HOW TO CHOOSE TESTS FOR YOUR LYME DISEASE PATIENTS

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Brief history of Lyme disease

- 1970s- community outbreak of neuropsychiatric symptoms, arthritis and facial palsy in children and adults in Lyme Connecticut
- In the early 1980s NIH tick researcher Willi Burgdorfer discovered a spirochete in the ticks he collected from Eastern Long Island, NY
- This was determined to be the organism that causes Lyme and was named Borrelia burgdorferi in honor of Willi
- By 1982-3 simple serologies were developed as a diagnostic test based upon this NY strain
- This strain, collected from a tick (not a human), now lab-adapted, is the basis of all FDA-approved Lyme serologic test kits used by big commercial labs **(Lab strain "B31")**
- However, many patients who fit the clinical picture of Lyme did not have a positive test (were seronegative)

Testing based upon a single strain of a single species from a NY tick does **not** reflect the true diversity of Lyme pathogens across the USA

Co-infections were not even considered

DIVERSITY OF TICK-BORNE PATHOGENS
Clinical "Lyme" is more than a Borrelia infection

A 2018 study of 10,000+ patient samples tested at IGeneX:

- 37.3% were positive for Babesia species
- 32.1% for Lyme Borrelia
- 27.7% for TBRF Borrelia
- 19.1% for Bartonella
- 16.7% for Anaplasma
- 12.8% for Rickettsia
- 6.9% for Ehrlichia

Concurrent infections

- 40% tested positive for two pathogens
- 15% tested positive for three
- 4.6% tested positive for four
- 0.7% tested positive for five

PART ONE:
BASICS OF TESTING

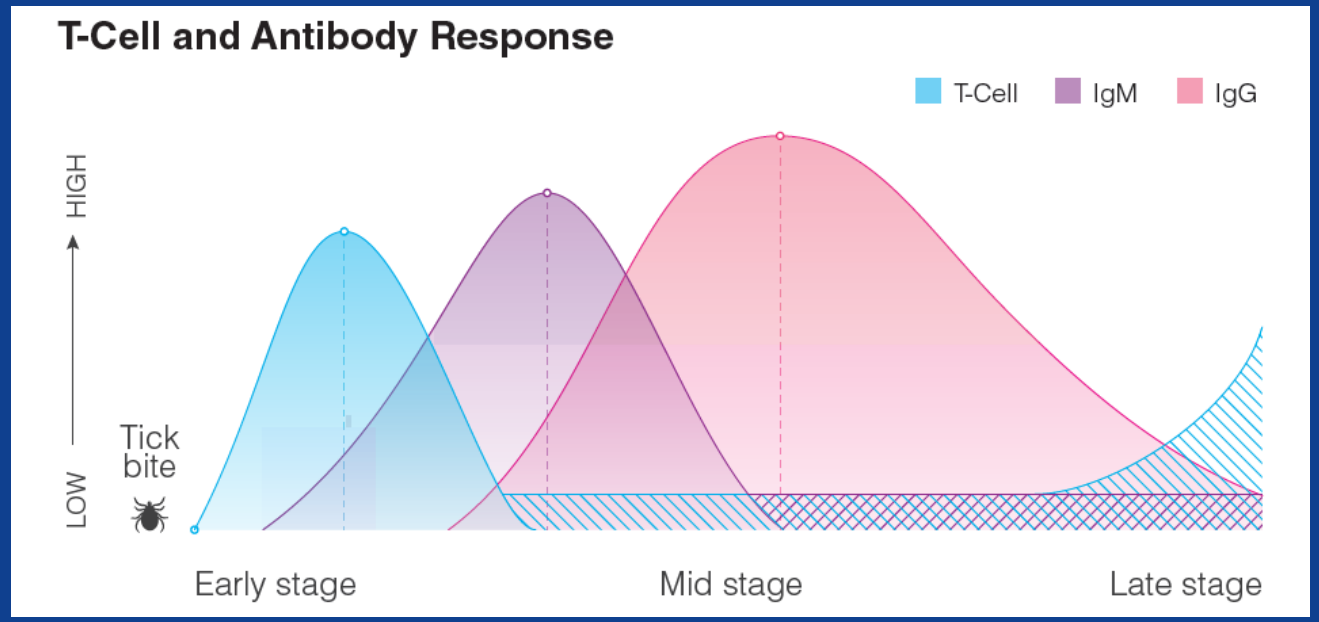
Indirect testing methods

Designed to detect host reactivity to an infection

- Humoral immunity (B-cells): measure antibody levels (serologies)
- Cellular immunity (T-cells): measure activation of T-cells after exposure to an antigen
- A positive result generally indicates prior exposure and not necessarily an active infection
- However B-cell and T-cell responses can vary with treatment so serial testing may be helpful-
  - Detectable antibody levels initially rise with helpful treatments, level off during ongoing treatment, then diminish as the patient recovers.
  - Full recovery results in a negative serology over time; a persisting positive serology may be cause for concern

Time course of immune response

- T-cells respond first, then tapers off, but may again respond late into the illness
- IgM appears next; may persist in a subset of patients but can rise again in late disease
- IgG appears last and while usually present, may not appear if IgM persists



A small percentage of patients produce T-cells during the middle stage, with a spike in the late stage of the disease

A small percentage of patients produce IgM antibodies throughout the entire stage of the disease.

Indirect testing methods

Except for early treatment of the initial infection, antimicrobial treatments will generally not affect results

- Exception is in advanced disease with high pathogen loads- antigen excess and resulting immune complexes can trap and mask free antibody. Here, effective antimicrobial treatments will decrease pathogen load and free up antibodies
- Seroconversion seen in 36% after treatment effective enough to impact symptoms
- This applies to serologies and not to T-cell based assays

Serologies are often the first choice

Because serologies are readily available and can give results quickly, they are the most commonly prescribed tests

- Potential shortcomings of serologic tests have been mostly overcome with the advanced testing methods now available from IGeneX, thanks to:
  - Recombinant technology
  - Extensive research
  - Recognition of the wide diversity of organisms, their many species and even subspecies, and their impact on test design
  - Ongoing validation and improvements thanks to recognition that clinical samples often are more useful and valid than commercially supplied vials of unknowns!!

Types of serologies

Standard, old-tech

- IFA- Immunofluorescent assay.
  - Organisms are bound to a slide, then patient serum is added. If antibodies are present, they will bind to the fixed organism. Then a fluorescent dye is added that binds to the human antibodies. Positives are detected by visualizing this fluorescence.
  - Manually read- not very exacting and subject to technical issues and operator error
  - False positives and false negatives- sensitivity varies from 50%-70%, specificity can be less than 75%

Single species per test!!

Types of serologies

Standard, old-tech

- ELISA- Enzyme linked immunosorbent assay
  - This is basically an automated IFA, read by a machine
  - More consistent but not a major advance in accuracy
  - Single species
- Microarrays
  - Basically is an ELISA but may use multiple wells to test an individual specimen.
  - Usually single species

Accuracy of both is similar to the IFA- sensitivity 50% to 70%, specificity varies, often 70%-80%

Types of serologies

Western blot

- Full complement of antigens from lysed lab strains are separated on a paper strip using electrophoresis
- Result is a “band” or dark spot on the test paper
  - Intensity of the band is dependent on antibody level and on the health of donor culture- can cause variable and inconsistent results
  - Location of the band is how the antibodies are identified- but electrophoresis is inexact, and a lysed organism releases many nonspecific antigens- each of these compromises accuracy
- Accuracy is not much better than an ELISA- sensitivity 50% to 70%, specificity varies, often 70%-80%

Single species!

Advanced serologic tests from IGeneX

- Lyme Screen Assay (LSA)- first-tier screening test to be used in conjunction with the ImmunoBlot for Lyme Borrelia
  - Uses recombinant antigens known to be present in most pathogenic Lyme Borrelia
  - Replaces the IFA- simple yes-no result
  - Multispecies for Lyme- Bb sl
  - Far more sensitive (89%) and specific (94%) than IFA
- Broad Coverage Assay (BCA)- completely replaces the ELISA for Lyme and TBRF Borrelia
  - Recombinant technology
  - Can be a stand-alone diagnostic test
  - Simple yes-no result; will not identify species or separate out IgM and IgG
  - More sensitive (87%) and specific (91%) than the ELISA
  - Multiple species included for Lyme and TBRF Borrelia
  - Detects disseminated and late-stage disease

The IGeneX ImmunoBlot

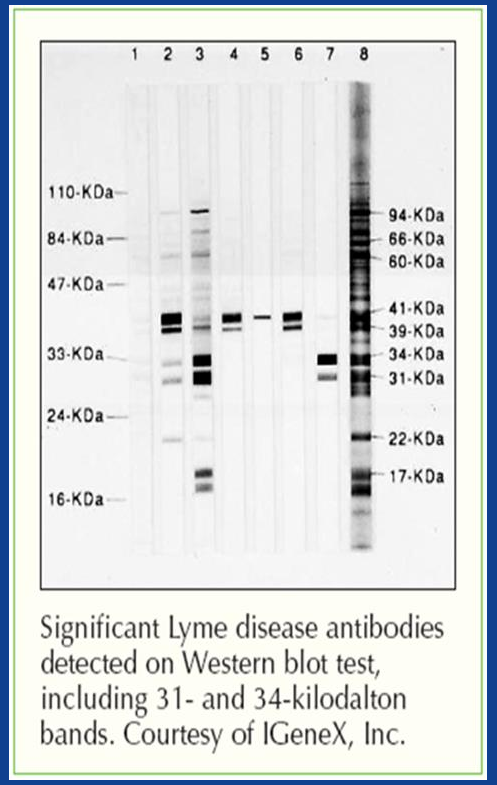
ImmunoBlot- The most inclusive and accurate serological test

- Includes a large number of recombinant antigens from multiple species and strains to ensure most comprehensive detection of pathogens and highest accuracy
- Separately reports IgM and IgG
- Will identify species in most cases
- “Unspeciated” results are true positives- inability to identify species usually is the result of multiple species being present, or presence of an atypical organism or an atypical host response
- So highly sensitive, is useful for even early Lyme Borreliosis (93% overall sensitivity using CDC “early Lyme” blinded specimens)
- Typical validation results- sensitivity 90% to 100%; specificity 97%-99%

Available for Lyme Borrelia, TBRF, Babesia and Bartonella

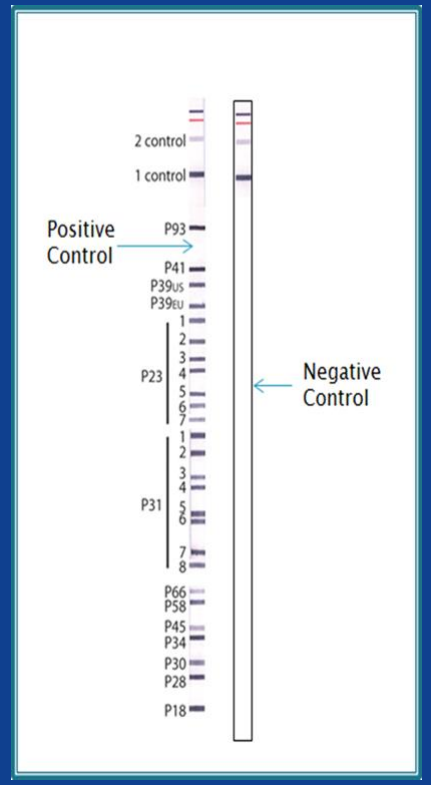
Western Blot vs. ImmunoBlot

Lyme WB- Based upon lysed cultures and electrophoresis- imprecise



Significant Lyme disease antibodies detected on Western blot test, including 31- and 34-kilodalton bands. Courtesy of IGeneX, Inc.

Lyme ImmunoBlot- Prints recombinant antigens onto the strip- precise location and quantity



Indirect test- T-cell response assay

IGeneX IGXSpot T-cell response assay measures cellular response

- A positive result indicates exposure to a pathogen
- The cellular immune response develops much earlier than the humoral response and the two are independent of each other
- Some patients remain sero-negative or seroconvert much later in chronic disease, but the T-cell response can be robust
- Thus, the IgXSpot is recommended for detection of very early infection, chronic disease, and in seronegativity
- When combined with the ImmunoBlot one gets a fuller picture of overall immune responsiveness

Available for Lyme Borrelia and Bartonella

Direct testing methods

Designed to detect the organism or its components

- A positive result generally indicates active infection
  - Tissue PCR may be the exception
- Best to do before treatment, or during symptomatic flares especially during treatment pauses
  - Antimicrobial therapy may decrease pathogen load and can decrease test sensitivity
  - Exception is the Lyme Dot-blot Assay urine antigen detection test
- When sensitivity is greater:
  - Very early in the infection before full immunity develops
  - In the immunocompromised
  - In more severe infections

Direct test- blood smear

Blood smear (in-hospital test for acute Babesia only)

- Giemsa-stained blood smear
- Not very sensitive- Limit of detection (LOD) is 0.5%
  - Means will detect organisms only when greater than ½ % of RBCs are infected
- Useful only during the first week of infection- after then, level of parasitemia decreases
- Will detect dead organisms- they can persist for a time after treatment
- Occasional confusion with platelets- specificity is operator-dependent
- Labor intensive and time consuming

Direct test- FISH

FISH- (fluorescent in-situ hybridization assay)- qualitative detection of ribosomal RNA of the pathogen directly on a blood smear

- Unlike a giemsa stain, the test uses fluorescent nucleic acid probes which bind to RNA sequences of the pathogen
- Limit of detection (LOD) is 0.001%
- Can identify organisms even if embedded in a biofilm!
- Since RNA doesn't persist after death of the organism, a positive result is a strong indicator of an active infection
- Genus-level detection to broaden species coverage

For Babesia and Bartonella

Direct test- PCR

PCR- detects the pathogen's nucleic acids (usually DNA) in a specimen

- Very low sensitivity in blood samples due to low numbers of organisms, PCR inhibitors, etc.
- However is very highly specific
- IGeneX improves test sensitivity by testing both whole blood and serum, and for Borrelia, is designed to detect both chromosomal and plasmid DNA
- Genus-level detection to broaden species coverage
- Can also test other body fluids (CSF, urine, etc.)
- Reasonable test for solid tissues (biopsies)- yield is higher than blood

Available for Lyme and TBRF Borrelia, Babesia, Bartonella, Anaplasma, Ehrlichia and the Rickettsias

Direct test- culturing- IGeneX cePCR

cePCR Culture

- Culture is always the Gold Standard, if done correctly
- cePCR is a two-week culture. The cultured organisms are then identified by a highly advanced PCR that has been 100% specific in validation studies using sequencing for confirmation
- Genus-level assay that ensures complete species coverage, including atypicals
- Sensitivity is at least a whole order of magnitude better than a plain PCR

Available for all the major tick-borne diseases except RMSF

Direct test- urine antigen detection

Lyme Dot-blot Assay (LDA)- urine antigen detection assay

Available for Lyme Borrelia (Bb sl)

- During active infection, Borrelia antigens and even whole organisms can be found in the urine
- Antigen spillage is intermittent, but generally is maximal during symptom flares, Herxheimer reactions and the menstrual cycle just before and during flow
  - PROTOCOL: for initial diagnosis, antibiotics on days 1-5; collect urine (3 specimens) on days 2, 4 and 6. For subsequent diagnosis, collect three samples during a symptom flare

Cross reacts with Leptospirosis and UTIs (routine urine culture is recommended when the Lyme specimen is collected)

New test news from IGeneX!!

AcuDart

- At-home Lyme disease detection test

iDart

- FDA-cleared Lyme IgG ImmunoBlot kit

AcuDart

Highly accurate at-home blood test kit for Lyme

- Based upon the IGeneX Broad Coverage Assay for Lyme Borrelia
  - Multi-species- Bb sl
  - Simple yes-no, but does not speciate or separately report IgM and IgG

Details:

- Patient can buy the test kit on-line from www.acudarthealth.com
- Fingerstick- then send it to IGeneX for processing
- Great for getting results while new patients are waiting for a visit, for testing between visits, and evaluating apparent recurrences of symptoms
- Positive results can then be confirmed and expanded upon by getting an ImmunoBlot
- Bulk pricing discounts available for practitioners

iDart FDA-cleared IgG Lyme ImmunoBlot

IGeneX's Laboratory Developed 2-Tier Lyme ImmunoBlot IgM and IgG Tests, approved by NYS, have been converted into the iDart Lyme ImmunoBlot IgM and IgG test kits. The iDart Lyme IgG ImmunoBlot Kit has been FDA-cleared as a stand alone test.

- Is a two-tier test!!
  - The kit includes the Lyme Screen Assay antigens band (LSA) to serve as tier-one
  - Then the ImmunoBlot serves as tier-two
- The ONLY FDA-cleared Lyme test kit that includes bands 31 and 34
- Interpreted using the IGeneX criteria, not the CDC/Dearborn criteria
  - Positive: if LSA is positive, and one or more bands from the following 2 or more groups: (P23, P31, P34, P39, P41 and P93) are present on the ImmunoBlot
- Will be available for New York State residents

iDart Lyme IgG ImmunoBlot

IGeneX Lyme ImmunoBlot interpretation criteria

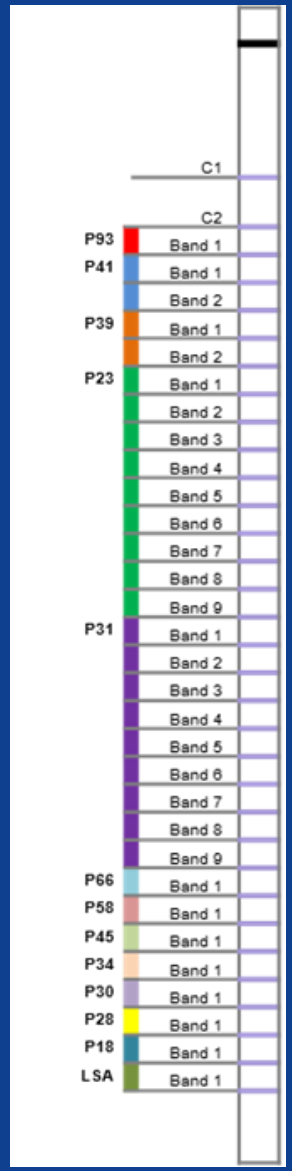
- Positive if LSA is positive
- and
- One or more bands from the following 2 or more groups: (P23, P31, P34, P39, P41 and P93) are present on the ImmunoBlot

Notice these “groups”-

- NINE different P31s
- NINE different P23s
- TWO P39s
- TWO P41s
- TWO different controls

Total of THIRTY ONE bands plus the controls

This is what makes the ImmunoBlot so much better than all other serologies



PART TWO:
APPLYING THIS TO PATIENT CARE

When to do which test….

Lyme Borreliosis- ALWAYS CONSIDER TBRF!!

Lyme: B. Burgdorferi *senso lato* (Bb sl)

B. burgdorferi B31 (Bb ss)

B. burgdorferi 297

B. californiensis

B. mayonii

B. afzelii

B. garinii

B. spielmanii

B. valaisiana

Tick-borne relapsing fever Borrelia (TBRF)

B. hermsii

B. miyamotoi

B. turcica

B. turicatae

B. coriaceae

B. parkeri

B. texasensis

- Species in red represent those that large commercial labs test for
- But the rest are also infecting USA patients and are included in IGeneX testing

Borrelia testing

Early disease (atypical EM, “summer flu”, etc.)

- ImmunoBlot- Both Lyme and TBRF- 90%-93% sensitivity
- IGXSpot T-cell response assay- available for Lyme Borrelia
- If immune compromised- cePCR (spirochetemia is higher early, before immune response matures)- Lyme and TBRF Borrelia

Disseminated but not chronic, with intact immunity

- ImmunoBlot; adding cePCR will increase yield
- Newborns and patients with access issues- Lyme Dot-Blot assay (urine antigen test)

Borrelia testing

Chronic Lyme, prominent co-infections and immunocompromised

- ImmunoBlot + cePCR culture
  - Studies show that combining a direct test with an indirect test will increase yield significantly in this patient group
- ImmunoBlot + IGXSpot T-cell response assay
  - Gives info on both arms of the immune response
- Always reasonable to add the Lyme Dot-Blot Assay urine antigen test if situation favors it- ongoing symptoms with episodic flares. Test when symptoms are increasing

Babesia testing

- Babesia are notoriously difficult to detect by lab testing
  - Circulating organisms rapidly decrease in number- sequestration in organs and blood vessels- impairs direct testing
  - Immune response can be suppressed- impairs indirect tests
  - Multiple species exist so broadest coverage is required
  - Solution is to combine methods to achieve highest yield
- Initial infection
  - Direct blood smear useful during the first week; FISH is far better
  - ImmunoBlot reactivity may appear within the first two weeks
  - cePCR culture can give high yield and also gives broadest range of species detection

Babesia testing

Disseminated and chronic infections

- Here, most sensitive approach is to combine ImmunoBlot + FISH + cePCR
  - Even so, cases may be missed
- Tricks to increase yield
  - If Borrelia are also present, treating this effectively will improve immune status so repeat testing for Babesia can uncover seronegative Babesiosis
  - Likewise, if clinical diagnosis warrants Babesia treatment, as the infection lessens and the immune system heals, then seronegative Babesiosis may seroconvert
  - Use of fibrinolytics may release sequestered Babesia, making FISH and cePCR more sensitive

Bartonella testing

Bartonella are considered “stealth pathogens”, meaning that they can be difficult to detect with routine testing

- Notorious cause of “fever of unknown origin” and “culture-negative endocarditis”
- Over 40 species exist and co-infections with two or more Bartonella can occur in an individual patient
- Just as with Borrelia and Babesia, this infection can be immunosuppressive, rendering serologies less sensitive
- Routine blood PCR testing has a dismal sensitivity of only 6%!

Bartonella testing

All stages of Bartonellosis require comprehensive testing to document infection

- Always test with ImmunoBlot
  - Broad species coverage and major species will be named
  - Excellent sensitivity
  - Shows immune reactivity to the infection
  - Adding the IGXSpot T-cell reactivity test adds further info on immunity
- Adding direct tests will increase yield, especially in advanced disease and chronic infections:
  - FISH and cePCR culture- highly sensitive, these also offer genus-level detection to expand the ability to detect atypical species

Rickettsia family

Labs are seeing an increase in incidence of all of the Rickettsias!

- Acute infection can be rapidly fatal, but also chronic infections have been documented
- Nonspecific testing clues- low WBCs, low platelets, and elevated LFTs

Ehrlichia, Anaplasma and Rickettsias

- Serology (IFA) and the cePCR culture are available

Rocky Mountain Spotted Fever

- Serology (IFA) and standard PCR (culturing not allowed unless lab is certified for Biosafety Level 4)

Best advice is to use all available methods when testing for these

Pregnancy and the TBDs

Potentially a double infection! Mother and baby

- All the major TBDs can cross the placenta and infect the child
  - However, placentas may block this transfer of pathogens
- Mother- MUST maximize testing
  - Immunoblots + cePCR culture + FISH if applicable
- Baby- Can avoid difficult and traumatizing blood draws by testing urine
  - cePCR culture of urine at birth and at any time suspicious symptoms or signs appear (can test for Lyme, TBRF, Bartonella, Babesia and the Rickettsias)
  - Dot-blot Assay Lyme urine antigen test at birth and if negative, monthly for 6 to 12 months (Lyme)
- Placenta- microscopy (pathologist), PCR and cePCR cultures

Can testing indicate whether treatment is working?

While there is no test for cure, there are ways to judge treatment effectiveness

- During treatment, serologic response will initially INCREASE if medications are lowering pathogen load
- Later, serologic response will level off
- If there has been only an IgM response, appearance of IgG is a sign of improving health and recovery
- After treatment ends, expect antibody levels to diminish and eventually disappear. A persisting positive serology usually indicates persistence of pathogens


Thank you!
Now time for Q&A