REFLEX TESTING FOR BORRELIOSIS

Joseph J. Burrascano Jr. M.D.

Jyotsna Shah, PhD

PRESENTATION TOPICS

- OVERVIEW OF BORRELIOSIS- *IT IS MORE THAN JUST LYME DISEASE*
  - DETAILED OVERVIEW OF BORRELIA SPECIES IN NORTH AMERICA
- BORRELIA TESTING
  - LIMITATIONS OF FDA-TEST KIT SEROLOGIES
  - THE PITFALLS OF THE CDC-RECOMMENDED CRITERIA
- REFLEX TESTING: MANAGING SERONEGATIVITY
  - FOR LYME DISEASE
  - FOR TICK-BORNE RELAPSING FEVER
  - IMPORTANCE OF TESTING FOR ALL BORRELIA (LD AND TBRF) AT THE SAME TIME
- RECOMMENDED TESTS AND TEST PANELS

BORRELIOSIS

PREVALENT AND SERIOUS!

- Potentially can become a chronic, debilitating illness
- Affects CNS, PNS, joints, heart, GI tract, cognition, mood and affect
- Can impair immunity and lead to opportunistic infections and worsen co-infections
- Can lead to an autoinflammatory state that mimics autoimmune disease
- Can impair detox pathways, causing the buildup of mycotoxins, heavy metals, pesticides, herbicides and more
- Can have a major negative impact on relationships- family, friends, work, school

So clearly we need to be able to secure a firm diagnosis

HISTORICALLY, LYME TESTING SEEMINGLY MISSED MANY CASES

- Clinically, we see and diagnose "seronegative Lyme"
- Many publications document that Lyme tests based upon FDA-test kits miss, on average, 50% of clinically defined cases
- Clearly lab diagnostics should perform better than a coin toss!

Study/Year	Sensitivity	Specificity
Schmitz et al, 1993	66%	100%
Engstrom et al, 1995	55%	96%
Ledue et al, 1996	50%	100%
Bakken et al. 1997	75%	81%
Trevejo et al, 1999	29%	100%
Nowakowski et al, 2001	66%	99%
Bacon et al, 2003	68%	99%
Coulter et al, 2005	18%	-
Wormser et al, 2008	14.1%	-

SO, WHAT IS REFLEX TESTING?

Reflex testing refers to how to handle borderline and seronegative results

Seronegativity is prevalent and a negative result does not rule out infection

- Know why it happens and what it may represent
- Know which tests to do when basic/screening tests are negative
- Understand what the newer testing technology offers and when to do what

WHY HAVE LYME TESTS BEEN SO INSENSITIVE?

THE USUAL, OBVIOUS REASONS:

Serologies must accept a tradeoff between sensitivity and specificity

- SENSITIVITY: Can someone have Lyme despite a negative test?
  - Impaired immunity is known to occur in advanced cases of Lyme, so the serum antibody levels may be too low to be detected by FDA-test kits
  - Immune complexes may form and trap antibody, potentially causing free antibody levels to be too low for these tests to detect
- SPECIFICITY: How sure are you that a positive result is a true positive?
  - Because non-Borrelia antibodies may be detected by these FDA-test kits, sensitivity cannot be turned up to overcome the above limitations

TEST INSENSITIVITY-
THE NOT SO OBVIOUS REASON #1

There are many more Lyme Borrelia out there infecting our patients!

- Borrelia burgdorferi senso lato
  - B. burgdorferi s.s. Strains B31 (tick-derived lab strain) and 297 (human strain)
  - B. afzelii
  - B. garinii
  - B. mayonii
  - B. californiensis
  - B. spielmanii
  - B. valaisiana
  - Potentially more….
- People and their pets travel, and birds transport exotic tick species

TEST INSENSITIVITY-
THE NOT SO OBVIOUS REASON #2

A WHOLE OTHER GROUP OF BORRELIA ARE CAUSING A SIGNIFICANT NUMBER OF LYME-LIKE ILLNESSES THAT ARE ALSO NOT BEING PICKED UP BY CONVENTIONAL LYME TESTS

TICK-BORNE RELAPSING FEVER!!

- B. hermsii
- B. miyamotoi
- B. turcica-like
- B. turicatae
- B. texasensis
- B. coriaceae
- …others possible

- The big news is that TBRF can present as a Lyme-like illness
- In fact, the classic relapsing-fever picture may be UNCOMMON!

YES, TBRF SPECIES ARE BEING FOUND IN NORTH AMERICAN PATIENTS

Lyme Disease: Diversity of Borrelia Species in California and Mexico Detected Using a Novel Immunoblot Assay. Fesler, MC et. Al. Healthcare 2020, 8,97; doi:10.3390/healthcare8020097

90 patients who met the clinical case definition of CHRONIC LYME DISEASE (not suspected to have TBRF)

- 46 positive for Bb s.l.
  - Bb s.s., B. californiensis, B. spielmanii, B. afzelii, B. garinii plus other Bb s.l. species that could not be identified
- 56 positive for TBRF- more than half!!!
  - B. hermsii, B. miyamotoi, B. turicatae, B. turcica, plus other TBRF species that could not be identified
- 8 positive for both Bb s.l. and TBRF

LYME DISEASE
Multiple species are present and are being missed

Results of Several Clinical Series

ACTUAL PATIENT SERIES: #1

36 seronegative patients from a single client: IGeneX Lyme ImmunoBlots were performed

- ImmunoBlot testing detected ten cases of B. mayonii, six each of B. spielmanii and B. californiensis, five B. garinii, two B. afzelii, one B. valaisiana and only one case of B. burgdorferi strain B31. Five cases could not be speciated.
- All of these patients are US citizens; fifteen of them have travelled widely
- Of all 36, only a single case of Bb s.s. B31 was found!
- Standard American FDA-test kit serologies are based upon this single species: B31

It is not surprising that so many were missed by FDA-test kit serologies

ACTUAL PATIENT SERIES: #2

- In another recent analysis of 39 client samples from American patients, Lyme ImmunoBlots detected:
- Eleven cases of B. spielmanii, six B. californiensis, three B. afzelii, two B. mayonii and two Bb ss strain B31
- There were four cases in which the ImmunoBlot identified unspeciated European Lyme Borrelia and eleven in which unspeciated Bb sl were found

If standard FDA-test kit serologies were performed as the sole serologic test, likely only the two B31 cases and possibly some of the Bb sl cases would have been identified. The rest of the 39 would have been incorrectly classified as negative (false negatives).

ACTUAL PATIENT SERIES: #3

- A total of eleven clinical Lyme cases were studied
- Of the eleven, seven had antibodies to B. spielmanii, four to B. californiensis, and one each to B. mayonii and Bb ss
- In this series as in the first one, the reason the total number of species is greater than the number of patients tested is because of co-infection with multiple Borrelia species
- In this series, two were seemingly co-infected with two species- one had antibodies to B. spielmanii plus B. mayonii, and one had them to Bb ss plus B. californiensis

If standard serologies were performed, not only is it possible that the majority of the results would have been falsely negative, the presence of Borrelia co-infections would have been totally missed.

WHAT ARE THESE OTHER LYME BORRELIA?

- B. valaisiana- Closest to afzelii; mainly Europe and far east; resists C'-mediated killing by a novel mechanism; has been detected in human CSF in patient with spastic paraparesis
- B. californiensis- Closest to B. americana and B. andersonii; Ixodes pacificus; California kangaroo rat; also found in Brazil
- B. mayonii- Closest to European species B. valaisiana and B. bisettii; patients (blood and synovial fluid), white-footed mice, red squirrels, and scapularis ticks in upper Midwest; resists C'-mediated killing; higher levels of spirochetemia; diffuse macular rash; nausea and vomiting; early neurological involvement
- B. spielmanii- Previously thought to be only found in Europe; ticks, mice and rats; can cause an EM rash (detected in skin biopsy of an EM); Three new types of ospC and a unique ospA; 12 plasmids (increased pathogenicity and variability)

TICK-BORNE RELAPSING FEVER

TBRF is Being Confused With Lyme Disease

The Classical Description of TBRF Can Be Misleading!

CLINICAL PRESENTATION OF "CLASSIC" TBRF

- "Recurring febrile episodes that last ~3 days and are separated by afebrile periods of ~7 days duration."
- "Each febrile episode involves a "crisis." During the "chill phase" of the crisis, patients develop very high fever (up to 106.7°F) and may become delirious, agitated, tachycardic and tachypneic. Duration is 10 to 30 minutes."
- "This phase is followed by the "flush phase", characterized by drenching sweats and a rapid decrease in body temperature. During the flush phase, patients may become transiently hypotensive. Overall, patients who are not treated will experience several episodes of fever before illness resolves."

BUT CAN ALSO PRESENT LIKE LYME

543 US PATIENTS WITH SUSPECTED LYME:

- 29% were positive for Ab to TBRF (even though only tested for only 2 species)

Cohort of 321 California residents:

- 38% were positive for Ab to TBRF (tested for only 2 species)

These patients did not have the "classic" acute TBRF presentation. Clinically, they resembled Lyme patients

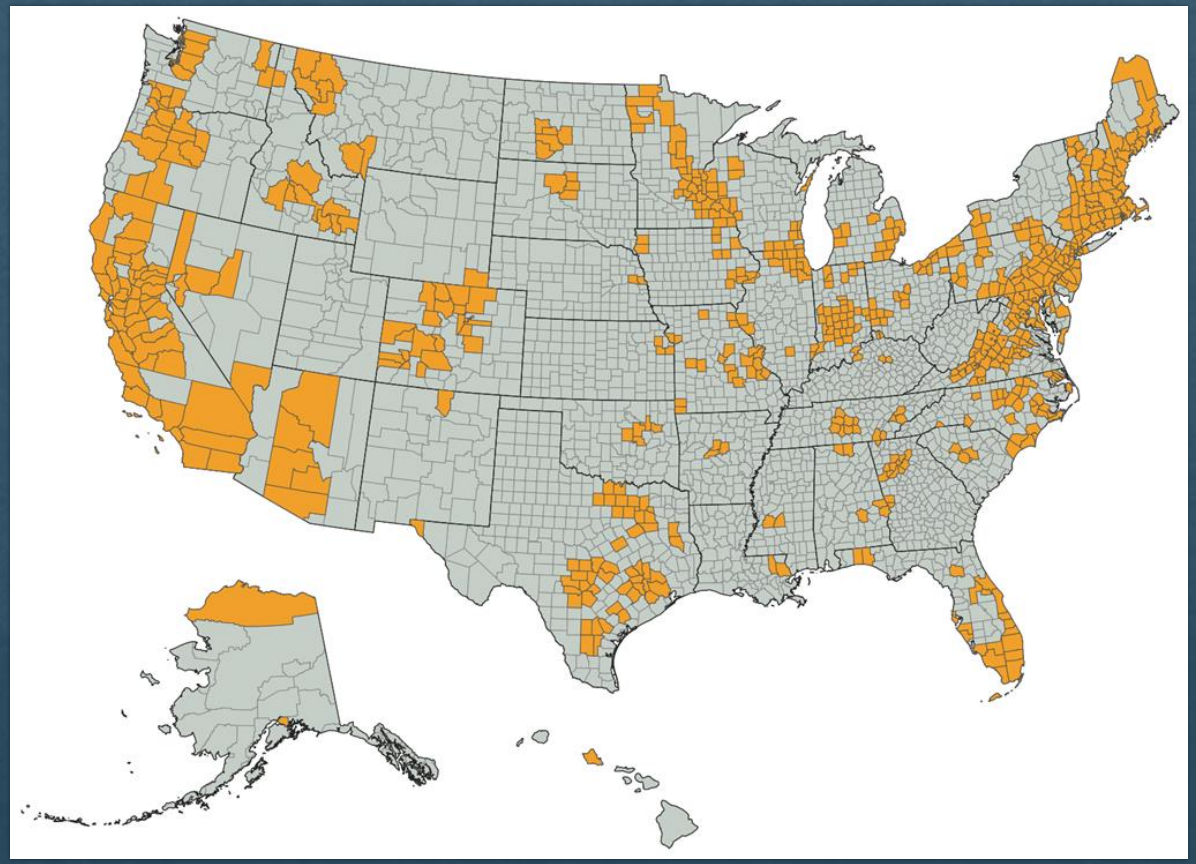
ANOTHER CAUSE OF "SERO-NEGATIVE LYME"?

DO WE NEED TO WORRY ABOUT TBRF?

Relapsing fever Borrelia in California: a pilot serological study. 2018. Middelveen, M.J. et. al.

- "In the USA, several species of RFB have been reported to cause disease in humans, including B. miyamotoi, B. hermsii, B. lonestari, B. parkeri, and B. turicatae, with most cases occurring in the western USA."
- "In the state of California, B. miyamotoi, B. hermsii, and B. parkeri have been shown to infect humans, and a fourth Borrelia species, B. coriaceae, infects ticks found in that state, although human infection has not yet been identified."

DO WE NEED TO WORRY ABOUT TBRF?



DO WE NEED TO WORRY ABOUT TBRF?

- "TBRF is typically considered a disease of outdoor enthusiasts and impoverished persons living in primitive conditions"
- "However, our study suggests emergence of B. turicatae in metropolitan areas"
- "Evidence indicates the endemicity of O. turicatae ticks in San Antonio, Dallas, and Austin, the seventh, ninth, and eleventh largest cities in the United States"
- "The University of Tennessee reported that in 2009, during fall hunting season, 58% of turkeys tested positive for B. miyamotoi"

TBRF: SOFT TICK VECTOR

ORNITHODOROS TICKS

- Do not live in the grass- live in crevices which can include wood cracks, leaf litter, caves and small- and medium-size mammal nests and dens- indoors and outdoors
  - Campers, hikers, cave explorers
  - Rodent-infested homes and cabins
  - May emerge when you start a campfire, wood stove or simply turn on the heat!
- Ornithodoros- The perfect vector!!
  - Ability to survive for decades
  - Because of transovarial passage it may serve as its own reservoir, and not need to feed on an animal to acquire or maintain infection
  - Wow!

RAPID FEEDING, BRIEF ATTACHMENT

- Attached ticks are rarely seen because Ornithodoros ticks are rapid feeders- attach for only 5 to 30 minutes
- Bites are painless and go unnoticed
- Transmission of B. turicatae occurs within seconds of the tick bite
  - *Transmission Dynamics of Borrelia turicatae from the Arthropod Vector. William K. Boyle et al. PLOS Neglected Tropical Diseases. April 3, 2014. https://doi.org/10.1371/journal.pntd.0002767*
- Can feed multiple times
- After feeding, ticks return to their crevice

CAN CONFUSE IXODES WITH ORNITHODOROS

Engorged Ixodes

Ornithodoros

TBRF- IMPORTANT FACTS

- Maternal-fetal passage well recognized and accepted
- Spontaneous abortion, premature birth, and neonatal death
- Louse-borne RF (B. recurrentis) transmission via mucous membranes!!
- Some TBRF species are immune to complement-mediated killing- just like Lyme Borrelia
- Acute Respiratory Distress Syndrome has been associated with B. hermsii (CDC)
- Prolonged QT interval has been reported with TBRF infection

TBRF CLINICAL PEARLS

Really is a migratory, multisystem illness that waxes and wanes just like Lyme

However:

- Disease cycles may be every two weeks and not every four as in Lyme
- More likely to have a low grade afternoon fever than Lyme
- If a fever is present, is more likely to be higher (Lyme rarely goes above 99.2)
- Greater incidence of GI upset and abdominal pain
- Stronger Herxheimers are possible

Absolutely no information on potential differences in treatment requirements!!

- *Is up to YOU to determine this <u>and</u> report your findings*

OK, SO HOW DO WE TEST FOR ALL THESE PREVIOUSLY HIDDEN BORRELIA?

Must begin with a little history…..

EVOLUTION OF LYME TESTING

LYME TESTING BEGAN WITH SIMPLE, LOW-TECHNOLOGY IMMUNOFLUORESCENT ASSAYS ("IFA")

- The original IFA was found to be inaccurate- many false negatives and some false positives- a tradeoff between sensitivity and specificity
- ELISA (enzyme-linked immunoassay) was developed- automated the IFA technology so it could be run by machine rapidly and with more consistency- but still prone to false positives and negatives
- While the CDC and some commercial labs claim the ELISA is totally accurate, we know this not to be the case
- So western blotting was developed for Lyme in an effort to increase accuracy

CDC AND THE WESTERN BLOT-1

- In an effort to improve accuracy, western blotting was introduced- supplemented the quantitative ELISA with a qualitative method that allows for interpretation by lab and user
- To standardize testing and make it more accurate, the CDC did two things-
  - created interpretation criteria for the Lyme western blot
  - insisted upon “two-tier” testing
- CDC’s interpretation criteria for western blots were developed for epidemiologic surveillance, and not for clinical diagnosis
- Although never meant for clinical diagnosis, government agencies, insurance companies and academic institutions ignore this and misapply these criteria to this day!

CDC AND THE WESTERN BLOT-2

Western blots are interpreted by reporting “bands”. Bands reflect an immune response to individual bacterial antigens.

A convention was convened to create interpretation criteria to specify which bands are significant and which ones to ignore

Problems:

- They include bands which are NOT specific to Lyme Borrelia- this can give rise to false positives
- They EXCLUDE bands that are very specific to Lyme Borrelia- gives rise to false negatives
- These criteria intentionally left out two very important and specific bands (31 and 34) to accommodate the then-upcoming Lyme vaccine
- Controversial! Several participants disagreed and one even walked out!

The result is unacceptably low accuracy

CDC AND TWO-TIER TESTING-1

Ideally, a diagnostic test should be both highly sensitive and very specific.

- The idea behind two tier testing is to begin with a very sensitive screening test- very sensitive, therefore will not miss any cases, at the cost of some false positives
- If the screening test is positive, then follow it with a very specific second test to confirm true positives and exclude false ones
- If the first test is negative, then the whole test is called negative and the second tier will not be done
- For this to work, the first tier must be 99% sensitive, and the second tier should be as sensitive but also 95% specific

CDC AND TWO-TIER TESTING-2

- The CDC version of Lyme two-tier test is to begin with an ELISA as tier one, and then use a western blot as tier two.
- PROBLEMS
  - The sensitivity of the "standard" Lyme ELISA (big labs and FDA test kits) is no better than a coin toss!
  - So as many as half of the cases are missed. This would never be acceptable for any other illness- not breast cancer, not HIV-AIDS, not anything- but politics prevail
  - And because the western blot is interpreted using the faulty CDC criteria, which limits sensitivity, even if there is a positive ELISA, many cases will still be missed because critical bands were omitted from the western blot

INSENSITIVE TESTS AND LYME DENIALISM

- The result- many Lyme patients are told they do not have Lyme-
  - are given other, incorrect diagnoses, or are told to just live with their symptoms, or are told they need psychiatric help
- Insurance companies may deny covering treatment if there is not a positive test
  - The treating practitioner's clinical judgement is negated by an anonymous insurance worker (many not even MDs) who never spoke to or examined the patient
- If the practitioner diagnoses Lyme on clinical grounds despite negative tests, then state medical Boards can and have charged them with misconduct and doctors have lost their licenses! These cases may be initiated by insurance companies who want to eliminate "expensive" doctors

LABORATORY TESTING AND THE FDA

"FDA Approval" is simply a licensing procedure- it is not intended to be a sign of test validation

- Test licensing is only needed if the test is made into a "kit" that is sold to other labs
- In contrast, lab test validation is performed by CLIA and is further supported by Medicare
- Individual states each must certify a lab when it opens, and inspections occur on a regular basis. May include unannounced visits.

LACK OF FDA APPROVAL DOES NOT MEAN AN INFERIOR TEST!!

…but there's more…

WHAT ARE THESE FDA-LYME TEST KITS?

- FDA approval is based upon comparability to a test standard
- Their test standard is an ELISA based upon the lab strain B31
  - Lab strains are Borrelia kept alive in continuous cultures
  - Over time, cultured Borrelia lose plasmids and lose pathogenicity so they no longer represent what actually is infecting patients
  - Strain B31 was derived from a tick, not from a human being, from USA's east coast
  - Good bet that this strain is not representative of Borrelia from other geographic areas

So any new FDA-approved Lyme test kit has to be only as good as the current FDA-ELISA!!!!

LAB TESTING FOR TBRF ALSO HAS LIMITATIONS

SEROLOGIES

- Most TBRF express p41- may give rise to a borderline or false positive Lyme ELISA
  - On a Lyme western blot, a single band 41 in a suspected Lyme patient may represent an unexpected TBRF infection
  - OspC is present in several TBRF species- another potential reason for borderline or false-positive Lyme serologies
- Large commercial labs: test for B. hermsii only!
- Can get a GLP-protein based ELISA for B. miyamotoi, but GLP is only one protein antigen and therefore prone to false negatives (like having just one band on a western blot)

PCR

- Commercial, test-kit PCR-sensitivity is poor (LOD 5,000/ml to125,000/ml)
- Comparison: IGeneX TBRF PCR: LOD is far better (100/ml to 500/ml)

CAN "SERONEGATIVE LYME" BE TBRF?

YES! Since TBRF often presents clinically as Lyme disease, but is missed by standard Lyme tests, the possibility is that Lyme seronegativity may simply be due to testing for the wrong species!

- NONE of the commercial test-kit Lyme IFAs, ELISAs, western blots, PCRs or T-cell tests have been validated for all of the Lyme Borrelia, or for any of the TBRF Borrelia
- Similarly, TBRF serologic testing has only been validated against two species, and you still have to order these two tests individually (hermsii and miyamotoi)

SOLUTION:

1. Awareness
2. Appropriate testing

APPROPRIATE TESTING- THE BORRELIA IMMUNOBLOTS

EXCLUSIVE TO IGENEX

- These represent important advances in testing technology
- Capable of detecting AND identifying multiple species of Lyme Borrelia
  - Positive results in many previously seronegative patients
- Capable of detecting AND identifying multiple species of TBRF
  - Likewise, can pick up many missed cases and identify most of them
- More sensitive, more specific and greater positive and negative predictive values than any other serologic method
- Game-changing sensitivity in early Lyme…

IGENEX IMMUNOBLOT TECHNOLOGY

Uses recombinant antigens that are specific to multiple individual species

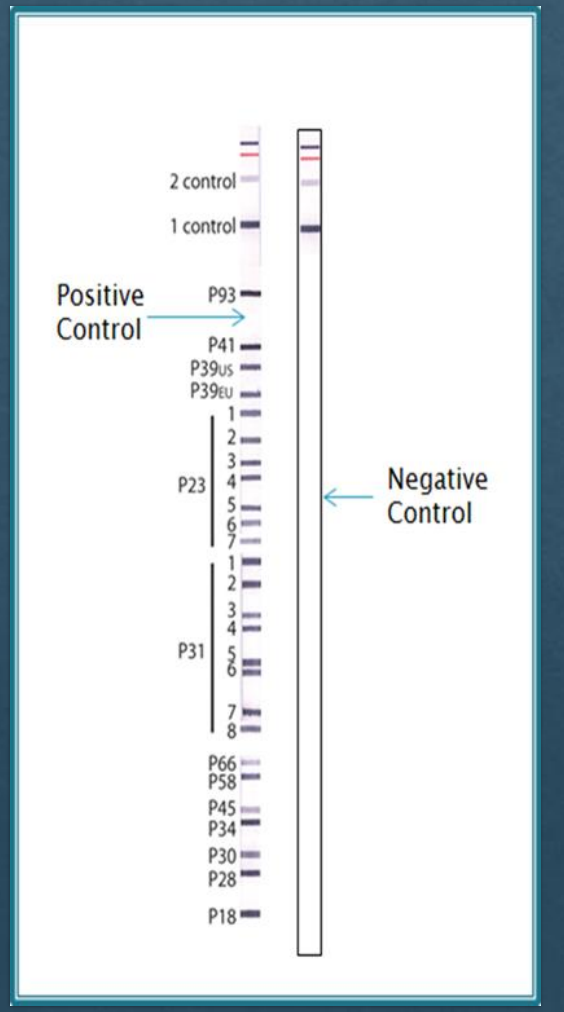
- A large variety of protein antigens are included
- Less likely to cross react with viruses, non-Borrelia bacteria and autoantigens
- Species-specific- no cross reactivity between RF and Lyme Borrelia

Unlike western blots, IGeneX ImmunoBlots deliver precise quantities of antigen to specific locations on the membrane- drastically improves accuracy and consistency

- Significantly increases real-world sensitivity
- Significantly increases specificity
- IgM and IgG

WHAT ARE RECOMBINANT ANTIGENS?

- Recombinant antigens are lab-created proteins that are identical to those of the pathogen being tested
- The DNA sequence of the bacterium that codes for this protein is inserted into and expressed by a host, often E. coli
- This results in the production of a pure protein antigen that can be used in the immunoblot
- In the IGeneX ImmunoBlots, many, many individual protein antigens are included to allow for this high degree of specificity, sensitivity, and breadth of species coverage



LYME IMMUNOBLOTS

So many advantages over all other serologic methods!

MAXIMAL SPECIFICITY

Samples	Negatives	Lyme IB (In-House)			Lyme IB (CDC)		
		IgM	IgG	IgM+IgG	IgM	IgG	IgM+IgG
CDC Set 1*	5	0	0	0	0	0	0
CDC Set 2*	20	0	1	1	0	0	0
PT Samples	11	0	0	0	0	0	0
Autoimmune	42	0	0	0	0	0	0
Viral Infections	46**	1	1	2	0	0	0
RPR +	28	0	2	2	0	1	1
Total False +		0	4	5	0	1	1
Total True -	152	151	148	147	152	151	151
Specificity %		99.3	97.4	96.7	100	99.3	99.3

*Western blot results were provided by CDC
**Out of 46 samples with antibodies to viruses, 11 had antibodies to CMV; 24 to EBV, 7 to HSV; and 4 to HCV
**Only 2 of 24 samples with antibodies to EBV were positive by Lyme IB
PT = proficiency test

LYME IB SENSITIVITY STUDIES

Samples Supplied by the CDC

Patients with:	n	2-tier Serological Testing for LD (ELISA follwed by Western blots)				Lyme ImmunoBlots		
		IgM	IgG	G+M		IgM	IgG	G+M
Early Lyme Acute (Stage 1)	15	20.0%	0.0%	20.0%		66.7%	46.7%	93.3%
Early Lyme Convalescent (Stage 1)	15	66.7%	33.3%	80.0%		86.7%	46.7%	100.0%
Neurological Lyme (Stage 2)	9	100.0%	55.6%	100.0%		100.0%	77.8%	100.0%
Lyme arthitis (Stage 3)	10	10.0%	100.0%	100.0%		30.0%	100.0%	100.0%
Total	49	46.9%	40.8%	69.4%		71.4%	63.3%	98.0%

GAME CHANGER!

IGeneX Lyme ImmunoBlot picked up 93% of early cases!!

No other test *of any kind* has been demonstrated to do this

- *Late-appearing IgM is significant (remember the 99.3+% specificity)*

IGENEX LYME IMMUNOBLOT: SUMMARY

Provides vital information not previously available

- Unprecedented specificity
- Unprecedented overall sensitivity
  - Helps detect presence of infection in patients who otherwise could have “seronegative Lyme”
- Game changing sensitivity in early Lyme
- Ability to identify multiple clinically relevant Bb s.l. species and Borrelia co-infections
  - Testing for these other species is NOT available anywhere else
- Does not cross-react with TBRF Borrelia
- Rapid turnover/quick results

IGENEX BROAD COVERAGE LYME ASSAY

Single serological test that covers multiple, clinically relevant Lyme Bb s.l. species

- Highly accurate replacement for standard serologies (IFA, ELISA)
- Single result includes IgM and IgG
- Simple yes-no results- makes interpretation easy
- But does not identify species- need to do an ImmunoBlot to get this info
- Cost effective
- Rapid turnover

IGENEX LYME PCR

BIG ADVANTAGE: The IGeneX Lyme PCR is GENUS-SPECIFIC

- This broad coverage has the ability to detect many different species of Bb s.l.
  - Large commercial labs- look for just a single species (Bb s.s., lab strain B31)
- IGeneX runs FOUR PCRs for Lyme with every sample:
  - Genomic and plasmid
  - Whole blood and serum
  - Massively increases real-world sensitivity
- Clinical tip: When to order PCRs
  - During early/acute stages of the disease
  - In the immunosuppressed
  - In the very ill, late stage patients

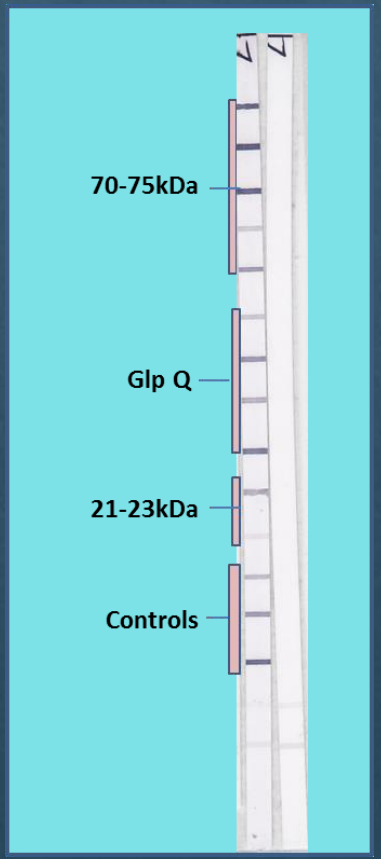
TBRF IMMUNOBLOTS

Inclusiveness and accuracy not available anywhere else

BETTER BY DESIGN

IGeneX TBRF ImmunoBlot uses multiple recombinant antigens:

- Able to detect the most commonly found TBRF species, not just B. hermsii or B. miyamotoi
- A large variety of protein antigens are included, not just one, as in large-lab testing for B. miyamotoi
- Significantly increases real-world sensitivity
- Significantly increases specificity
  - Less likely to cross react with viruses, non-Borrelia bacteria and autoantigens
  - Species-specific- no cross reactivity between RF and Lyme Borrelia
- IgM and IgG



EXAMPLE OF WHAT IS BEING SEEN

SUMMARY OF TBRF-POSITIVE PATIENTS (n=62)	
TBRF Species	**TBRF IB (+)**
B. hermsii	7
B. miyamotoi	13
B. turicatae	13
B. turcica-like	2
TBRF Borrelia species	26
B. hermsii + B. miyamotoi	1

TBRF IMMUNOBLOT SPECIFICITY

Sample Types	N	**IgM (+)**	**IgG (+)**	**IgM or IgG (+)**
Endemic area control	10	0	0	
Fibromyalgia	5	0	0	
Mononucleosis	9	0	0	
Multiple sclerosis	5	1	0	1
Non-endemic area control	14	0	0	
Periodontitis	5	0	0	
Rheumatoid arthritis	14	0	0	
Syphilis	13	1	2	2
HIV-1 infection	4	0	0	
Cytomegalovirus infection	5	0	1	1
Autoimmune and Allergy	33	1	0	1
Borrelia burgdorferi infection	12	0	0	
Bartonella henselae infection	7	0	0	
Human granulocytic anaplasmosis	16	0	0	
Babesia microti infection	14	0	0	
Babesia duncani Infection	41	0	0	
Human monocytic ehrlichiosis	5	0	0	
Total False (+)	0	**3**	**3**	**5**
Total True (-)	212	209	209	207
Specificity		**98.6%**	**98.6%**	**97.6%**

TBRF IMMUNOBLOT SENSITIVITY

TBRF ImmunoBlot: Sensitivity Study Summary 15 PCR + Patients- 7 patients: 2 samples/patient; 1 patient: 1 sample						
Sample Type	Number	IgM (+)	IgG (+)	IgM & IgG (+)	Total (+)	**Sensitivity**
1st Sample (acute)	15	7	1	2	10	**66.7%**
2nd Sample (convalescent	7	4	1	2	7	**100%**

PCR-Positive patients

- First sample refers to **early disease**, when serologies are expected to be nonreactive. Note however **the sensitivity of 66.7%**.
- Second sample, a **convalescent** sample, shows **100% sensitivity**
- IgM may be positive as early as two weeks after onset of infection
  - *Late-appearing IgM is significant (remember the 98.6% specificity)*

IGENEX TBRF IMMUNOBLOT: SUMMARY

Provides vital information not previously available

- Accurately identifies TBRF-
  - Especially important in diagnosing “Lyme-like illnesses”
  - Positive results for select cases of “seronegative Lyme”
- Useful in early disease
- Ability to identify multiple clinically relevant TBRF species
  - Maximizes sensitivity
  - Tests for species that is not available at any other lab
- Does not cross-react with Lyme Borrelia
- Rapid turnover

IGENEX BROAD COVERAGE TBRF ASSAY

Single serological test that covers multiple, clinically relevant TBRF species

- Highly accurate replacement for standard serologies
- Think of it as a better IFA or ELISA
- Single result includes IgM and IgG
- Simple yes-no results- makes interpretation easy
- But does not speciate- need an immunoblot to do this
- Cost effective
- Rapid turnover

IGENEX TBRF PCR

BIG ADVANTAGE: The IGeneX TBRF PCR is GENUS-SPECIFIC

- Therefore may detect many different species of TBRF
  - Large commercial labs- look for just a single species- B. miyamotoi
- Pos or Neg results- will only speciate if B. miyamotoi is present
- Clinical tip: When to order PCRs
  - During early/acute stages of the disease
  - In the immunosuppressed
  - Very ill, late stage patients

SUMMARY: SUGGESTED TESTING

TEST FOR BOTH LYME AND TBRF IN ALL YOUR PATIENTS

ImmunoBlot is always the first choice in patients with intact immunity

- Good for all stages of disease, with unprecedented sensitivity in early disease
- Late positive IgM is still significant!!

Broad Coverage assays

- Just like the ImmunoBlots, are very sensitive and specific, with extended species coverage
- Cost effective replacement for the ELISA and IFA

PCR

- **Add the PCR** to catch those who may not have detectable levels of free antibody
  - Very early disease; late-stage disease; immunocompromised
- Reciprocal relationship between serologies and PCR due to this immune system effect
- By doing both a serology and a PCR, you also get a picture of immune responsiveness

TESTING PANELS

- Designed to make ordering easy!
  - Combines the most popular tests into a single, easy to order choice
  - Good "memory jogger" so you are more likely to be complete in your assessments
- Also results in huge discounts over ordering tests individually

Some examples:

- LTP1- LYME/TBRF PANEL 1
  - Includes Lyme and TBRF Borrelia
  - Adds a screening IFA to satisfy two-tier requirements needed for some cases of insurance coverage
  - Saves $204.50 over ordering tests individually
- TBD6- TICK BORNE DISEASE PANEL 6
  - LB, TBRF, Bab microti and duncani, Bartonella, HME, HGA, R. rickettsia, R. typhi
  - Includes IFA, ImmunoBlot, PCR, FISH; serum + whole blood
  - Saves $1,308.50!!

IN CLOSING….

- Patient's symptoms are real and it is up to us, the practitioners, to help diagnose them properly
- Appreciation of many different types of pathogenic Borrelia will help us to be better diagnosticians
- FINALLY- Immunoblots and related broad-spectrum tests give us a potential explanation for all this seronegativity!
- Take advantage of the test panels- money saved will allow you to include more tests for more pathogens
- Please tabulate your findings and share them- many unanswered questions on how these other Borrelia present and respond to treatment

Remember- Better testing is better for the patients, and better for you!

Many, many thanks for all that you do!