"Diagnosing and Laboratory Testing of Lyme Disease and Tick-borne Diseases"

By
Doug Fearn, Vice President
&
Harvey Kliman, President
Lyme Disease Association of Southeastern Pennsylvania, Inc.

Lyme disease requires a CLINICAL DIAGNOSIS

Many other diseases require a clinical diagnosis

Clinical Diagnosis

1. Exposure to ticks
2. Signs
3. Symptoms

Exposure to ticks

- Lyme-endemic area?
- Tick found?
- Tick kept?

Signs

(Things a doctor can detect)

- Bull's eye rash?
- Fever?
- Swollen joints?

Symptoms

(Things you report)

- Flu-like symptoms
- Aching joints
- Headache
- Stomach problems (in children)
- Light/Sound sensitivity
- Malaise

Laboratory tests only support the clinical diagnosis

- Provide security to the diagnosing physician
- Help convince the skeptical patient
- Supporting evidence for health insurance company

Lyme disease requires a CLINICAL DIAGNOSIS

First Principles of Testing

- Lyme disease is a clinical diagnosis
- Testing serves to support the diagnosis (CDC statement)
- Other tick transmitted disease tests may be more reliable, but in general have most of the same drawbacks as the Lyme tests.

Two Basic Test Types

- **Antibody detection tests**
  - ELISA (Lyme titer)
  - C6 Peptide Test (one specific antibody)
  - Western blot
- **Direct detection**
  - Microscope observation of spirochetes
  - Culture (grow spirochetes in Petri dish)
  - PCR (polymerase chain reaction) finds DNA
  - Antigen detection

Antibody Tests

- **Definitions help:**
  - **Antibody** (remember BODY) these are white blood cells made by your BODY to attack foreign objects.
  - **Antigen** – these are the foreign objects, whole microbes or pieces of them that the immune system can recognize and attack
    - Examples for Borrelia burgdorferi, the Lyme spirochete, are OspA – outer surface protein A, OspB & OspC.

ELISA test

- Designed to detect all antibodies as a group
- Most labs use test kits based on only one strain of Borrelia, B31
- Result is a test that only finds 40 to 65% of infected people
- IGeneX Labs Inc is arguably the best lab for Lyme testing today. The lab uses 3 strains of Borrelia in antibody tests.

Sensitivity & Selectivity

Definitions again:

- **Sensitivity** of a test is the measure of what percent of true infections are detected as positive. 95% is the criterion for a screening test.
- **Selectivity** is the measure of how accurately the test correctly identifies only the target disease. 95% is an excellent test misidentifying only 5% of true infections.

Back to the ELISA test

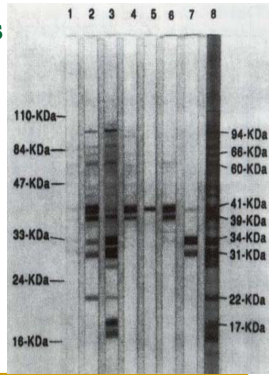
- The sensitivity is 65% - low for an initial test
  - Means 35% of true infections are missed
- Selectivity is also about 65%
  - Means that of those positives, up to 35% might be positive from another microbe or disease
- Either way, the ELISA is either 65% OK or 42% OK (65% of 65%) - NOT GOOD!

Western blot

- Also an antibody test, but it displays each antibody separately
- Sensitivity depends on interpretation of bands
  - CDC criteria of 5 critical bands ~ 50% est.
  - Using 2 of the most selective bands ~85% or better
- Antibody tests depend on the person's immune system to produce antibodies in response to infection.

Western blot Bands

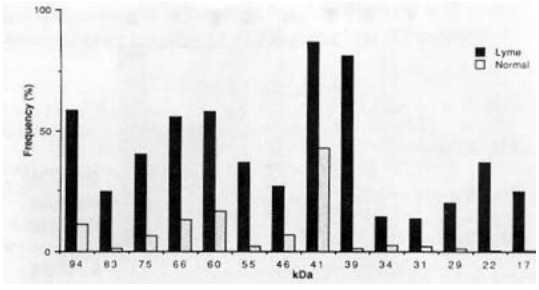
- Image shows bands
- Intensity varies from band to band and is subject to interpretation as to positive + or ++ or +++ or equivocal +/- (Different technicians can make different judgements)



Western blots of B. burgdorferi B31 strain: The blots were: (1) stained with amino black, (2) reacted with rabbit antisera, (3) with goat antisera, (4-7) with various monoclonal antibodies, and (8) with pooled patient sera. Reproduced by permission from Ma B, Christen B, Leung D, Vigo-Peltrey C. Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against B. burgdorferi. J Clin Microbiol 1992; 30: 370-76.

Western blot bands

Sensitivity of bands – N. Harris, PhD – IGeneX, Labs Inc.



Comparison of antibody reactivity to various B. burgdorferi *antigens. The dark bars are from 186 patients with clinically confirmed Lyme disease and the light bars are from 320 normal controls. Reproduced by permission from Ma B, Christen B, Leung D, Vigo-Peltrey C. Serodiagnosis of Lyme borreliosis by Western immunoblot: reactivity of various significant antibodies against B. burgdorferi. J Clin Microbial 1992; 30: 370-76.*
Nick S Harris, An Understanding of Laboratory Testing for Lyme Disease, Journal of Spirochetal and Tick-borne Diseases, Volume 5, Spring/Summer 1998

False Positives and Negatives

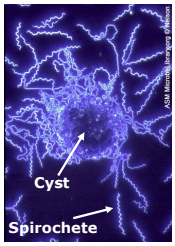
	Patient Infected	Patient NOT Infected
Test Result POSITIVE	**True** Positive	**False** Positive
Test Result NEGATIVE	**False** Negative	**True** Negative

Direct Detection Tests

- Microscope observation of spirochetes
- Culture (grow spirochetes in Petri dish)
- PCR (polymerase chain reaction) finds DNA
- Antigen detection

Direct Detection

- Observation with microscope
  - Spirochetes hard to see – very small and hard to stain to make visible
  - Few spirochetes to be seen anywhere
    - In fluids – very few
    - In tissues – not east to sample



Direct Detection

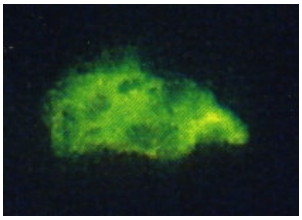
- Culture
  - Relatively difficult
  - Few labs able to do it routinely
  - Slow response time – 2 or more weeks
  - Definitive if positive
  - Often easiest from EM skin rash

The Spirochete in Culture

(Borellia burgdorferi – Bb cultured at Matman Labs 6/02 – closed in 2002. Bowen labs may be doing cultures in FL)

- Photomicrograph of spirochete and pleomorphic mass cultured from blood (so-called **cystic form** a no-cell-wall form)

After 12 weeks of oral and 6 weeks of IV antibiotics



PCR test for Borrelia DNA

- Positive if positive result
- 100% selectivity, but only 10% sensitivity in blood
  - Means only 1 in 10 blood PCR tests are positive for infected patients
  - Lyme spirochetes survive best in tissues not fluids
- Not officially accepted by the FDA or CDC as positive for Lyme disease – considered experimental. This is RIDICULOUS!

Antigen tests

- IGeneX Inc. has developed a way to detect parts of the spirochete in urine and serum
- Not approved (yet) by FDA – considered experimental

See www.igenex.com

Other Tick Transmitted Diseases

- Bartonella, Babesiosis, Ehrlichiosis (HME), Anaplasma (HGE), Rocky Mountain
- Antibody tests are generally more reliable than Lyme tests
- Babesiosis IFA test less sensitive than Fluorescent In-Situ Hybridization (FISH) of Igenex labs by 100X (but not approved by the FDA yet)
- PCR tests similar to Lyme – high selectivity but low sensitivity except maybe Babesiosis
  - Babesia microti target red blood cells and are easier to find

Summary

- Lyme disease is a clinical diagnosis
- Tests serve to support a diagnosis
- Many people do not test positive
  - Some turn positive after antibiotic treatment
  - Depends on one's immune system response
- Tests cannot PROVE you DO NOT have Lyme disease
- PCR **can prove** you have it, but…

The End

- Slides Presented by Douglas W. Fearn and Harvey L. Kliman, Ph.D. at the monthly LDASEPA meeting on July 20, 2005.