



# IS IT LYME DISEASE, or TICK-BORNE RELAPSING FEVER?

**Webinar Presented by**  
Joseph J. Burrascano Jr. M.D.  
Joined by Jyotsna Shah PhD for the Q&A

*January 2020*



# Presenters

## **Joseph J. Burrascano Jr. M.D.**

- Well-known pioneer in the field of tick-borne diseases, active since 1985
- Founding member of ILADS and ILADEF
- Active in physician education on all aspects of tick-borne diseases



## **Jyotsna Shah, PhD**

- President & Laboratory Director of IGeneX Clinical Laboratory
- Over 40 Years of Research Experience in Immunology, Molecular Biology & Microbiology
- Author of Multiple Publications & Holds More Than 20 Patents
- Member of ILRAD as a Post-Doctoral Scientist
- Started the First DNA Sequencing Laboratory in E. Africa





# Poll Question

Before we begin, we'd like to ask a poll question.

Which one of these *Borrelia* causes Tick-Borne Relapsing Fever (TBRF)?

- a) *B. mayonii*
- b) *B. turicatae*
- c) *B. burgdorferi*
- d) *B. andersonii*
- e) *B. garinii*



# Poll Question

Before we begin, we'd like to ask a poll question.

Which one of these *Borrelia* causes Tick-Borne Relapsing Fever (TBRF)?

- a) *B. mayonii* - Lyme
- b) *B. turicatae* - TBRF
- c) *B. burgdorferi* - Lyme
- d) *B. andersonii* - Lyme
- e) *B. garinii* – Lyme strain in Europe



# What is TBRF?

- Has been defined by clinical presentation
- Has been defined by tick vector
- Has been defined by genetics
- Has been defined by serotype

## **BUT**

- Each of these has exceptions and limitations!



# 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.”



# Classic TBRF: Cycles

- TBRF *Borrelia* express surface antigens that undergo cyclic changes
- Cycles of disease are caused by regular variation of these antigens
- These antigens are coded for by plasmid genes (VSP and VLP)
- The ability of a single spirochete to switch expression among antigenically distinct VSP and VLP genes allows escape from an individual host's immune response and can cause late-appearing IgM antibodies
- Alteration of these proteins prevents elimination of the spirochetes by the immune system, leading to recurrent febrile episodes.
- Allelic polymorphism or genetic variability of VSP and VLP genes within the total spirochete population may help to evade herd immunity



# But can also Present like Lyme!

- 543 US patients with suspected Lyme:
  - 29% were positive for Ab to TBRF (tested for 2 species)
- Cohort of 321 California residents:
  - 38% were positive for Ab to TBRF (same 2 species)

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

**Sero-negative Lyme?**





# Soft Tick Vector

## ORNITHODOROS TICKS

- These ticks have a life span of **10 to 20 years** and can endure starvation for >5 years
- Once infected, the ticks remain infected for the rest of their lives!
- “*B. turicatae* is maintained transovarially ...”
- Meaning that larvae may transmit infection



# Soft Tick Vector

## ORNITHODOROS TICKS

- Attached ticks are rarely seen because these ticks are rapid feeders- **attached for only 5 to 30 minutes**
- Bites are **painless** and go unnoticed
- Transmission of *B. turicatae* occurs **within seconds** of the tick bite
- Can feed **multiple times**
- **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**
- After feeding, ticks return to their crevice
- Campers, hikers, cave explorers; rodent-infested homes and cabins. May emerge when you start a campfire, wood stove or simply turn on the heat!



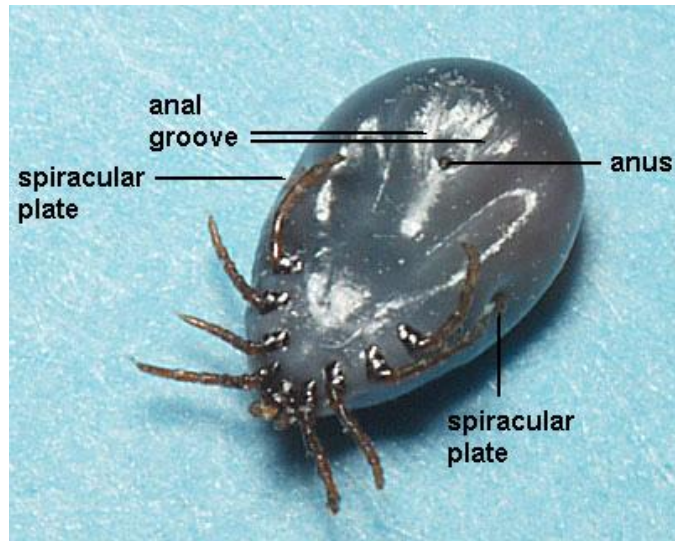
# Ornithodoros Ticks





# Engorged *Ixodes*

Might be confused with *Ornithodoros*





# But Some Species of TBRF Have Hard Tick Vectors

- *B. miyamotoi* - vector is *Ixodes*
- *B. lonestari* - vector is *Amblyomma americanum*
- Others??



# Complex Genetics (No Surprise)

- Can characterize an organism by the genetic sequences
- Sequencing is typically done on a specific gene- for example, the flagellin gene
- Can also sequence other genes and even the telomere
- Problem is, the family tree that results looks different for each method, making precise groupings of families of *Borrelia* impossible
- Serotyping (based on expressed surface antigens) is tricky due to antigenic variation- so to be accurate, need to measure multiple types of each surface antigen. This is the basis of advanced immunoblotting.



# TBRF - Important Facts

- Transmission within **15 seconds** of tick bite
- **Maternal-fetal passage** well recognized and accepted
  - Spontaneous abortion, premature birth, and neonatal death
- Louse-borne RF (*B. recurrentis*) transmission via **mucous membranes!!**
- **Acute Respiratory Distress Syndrome** has been associated with *B. hermsii* (CDC)
- Ornithodoros- because of transovarial passage of *Borrelia* and its ability to survive for decades, it may **serve as its own reservoir**, and not need to feed on an animal to acquire or maintain infection
- Some TBRF species are immune to complement-mediated killing
- Prolonged QT interval has been reported with TBRF infection



# 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. turicata* 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***”





# Do we Need to Worry About TBRF?

**Relapsing fever *Borrelia* in California: a pilot serological study. 2018.  
Marianne J Middelveen, Jyotsna S Shah, Melissa C Fesler, Raphael B Stricker.**

- “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.”



# “High prevalence of tick-borne co-infections in patients with Lyme-like symptoms” Abstract, ILADS 2019

“We reviewed results of over 10,000 patients tested for the presence tick-borne disease pathogens by direct or indirect tests.”

Table 1: Patients Positive for exposure to Tick-Borne Pathogens	
Pathogen	% (+)
Babesia	37.3%
Borrelia burgdorferi	32.1%
Tick-Borne Relapsing Fever Borrelia	27.7%
Bartonella	19.1%
Anaplasma phagocytophilum ( HGA)	16.7%
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Ehrlichia chaffeensis (HME)	6.9%

Table 2: Percentage of Lyme patients with one or more co-infection	
One Co-infection	40%
Two Co-infections	15%
Three Co-infections	4.6%
Four Co-infections	0.7%



# TBRF in Suspected Lyme Patients

## 543 US patients with suspected Lyme

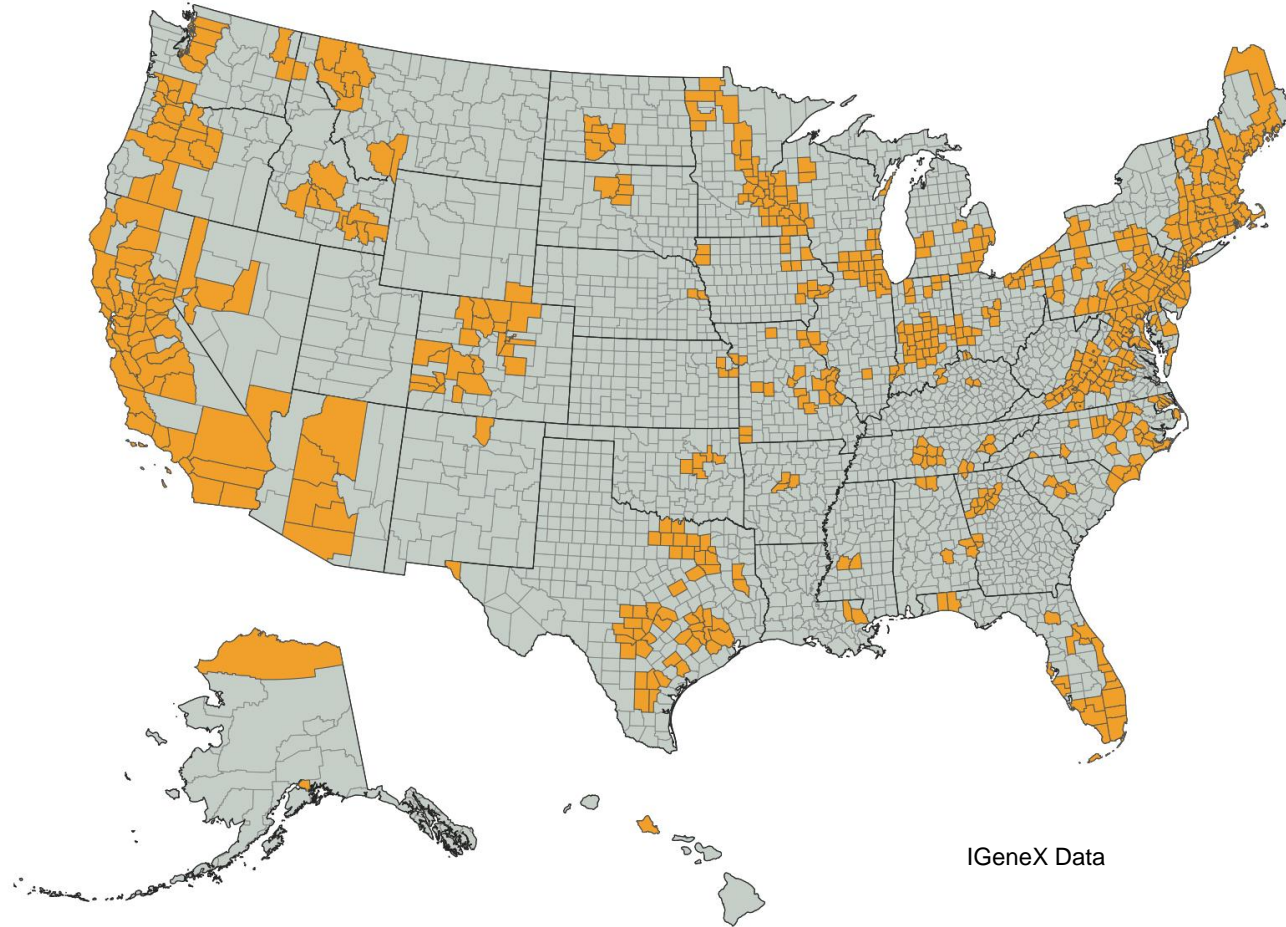
- 32% were positive for Ab to LB
- **22% were positive for Ab to TBRF**
  - Only tested for 2 species- *hermsii* and *turcica*
- 7% were positive for Ab to both

## California cohort- 321 patients

- 33% were positive for Ab to LB
- **27% were positive for Ab to TBRF (2 species)**
- 11% were positive for Ab to both



# TBRF Reported in 49 of 50 States





# World-wide Distribution

- TBRF has also been reported in **Central and South America**
- *Borrelia hispanica*, *B. persica*, and *B. miyamotoi* are important causes of TBRF in **Europe and Asia**
- *B. hispanica*, *B. crocidurae*, and *B. duttonii* are important causes of TBRF in **Africa**



# Clinical Presentations

## Two types

- **Classic presentation- acute-** fever, chills, headache, myalgias, arthralgia, nausea
- **Lyme-like presentation**
- ***B. miyamotoi*** seems to be a blend of the two
  - “*Borrelia miyamotoi* disease”
- Note that GI involvement is more common in TBRF than in Lyme
- Classically presenting TBRF may be confused with Rickettsia, viruses, Babesia and Malaria



# Lyme and Relapsing Fever Symptoms

Manifestation	% Patients			
	<i>B. miyamotoi</i> (2009) (n=46)	<i>Relapsing Fever Borrelia</i> (CDC)	<i>B. garinii</i> (2009) (n=21)	<i>B. burgdorferi</i> (1991-2008) (n=92)
EM	9	18	91	89
Multiple EM	0		14	7
Fever	98	100	67	32
Fatigue	98		86	74
Headache	89	94	57	63
Chills	35	88	10	43
Myalgia	59	92	52	63
Artralgia	28	73	29	62
Nausea	30	76	10	24
Vomiting	7	71	5	7
Neck Stiffness	2	24	0	38



# TBRF Species (so far...)

1. *Borrelia americana*
2. *Borrelia anserina*
3. *Borrelia coriaceae*
4. *Borrelia crocidurae*
5. *Borrelia duttonii*
6. *Borrelia hermsii*
7. *Borrelia lonestari*
8. *Borrelia miyamotoi*
9. *Borrelia parkeri*
10. *Borrelia persica*
11. *Borrelia recurrentis*
12. *Borrelia sinica*
13. *Borrelia theileri*
14. *Borrelia turcica*
15. *Borrelia turicatae*
16. *Borrelia valaisiana*
17. *Candidatus Borrelia texasensis*
18. *Candidatus Borrelia johnsonii*





# Important TBRF Species in USA

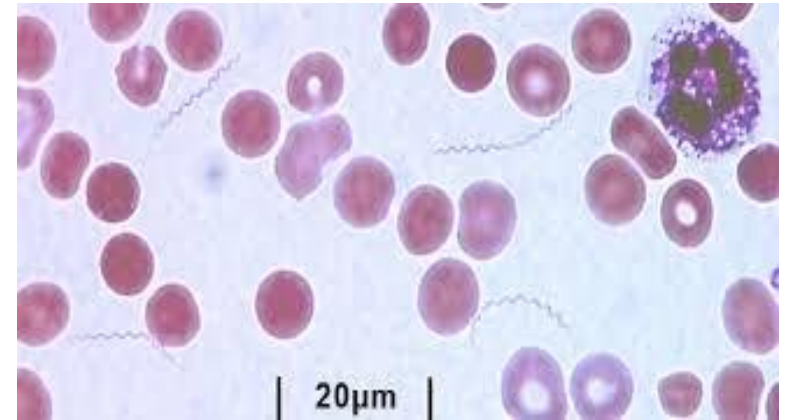
1. *B. hermsii*
2. *B. miyamotoi*
3. *B. turcica*-like
4. *B. turicatae*
5. *B. coriaceae*?
6. *B. parkeri*
7. *Candidatus Borrelia johnsonii*?



# Laboratory Testing

## **BLOOD SMEARS**

- Some TBRF species may be visible on blood smears
- Only in acute stage of a crisis
- Cannot determine species
- False positives
- Positive smear may be incorrectly diagnosed as Lyme





# Laboratory Testing

## PCR

- Large commercial labs- for *B. miyamotoi* only
- IGeneX PCR- Genus-specific
  - Therefore may detect many different species of TBRF
  - Can identify and report *B. miyamotoi*
- PCRs only acceptably sensitive during early or acute stages of the disease and in the immunosuppressed, including very ill, late stage patients



# Laboratory Testing

## **SEROLOGIES**

- Most TBRF express p41
  - May give rise to a 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 false-positive Lyme serologies



# Laboratory Testing

## SEROLOGIES

- Large commercial labs- for *B. hermsii* only!
- Can get a GLP-protein based ELISA for *B. miyamotoi*, but is only one protein antigen and therefore prone to false negatives
- IGeneX offers a variety of advanced serologic tests for TBRF
  - **ImmunoBlots**- provides the most information and is the gold standard
  - **Broad Coverage Antibody Assay**- simple and cost effective but still better than the Large-Lab options



# The IGeneX TBRF ImmunoBlot

The most advanced TBRF diagnostic test  
available to clinicians



# Advantages of the ImmunoBlot

- Uses **recombinant antigens** that are specific to multiple individual species
- Unlike western blots, IGeneX ImmunoBlots deliver **precise quantities** of antigen to **specific locations** on the gel- drastically improves sensitivity and specificity
- **A large variety of protein antigens** are included, not just one, as in large-lab testing for *B. miyamotoi*
- Antigens reflect the **seven most commonly found TBRF species**, not just *B. hermsii* or *B. miyamotoi*



# Advantages of the ImmunoBlot

## **USES RECOMBINANT PROTEIN ANTIGENS AND INCORPORATES UNIQUE Ag DELIVERY AND DETECTION METHODS**

- 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



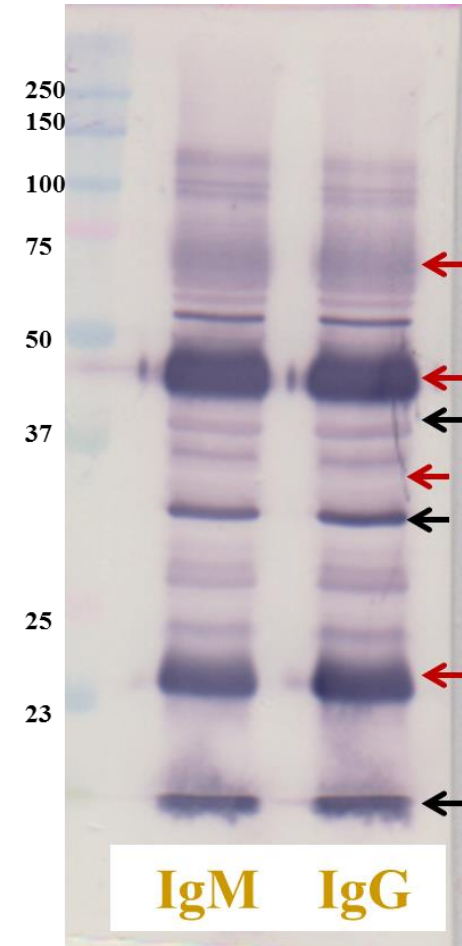


# WB: How Bands are Identified

## IDENTIFICATION IS BASED UPON POSITION: MIGRATION-DEPENDENT

*However:*

- Migration is not an exacting process and the variable location of the bands can make identification of individual antigens very difficult- may not line up.
- Also, some nonspecific or unimportant proteins may co-migrate with important *Borrelia* proteins, and the WB cannot distinguish these.



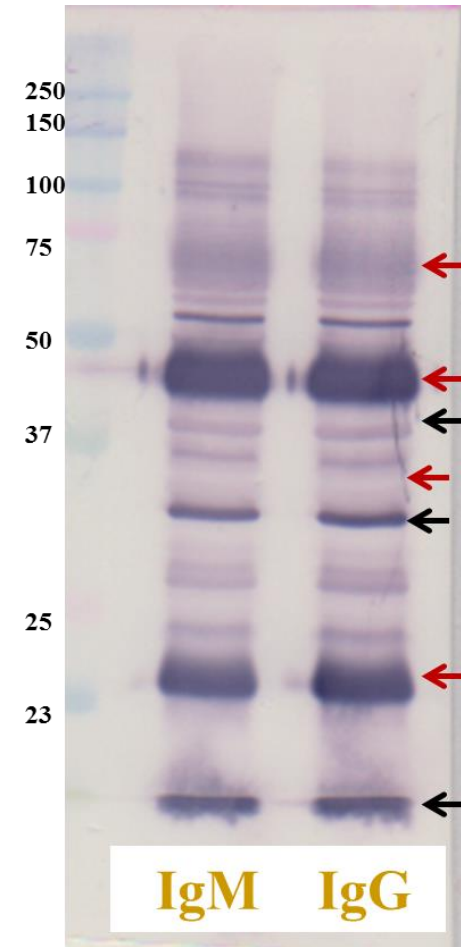


# WB: How Bands are Scored

## SCORING (POS OR NEG) IS BASED UPON BAND INTENSITY

*However:*

- How dark must a band be to be called positive?  
Indeterminate? Because protein content can vary, the band intensity can vary, potentially causing false positives and false negatives.
- What does a broad band mean?
  - More antigen?
  - Multiple co-migrating antigens?
  - Stronger host reaction?
  - ??





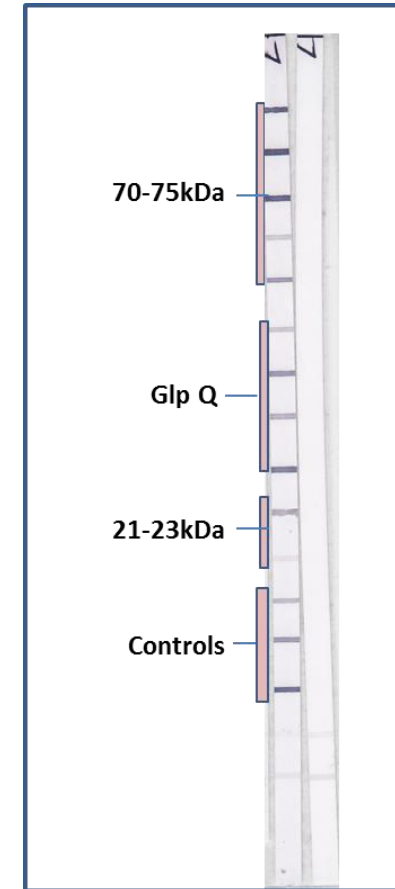
# Scoring the ImmunoBlot

## PRECISE AMOUNT OF ANTIGEN

- Banding intensity is no longer source-dependent
- Positive bands are more clearly displayed
- Reduces false positives and false negatives

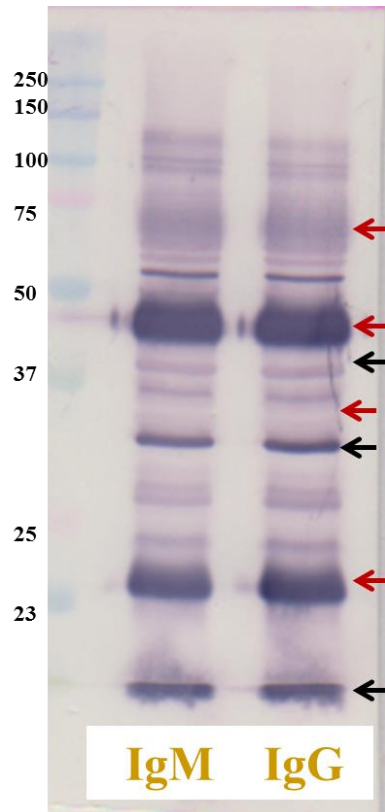
## PLACED AT SPECIFIC LOCATIONS

- Band locations are no longer migration-dependent
- Know exactly what each positive band represents
- No longer an issue with co-migration of other, non-TBRF antigens (viruses, other bacteria, autoantibodies)

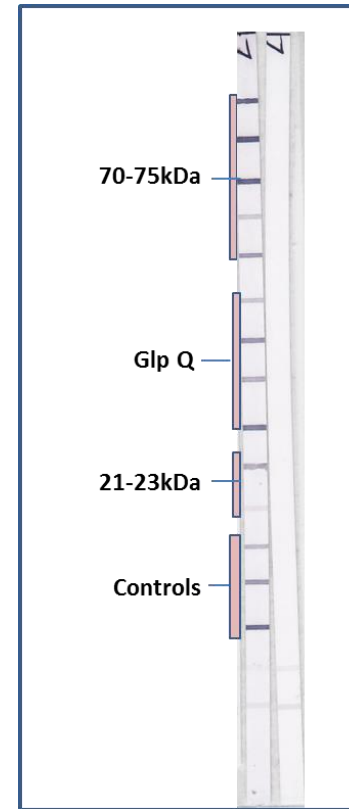


# WB vs. IGeneX ImmunoBlot

TBRF Western Blot



TBRF ImmunoBlot





# TBRF ImmunoBlot

**MULTIPLE** species of TBRF *Borrelia* are included

Between IgM and IgG, one set of IGeneX TBRF ImmunoBlots deliver the equivalent of **TWELVE** individual tests!

**SPECIES:**

*B. hermsii*

*B. miyamotoi*

*B. turcica-like*

*B. turicatae*

*B. coriaceae*

*B. parkeri*



# Example of What is Being Seen

<b>Summary of TBRF ImmunoBlot Positive Patients (n=62)</b>	
<b>TBRF Species</b>	<b>TBRF IB (+)</b>
<i>B. hermsii</i>	7
<i>B. miyamotoi</i>	13
<i>B. turicatae</i>	13
<i>B. turcica-like</i>	2
<i>TBRF Borrelia sp.</i>	26
<i>B. hermsii</i> and <i>B. miyamotoi</i>	1



# 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
1 <sup>st</sup> Sample (acute)	15	7	1	2	10	<b>66.7%</b>
2 <sup>nd</sup> Sample (convalescent)	7	4	1	2	7	<b>100%</b>

### 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**
- Late-appearing IgM is not uncommon



# 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
<i>Borrelia burgdorferi</i> infection	12	0	0	
<i>Bartonella henselae</i> infection	7	0	0	
Human granulocytic anaplasmosis	16	0	0	
<i>Babesia microti</i> infection	14	0	0	
<i>Babesia duncani</i> Infection	41	0	0	
Human monocytic ehrlichiosis	5	0	0	
Total False (+)	0	3	3	5
Total True (-)	212	209	209	207
<b>Specificity</b>		<b>98.6%</b>	<b>98.6%</b>	<b>97.6%</b>





# IGeneX TBRF ImmunoBlot Summary

## Provides vital information not previously available

- Accurately identifies TBRF- especially important in diagnosing “Lyme-like illnesses”
  - Does not cross-react with Lyme *Borrelia*
- Ability to identify multiple clinically relevant TBRF species
- Useful in detecting early disease
- Highly sensitive
- Highly specific
- Rapid turnover



# IGeneX Broad Coverage TBRF Antibody Assay

Simple and cost effective



# IGeneX TBRF Broad Coverage Antibody Assay

**Single serological test that covers multiple, clinically relevant TBRF species**

- Highly accurate replacement for standard serologies
  - Think of it as a better ELISA
- Single result includes IgM and IgG
- Simple yes-no results- makes interpretation easy
- Rapid turnover
- Cost effective



# Using These New Assays

- **The Broad Coverage Antibody Assay offers a more sensitive, highly specific and broad-spectrum alternative to standard serologies**
  - Tests for multiple TBRF *Borrelia* species
  - Very cost-effective
  - Simple yes-no interpretation; includes both IgM and IgG
- **The IGeneX ImmunoBlots include broad, multi-species coverage, BUT CAN ALSO IDENTIFY SPECIES\***
  - Allows the clinician to identify patterns of presentation and treatment response associated with specific species of TBRF *Borrelia*
  - Provides information not available anywhere else
  - Highly sensitive and specific
  - Extremely useful in selected early cases
  - Replacement for the western blot and all other standard serologies

\* Speciation not available in New York



# Testing for TBRF

- **In patients with intact immunity, the TBRF ImmunoBlot is always the first choice**
  - Good for all stages of disease
  - Late IgM is still significant
  - Two or more reactive band groups is read as positive
  - One reactive band group is considered a borderline result
- **Immunocompromised**
  - Add the TBRF PCR to catch those who do not have detectable levels of free antibody
  - Reciprocal relationship between serologies and PCR due to this immune system effect
  - By doing both a serology and a PCR, you get a picture of immune responsiveness



# Why Test Panels? Results from 10,000 Patients

<b>Table 1: Patients Positive for exposure to Tick-Borne Pathogens</b>	
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<b>Table 2: Percentage of Lyme patients with one or more co-infection</b>	
One Co-infection	40%
Two Co-infections	15%
Three Co-infections	4.6%
Four Co-infections	0.7%



# Why Test Panels

- Nearly all Lyme patients, especially the more ill ones, have co-infections
- In light of the high prevalence of TBRF, which may present acutely and with high fever, more important than ever to distinguish this from acute rickettsial and acute Babesia infections
- Many reports of nervous system involvement by TBRF, making it difficult to separate clinically from tick-borne viral encephalitis
- Positive blood smears for spirochetes or protozoa require more definitive lab testing for confirmation

# What About Treatment?

A blue-tinted photograph of a laboratory. In the foreground, a scientist in a white lab coat is seated at a workstation, looking at a computer monitor. To the left, there is a large piece of laboratory equipment with several petri dishes on top. In the background, another scientist is visible, also working at a workstation. The overall scene is a busy laboratory environment.





# Treating TBRF

- As far as we can tell, treatment regimens for TBRF and Lyme are similar
  - Very few studies on antibiotic susceptibility
  - TBRF can be a chronic illness
  - TBRF can involve the central nervous system
  - TBRF can involve the joints
  - TBRF can induce chronic fatigue
- **BE CAREFUL!** In the classical form of TBRF, if treated during a crisis, **severe Herxheimer** reactions may ensue- hypotension, cardiac arrhythmias, etc. so be careful!!



# Concluding Thoughts

- TBRF is newly recognized as being far more prevalent than previously thought
- May be responsible for many cases of seronegative Lyme
- More difficult to clinically identify this without laboratory confirmation
- Test with ImmunoBlots to learn if there are any important differences among the various *Borrelia* regarding presentation and response to treatment
- Keep records, share your info, and publish!



# More Information

## Line Immunoblot Assay for Tick-Borne Relapsing Fever and Findings in Patient Sera from Australia, Ukraine and the USA

- Published in October 2019
- Lead author is Dr. Shah from IGeneX
- Available on [www.igenex.com](http://www.igenex.com)



healthcare



Article

## Line Immunoblot Assay for Tick-Borne Relapsing Fever and Findings in Patient Sera from Australia, Ukraine and the USA

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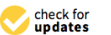
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**Abstract:** Tick-borne relapsing fever (TBRF) is caused by spirochete bacteria of the genus *Borrelia* termed relapsing fever *Borreliae* (RFB). TBRF shares symptoms with Lyme disease (LD) caused by related Lyme disease *Borreliae* (LDB). TBRF and LD are transmitted by ticks and occur in overlapping localities worldwide. Serological detection of antibodies used for laboratory confirmation of LD is not established for TBRF. A line immunoblot assay using recombinant proteins from different RFB species, termed TBRF IB, was developed and its diagnostic utility investigated. The TBRF IBs were able to differentiate between antibodies to RFB and LDB and had estimated sensitivity, specificity, and positive and negative predictive values of 70.5%, 99.5%, 97.3%, and 93.4%, respectively, based on results with reference sera from patients known to be positive and negative for TBRF. The use of TBRF IBs and analogous immunoblots for LD to test sera of patients from Australia, Ukraine, and the USA with LD symptoms revealed infection with TBRF alone, LD alone, and both TBRF and LD. Diagnosis by clinical criteria alone can, therefore, underestimate the incidence of TBRF. TBRF IBs will be useful for laboratory confirmation of TBRF and understanding its epidemiology worldwide.

**Keywords:** borreliosis; line immunoblots; Lyme disease; relapsing fever; relapsing fever *Borreliae*; serological diagnosis

**NOW TIME FOR  
QUESTIONS**

