IGeneX Inc.

Bartonella for Clinicians

Laboratory testing and introducing the IGeneX Bartonella ImmunoBlot

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

July 2021

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

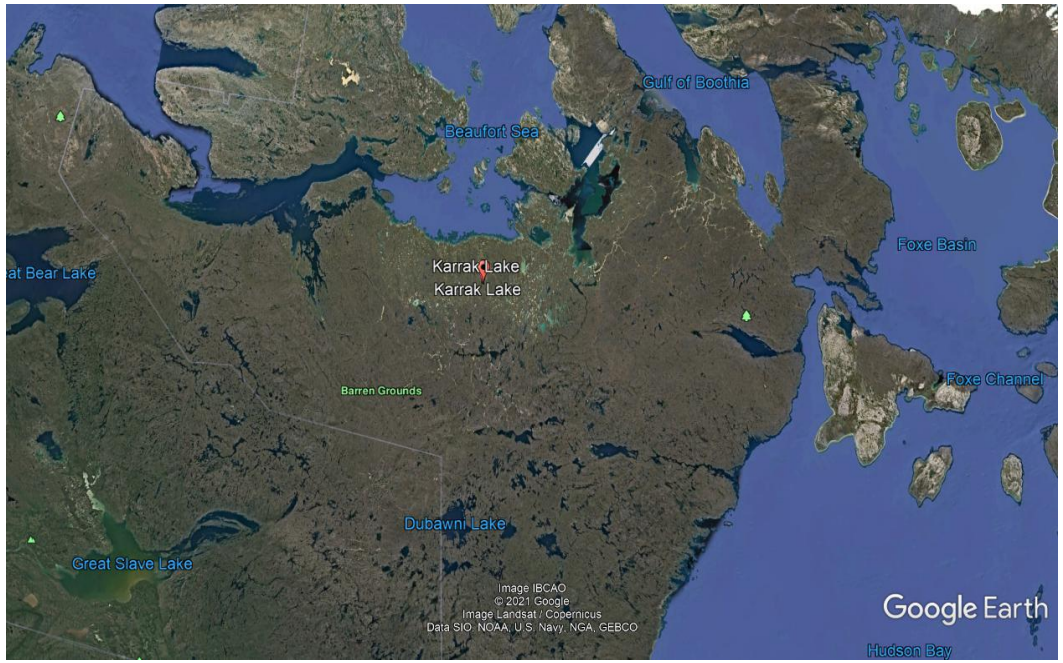
- President & Laboratory Director of IGeneX Clinical Laboratory
- Over 40 Years of Research Experience in Immunology, Molecular Biology & Microbiology
- Author of Multiple Publications & Holds More Than 20 Patents
- Member of ILRAD as a Post-Doctoral Scientist
- Started the First DNA Sequencing Laboratory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

Bartonella

- **Over 30 species** so far recognized, and a patient can harbor more than one species!
  - Gram negative facultative intracellular parasite but can also be found extracellularly in blood and tissues
  - Major biofilm builder- even intravascular biofilms
  - Mammals and birds
- **Many ways** to acquire an infection:
  - Vectors: ticks (both hard ticks and soft ticks- bat ticks in NJ), fleas, mosquitos, biting flies, mites, red ant!
  - Dog and cat bites and scratches
  - Needle sticks
  - Maternal-fetal
- **Worldwide** distribution- even found far above the arctic circle!

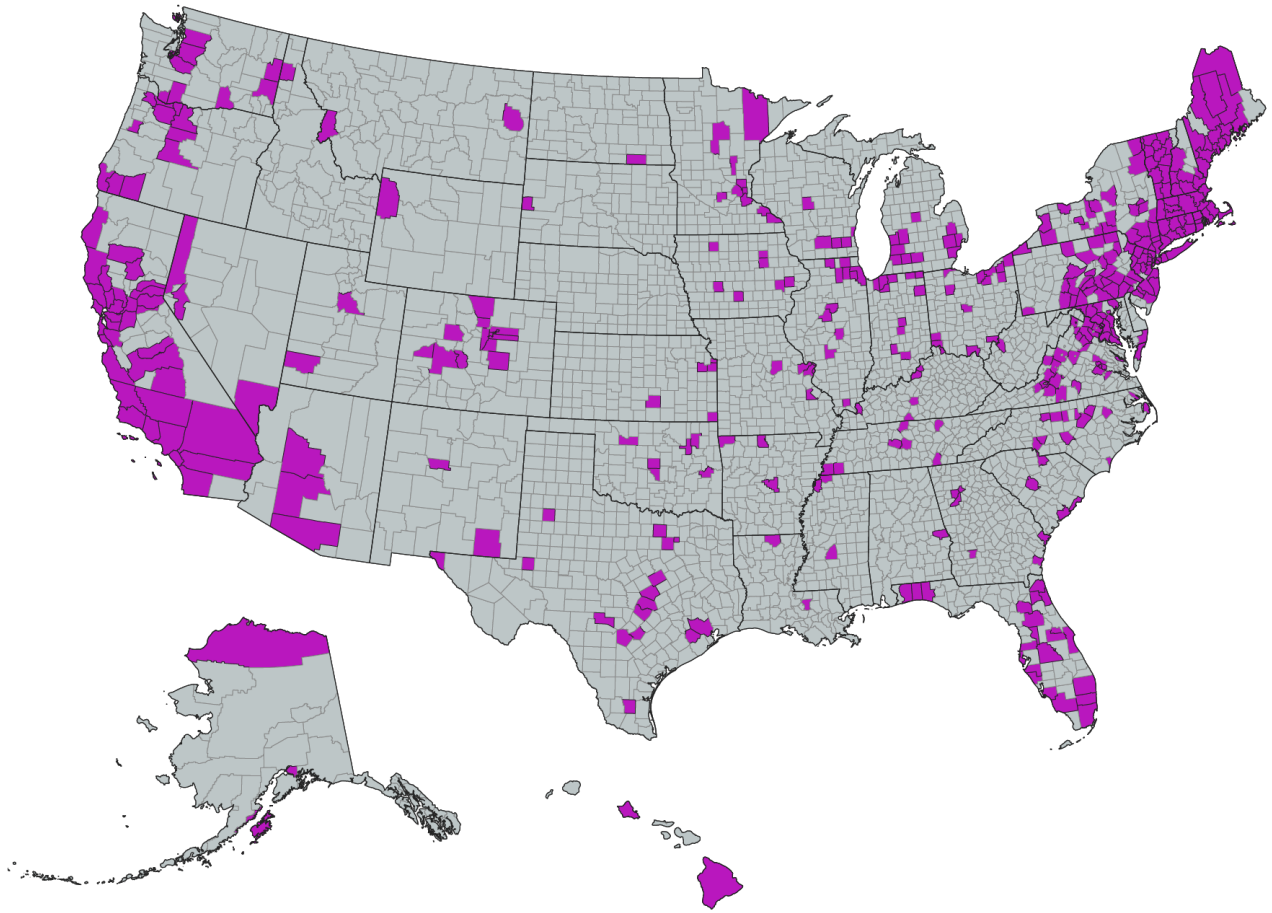
YIKES!

Buhler, K.J., Maggi, R.G., Gailius, J. *et al.* Hopping species and borders: detection of *Bartonella* spp. in **avian nest fleas and arctic foxes** from Nunavut, Canada. *Parasites Vectors* **13,** 469 (2020). https://doi.org/10.1186/s13071-020-04344-3



Not just the Artic!

IGeneX has detected Bartonellosis in 49 states



Bartonella Species

- Most common species are *B. henselae*, *B. quintana*, *B. elizabethae*, *B. washoensis*, *B. vinsonii*, *B. koehlare*, and *B. alsatica*
- Referred to as Cat Scratch Disease (*B. henselae*) or Trench Fever (*B. quintana*)

Can you get Bartonella from a tick bite?

YES!

Scalp Eschar and Neck Lymphadenopathy Caused by *Bartonella henselae* after Tick Bite.
Emmanouil Angelakis, Celine Pulcini, Julie Waton, Patrick Imbert, Cristina Socolovschi, Sophie Edouard, Pierre Dellamonica, and Didier Raoult. Clinical Infectious Diseases 2010; 50:549–51. © 2010 by the Infectious Diseases Society of America. 1058-4838/2010/5004-0015$15.00. DOI: 10.1086/650172

Vector Competence of the Tick Ixodes ricinus for Transmission of Bartonella birtlesii. Caroline Reis, Martine Cote, Danielle Le Rhun, Benoit Lecuelle, Michael L. Levin, Muriel Vayssier-Taussat, Sarah I. Bonnet. PLoS Negl Trop Dis 5(5): e1186. doi:10.1371/journal.pntd.0001186

- "The nymphs successfully transmitted B. birtlesii to naıve mice as bacteria were recovered from both the mouse blood and liver at seven and 16 days after tick bites."
- **"Consequently, bartonelloses should be now included in the differential diagnosis for patients exposed to tick bites."**

"Histochemical staining showed the presence of bacteria in **salivary glands** and muscle tissues of partially engorged adult ticks."

- Rapid transmission??

The better known Bartonella species, their hosts, and their vectors

Bartonella Species	Host (s)	Vector(s)
B. henselae	Cat, human, dogs, horses	Fleas, lice, ticks, spiders
B. quintana	Humans, macaques, cats, dogs	Human body lice, fleas, bed bugs
B. bacilliformis	Humans	Sandflies, fleas
B. koehlerae	Cats, dogs, humans	Fleas
B. vinsonii ssp. berkhoffi	Dogs, horses, foxes, humans	Fleas, ticks
B. bovis	Cattle, cats, dogs, human	Biting flies, ticks
B. clarridgeiae	Cats, dogs	Fleas, ticks
B. rattimassiliensis	Rats	Fleas
B. tamiae	Rats, humans	Mites
B. tribocorum	Rats	Fleas
B. rousetii	Bats	Bat flies
B. schoenbuchensis	Cattle	Biting flies, ticks
B. chomelii	Cattle	Biting flies, ticks
B. doshiae	Rats, humans	Fleas
B. grahamii	Mice, humans	Fleas
B. birtlesii	Mice	Fleas
B. mayotimonensis	Bats, humans	Bat flies, fleas, ticks
B. elizabethae	Rats, humans, dogs	Fleas
B. washoensis	Dogs, humans	Fleas, ticks
B. rochalimae	Dogs, humans	Fleas, ticks
B. vinsonii ssp. arupensis	Dogs, humans	Fleas, ticks
B.melophagi	Sheep, humans	Sheep keds

The table has been adapted from Breitschwerdt, 2017.

Clinically challenging

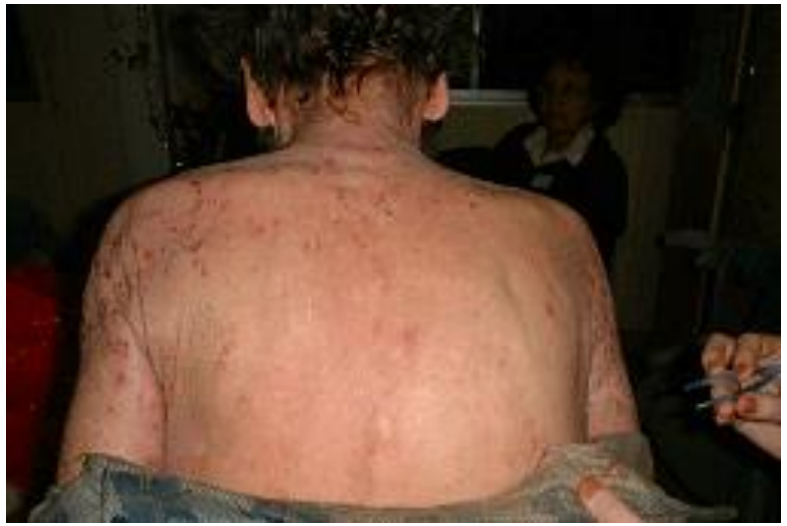
- Difficult to diagnose- most symptoms are nonspecific and traditional testing is notoriously insensitive
  - Notorious as a cause of FUO
- Can be isolated from blood, spinal fluid, GI tract including the appendix, liver, skin, bone, cartilage, lymph nodes, stem cells, heart valves…
- Documented *in utero* transmission (doi:10.1128/JCM.00326-10)
- Can drive autoimmunity
- Can drive angiogenesis by activating host VEGF pathway

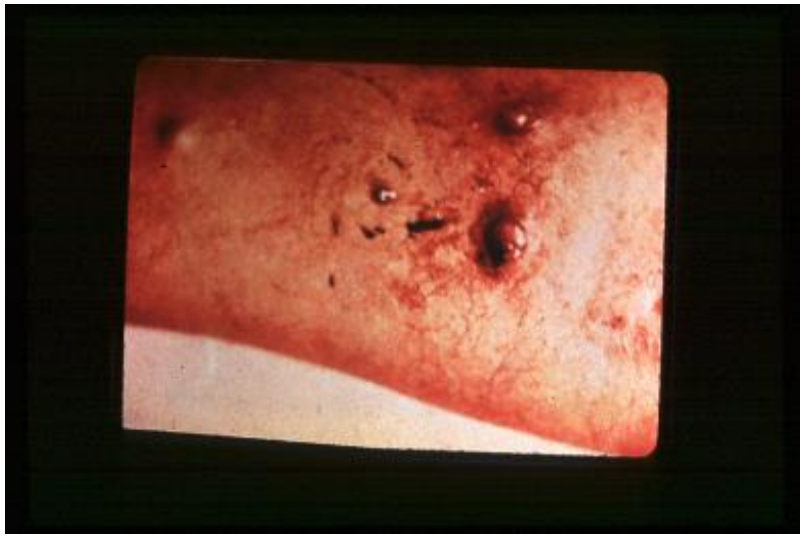
Bartonella Clinical Picture

- **CNS symptoms** out of proportion to physical- **CNS irritability**
  - Anxiety, rage attacks, antisocial behavior, tremors, seizures, ataxia, insomnia, depression, dementia, hallucinations, schizophrenia
- **Peculiar skin manifestations** in some- “Bartonella tracks”
  - Can find organisms in these rashes (mix of Bartonella and Borrelia)
  - Clinical reports associate Bartonella co-infection with Morgellons
  - Baciliary angiomatosis
- **Multiple target organs-** but varies among individuals
  - GI: gastritis, heartburn and abdominal pain
  - Connective tissues: tender skin nodules, sore soles, tendonitis, joint and bone pain
  - Eyes: uveitis, retinitis, retinal artery thrombosis
  - Others: headache, neuropathy, fatigue
- AM fevers, light night sweats
- Persistent CNS symptoms despite Lyme Rx and rapid return of symptoms if treatment ends too soon

Bartonella in the skin

BACILLIARY ANGIOMATOSIS
Red bumps - may form scabs





Bartonella “Tracks”

Resemble stretch marks but are red and do not follow skin planes

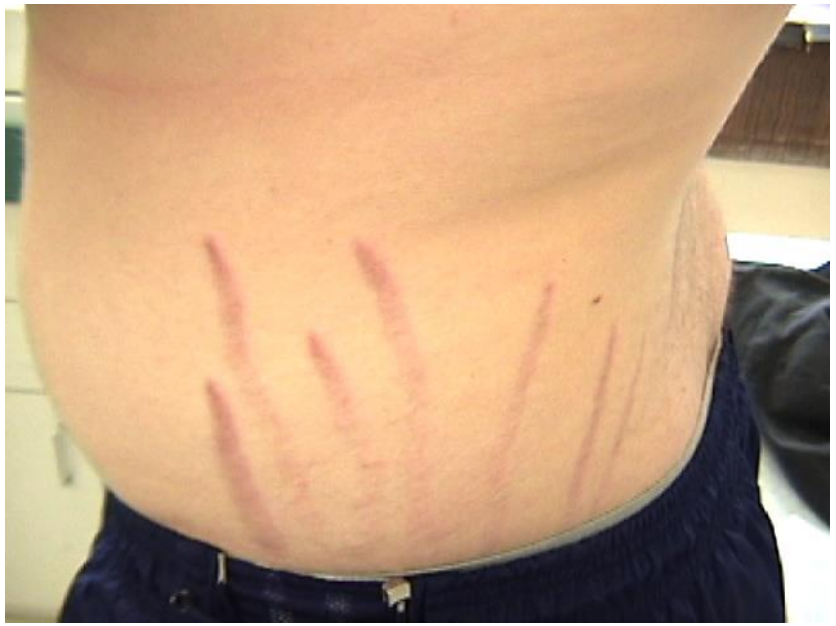


Photo taken by Dr. Martin Fried, with thanks to the Lyme Disease Association

Bartonella “Tracks”

Under the arm

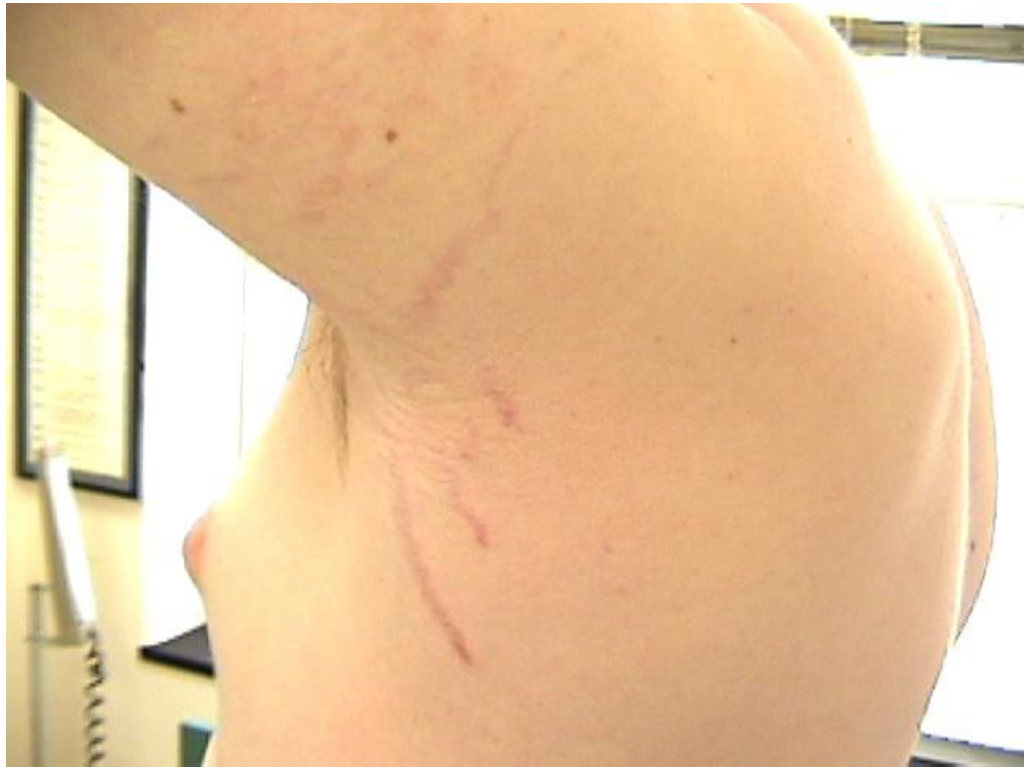


Photo taken by Dr. Martin Fried, with thanks to the Lyme Disease Association

Bartonella “Tracks”

Lower back

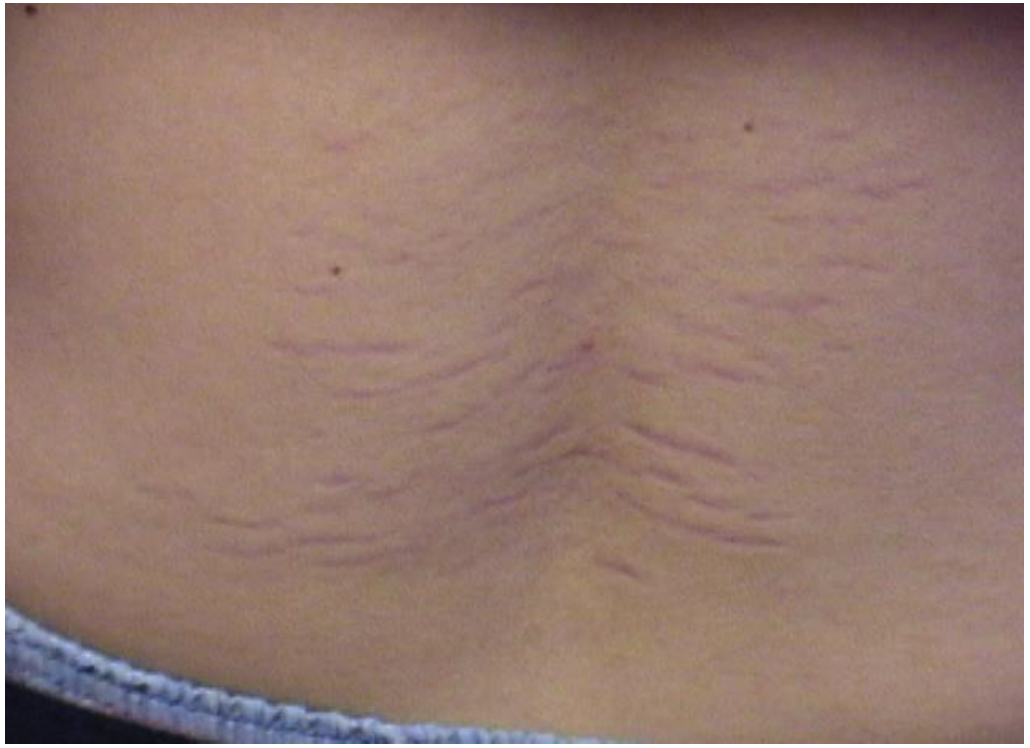


Photo taken by Dr. Martin Fried, with thanks to the Lyme Disease Association

Bartonella - mixed rashes

Mixed rash - Linear and Papular

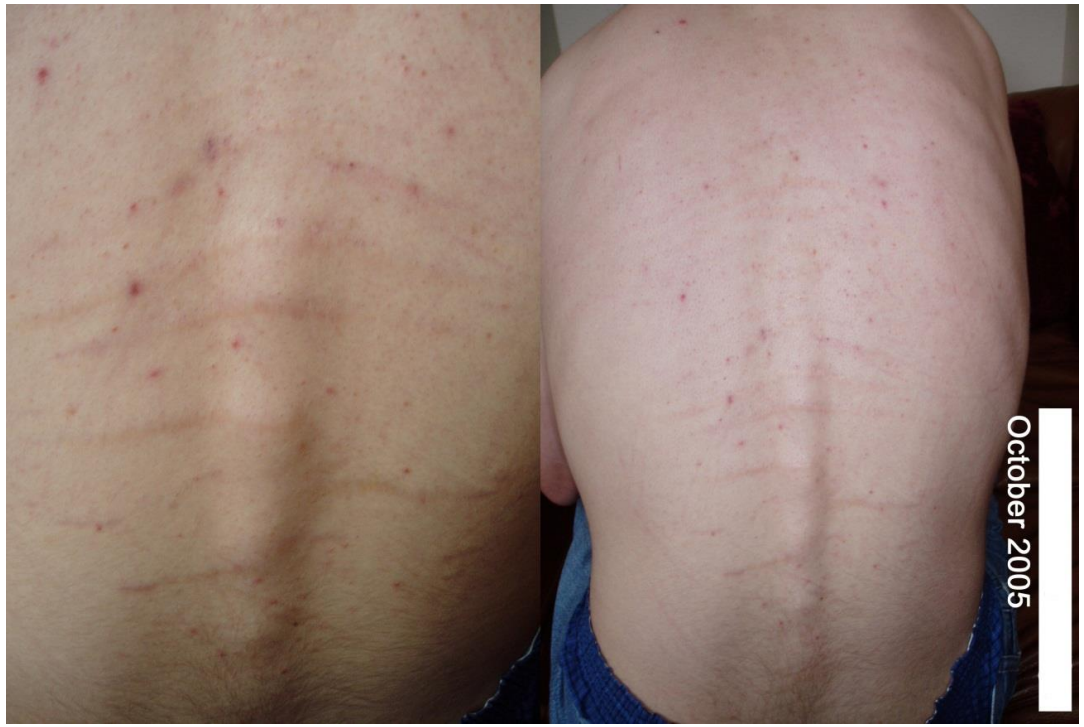


Photo taken by Dr. Martin Fried, with thanks to the Lyme Disease Association

Laboratory testing

- PCR
  - qPCR
  - ddPCR
- Serologies
  - IFA/ELISA
  - Western blot
  - ImmunoBlot- Brand New!
- FISH (Fluorescent In-Situ Hybridization)

Bartonella PCR- is insensitive

- Sensitivity of qPCR is low (6/112) when testing patient blood and enrichment blood culture samples. (5% sensitivity even including pre-culturing!)

- “Unfortunately, the sensitivity associated with conventional and qPCR methodologies utilized for Bartonella spp. DNA detection are poor due to the low bacterial load present within most biological samples, the long time required for bacterial growth in enrichment culture, and the presence of PCR-inhibitory components (such as anti-coagulants, hemoglobin, and high concentrations of host DNA) found within blood and tissues.”

Maggi. https://doi.org/10.1016/j.mimet.2020

New PCR technology

DROPLET DIGITAL ePCR

- Blood samples only
- A fluorescent-labelled PCR probe is introduced to the sample; blood sample is broken up into tiny droplets- a nanoliter!
- The DNA in each drop is amplified then each droplet is scanned for fluorescence by a method similar to flow cytometry

RESULTS

- “The ddPCR sensitivity (53/112) was significantly better than qPCR (6/112) when testing patient blood and enrichment blood culture samples”
- “Sixfold more sensitive than qPCR” (**30% sensitivity**)
- Sensitivity 47% (when you include all samples- straight blood draw and cultured patient blood, sampled at 7, 14 and 21 days)

Maggi. https://doi.org/10.1016/j.mimet.2020

Bartonella serologies - are insensitive

- Standard, FDA-kit serologies (big commercial labs) and even in-house tests reportedly are insensitive
  - Usually IFA; ELISA in Europe
  - Usually validated for **one serotype** of **one species** (*B. henselae* most common; *B. quintana* in a few)
  - **Sensitivities (IgG) range from 28% to 91%**

- *Higher sensitivities were only seen in those with endocarditis or marked lymphadenopathy*

Bartonella serology case series

Clinical series: six patients, all with severe neurological dysfunction

- Clinical diagnoses, but after repeated testing, all eventually were PCR positive
- Three of the six never were positive on CDC serology (what does this mean?)
  - Patient 3- tested SIX times, all samples seronegative
  - Patient 4- tested three times- all samples seronegative
  - Patient 5- one sample
- One other patient was initially seronegative but later seroconverted

Breitschwerdt doi:10.1128/JCM.00832-08

Bartonella serologies - low specificity too!

FALSE POSITIVES!!

- **Specificity only 27% to 68% (IgG)**
  - Chlamydia- 36% of positive Bart serologies were in fact due to this
  - EBV 24%
  - CMV 24%
  - Coxsiella 20%
  - Strep pyogenes 19%

Vermeulen DOI 10.1099/jmm.0.015248-0

- Also:
  - F. tularensis
  - Toxoplasma gondii

Why such poor results - 1

- Familiar story: There are over 30 species, 13 of which are of clinical significance to humans, but standard serologies usually are validated for ONE species, and often only one serotype of that species.
  - “low test sensitivity is due to regional distribution of different Bartonella species”

- Immune evasion: “Bartonella spp. can invade several cell types,[and] evade the host's immune system, often leading to long delays in seroconversion and negative serology test results.”

Why such poor results - 2

With all serologies, there is a tradeoff between sensitivity and specificity.

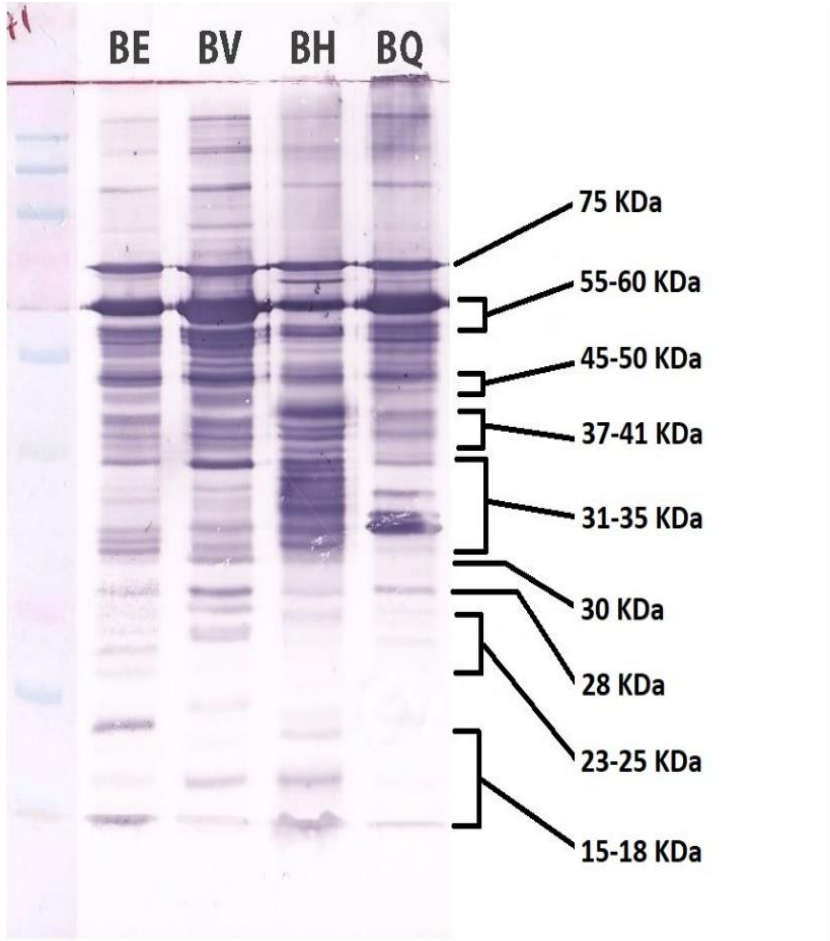
- Is affected by the selection of a cutoff value that would indicate a positive test
- Lower the cutoff and it becomes more sensitive but less specific
- Raise the cutoff and it becomes more specific, but loses sensitivity

Western blot is a clear improvement

An IGeneX exclusive

- Four (IgM) to six (IgG) times more sensitive than the IFA
- Bartonella Western Blot Panel includes four clinically relevant species of Bartonella.
  - *B. elizabethae*
  - *B. henselae*
  - *B. quintana*
  - *B. vinsonii*
- Reports both IgM and IgG
- To date, has been the most sensitive and specific serology available for Bartonella species

Bartonella Western blot



ADVANCING BEYOND THE WESTERN BLOT

Introducing the latest testing advance

The Bartonella ImmunoBlot!

- ImmunoBlotting has revolutionized testing for *Borrelia* and for COVID (SARS CoV-2)
  - **Multispecies detection**
  - **Highest sensitivity**
  - **Highest specificity**
- *Now, IGeneX is pleased to announce that Bartonella will soon be joining the ImmunoBlot revolution*

COMPARING A WESTERN BLOT TO AN IMMUNOBLOT

Advanced technology that overcomes the limitations of conventional serologies

Western blot

How it is made is the basis of its limitations

For a WB, Bartonella are grown in culture, lysed, and then using electrophoresis, the protein antigens are separated by size and transferred onto a membrane strip.

PROBLEMS:

- Antigens are derived from cultured samples (Vero cells or liquid media)
  - Limited number of species are available for culture
  - Variability in culture conditions can affect which antigens are produced and their quantity
  - Test result may be affected by other laboratory variables such as reagent concentrations and temperature
- Making multiple western blots to cover multiple species is not practical to manufacture, and multiplies costs

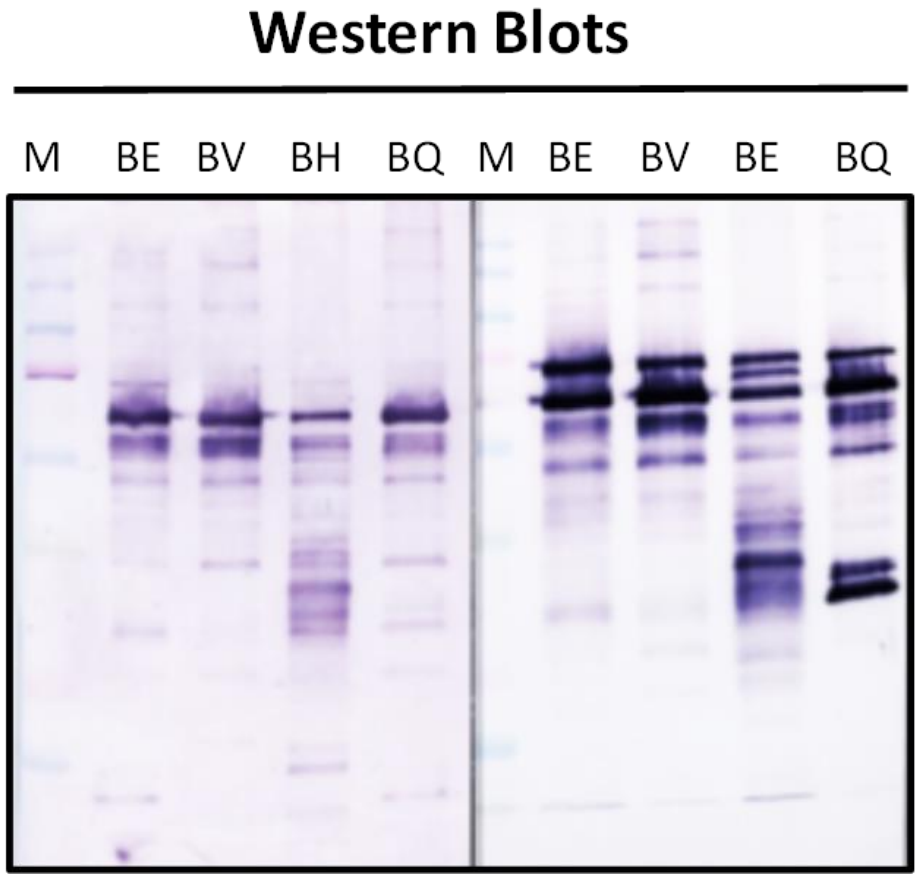
Bartonella Western blot – How bands are identified

IDENTIFICATION IS MIGRATION-DEPENDENT

- If Bartonella-specific antibodies are present in the patient's sample, they will be bound to the corresponding antigen(s) on the strip and appear as dark bands on the membrane
- Identified by where they end up on the strip

PROBLEMS:

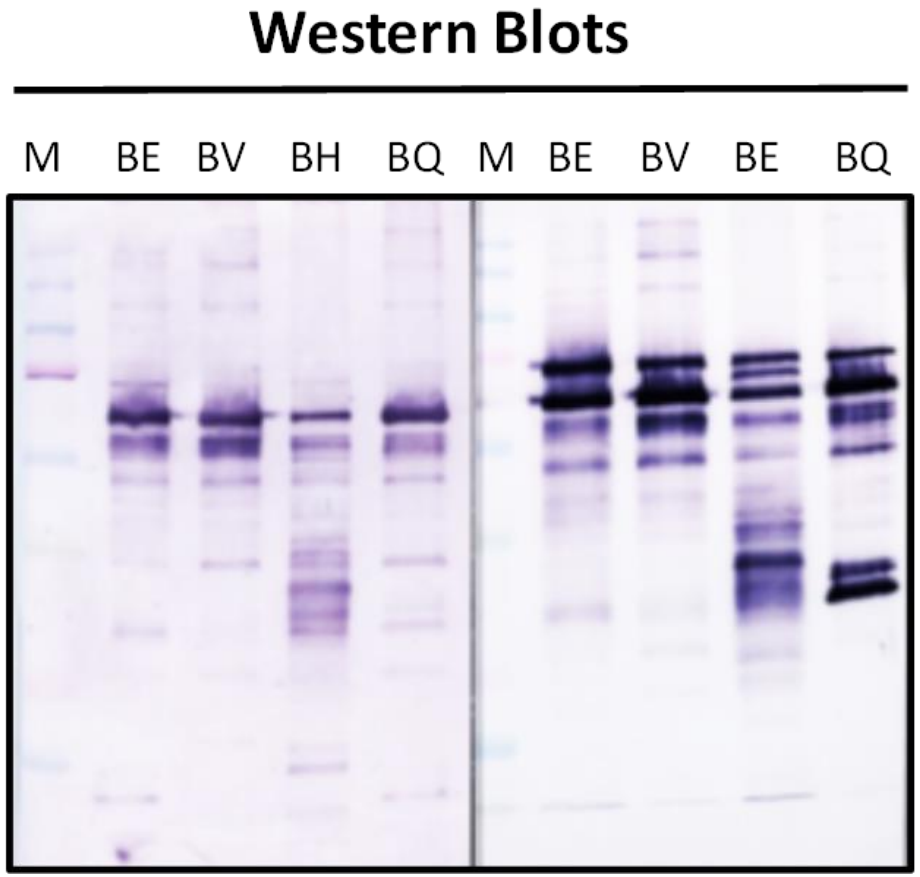
- Sometimes difficult to exactly know how they line up
- With the electrophoresis, some nonspecific or unimportant proteins may co-migrate with important Bartonella proteins, and the WB will not distinguish these others.



Bartonella Western blot – scoring the results

SCORING IS BASED UPON BAND INTENSITY

- **PROBLEM**: Because protein content of any given culture can vary, the band intensity can vary, potentially causing false positives and false negatives
- How distinct must it be to be scored as positive?
- Not every antigen is always produced in every culture, so some bands may not appear



Replacing the Western Blot

Bartonella will soon be joining the ImmunoBlot revolution!

What is an ImmunoBlot?

It is a serological test for infection, but it is fundamentally different from other serologies

- The difference is that it uses pure, specifically created **recombinant proteins** as the test antigens, and not proteins from cultures of specimens grown in the lab
- Result is ability to increase sensitivity without sacrificing specificity
- Allows for identification of a broad range of clinically important Bartonella species
- Can be made genus and species-specific

IGeneX Bartonella ImmunoBlot

Overcomes WB Limitations

Uses antigens derived from recombinant proteins:

- Significantly increases sensitivity
  - Are able to include antigens from a variety of clinically important species
  - 22 Bartonella specific recombinant antigens and 2 controls are present on every strip at a predetermined position
- Much greater specificity - no band overlap or co-migration
- No tradeoff between sensitivity and specificity
- Renders Bartonella western blots obsolete and is meant to replace them

Bartonella ImmunoBlots are more sensitive than Bartonella Western Blots

Patient	Collection Date	Bartonella Western Blot		Bartonella ImmunoBlot	
		IgM	IgG	IgM	IgG
JP	9/15/2020	Neg	Neg	BE	BE/BV
GR	8/11/2020	Neg	Bart SP/BE	Neg	BE/BV
GG	7/13/2020	Bart sp.	Neg	BE/BH	Neg
AE	10/15/2020	Neg	Neg	BE	Neg

* Bartonella FISH (+); BE - Bartonella elizabethae; BV - B. vinsonii; BH - B. henselae; BQ - B. quintana; Bart sp. - Bartonella species

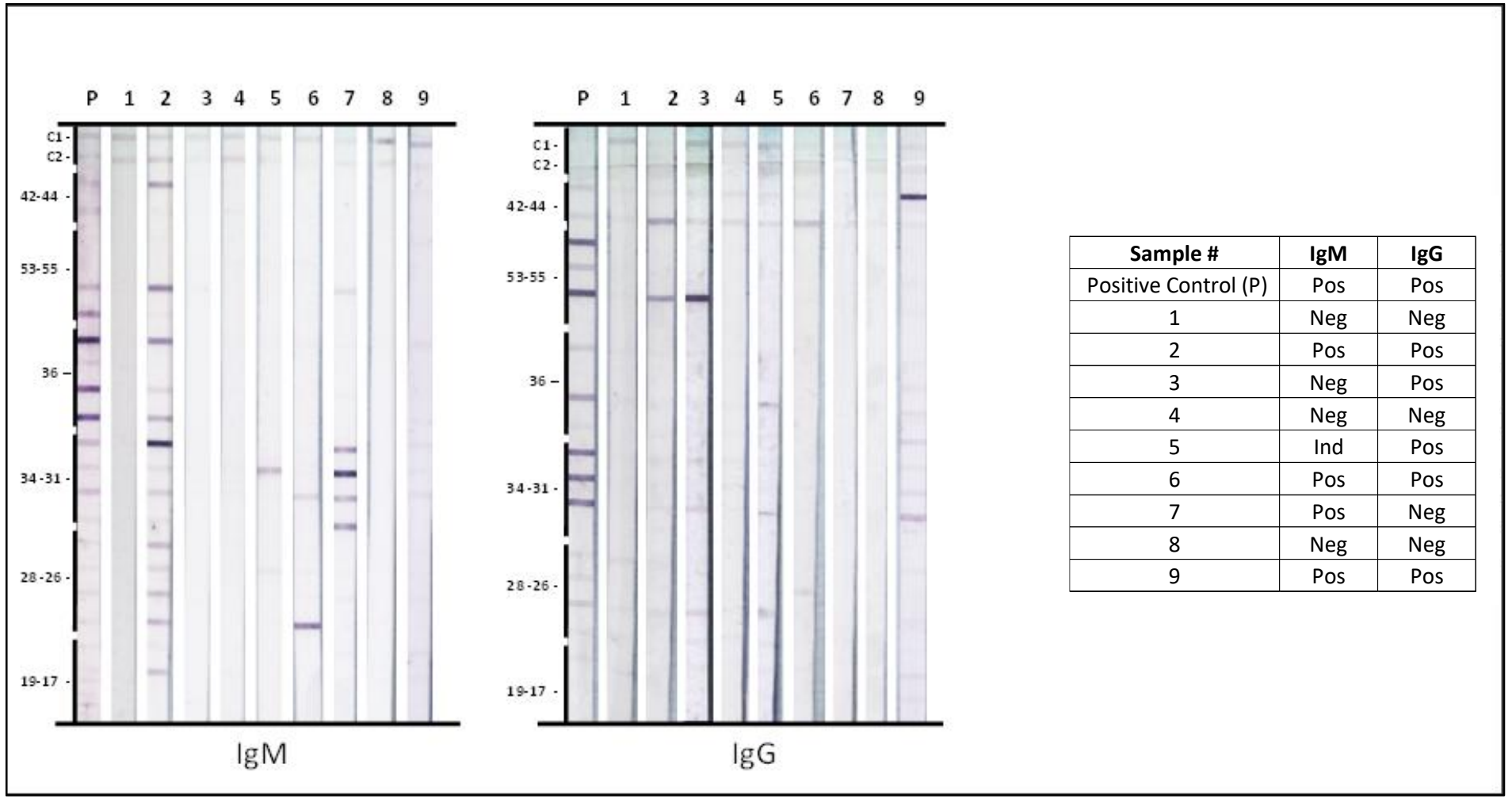
How the Bartonella ImmunoBlot is made

In the immunoblot, specific, important recombinant proteins are created. Then a precise amount is sprayed directly onto the membrane as distinct lines at specific locations.

- **Precise Amount**
  - Banding intensity is no longer variable and culture-dependent
  - Positive bands are more clearly displayed
  - Reduces false positives and false negatives
- **Specific Locations**
  - Band locations are no longer migration-dependent
  - Know exactly what each positive band represents
  - No longer an issue with co-migration

Because location and concentration are controlled, results are far more consistent and specificity improves.

Bartonella ImmunoBlots



Sample #	IgM	IgG
Positive Control (P)	Pos	Pos
1	Neg	Neg
2	Pos	Pos
3	Neg	Pos
4	Neg	Neg
5	Ind	Pos
6	Pos	Pos
7	Pos	Neg
8	Neg	Neg
9	Pos	Pos

Bartonella IB TBD Specificity Study

Specificity Study (n=30)			
Sample Type (Positive for antibodies to)	**N**	**Bart IB IgM**	**Bart IB IgG**
Borrelia burgdorferi (BB)	5	0	0
BB +Tick-Borne Relapsing Fever *Borrelia*	2	0	0
BB + *Babesia*	3	0	0
BB and *Anaplasma phagocytophilia*	1	0	0
Babesia	7	0	0
Controls	12	0	0
Specificity		**100%**	**100%**

Bartonella IB TBD Validation Study (n=46)

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

Specificity

IgM – 100%

IgG – 100%

Sensitivity

IgM – 75%

IgG – 88.9%

Overall (IgM+IgG)– 100%

ANOTHER IGENEX EXCLUSIVE-

Bartonella FISH

Fluorescent In-Situ Hybridization assay

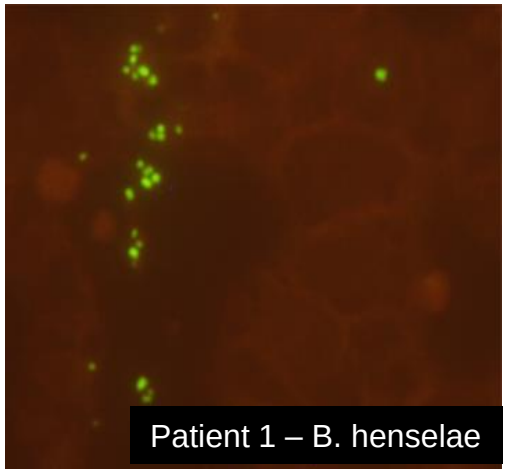
Bartonella FISH - Fluorescent in-situ hybridization

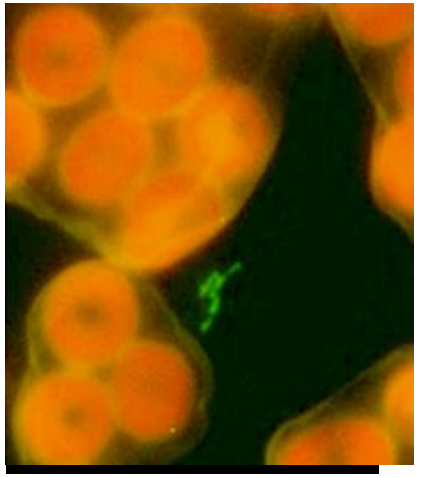
FISH detects presence of pathogen **RNA** - reported as positive or negative

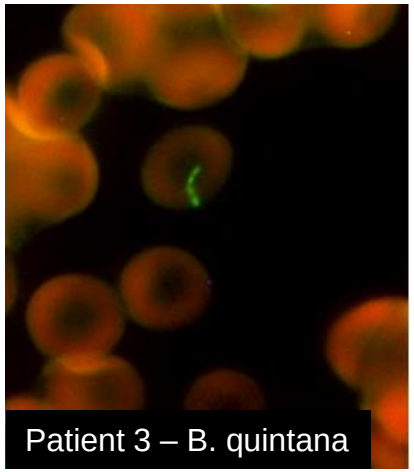
- Specific fluorescent RNA stains are applied to a blood smear for direct visualization
- RNA does not persist post-infection- disappears as soon as pathogen dies
- ***Able to detect pathogens even if embedded in biofilms!!***
- Can be designed to be **genus-specific**, increasing breadth of species detection
  - validated to detect *B. henselae, B. quintana, B. vinsonii, B elizabethae, B grahamii* and *B. berkhofii*
  - others possible

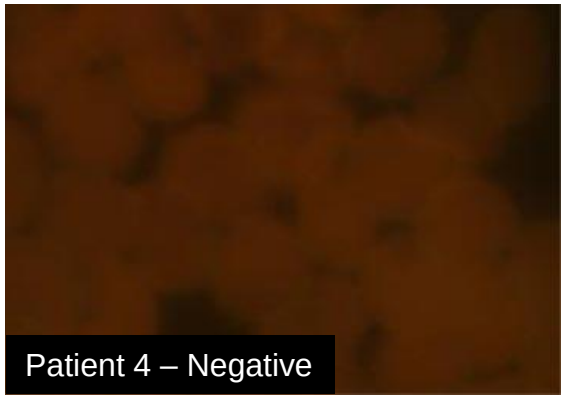
Bartonella FISH - patient samples

All three positives confirmed by sequencing









Bartonella FISH performance

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

Guidelines on use of Bartonella FISH

- Early in infection, parasitemia is highest because antibodies have not yet formed
- Intermediate in infection, as immune responsiveness matures, parasitemia is low
- Late in infection, especially in the co-infected TBD patient, immunity may weaken and parasitemia can increase
- Same is true if immunosuppressed

Therefore useful in early and very late disease, and in any situation in which there is poor B-cell response

Bartonella IgXSpot - T-cell response assay

- IgXSpot uses the ELISPOT method- is based upon host T-cell response
- Detects the production of interferon-gamma from T-cells previously sensitized to Bartonella
- Genus-specific so can detect the major species of clinical concern: *B. henselae, B. quintana, B. vinsonii, B elizabethae* and *B. berkhofii*
- Because T-cell responses occur much sooner than B-cell responses, the IgXSpot is especially useful in early disease
- T-cell responses are independent of serological results, so the IgXSpot is useful in not just early disease, but also in very late disease and in those with compromised B-cell function
- When combined with the ImmunoBlot, provides information on the full spectrum of patient's immune response to infection and stage of disease

Bartonella - Recommend a panel approach to testing

While these advanced tests represent huge improvements over standard serologies and PCR, not every case will be picked up by every test

- B-cell dysfunction can render serologies less sensitive, but will not affect ELISPOT (IGeneX IGXSpot) and may even allow the FISH to detect more cases
- Low parasite load can render direct tests such as FISH and PCR less sensitive, so serologies (ImmunoBlot) and ELISPOT (IGXSpot) can be more useful in this situation
- Early disease (less than one month) favors direct tests- FISH and PCR
- Very late disease, especially in the ill, co-infected patient, is a diagnostic challenge and multiple methods may be needed
  - **Because the specificity of the ImmunoBlot, FISH and PCR are very high, if results are discordant, trust the positive result**
- IGeneX offers many testing panels that simplify test choices and result in a very significant cost savings

I suggest you become familiar with the IGeneX test panels!!

Bartonella - Why a panel approach?

N	130	% (+)
FISH	15	11.5%
WB	51	39.2%
IgX	23	17.7%
Overall	69	53.1%

Note: ImmunoBlots are more sensitive than Western Blots. Therefore we expect the overall sensitivity to improve using FISH, IB and IgX spot test.

Remember, using insensitive tests can cause trouble!!

- Because of seronegativity:
  - Missed diagnoses
  - Incorrect diagnoses, especially neuropsychiatric ones
  - Patients are told they need psychiatric help!!
  - Patients are told to just live with their symptoms ☹
- Because of seronegativity:
  - Insurance companies may deny covering treatment
- Because of seronegativity:
  - If the clinician diagnoses Bartonella on clinical grounds despite negative tests, then state medical Boards may pay a visit!

THEREFORE YOU MUST INSIST UPON USING THE MOST ACCURATE TESTS AVAILABLE!

IGeneX is here to help by offering the highest quality and broadest range of Bartonella tests than any other lab


THANK YOU!!
Q & A Session to follow-
Hosted by Drs. Burrascano and Shah