an auto-antibody against cardiolipin has been clearly established in Lyme patients with Lyme carditis.

Perhaps, in addition to other Lyme tests, we should also be tissue typing patients and searching for auto-antibodies? Tissue typing requires a small blood sample, and costs about \$200.

## **Western Blot**

The Western Blot essentially makes a map of the different antibodies the immune system produces to the bacteria. The map separates the antibodies by the weight of their respective antigens and are reported in units called kilo daltons or kDa. For example, a Western Blot may report bands at 22, 23, 25, 31, 34, 39, and 41 kDa. Each of these bands represents an antibody response to a specific protein found on the spirochete. The 41 band indicates an antibody to the flagella 41 kDa protein and is nonspecific. The 31 kDa band represents the OSPA protein and is specific for just a few species of *Borrelia*, as is the 34 band OSPB, and 23 kDa OSPC.

In 1994, the Association of State and Territorial Public Health Laboratory Directors, under a CDC grant, decided that there should be consistency between labs reporting Lyme disease Western Blots, and that a specific reporting criteria should be established. The consensus committee, chaired by Dr. Michael Osterholm, Ph.D., MN, set nationwide standards for Western Blot reporting. This sounds good, but one could argue they made a bad situation worse. Prior to the hearing, virtually every lab had accepted bands 22, 23, 25, 31, and 34 kDa as specific and significant, and reported them as positive for exposure to *Borrelia burgdorferi*. Not only are these bands specific for *Borrelia* species, but they represent all of the major outer surface proteins being used to develop the Lyme vaccines. The committee, without any clear reasoning, disqualified those bands as even being reportable.

After the consensus meeting, those bands were no longer acceptable. The result was that what had been a fair-to-good test for detecting Lyme disease had now become poor, arguably useless. Many scientists have questioned these new reporting criteria, and several wrote letters of protest to both the committee and to laboratory journals. Many labs stopped reporting the actual bands and instead, simply reported the test as positive or negative, thus preventing any further interpretations. (90)

How badly did the Lab Directors bootstrap this test? The following is an analysis of the new guidelines presented as an abstract and lecture at the 1995 Rheumatology Conference in Texas, chaired by Dr. Alan Steere, MD. (1995 Rheumatology Symposia Abstract #1254, Dr. Paul Fawcett, et al.)

This was a study designed to test the recently proposed changes to Western Blot interpretation by the Second National Conference on Serological Testing for Lyme Disease, sponsored by the CDC. The committee proposed limiting the bands that could be reported in a Western Blot for diagnosis of Lyme disease. Out of a possible 25 bands, 10 specific bands were selected as being reportable. An IgG Western Blot must have five or more of these bands: 18, 21, 28, 30, 39, 41, 45, 58, 66 and 93 kDa. An IgM Western Blot must have two or more of the following three bands: 23, 39, 41.

Conspicuously absent are the most important bands, 22, 23, 25, 31, and 34, which include OSPA, OSP-B and OSP-C antigens - the three most widely accepted and recognized Bb antigens. These antigens were the antigens chosen for human vaccine trials. This abstract showed that, under the old criteria, all of 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. (The number of false positives under both criteria was zero percent.) That means 46 children who were all symptomatic would probably be denied treatment! That's a success rate of only 31%.

*\*Note: A misconception about Western Blots is that they have as many false positives as false negatives. This is not true. False positives based on species specific bands are rare.*

The conclusion of the researchers was: "the proposed Western Blot reporting criteria are grossly inadequate, because it excluded 69% of the infected children."

## **Elisa Test**

The Enzyme-Linked Immunosorbant Serum Assay is the simplest, least expensive, easiest to perform, and most common Lyme test ordered. It is a test based on detecting the antibodies that our bodies make in response to being exposed to *Borrelia burgdorferi* (Bb). It is a preferred test by laboratories, not because it is more accurate than other Lyme tests, but because it is automated. Many different patient samples can be performed by a single machine simultaneously. This allows for a faster turnover, less costs, and theoretically, standardized test results that are consistent from lab to lab.

We are told by manufacturers, health departments and clinics that the Lyme ELISA tests are good, useful tests, but in two blinded studies that tested laboratories for accuracy, they failed miserably. Lorie Bakken, MS/MPH, showed in her studies that there was not only inaccuracy and inconsistency between competing laboratories, but also between identical triple samples sent to the same lab. In other words, identical samples often resulted in different results! In the first study, forty-five labs correctly identified the samples only 55% of the time.

In the latest study by the College of American Pathologists, 516 labs were tested. The overall result was terrible! There were almost equal numbers of false positives as false negatives. Overall, the labs were 55% inaccurate. The labs could only give a correct result 45% of the time. You are actually better off to flip a coin!

The basis of the ELISA test is that it can be primed to be very specific for particular antibodies. This is done by taking a laboratory sample of the Lyme bacteria and breaking the sample down into fragments. These fragments, or antigens, are then embedded on the side of a reagent vessel like a test tube. Then the patient's serum is added, and any free (non-complexed) antibodies specific for the test strain will then bind to the antigens, which are linked to special enzymes that will change color when antibodies are present. The sample is continually diluted until the reaction no longer occurs and no color change can be detected. The sample is then reported as a dilution ratio, such as one part serum to 256 parts water, or 1:256.

The ELISA test sounds simple and straight forward, but it has a couple of major flaws. *Borrelia* species are some of the most polymorphic bacteria known to exist. In other words, most *Borrelia* species can significantly change its surface proteins enough during cell division as to evade our immune system, and may differ from laboratory strains enough to result in negative tests, even if antiBb antibodies are present! In Europe, this problem is intensified because they have recognized three species of *Borrelia* that cause Lyme disease, and so they have available three separate ELISA tests. The questions in America are: 1) Have we recognized all the strains and species of *Borrelia* that cause Lyme disease symptoms, and 2) are we incorporating them into our tests? The answer is no. Convenience and expedience has chosen that we don't prime our ELISA tests with wild strains, but use a laboratory strain.

When a lab reports that their ELISA test has had high specificity and high sensitivity, it is usually interpreted by doctors as being a more accurate test, but the doctors don't know what the lab is actually measuring. One of the hidden problems of serologic Lyme tests is the fact that the tests must be primed with a source of bacteria to create the reactions with the patient's antibodies. To do this, virtually all labs rely on a laboratory strain of Bb known as strain B-31. Taking purified antigens from strain B-31 and injecting them into mice, they

then can extract a monoclonal antibody to each antigen, or a polyvalent antibody soup. This antibody is concentrated and purified, and then added to the ELISA test to test the efficacy and performance of the test. Unlike the wild strains, B-31 grows well in culture, and this makes it a perfect choice as a consistent and inexpensive source of Bb. But the affinity the mouse monoclonal antibody has to B-31 antigen is quite different from the affinity the patients' antibodies have to the same antigen. This means the test may register as negative because the test cannot detect the slightly different antibody profile that a wild strain of Bb can produce. In other words, the labs are really comparing apples to oranges! This is why, when the American College of Pathologists used human sera to test the accuracy of 516 different laboratories ELISA tests nation wide, the overall accuracy was only 45%.

In the quest for specificity, most ELISA tests have become so specific that the test may fail to detect antibodies from related strains of *Borrelia*. This would include different genospecies that cause Lyme disease, as well as different *Borrelia* species that cause Tickborne Relapsing Fever. Would a cross reaction to the *Borrelia* species that cause Tickborne Relapsing Fever be so bad?

The real Achilles' Heel of an ELISA Test is that it can only detect free antibody. It cannot detect any antibody that has become complexed with antigen.

The ELISA test depends on the active, free antibodies to attach to the free antigens that have been embedded on the walls of the test tube. If the antibodies in the serum being tested are already attached to antigens, then the enzyme reaction cannot take place. If we think of antibodies as sort of keys that fit into locks, and that on the surface of the bacteria are specific locks we now call antigens, you can see that once a key is inserted into a lock, the key is no longer available to open any other locks.

What makes this test so misleading is that many doctors accept high readings as an indication that the patient must really be sick. This logic is exactly backwards. If a patient is really infected with lots of bacteria, that means they have a lot of bacterial antigens floating around in the blood that are complexing free antibodies. So, as free antigen increases, free antibody decreases. Since the ELISA test detects only free antibody, a negative test might actually indicate a more serious infection. Many times, I have seen totally asymptomatic patients with ELISA titers over 1000 be treated as though they were on death's doorstep simply because they had a high titer, while patients with borderline titers who are practically disabled are ignored, because a low titer is perceived as meaning less infected! These conclusions are erroneous and actually opposite to the truth, which is that a high titer means greater natural immunity.

This phenomena can actually be observed by using vaccines. If a patient has been vaccinated for a disease like tetanus, they will carry a high titer of free antibodies. If you try to measure those antibodies an hour after a booster shot is given, they will test negative. This is because the injected tetanus antigen complexes all available free antibody before the body can make more, so the measurable free antibody level drops.

The nature of all antibody is to seek out the proper antigen. The level of free antibody available is variable and often inadequate for the amount of antigen available. As antigen increases (i.e. The bacteria are dividing faster than the immune system can handle), free antibody drops.

What a high ELISA test may be a better indicator of is what level of immunity is the patient capable of mounting against this infection? A high titer is the same thing as saying the patient has a high natural immunity, and a low can mean that the patient may be overwhelmed by infection.

In one year-long study by Dr. Sam Donta, MD, done on chronic Lyme patients, the initial

ELISA tests proved to be more than 66+% inaccurate (1996 LDF Conference lecture). Other researchers have also found the ELISA tests to be inaccurate. Using a 45-panel diagnostic testing protocol from the NIH for testing the efficacy of the ELISA and Western Blot, researchers found the accuracy of the Lyme ELISA varied from about 50-75%, and were routinely inconsistent. The CDC's ELISA test did no better on average than any other ELISA. It is the CDC ELISA test which is used for surveillance of emerging Lyme disease in the United States, yet the test was correct only about two out every three tests. Too often, a single negative ELISA test can prevent a sick patient from getting treatment, even despite having serious symptoms!

In my opinion, the ELISA test is worthless as a diagnostic tool in Lyme disease. It is inconsistent and inaccurate, and should be discontinued as a tool to diagnose Lyme. If the NIH and CDC truly believe, as they've stated, that the diagnosis of Lyme disease is to be made on the basis of symptoms, then these tests should be temporarily banned until each manufacturer can prove efficacy using human serum.