CLINICAL DIAGNOSIS AND LABORATORY TESTING FOR THE MAJOR TICK-BORNE INFECTIONS

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PART ONE- GENERAL CONSIDERATIONS

- Brief history of Lyme
- Recognition of co-infections
- Rapid dissemination of pathogens
- Deep dive into testing methods

PART TWO- THE MAJOR TBDs

- Lyme
- TBRF
- Bartonella
- Babesia
- Ehrlichia and Anaplasma
- RMSF

BRIEF HISTORY OF LYME DISEASE-1

- 1970s- community outbreak of arthritis and facial palsy in children and adults in Lyme Connecticut
- After years of requests from local resident Polly Murray, finally the CDC sent an investigator- an immunologist/rheumatologist, and not an expert in infectious diseases
- Initially he thought it was an autoimmune disease, not an infection, but this bias still exists: many still think of chronic forms of this disease to be immune mediated and not infectious.
- In the early 1980s, NIH tick researcher Willi Burgdorfer discovered a spirochete in the ticks he collected from Shelter Island, NY (is 20 miles from Lyme CT and along a bird flyway- birds carry and disseminate ticks)

BRIEF HISTORY OF LYME DISEASE-2

- Eventually the spirochete was named after Willi Burgdorfer- “Borrelia burgdorferi”
- By 1982-3 simple serologies were developed as a diagnostic test based upon this NY strain of Borrelia- this strain, now lab-adapted, is the basis of all FDA-approved Lyme serologies (Lab strain B31)
- However, many patients who fit the clinical picture of Lyme did not have a positive test (were seronegative)- thus began a huge diagnostic problem that continues to this day.
- Later in the 1980s it was discovered that ticks may contain many other pathogens that are transmitted along with the Lyme spirochete
- First Babesia, then Ehrlichia, then Anaplasma, then Bartonella, then other species of Borrelia (other than B. burgdorferi)
- It became clear that while many were seronegative due to poor test quality and perhaps by the use of this restrictive lab strain, it also became clear that many might have had one or more of these other pathogens and not Lyme

DIVERSITY OF TICK-BORNE PATHOGENS

A 2018 study of 10,000+ patient samples tested at a reference lab:

- 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

Co-infections

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

RAPID TRANSMISSION OF PATHOGENS

- W. Burgdorfer: “5-10% of ticks that are carrying Lyme Disease have a systemic infection and have the microbes in their saliva and can transmit it as soon as they bite.”
- Relapsing fevers can be transmitted in as little as one minute (soft-bodied ticks)
- Bartonella is found in tick saliva so rapid transmission is likely
- Rickettsias (Ehrlichia, Anaplasma, RMSF) and arboviruses are also present in tick saliva
- Also:
  - Improper tick removal
  - Infected tick feces

LABORATORY TESTING- Three main issues:

Sensitivity-

- Don’t want to miss cases

Specificity-

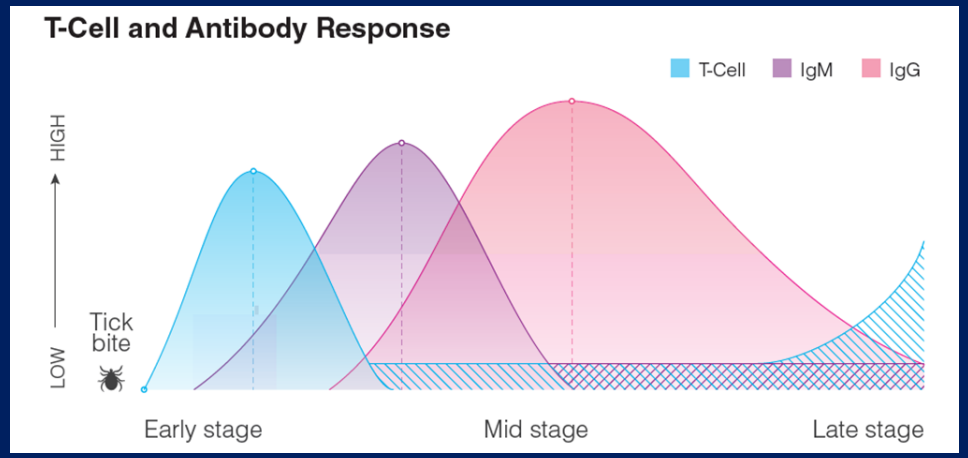
- Don’t want false positives

Broad coverage-

- Must be able to test for as many potential pathogens as possible- many new species are being documented

TIME COURSE OF IMMUNE RESPONSE

- T-cell response- is the earliest to react and is most sensitive then; sensitivity drops off but can increase in late, chronic stages even if seronegative (T-cell response is independent of B-cell response)
- IgM reacts next, and while its levels usually diminish, in a subset of patients, IgM response may persist
- IgG appears last; may persist or drop off. Absent IgG response often seen in late, chronic infections
- Paradoxically, the more ill, the less likely to have a positive IgG



SEROLOGIES- IFA AND ELISA

- IFAs and ELISAs are made from either whole cell sonicates or are made from one specific antigen
  - Example- in Lyme, use either a sonicated whole Borrelia, usually lab strain B31, or use just the flagellin (p41) antigen
  - Example- In TBRF, the ELISA for B. miyamotoi targets only one antigen- GLpQ
- Whole cell is too nonspecific and single antigen is too insensitive
  - Example- In Lyme, p41 is also not specific as most spirochetes and some other bacteria express this
- Can only reliably test for one species at a time

SEROLOGIES- Western Blots

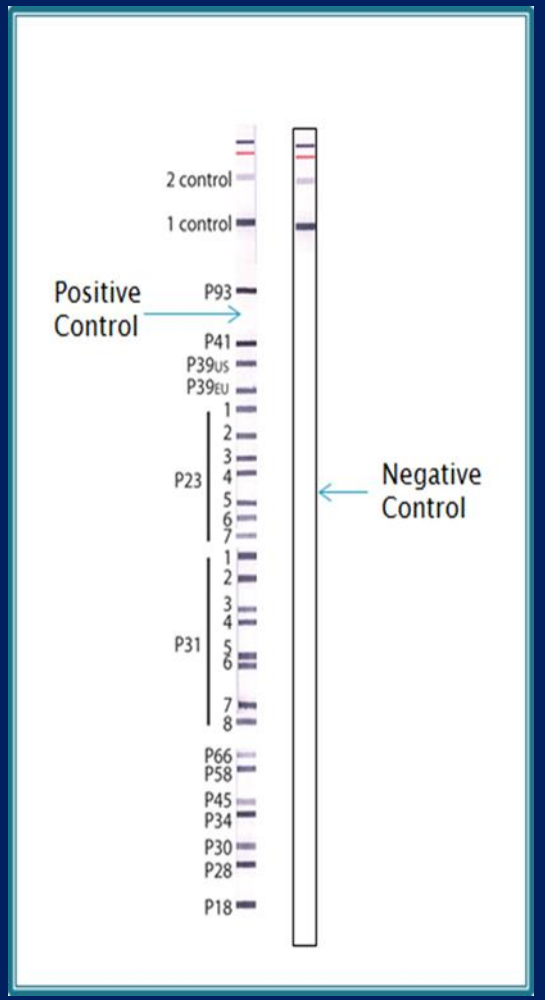
- Western Blot should be better as it can display multiple antigens, letting us choose ones that are more specific
- However, several problems:
  - Made from cultured lab strains of the organism
  - Organisms are lysed- many antigens are released and many are nonspecific
  - Are able to detect only one species at a time
  - In Lyme disease it is usually based upon only lab strain B31 which does not represent the presence of Bb sl and all of the important TBRF species
  - Also, in Lyme the CDC (and insurers, among others) insist upon using “CDC case definition surveillance criteria” that specifies which bands to include and ignore, and a minimum number of bands that must be present to call the test positive
  - *These criteria exclude key Borrelia bands but include several nonspecific bands*
- Result is poor sensitivity and poor specificity, plus limited species coverage

SEROLOGIES- ImmunoBlot

It is fundamentally different from the western blot and all other serologies

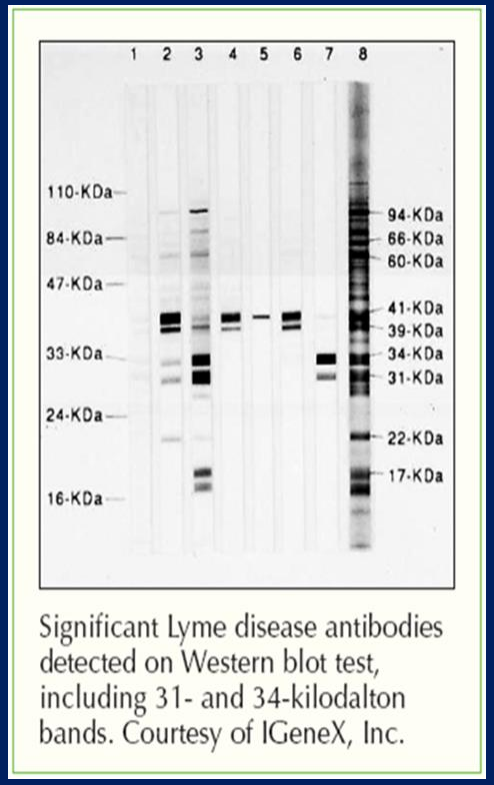
- Uses recombinant antigens, not lysed organisms
- Antigens are printed on the strip (instead of electrophoresis)
  - At precise locations- so confusion with foreign antibodies far less likely
  - Band intensity- is no longer dependent on culture viability
- RESULT: Increased sensitivity, increased specificity and ability to detect multiple species
  - *In Lyme, can detect all Bb sl*
  - *In TBRF, can detect all major pathogenic species known to exist in USA patients*
  - *In Bartonella and Babesia, can detect multiple species and name the major ones*

Highest sensitivity and specificity of any serological test



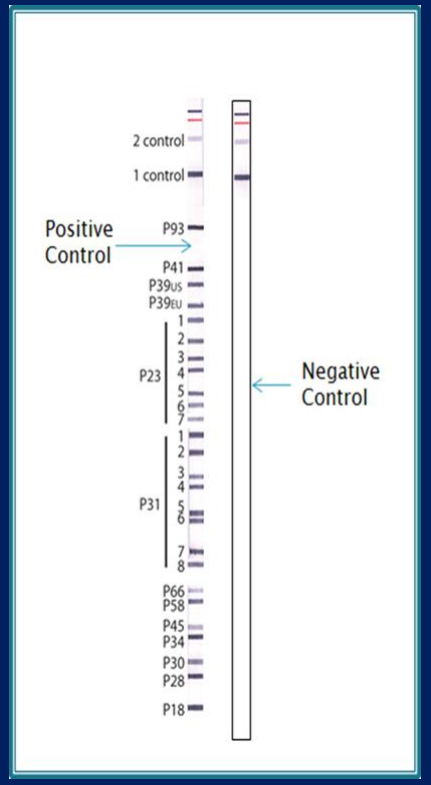
SEROLOGIES- Western Blot vs. ImmunoBlot

Lyme WB- Based upon electrophoresis- technically difficult to read



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



SEROLOGIES- OTHER CONSIDERATIONS

- A positive result means free antibody is present and has been detected
- However, free antibodies may NOT be present:

1. Antigen excess- all antibodies are bound up in immune complexes and none are free to be detected
2. Immune deficiency- patient is not making enough antibody to be detected
3. Stealth organisms/hidden organisms that are not eliciting an antibody response

T-CELL RESPONSE ASSAYS

- Reflects past exposure to an organism by measuring T-cell response
- Method:
  - Patient blood must be handled carefully to keep T-cells viable by the time they arrive in the lab, and lab must process the specimen promptly
  - Antigens of the organism to be tested are introduced into the cell culture
  - If the T-cells had been previously exposed to this organism due to past infection, then the T-cells will activate
  - Activation can be assessed by incorporation of radiopharmaceuticals or by liberation of interferons (ELISPOT method measures production of interferon-gamma by the T-cells)

T-CELL RESPONSE ASSAYS

Clinical features-

- Reactivity appears very early, tapers off, then may reappear late in chronic illness
- Because T-cell responses are independent of B-cell responses, can be positive in seronegative patients- in early, chronic and B-cell dysfunction
- Can be designed to offer genus-level detection (IGeneX)- broadens coverage.
- Sensitivity and specificity each are about 80% when tested within its desired time window
- When combined with the ImmunoBlot, provides information on the full spectrum of patient's immune response to infection and stage of disease

ANTIGEN CAPTURE

- Direct assay (urine, CSF) to detect presence of antigens from the organism in question- Lyme disease only
- Can use one or several antigens from the pathogen
  - Lyme Dot-blot (IGeneX)- Multispecies (Bb sl); multiple antigens 31, 34, 39, and 93 kDa
  - Nanotrap (Galaxy)- Multispecies (Bb sl) but one antigen- Bb OspA (31 kDa)
- Extremely helpful when impractical to draw blood (poor access, newborns, etc.)

ANTIGEN CAPTURE

- Antigen spillage is not constant- varies widely
- Spillage and therefore sensitivity tracks symptom severity- symptom flares, Herxheimers, menses
  - To increase sensitivity, some clinicians pre-treat with antibiotics to induce a Herxheimer
  - They usually collect three samples to increase yield
  - Specific as long as there is no UTI, so recommend doing a concurrent U/A and urine culture
  - If only one of the three samples is positive, believe the positive one

FLUORESCENT IN-SITU HYBRIDIZATION ASSAY (FISH)

FISH detects presence of pathogen RNA – is a direct-detection test

- Specific fluorescent RNA stains are applied to a blood smear for direct visualization
- RNA does not persist post-infection- disappears as soon as pathogen dies, so a positive means infection is present
- Able to detect pathogens even if embedded in biofilms!!
- Is designed to be genus-specific (IGeneX), increasing breadth of species detection
- Available for Bartonella and Babesia (IGeneX)

FLUORESCENT IN-SITU HYBRIDIZATION ASSAY (FISH)

Clinical-

- Pathogenemia is high early in the infection, before effective immunity develops- positives can appear very early in disease
- Pathogen load also increases very late in the infection as immunity declines and the organisms adapt to the host- another time when this test can be very helpful
- Highly specific, so a positive result should not be dismissed, but a negative does not rule out infection

POLYMERASE CHAIN REACTION (PCR)

- PCR is a direct detection assay that looks for presence of nucleic acids (usually DNA) of the organism in the specimen
- Can test blood, other body fluids and biopsy samples
- If enough DNA is found, then direct sequencing can be done to confirm identity of the pathogen
- PCRs can be crafted to offer genus-level detection (IGeneX, Galaxy)
- PCR testing is available for most of the TBDs and many viruses
  - Borrelia, Babesia, Bartonella, Ehrlichia, Anaplasma, RMSF, others

POLYMERASE CHAIN REACTION- Process

- A target segment of the nucleic acid is amplified
  - This is key- the entire genome is not amplified- just a segment of it is
  - Therefore this is a test to detect the presence of the NA, and not to identify the pathogen
  - Identification involves NA sequencing- but here again, usually only a fragment of NA is sequenced. Which segment is used may have major impact on identification.
- Amplification is to increase the minute quantities of NAs in clinical specimens to a quantity large enough to be detected

POLYMERASE CHAIN REACTION- Sensitivity

- In TBD testing, Blood PCR is notoriously insensitive-
  - There are PCR-inhibitors in blood- heparin, host DNA, hemoglobin
  - Pathogen load is often too low to detect especially with these inhibitors
  - Pathogenemia is often intermittent in TBDs
- Ways increase sensitivity:
  - Draw and test larger blood volumes and/or collect multiple specimens over time
  - Test when pathogenemia is expected to be greatest
    - Varies by pathogen, but generally is highest during flares
    - Should not be done while on antimicrobials
  - Use fluids that do not have lots of inhibitors (CSF, urine)
  - Remove inhibitors- requires careful specimen preparation and pre-culturing
  - Test tissues, not fluids

CULTURING

Huge technical limitations to culturing the TBDs

- TBDs are adapted to thrive in living organisms, not artificial culture media
- Pathogens are not always present in the blood sample
- TBDs all grow very slowly, so culturing may have to be extended many weeks to get a positive result
- With long culturing intervals, other pathogens which may be present can overgrow and spoil the culture
- Once cultured, how do you confirm identity of what has grown?
- Lab issues- complex methodology, labor intensive, time consuming, contamination risk: High cost!

IGENEX CULTURE-ENHANCED PCR (cePCR™)- New!!

Available from IGeneX for Borrelia (Lyme and TBRF), Bartonella, Babesia, Ehrlichia and Anaplasma

Took over two years of research and development, and many hundreds of samples were used

- Blood sample is held in proprietary culture medium for two weeks
- After two weeks, sample is tested by sensitive and validated PCR
- Genus level reporting- broadens number of pathogens being detected, but will not identify species
- Each type of pathogen requires a different culture medium, so tests must be ordered individually

CULTURE-ENHANCED PCR (cePCR)

How did IGeneX validate positive cultures?

- In a clinical lab, PCR is best choice (available; proven technology)
- But PCR needed to be optimized and then validated
  - PCR inhibitors in peripheral blood are neutralized or removed
  - PCR process is rigidly standardized and controlled
- Validation of the PCR
  - During development, ALL positive samples were sent to an outside reference lab for sequencing to confirm identity
  - In addition, to further confirm the pathogen was really present, reverse western blots were performed using recombinant technology

CULTURE-ENHANCED PCR (cePCR)

Specificity:

- All sequencing results matched initial PCR determination
- All reverse western blots matched exactly the results of sequencing

Sensitivity:

- Difficult to report sensitivity, as there is no gold standard to compare it to
- Reports that culture-enhanced PCRs increase sensitivity over standard PCRs by a factor of 6 to 10

CULTURE-ENHANCED PCR (cePCR)

Genus level diagnosis- broad species coverage

So broad, in fact, that an unusual species of Anaplasma was detected in a human patient:

Anaplasma platys (formerly Ehrlichia platys)

- Is a tick-borne intracellular bacterium that infects platelets, resulting in infectious cyclic thrombocytopenia in dogs.
- Report of A. platys in hard ticks in China- *Rhipicephalus microplus*
- Also reports of this infecting cattle
- Four cases reported of human infection

SUMMARY: Optimizing testing using indirect tests

Indirect tests- serologies and T-cell response assays

Key is to use these when immune response is expected to be highest

- Early disease- T-cell response assay, ImmunoBlot
- Disseminated but not chronic, with intact immunity: ImmunoBlot
- Late, chronic infection: ImmunoBlot + T-cell response assay
  - If immune deficiency is suspected, then add direct test(s)
- Even if immunity is compromised, always useful to do an immunoblot to document antibody response
  - With ongoing treatment, can see a paradoxical rise in antibody levels as the pathogen load decreases and the immune system heals

SUMMARY: Optimizing testing using direct tests

Direct tests: Culture, FISH, Urine antigen capture, PCR

Key is to use these when pathogen load is expected to be highest

- Higher load early in the infection, before effective immunity develops
- Higher load during symptom flares
  - This includes during periodic flare-ups seen in Borrelia infections (q2-4 weeks)
- Higher load at specific times of the day
  - Borrelia- early afternoon and during chill phase
  - Babesia- during chill phase
  - Bartonella- not known
- Antimicrobials
  - If on treatment, no meds for long enough for the organisms to re-emerge, but do NOT stop needed treatment just to do a test!!
  - If not already on treatment, some pre-treat to enhance pathogen release. Others recommend physical measures such as massage, sauna, etc. (anecdotal and not proven)

PART TWO- Applying this to the major TBDs

- Lyme
- TBRF
- Bartonella
- Babesia
- Ehrlichia and Anaplasma
- RMSF

LYME DISEASE

The most common vector-borne infection in the USA

- Can live in tissues, inside cells and transits through the blood stream
- Evades host immunity: inhibits and kills B- and T-cells; inhibits maturation of natural killer cells from CD56 to CD57
- Able to shift into multiple morphologic forms that help it to evade immunity and resist antibiotic treatments
- Capable of reverting into a dormant, “stationary phase” to further evade immunity and antibiotic treatment
- Lab testing can miss cases
- Can persist and become chronic despite antibiotic treatments

RAPID DISSEMINATION OF BORRELIA

Borrelia rapidly disseminate after the tick bite

- Appear in the CNS within hours to days

But spinal fluid serologies are terribly insensitive-

- Spinal tap- in Lyme meningitis, the most acute form of neurologic Lyme, only 9% had + CSF antibodies *(Coyle, SUNY at Stony Brook)*

SUMMARY:

- All cases of disseminated Lyme involve the CNS
- Negative CSF serology does NOT rule out CNS infection
- Neurologic Lyme is being vastly underdiagnosed (case definition vs clinical reality)

PATIENT HISTORY

Well patient who became ill after potential exposure

- Often begins with nonspecific, viral-like symptoms
- These persist, and new ones appear
- Then symptoms become more focused on individual organ systems- musculoskeletal, peripheral and central nervous systems, heart and skin
- Can progress to whole-body, multisystem illness
  - Fatigue, headaches, confusion, memory issues
  - Peripheral and autonomic neuropathy; cranial nerves, vagal tone
  - Joint pain with or without swelling
  - Myocarditis with rhythm and conduction disturbances
  - Chronic inflammation but immune suppressed

PATTERN RECOGNITION

Lyme Borrelia undergo regular shifts in gene expression and antigen presentation EVERY FOUR WEEKS

- Symptoms wax and wane on a monthly basis
- Can result in changes in type of symptoms
- Can result in symptoms that migrate

Is the basis of the classic Lyme presentation:

- Multisystem
- Migratory
- Cyclic

CRITICAL FOR DIAGNOSIS AND TREATMENT!!

PHYSICAL EXAM

SUBTLE SIGNS ARE ALWAYS PRESENT- BUT YOU HAVE TO LOOK!

- Appearance, cognition and speech
- Rashes, skin temperature and color
- Orthostatic hypotension- and observe change in pulse
- Temperature record- low in AM, high in afternoon
- Neuro- cranial nerves incl. gag and corneal reflexes, EOMs; DTRs- absent/decreased, delayed, brisk, clonus
- Joints- synovial thickening, tenderness, heat, redness, effusions, ROM
- Muscles and tendons- nodules, tenderness, weakness
- Nuchal rigidity; “Lyme shrug”
- Hepatosplenomegaly, lymphadenopathy

DON’T FORGET THAT YOU ARE ALSO LOOKING FOR OTHER POSSIBLE DIAGNOSES!

ERYTHEMA MIGRANS
The characteristic Lyme rash

INCIDENCE MAY BE LESS THAN 50%

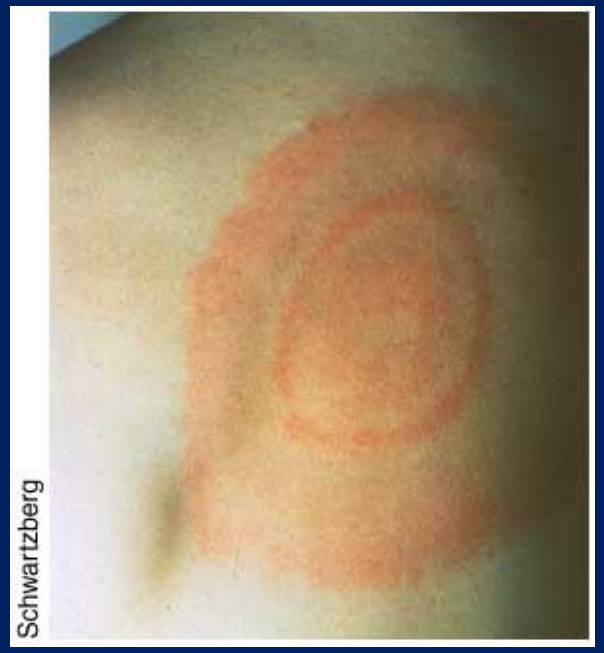
- Circular-ovoid rash that expands over time
- But can be atypical
  - many different appearances
  - because they follow skin planes, are rarely circular
  - the most common reaction is a small raised bump at the bite site that may persist for several days but not expand
- May be hidden (scalp, back, groin)
- Painless, raised, warm
- Rarely itch
- Disappears in days to weeks EVEN IF NOT TREATED!

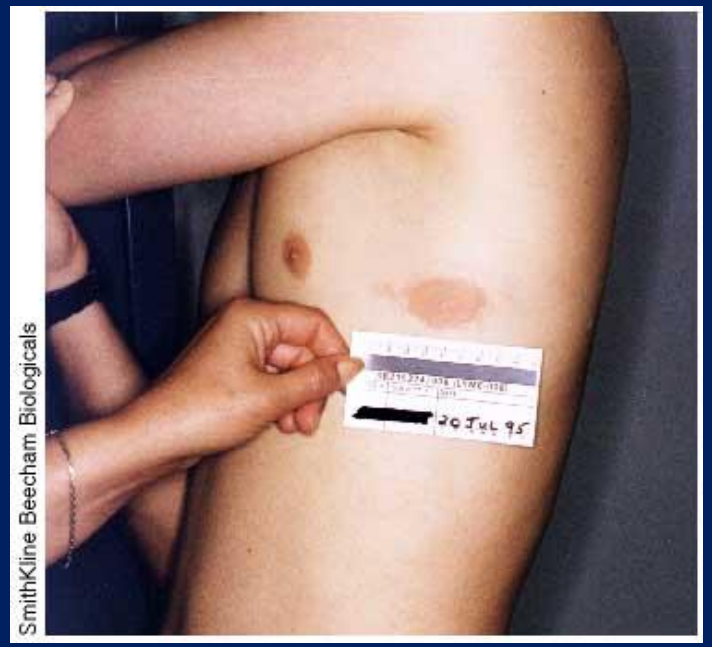
ERYTHEMA MIGRANS

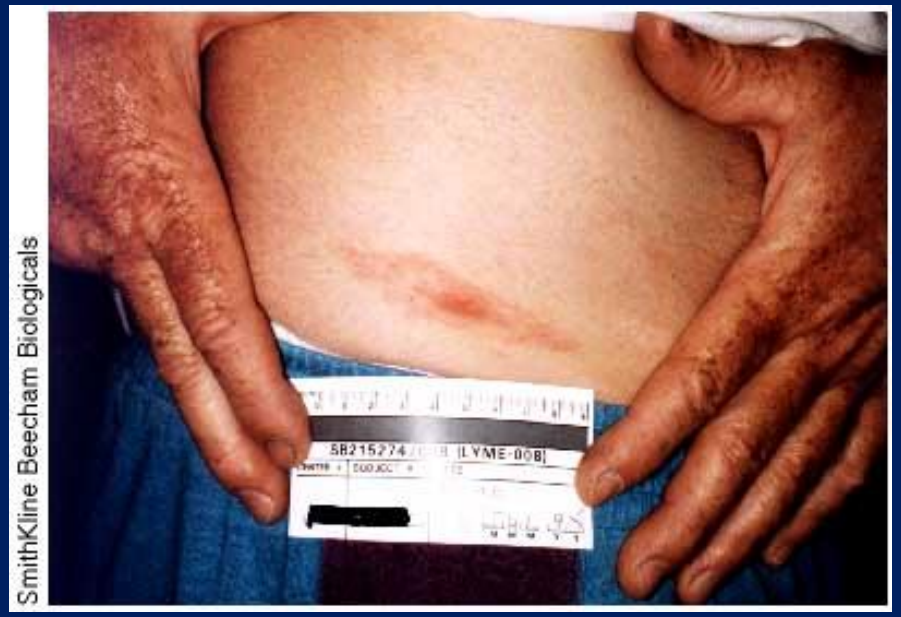
Classical “bullseye” rash is actually quite rare!

Uniform Rash

Not always circular

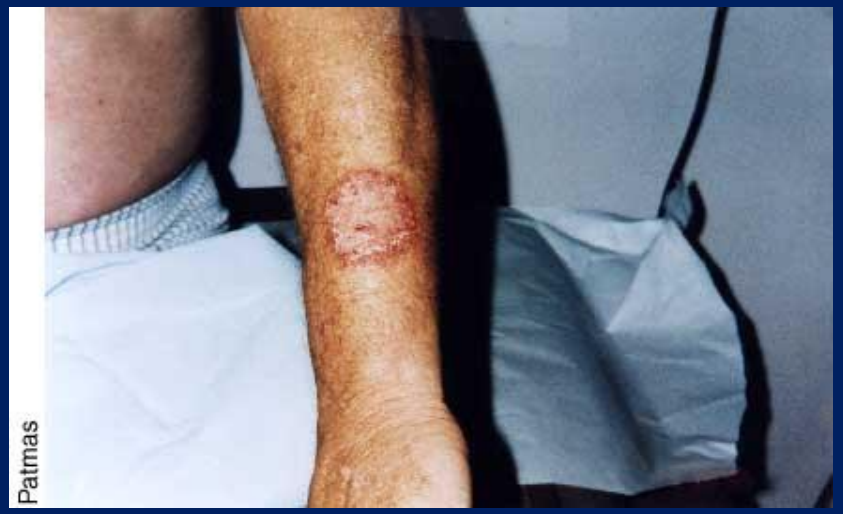




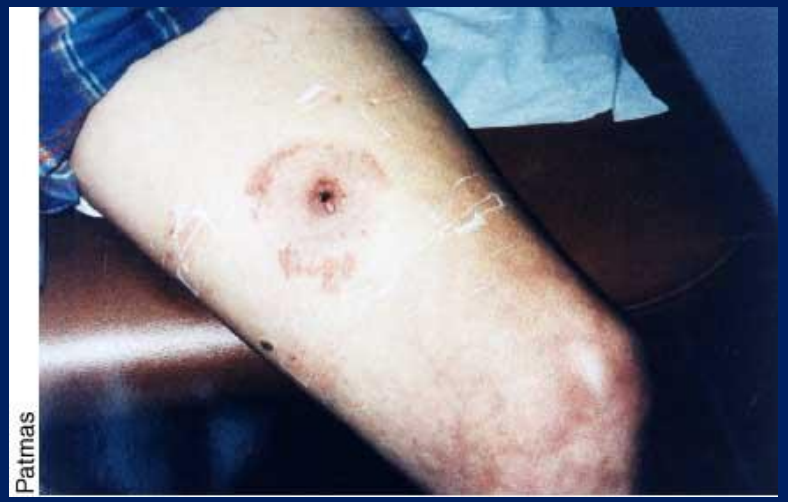


OTHER RASHES
Not Lyme Disease

RINGWORM (Tinea)
Scaly center
Not raised or warm

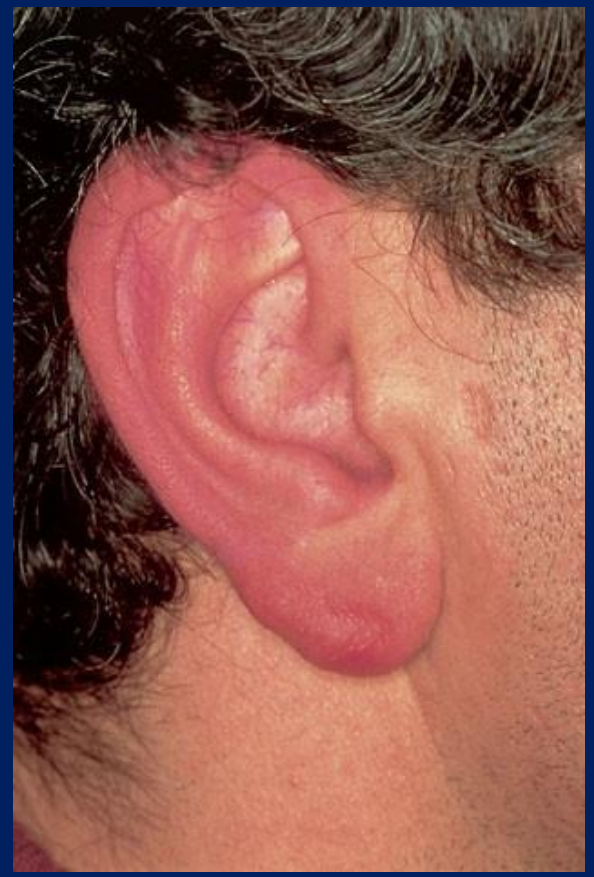


SPIDER BITE
Painful!
Necrotic center



BORRELIA LYMPHOCYTOMA

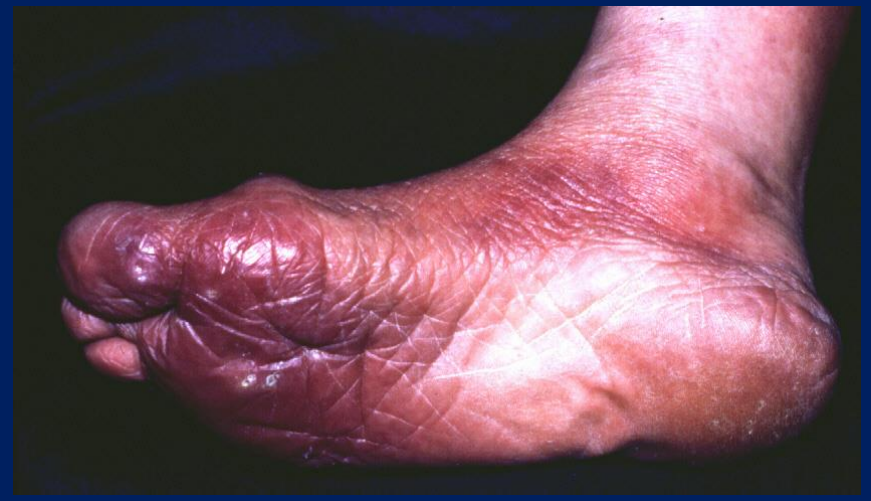
- Hot, red, swollen and tender
- Usually involves the ear; may also involve the nipple
- Site of active infection with B. burgdorferi



ACRODERMATITIS CHRONICA ATROPHICANS (ACA)

Appears late in the illness

- Usually associated with *B. afzelii* but some cases associated with *B. garinii* have been reported
- Inflamed skin slowly evolves into thinned, atrophic skin and sclerotic patches are possible
- Underlying neuropathy



LABORATORY TESTING FOR LYME DISEASE

- “Standard” serologies- IFA and ELISA
- Western Blot
- ImmunoBlot
- Lyme Screen Immunoassay (new)
- T-cell response assay
- Urine antigen capture (CSF too)
- Standard PCR
- Culture-enhanced PCR (cePCR) (new)

LYME ELISA- SHOCKINGLY INSENSITIVE

Most commercial ELISAS are based upon a lab strain called B31

- ELISA- Sensitivity averages 49% (range 29% to 75%) *(Stricker, BMJ 2007; 335 (7628): 1008)*

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%	-
MEAN TOTAL	49.01%	96%

Trade-off between Sensitivity and specificity

1. Schmitz et al. Eur J Clin Microbiol Infect Dis. 1993;12:419-24
2. Engstrom et al. J Clin Microbiol. 1995;33:419-27.
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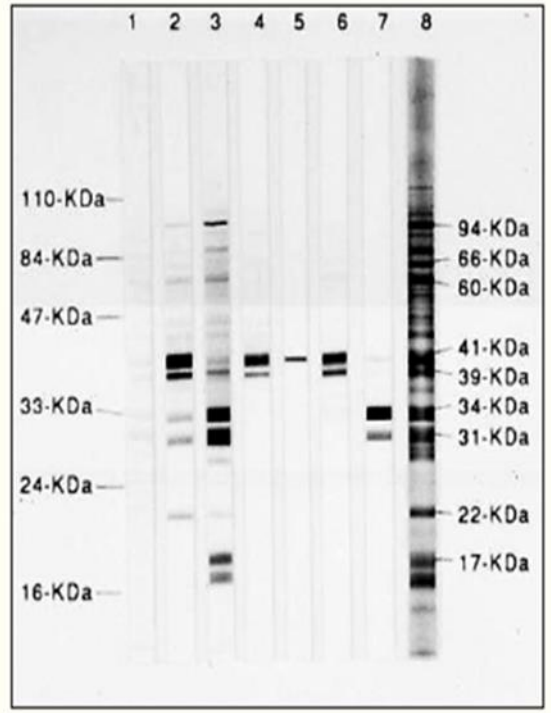
9. Wormser et al. Clin Vaccine Immunol. 2008;(10):1519-22.

LYME WESTERN BLOT

- Cultured Lab strains of Borrelia are lysed, antigens are separated by electrophoresis and then transferred to a membrane strip
- Patient serum is added, and if antibodies are present they will bind to the antigens and a dark band will appear on the strip where the antigens ended up after electrophoresis
- Interpretation is based upon whether or not a band is present, its location, and its intensity
- Disadvantages-
  - Band location is migration-dependent- uses electrophoresis
  - Confusion with nonspecific antibodies is possible
  - Band intensity is culture-dependent and not always consistent
  - Overall sensitivity 50%-70%- not much better than the ELISA



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

CDC AND THE LYME WESTERN BLOT

CDC developed interpretation criteria that are for epidemiologic surveillance and not for clinical diagnosis!

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
- The result is unacceptably low accuracy

Do not use CDC criteria for diagnosis! Only surveillance.

TWO-TIER TESTING

- 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 FOR LYME

The CDC Lyme two-tier test must begin with an ELISA as tier one, and then use a western blot or another ELISA as tier two

- PROBLEMS
  - The sensitivity of the ELISA 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 are still missed
  - Illogical to use another ELISA as tier two- second one is no better than the first
- Also for SURVEILLANCE and not for clinical diagnosis

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 hospitals and other labs
- In fact, in the case of Lyme, FDA-approved test kits are based upon lab strain B31 and are known for their insensitivity
- Lab test validation is performed by others- CLIA, Medicare, individual states, CAP

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

- In fact, "Laboratory-developed tests" often use methods that are more accurate than the FDA-approved ones

BORRELIA SPECIES IN USA

B. Burgdorferi *senso lato* (Lyme)

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 must be included when testing

LYME IMMUNOBLOT- Technology

- Recombinant technology makes this method more sensitive and more specific than other serologies
- Examples-
  - Able to detect IgM and even IgG in early Lyme, with a combined sensitivity of 93%
  - A positive IgM, even in late disease, is 97% specific- no longer can dismiss this
- Uses recombinant antigens from multiple species, broadening the number of Lyme Borrelia that can be detected
  - In Lyme, can detect all Bb sl
  - In TBRF, can detect all major pathogenic species known to exist in USA patients
- Basically, if free antibody is present, you will get a positive result

LATEST VALIDATION RESULTS ON THE LYME IMMUNOBLOT

Blinded testing using CDC-supplied test samples

- Samples included positives, negatives, other illnesses, early Lyme and later stages of Lyme
- *Laboratory (IGeneX) criteria were used, not CDC-criteria*

RESULTS

- 100% specificity- NO false positives despite using in-house developed criteria
- 90% sensitivity- This includes samples from all stages of infection, males and females, and a wide age range- good agreement with previous reports
- Vastly superior to two tier testing, and better than any other reported testing method

PERFECT EXAMPLE OF A LABORATORY-DEVELOPED TEST THAT OUTPERFORMS FDA-LICENSED TESTS

NEW TEST! IGENEX LYME SCREEN
IgM and IgG Immunoassays

REPLACES LYME IFA (Immunofluorescence assay) effective 02/01/2023
Why? *Better sensitivity and specificity*

Overall Clinical Sensitivity -IgG and/or IgM					
Disease Stage	N	IgM	IgG	Overall	Sensitivity
		Positive	Positive	Positive	% Positive
Early Lyme	28	16	16	21	75%
Neuo-cardiac Lyme	8	8	8	8	100%
Lyme arthritis	8	8	8	8	100%
Total Samples	44	32	32	37	84.09%

Clinical Specificity Summary			
Disease Stage	**Total Samples**		
	N	Positive	Specificity
Fibromyagia	8	1	88%
Healthy endemic	16		100%
Healthy non-endemic	16		100%
Mononucleosis	8	1	88%
Multiple sclerosis	8		0%
Rheumatoid arthritis	8		0%
Severe periodontitis	8	2	75%
Syphilis	8	1	100%
Overall	**80**	**5**	**93.8%**

Note: Samples used in this study were provided by CDC

IGENEX LYME SCREEN
IgM and IgG Immunoassays

Replaces the current Lyme IFA screen in all IGeneX panels at no additional cost

Example Panels

IB1 - Lyme ImmunoBlot Panel 1
~~Lyme IgG/IgM/IgA Screen~~, Lyme IB IgM & IgG

TBD6IB - Tick Borne Disease Panel
FISH: Babesia & Bartonella
IFA (IgM & IgG): ~~Lyme IgG/IgM/IgA~~, HME, HGA, R. rickettsii/typhi IgG
ImmunoBlot (IgM & IgG): Lyme, TBRF, Babesia, Bartonella
PCR: Lyme serum & whole blood, TBRF serum & whole blood

TICK-BORNE RELAPSING FEVER (TBRF)- *Unexpected clinical presentation!*

Classic TBRF- an acute illness that includes a high fever and severe malaise, lasting for just a few days, and ending with severe sweats and weakness

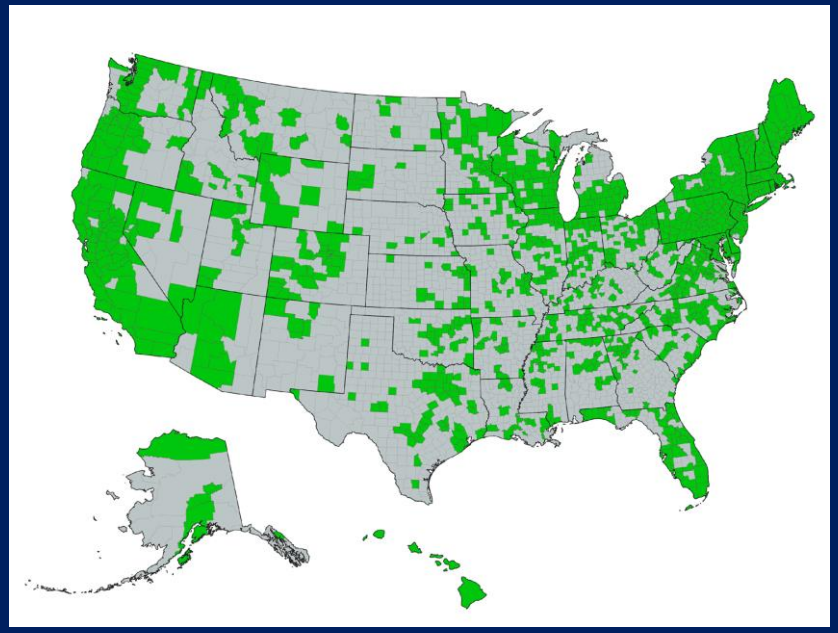
- This is followed by several days of relative wellness, then the acute symptoms recur and repeat every 5 to 7 days

However, this is not the case with a large number of patients, who in fact present as Lyme disease

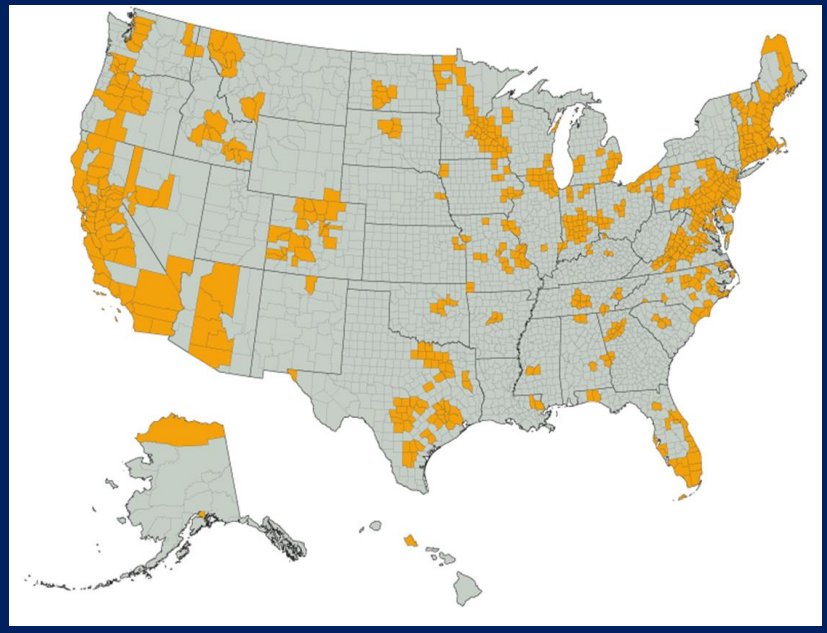
- Diagnostic uncertainty may result because Lyme tests do not detect TBRF

LYME AND TBRF ANTIBODIES ARE WIDESPREAD

LYME



TBRF



TBRF IS SURPRISINGLY COMMON!

543 US patients with suspected Lyme:

- 32% were positive for Antibodies to Lyme Borrelia
- 22% were positive for Ab to Relapsing Fever Borrelia
- 7% were positive for Ab to both LB and RFB

- Clinically, they ALL resembled Lyme patients, not “relapsing fever” patients

CONCLUSION: Lyme testing must also include TBRF

CAN THIS BE "SERONEGATIVE LYME"?

Possibility is that 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 the Lyme Borrelia, or for TBRF
- Similarly, commercial TBRF serologic testing has only been validated against two species (hermsii and miyamotoi, and each test has to be ordered individually)

Solutions-

- For serologies, use ImmunoBlots as they are inclusive of multiple species
- For direct testing, use the Culture (cePCR) as it offers genus-level detection

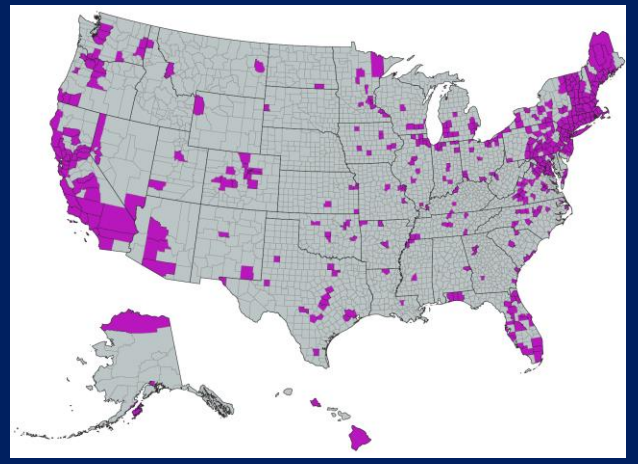
TESTING RECOMMENDATIONS- Borrelia

- Always test for both Lyme and TBRF for initial diagnosis and for re-evaluations-
- Use tests that can detect the broadest range of species
  - Lyme- Bb sl (ImmunoBlot, Culture, IGeneX urine antigen capture, IGeneX T-cell response assay)
  - TBRF- maximal species coverage (ImmunoBlot, Culture)
- Often need to combine multiple testing methods (test panels)
  - ImmunoBlots + Culture (cePCR)- indirect + direct
  - Option to add urine antigen testing and T-cell response
  - Synovial biopsy with PCR testing has a reasonably good yield

BARTONELLA-
Documented in at least 49 states

Extremely common in Lyme/TBD patients

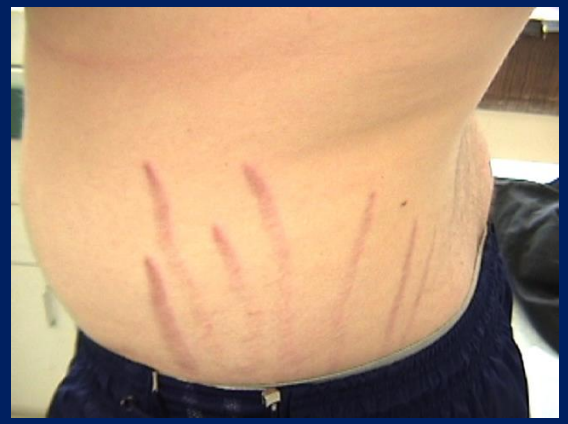
- Is easily confused with Lyme
- Over 45 species known to exist!!
- Many ways to acquire an infection:
  - Common vectors: fleas, mosquitos, biting flies, mites, red ants
  - Now demonstrated that ticks may also transmit Bartonella
  - Animal bites and scratches, needle sticks, maternal-fetal
- Worldwide distribution- even found far above the arctic circle!

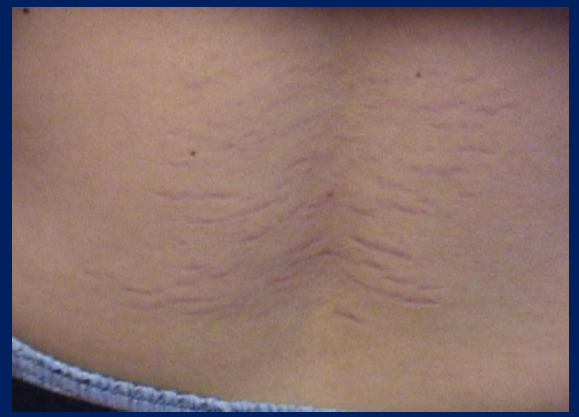


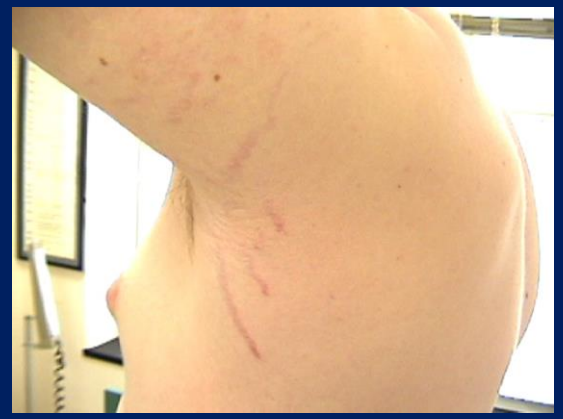
BARTONELLA- CLINICAL PICTURE

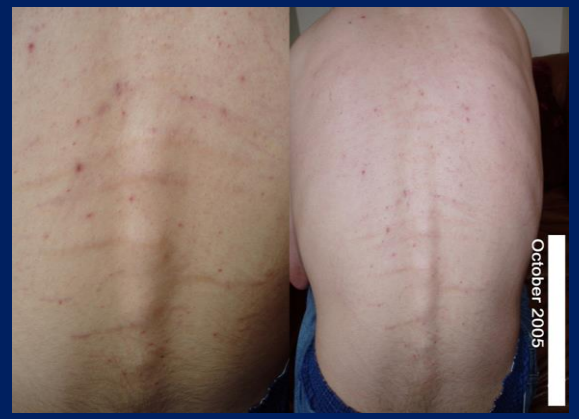
- CNS- irritability and global dysfunction
  - Anxiety, rage attacks, panic attacks, insomnia, depression, tremors, seizures, ataxia, antisocial behavior, hallucinations, schizophrenia, dementia
- Eyes- uveitis, retinitis, retinal artery and vein thromboses
- Regional lymphadenopathy
- Connective tissues: tender nodules (skin, along fascia), sore soles, tendonitis, bone pain, painful joints without synovial swelling
- Peculiar skin manifestations:
  - “Bartonella tracks” (like atypical stretch marks)
  - “Bacilliary angiomatosis” (red bumps that may scab)
- GI involvement
  - Gastritis, mesenteric lymphadenitis, peliosis hepatis

BARTONELLA RASHES

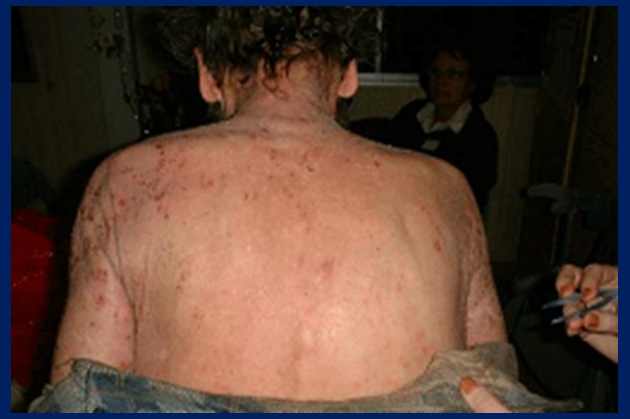


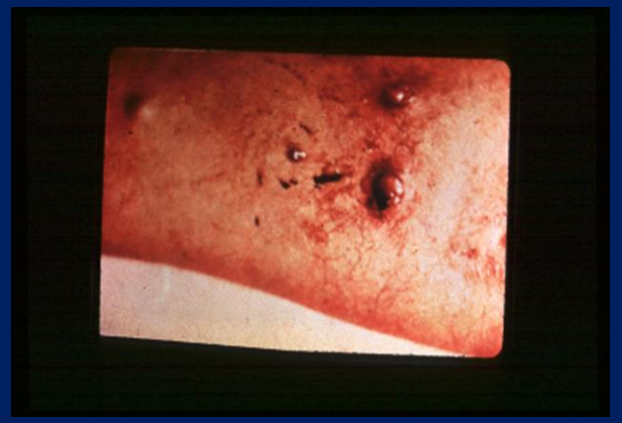






BARTONELLA TRACKS





BACILIARY ANGIOMATOSIS

BARTONELLA TESTS

- IFA- old technology; designed to detect only B. henselae.
- ImmunoBlot : More sensitive and designed to detect multiple species
- FISH (Fluorescent in-situ hybridization)- Direct visualization via fluorescent RNA probe; is genus-specific thus offers extended species coverage. *Also can detect Bartonella hidden in biofilms*
- Standard PCR – Detects presence of DNA of the organism after amplification; useful but of limited sensitivity
- Culture (cePCR)- increases sensitivity and overcomes many of the technical limitations of standard PCRs; genus-level detection allows for broad coverage

BARTONELLA TESTING- Recommendations

Notoriously difficult to detect!

- Because of stealth features, no single test is 100% sensitive
- Also, multiple species are infecting our patients
- Therefore need highest sensitivity and broadest species coverage

Testing by multiple methods is recommended

- ImmunoBlot + FISH + Culture (cePCR)
- If there is a known B-cell functional defect, substitute a T-cell response assay for the ImmunoBlot

BABESIOSIS

Malaria-like intra-erythrocytic parasite

- Is the most common co-infection in Lyme patients
- Causes fever, sweats, headache, air hunger, cough, profound fatigue, balance issues and cognitive dysfunction
- Many other symptoms overlap with Lyme and TBRF
- Transmitted by the same tick that transmits Lyme
- The two dominant species in the USA are B. microti and B. duncani
- B. MO-1, B. odocoilei, B. divergens- also occasionally seen
- Rarely, atypical apicomplexa can also be found in humans

BABESIA TESTING

- Stained blood smear- Done in hospitals- only useful within first week of infection
- FISH- Qualitative detection of Babesia ribosomal RNA directly in a blood smear
  - Far more sensitive than standard smear; can detect organisms in biofilms; genus-level test so has broad coverage
- IFA- Insensitive and outdated; need separate IFAs for B. microti and for B. duncani; not available for other species
- Immunoblot- Far more sensitive than IFA and offers broad species coverage
- Culture (cePCR)- is a genus-level test so it can detect at least microti and duncani- (others have been detected)

BABESIA TESTING- Recommendations

Notoriously difficult to detect!

- Because of complex parasite biology, no single test is 100% sensitive
- Also, now finding atypical species previously not expected
- Therefore need highest sensitivity and broadest species coverage

Testing by multiple methods is recommended

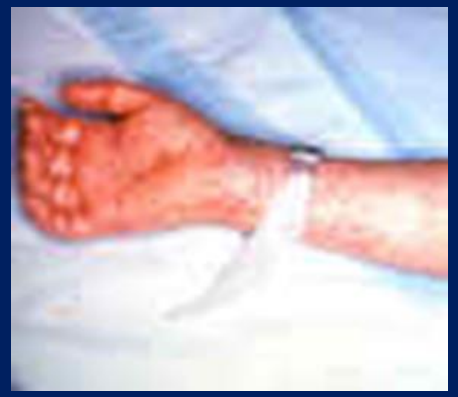
- ImmunoBlot + FISH + Culture (cePCR)
- If there is a known B-cell functional defect, substitute a T-cell response assay for the ImmunoBlot

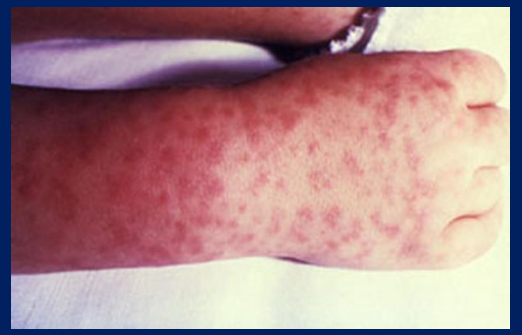
RICKETTSIA FAMILY

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

Anaplasma, Ehrlichia and Rocky Mountain Spotted Fever

- Acute fever, headache, myalgias, malaise
- Often associated with low WBCs, low platelets, and elevated LFTs
- RMSF rash- vasculitic; blanches with pressure and refills from center; includes palms and soles;
- Rash occasionally seen in the others (<5%)





RICKETTSIA FAMILY- Testing

Ehrlichia and Anaplasma

- Serology (IFA)
- NEW! Culture (cePCR)- replaces standard PCR

RMSF

- Serology (IFA)
- 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

CONCLUSIONS

- TBD test reliability is not optimum, but advances are being made
- Knowing the performance characteristics of each test will help you choose which ones to order and when
- Knowing pathogen behavior can be helpful too
- Highest yield is obtained when a panel of tests is ordered that collectively utilizes different methodologies (indirect + direct)
- Always best to use labs that are transparent with their test performance, have publications and seek acceptance in all states

Bottom line is that clinical judgement must prevail


THANK YOU!
And best wishes, from Dr. B