How to best utilize laboratory testing for tick-borne diseases

Joseph J. Burrascano Jr. M.D.

Lyme Bytes 2024

Laboratory testing for TBDs

Why test?

- Symptom overlap
- Documentation

What is necessary:

- 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- Most patients are co-infected, and many new species are being documented all the time

Many “Lyme” patients are co-infected with other TBDs

Below is data from >10,000 patients showing exposure to multiple pathogens

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%
Rickettsia	12.8%
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%

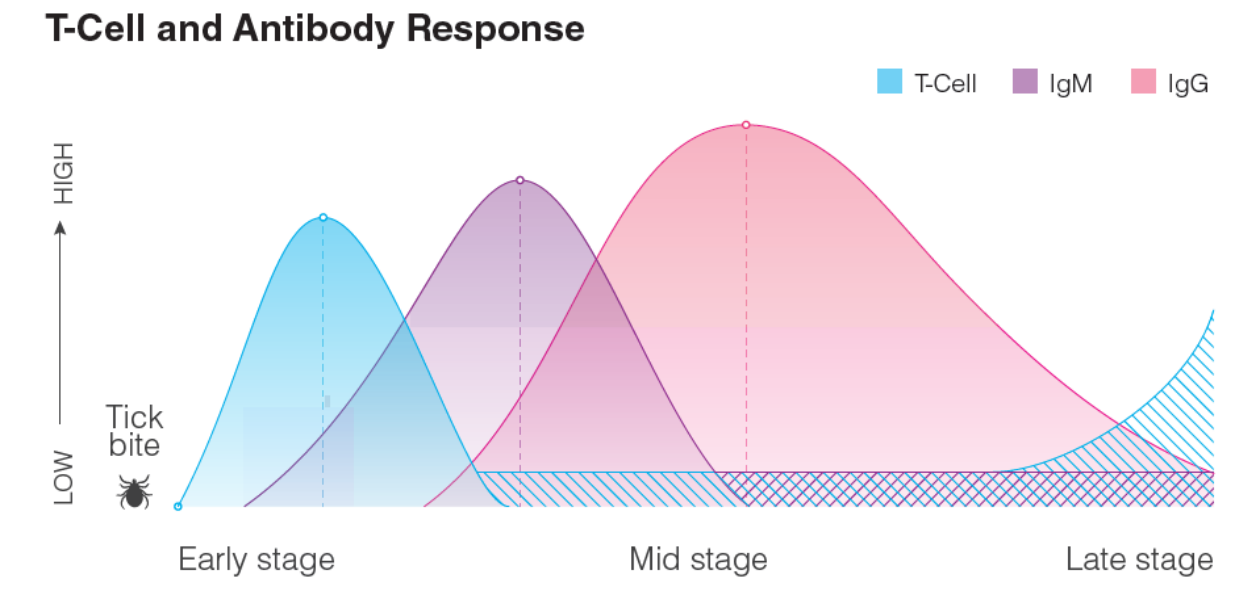
Note: This data does not show active disease. It only demonstrates that pathogen antibodies or DNA or RNA was present in the clinical samples.

CLINICAL GUIDE

INFECTION	ONSET	CYCLES	SYMPTOMS	HEADACHE	FEVER	SWEATS	RELAPSE
LYME	Gradual	4 weeks	Multisystem Migratory, cyclic Joints	Nuchal “Lyme shrug”	Afternoon, Low-grade	No	Slow (weeks)
BARTONELLA	Gradual	No	Excitatory Soft tissues Lymphadenopathy	No	Morning- Low-grade	Light	Rapid (days)
BABESIA	Can be abrupt	5-7 days	Tippy, air hunger/cough Worsens everything	Band-like, Migraine-like	Any time, Can be high	Drenching	Slow (weeks to months)
RICKETTSIAS	Abrupt	No	Acute flu Muscles	Knife in the eyes	Constant, High	Acutely	Gradual

Understanding immune response is crucial when prescribing tests

- Early after infection, T-cell response is the strongest, making T-cell reactivity assays (IgXSpot) the most effective early test.
- IgM and IgG antibodies develop soon afterwards, at which time ImmunoBlots become most effective.
- In a subset of patients, IgM response may persist
- Absent IgG response often seen in late, chronic infections- Paradoxically, the more ill, the less likely to have a positive IgG
- T-cell response may also re-appear later.
- Direct tests are mostly positive in early disease, but may be positive at any stage, especially in the immune-deficient



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

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

T-cell response assays- IGXSpot

Reflects past exposure to an organism by measuring T-cell response

Clinical features-

- Live cell testing- MUST BE A FRESH SAMPLE!!
- 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 (IGXSpot- 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

IGXSpot is available for Lyme and TBRF Borrelia, and Bartonella

Serologies

Widely available, rapid turn around, can be engineered to increase accuracy and broaden species coverage

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

Is why many will add other tests to their serology screens- T-cell assays and/or direct tests (culture, FISH, PCR)

Fluorescent in-situ hybridization assay (FISH)

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

- 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!!
- The IGeneX FISH is designed to be genus-specific, increasing breadth of species detection
- 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

Available for Bartonella and Babesia

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

- Highly specific but very poor sensitivity for blood specimens
  - Believe a positive test, but a negative result never rules out infection
- Can test blood, other body fluids, and biopsy samples
- The IGeneX PCRs are crafted to offer genus-level detection
  - This allows for detection of multiple species

PCR testing is available for most of the TBDs and many viruses

- Borrelia, Babesia, Bartonella, Ehrlichia, Anaplasma, RMSF, others

PCR vs. FISH

FISH detects pathogen RNA - reported as positive or negative

- RNA does not persist post-infection- *disappears as soon as pathogen dies*
- Can be designed to be **genus-specific**, increasing breadth of species detection
- *Able to detect pathogens even if embedded in **biofilms**!!*

PCR detects pathogen DNA- reported as positive or negative

- Some believe DNA can persist post-infection and a positive PCR may reflect dead pathogens or their fragments-
  - Our blood contains potent DNAses that clean up circulating DNA
  - However DNA in tissues MAY persist after death of the organism
- Sensitivity in blood samples is low due to circulating inhibitors to the PCR method
  - PCR inhibitors- hemoglobin, heparin, and high concentrations of host DNA

Culturing is the gold standard

Culturing ("cePCR") is available from IGeneX for Borrelia (Lyme and TBRF), Bartonella, Babesia, Ehrlichia, Anaplasma and Rickettsias

- Blood sample is held in proprietary culture medium for two weeks
- After two weeks, sample is tested by a sensitive and validated PCR
- Genus level reporting
  - Broadens number of pathogens being detected, but will not identify species
- Specificity- 100% in validation testing
- Sensitivity- *at least* 10X that of a PCR

UNUSUAL SPECIES HAVE BEEN DETECTED!!

- Has already solved "mystery cases"

Each type of pathogen requires a different culture medium, so tests must be ordered individually

Optimizing testing using indirect tests

Indirect tests- ImmunoBlot 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)
- Can test while on antibiotics

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

Optimizing testing using direct tests

Direct tests: Culture, FISH, 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
- 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)

LYME DISEASE- Borrelia burgdorferi s.l.

The most common vector-borne infection in the USA

- Can live in tissues, inside cells, and transits through the blood stream only intermittently
  - Makes direct testing less sensitive
- Evades host immunity:
  - Inhibits and kills B- and T-cells; inhibits maturation of natural killer cells
  - May render indirect tests less sensitive
- Can persist and become chronic despite antibiotic treatments
  - Here, is both a lower pathogen load and weaker immune response-
  - Is why combining indirect and direct tests are necessary to maximize yield

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

TBRF may be clinically indistinguishable from Lyme

CASE SERIES:

- 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

Serologic testing for Lyme disease

- "Standard" serologies are notoriously insensitive and detect only one species (typically Bb ss, strain B31):
  - Commonly made from lysed lab strains- imprecise, "dirty"
  - IFA and ELISA-Sensitivity 50%
  - Western Blot- Sensitivity 50%-70%
- **Recombinant assays** are FAR better- highest sensitivity and specificity and can be engineered to detect multiple species
  - **Lyme Screen Assay** (LSA, IGeneX)- replaces the IFA and ELISA
  - **Broad Coverage Assay** (BCA, IGeneX)- simplified way to ensure broad species coverage
  - **ImmunoBlot**- Multispecies capability, separate IgM and IgG reports; will identify the major species

Lyme ELISA- what the data shows

Most commercial Lyme ELISAS are based upon lab strain B31

- ELISA- Sensitivity averages 49% *(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%

MULTI-WELL AND MICROARRAY TESTING

- Technology used in these tests is a modification of the ELISA and are no more sensitive or specific!!

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.
3. Ledue et al. J Clin Microbiol. 1996;34:2343-50.
4. Bakken et al. J Clin Microbiol 1997; 35(3): 537-543.
5. Trevejo et al. J Infect Dis. 1999;179:931-8.
6. Nowakowski et al. Clin Infect Dis. 2001;33:2023-7.
7. Bacon et al. J Infect Dis. 2003;187:1187-99.
8. Coulter et al. ., J Clin Microbiol 2005; 43: 5080-5084.
9. Wormser et al. Clin Vaccine Immunol. 2008;(10):1519-22.

CDC Lyme laboratory diagnosis

CDC criteria are for epidemiologic surveillance and not for clinical diagnosis!

Western blot interpretation criteria

- They include bands which are NOT specific to Lyme Borrelia- this can give rise to false positives (many bands!!)
- They EXCLUDE bands that are very specific to Lyme Borrelia- gives rise to false negatives (31 and 34)
- Require a minimum of 5 bands on the IgG western blot- not all patients will have this

Two tier testing

- First tier- an insensitive ELISA. If positive, then another, different ELISA or a western blot

SIGNIFICANTLY INSENSITIVE AND NOT FOR CLINICAL DIAGNOSIS!

Lyme ImmunoBlot is now FDA cleared!

This testing method, used by IGeneX for years, has been converted into a test kit that has received *full FDA clearance*

- **Considered a Modified Two Tier Test**, this kit consists of a combination of the Lyme Screen Assay (LSA) as tier one, with the Lyme IgG ImmunoBlot as tier two.

ImmunoBlot interpretation is based upon the "IGeneX Criteria", and not CDC criteria

- **Only two bands are needed** on the ImmunoBlot to be considered positive if the LSA is positive
- **Bands 31 and 34 are included**- *this is the only FDA-cleared Lyme serological test that includes these bands*
- Sensitivity 93%-100%, specificity 97%-99%

IGeneX Inc. wishes to thank the Bay Area Lyme Foundation, the Centers for Disease Control and Prevention and the entire IGeneX, Inc. staff.

And a big Thank You to the many practitioners who have believed in and supported IGeneX over the years.

Guidelines for testing for Borrelia-1

Always consider TBRF in your Lyme patients- ignoring TBRF testing can lead to "seronegative Lyme"

- Simple screening test- **Broad Coverage Assay** (BCA, IGeneX)
  - Simple yes-no answer; does not separately report IgM and IgG or name species
- Most comprehensive serological test- **Lyme and TBRF ImmunoBlots**
  - Will separately report IgM and IgG and name the major species found
  - If two-tier testing is required for Lyme, then add the LSA as tier one, and the ImmunoBlot will be tier two
- Adding the **IGXSpot T-cell response assay** can increase yield and also inform the status of the patient's immune system
- **Culturing (cePCR, IGeneX)** for Lyme and TBRF further increases yield and is capable of detecting even atypical species; strongly recommended for those with compromised immunity

Guidelines for testing for Borrelia-2

- **Early** disease (summer flu, atypical EM rash)
  - IGXSpot reacts first- within a few days
  - ImmunoBlot also excellent- picked up 93% of CDC-defined early cases in blinded testing
- **Established** Lyme, not chronic (immunity reasonably intact)
  - ImmunoBlot is always the first choice. Broad coverage assay (BCA) may be a cost-effective screening test. Adding the cePCR (culture) will increase yield
- **Chronic** Lyme and situations with suspected immune compromise
  - ImmunoBlot + cePCR (culture). Adding IGXSpot (T-cell response assay) will give additional information on immune status
  - Serial testing with the ImmunoBlot during treatment may help guide therapy

Bartonella

- Bartonellosis in the Lyme patient may be from any of a number of different Bartonella species, and more than one species has been found in individual patients
  - MUST insist on tests capable of detecting multiple species
- Lyme patients with a Bartonella co-infection pose diagnostic challenges due to symptom overlap and poor quality standard testing
  - Bartonella often goes overlooked so one must always consider Bartonella
  - Insist on only the highest quality, proven test methods

Bartonella tests

- **IFA:** old technology; designed to detect only one species- Bartonella henselae
- **ImmunoBlot** : More sensitive, more specific, and designed to detect multiple species
- **IGXSpot:** T-cell response assay; genus-level detection thus can 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**: Limited sensitivity- not recommended
- **Culture (cePCR)**: Genus-level detection allows for broad species coverage

Bartonella testing- ImmunoBlot

Bartonella ImmunoBlot (IGeneX)

- Very broad species coverage- Positive samples can detect multiple species and identify and report the following four: B. elizabethae; B. henselae; B. quintana; B. vinsonii
- Does not cross-react with other TBDs
- Highly sensitive and specific

Specificity

IgM – 100%

IgG – 100%

Sensitivity

IgM – 75%

IgG – 88.9%

IgM+IgG – 100%

Study Samples (n=46)		
Sample Type	N	Bart IB
Lyme Positive	5	0
Lyme +TBRF Positive	2	0
Lyme +Babesia Positive	3	0
Lyme + HGA Positive	1	0
Babesia Positive	7	0
Controls (Neg)	12	0
Bartonella FISH, IFA and Western blot Positive	5	5
Bartonella IFA and Western blot Positive	9	9
Bartonella FISH, Western blot Positive	2	2

Bartonella testing- FISH

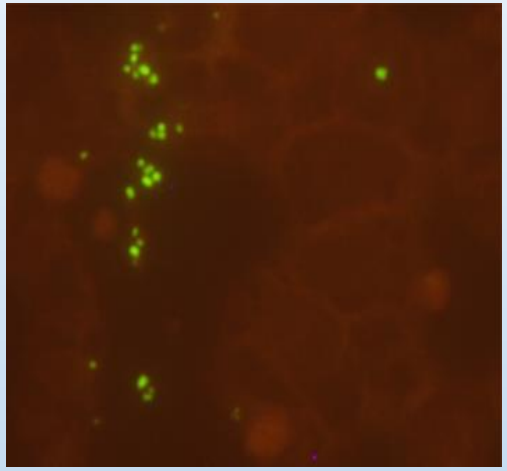
FISH (Fluorescent in-situ hybridization)- Direct visualization via fluorescent RNA probe

- Is genus-level test thus offers extended species coverage.
- *Also can detect Bartonella hidden in biofilms*
- Accuracy:
  - **Inclusivity study**- able to detect all tested Bartonella species (6, plus an atypical)
  - **Specificity study**- No cross reactivity with any other common TBD pathogen, nor with Plasmodia or Trypanosomes

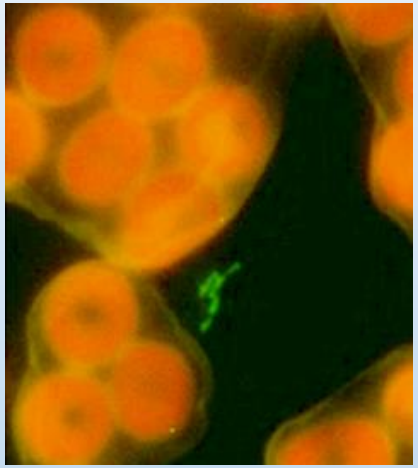
Specificity Study		
Exclusivity		
Specificity Study	100%	**Agents tested:**
		Babesia microti
		Babesia duncani
		Borrelia burgdorferi
		Plasmodium falciparum
		Trypanosoma cruzi
		Anaplasma phagocytophilum
		Ehrlichia chaffeensis
		Rickettsia rickettsii
		Rickettsia typhi
Inclusivity		
Inclusivity Study	100%	**Agents included as confirmed by PCR and DNA sequencing:**
		B. vinsonii
		B. spp.
		B. henselae
		B. quintana
		B. grahamii
		B. elizabethae

- No cross reactivity to these other pathogens
- Detected all five tested Bartonella species plus one other, unidentified Bartonella

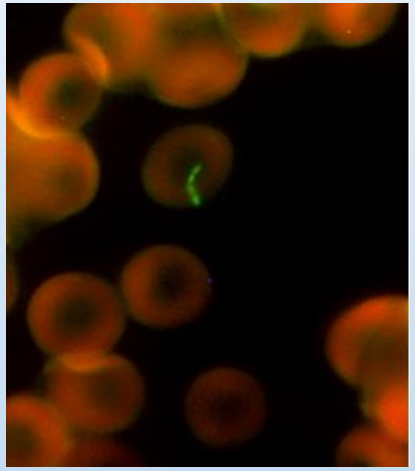
Bartonella FISH - patient samples



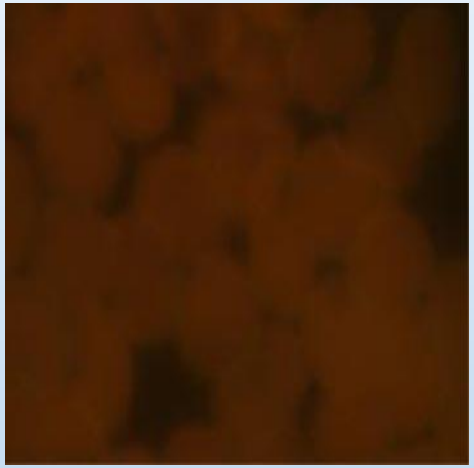
Patient 1 – B. henselae



Patient 2 – B. henselae



Patient 3 – B. quintana



Patient 4 – Negative

All three positives confirmed by DNA sequencing

Negative control

Bartonella cePCR (culture)

- Standard PCR –
  - Performance has been disappointingly poor-
  - limited sensitivity (6%) but highly specific
- Droplet digital PCR-
  - Improved sensitivity (30%)
- **Culture (cePCR)**-
  - Increases sensitivity further- at least 10X that of a standard PCR
  - Genus-level detection allows for broad coverage
  - Specificity 100% in validation studies
  - **Has replaced the PCR for these reasons**

Bartonella testing- Recommendations

Because of stealth features and potential for multiple species to cause infection, recommendation is to test by combining multiple methods

- ImmunoBlot + FISH + Culture (cePCR)
- If there is a known B-cell functional defect, add an IGXSpot (T-cell response assay)

Using a combination of direct tests (FISH, cePCR) plus indirect tests (ImmunoBlot for B-cell response, IGXSpot for T-cell response) you will be able to determine host immune response to this infection

- Extremely useful for initial assessment and for serial tracking of progress

Babesiosis

Babesiosis is the most common co-infection in Lyme patients

Many symptoms overlap with Lyme, TBRF and Bartonella so testing is extremely important

- The two dominant species in the USA are B. microti and B. duncani
- B. MO-1, B. divergens. B. odocoilei and at least 3 others are also occasionally seen
- Rarely, atypicals can be found in humans that would never have been detected with standard testing

Babesia Odocoilei

B. odocoilei- controversial!

- Found in many ticks all across North America
- Published case reports- cases found in Canadian and USA patients
- Multiple IFA-stained patient samples said to indicate odocoilei
- But a series of 460 Babesia-positive patient samples had DNA sequencing- did not find ANY B. odocoilei
- Confusion may relate to which primer sets are being used for PCR and sequencing, and/or nonspecificity of the IFA stain
- Immunoblot data shows a significant percentage of Babesia species in human patients are not microti or duncani. Could these be divergens and/or odocoilei?

Babesia testing

- **Stained blood smear-** Done in hospitals- only useful within first week of infection. Limit of detection 0.5% of RBCs infected
- **FISH**- Blood slides target specific rRNA sequences using fluorescent probes
  - Far more sensitive than standard smear; L.O.D. 0.001%
  - Can detect organisms in biofilms; genus-level test so has broad coverage
- **Immunoblot**- the most sensitive serological test, and offers broad species coverage; Validation studies: 100% specificity
- **Culture (cePCR)**- is a genus-level test so it can detect at least microti and duncani- (and others have been detected too)

Babesia ImmunoBlots showed no cross reactivity to other pathogens

FIRST SERIES- PATHOGENS TESTED

Lyme Borrelia

- Bb B31
- Bb 297

TBRF Borrelia

- B. hermsii
- B. turcica
- B. coriaceae
- B. mayonii

Bartonella

- B. elizabetheae
- B. henselae
- B. vinsonii
- B quintana

E. coli

SECOND SERIES

Anaplasma, Ehrlichia, Rickettsia, Bb, plus normal controls

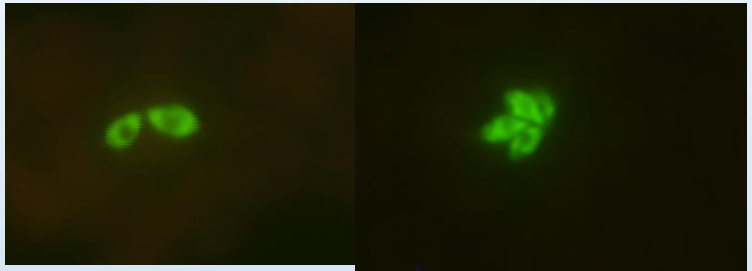
Clinical Specificity - Overall Summary						
Source		**Sample Types**	**n**	**IgM (+)**	**IgG (+)**	**IgM and/or IgG**
In-house	Set 4	Anaplasma phagocytophilum	4	0	0	0
		Ehrlichia chaffeensis	1	0	0	0
		Rickettsia	3	0	0	0
		Borrelia burgdorferi	1	0	0	0
	Set 5	Normal Controls	15	0	0	0
False Positive				0	0	0
True Negatives			24	24	24	24
Specificity			**100%**	**100%**	**100%**	**100%**

Babesia ImmunoBlot – Highly Sensitive

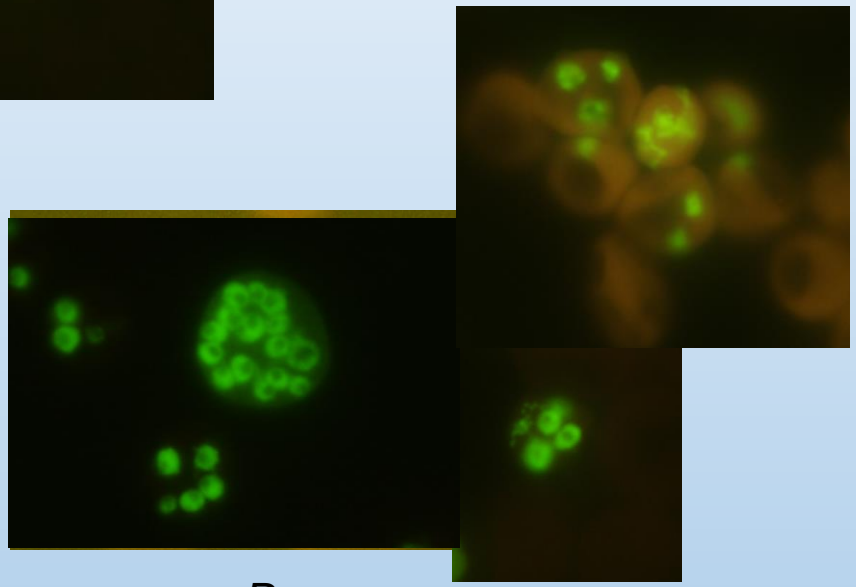
Babesia FISHand or IFA Positive Patients Serum Samples Tested by Babesia ImmunoBlots IgM and IgG					
Sample Types	**Samples tested**	**Babesia ImmunoBlots Results**			
		Genus (+)	**B. microti (+)**	**B. duncani (+)**	**Negative**
Set 1 (Babesia FISH Pos)	13	12	5	6	1
Set 2 (Babesia FISH Pos)	17	15	6	1	2
Set 3 (Babesia IFA Pos)	42	36	14	13	6
Total Samples	72	63	25	20	9
Sensitivity		**87.5%**	**34.7%**	**27.8%**	**12.5%**

Note that of 63 positive samples, 18 were NOT microti or duncani
Demonstrates ability to detect a broad array of species

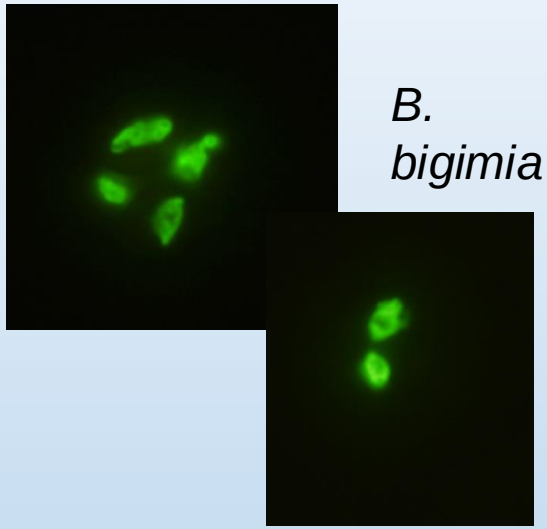
Babesia FISH detects Babesia at the genus level



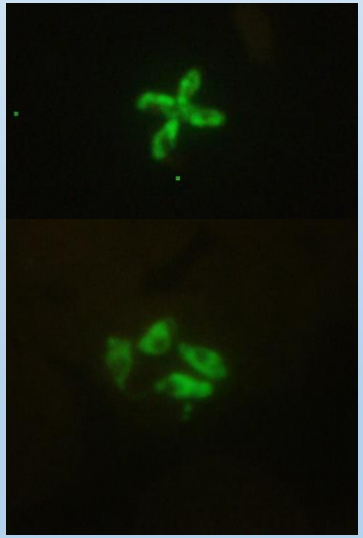
B. bovis



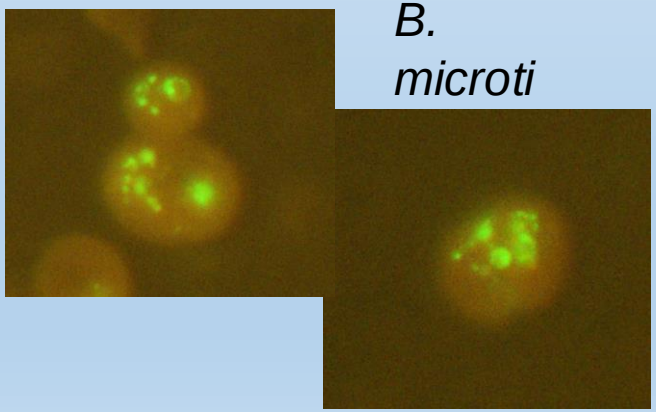
B. duncani



B. bigimia



B. divergens



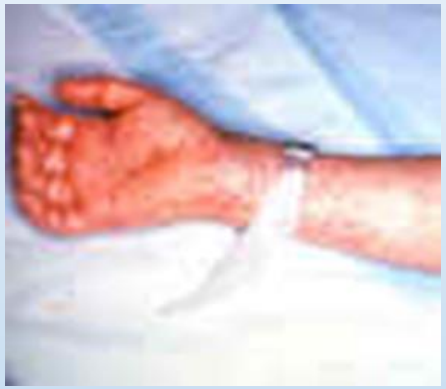
B. microti

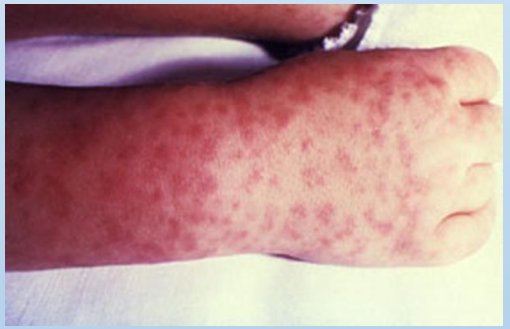
Rickettsia family

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

Anaplasma, Ehrlichia, Rocky Mountain Spotted Fever and other Rickettsias

- CAN BE FATAL!!
- 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, Anaplasma and Rickettsias

- Serology (IFA)
- 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

Testing by multiple methods increases accuracy-

Direct Testing **These tests look for the pathogen in the specimen**	**Indirect Testing** **These tests look for antibodies or T-cell reactivity in the blood**
Why use direct tests: • Not every patient has detectable antibodies • Patients may be immune-suppressed • Antibodies may be bound in immune complexes • Some patients are challenging blood draws (i.e. young kids) so urine specimens can be tested instead	**Why use indirect tests:** • Pathogens not always in the bloodstream • Superior at detecting early and/or late stage disease • Certain tests can detect T-cell response, an early disease indicator and are useful in B-cell immune deficiencies
Types of direct tests: • **Culture** (cePCR)- multiple sources possible • **FISH**- blood samples only • **Urine antigen capture** • PCR	**Types of indirect tests:** • **ImmunoBlot** • **IgXSpot** (T-cell response) • IFA, ELISA • Western Blot

TESTING GUIDE

TEST	METHOD	FEATURES	WHEN TO USE	ACCURACY
IFA, ELISA, WB	Serology	Single species	**Not recommended**	False negatives False positives
ImmunoBlot	Serology	Recombinant Ag's Multiple species	All stages	Maximal
T-cell response assay	Mitogen stimulation assay	Limited time window	Early and In B-cell dysfunction	Medium- depends on timing
PCR	DNA detection	Fluids and tissues	**Tissues only** if possible	Insensitive but very specific
Culture (cePCR)	Culture with pathogen ID confirmed by PCR	Blood and CSF	All stages but not if on treatment	Maximal
FISH	RNA-stained blood slide	The best test if biofilms are present	All stages but not if on treatment	Good
Urine antigen capture	Direct antigen detection	Lyme only	When blood draws are to be avoided	Good

LAST SLIDE: Tips to optimize testing

- Timing
  - Do direct tests (cePCR, FISH) when pathogenemia is highest
    - Early disease, disease flares, immune compromised
    - Do NOT do direct tests when currently on treatment
      - (Urine antigen assay is the exception)
  - Do indirect tests (ImmunoBlots, IGXSpot T-cell test) when immune response is highest
    - Disseminated illness, reasonably intact immunity
    - In chronic cases, indirect tests may become more sensitive as treatment continues
    - OK to do while on treatment
- Combine methods to maximize sensitivity
  - Direct test + indirect test
  - EXAMPLE: cePCR + Immunoblots/FISH


THANK YOU!!