IGeneX Inc.

Culturing to Support the Diagnosis of Tick-Borne Diseases

August 2023

Culture Testing for TBDs- Historical Perspective

- **Dr. Alan MacDonald was the first one to develop a viable Borrelia culture for clinical use (mid-1980s)**
  - This test was available to me in limited numbers
  - Demonstrated infection in patients not suspected of having Lyme, thus expanding the variety of possible clinical presentations
  - Demonstrated infection in patients who were seronegative
  - Demonstrated ongoing infection in patients despite previous treatment with then-standard courses of antibiotic therapy- including both oral and IV antibiotics
  - **Demonstrated that highest yield was obtained if blood was drawn during peaks of patient symptoms**
- Formed the basis of the present, ILADS-supported view of clinical Lyme disease

Culture Testing for TBDs- Historical Perspective

- **Dr. MacDonald then was able to demonstrate previously unappreciated effects of this infection-**
  - Association with birth defects in children of infected mothers
  - Association of congenital Lyme with stillbirth
  - Association of congenital Lyme with crib death
  - Association of chronic Lyme with Alzheimer's dementia
  - Association of chronic Lyme with other chronic neurological diseases

Culture Testing for TBDs- Historical Perspective

Many years later, Dr. Eva Sapi collaborated with Dr. MacDonald to optimize her Borrelia culture process

- Multiple advanced experiments were then possible, resulting in many important findings-
  - Borrelia produce biofilms
  - Biofilms were then demonstrated in lab cultures as well as in patient tissues
  - Presence of Borrelia in a variety of diseases
  - Adoption of vital assays that can document Bb growth and activity
  - Antimicrobial sensitivity testing
  - Presence of Borrelia in patients with post-treatment, chronic Lyme symptoms

Culture Testing for TBDs- Lyme and Co-Infections

- **These advances were critically important for advancing the spectrum of Lyme Borreliosis, but what about the co-infections?**
  - Possibly as many as one-third of patients diagnosed with Lyme Borreliosis do not have Lyme, but have tick-borne relapsing fever, which may present identically to classic Lyme
  - Babesiosis is the most commonly found co-infection in patients with chronic Lyme
  - Anaplasma, Ehrlichia and the Rickettsias are generally the most prevalent pathogens found in ticks
  - Bartonella co-infections are very common in patients with chronic Lyme, and recent reports suggest that ticks can carry and transmit Bartonella
  - Biting flies, fleas and possibly mosquitos have variously been implicated in spreading infections also known to be harbored by ticks
- Unfortunately, advanced culture testing had not been clinically available for any of these until now

Culture Testing- Importance

Culturing is a direct test- a positive result indicates the infection was present and active the day the blood was drawn

- Just as it revolutionized my view of Lyme, an accurate and readily available culture test can dramatically improve patient care:
  - Document TBDs as an unexpected cause of many chronic disease states
  - Document infection in patients for whom other testing methods were non-reactive
  - Document ongoing infection in chronically ill patients
  - Uncover co-infections that were previously not suspected
  - Document treatment failures
  - Document reinfections
  - Provide insight on the value of various treatment methods

Culture Testing of the Major TBDs- Difficult to do!!

Culturing is the gold standard for diagnosing Tick-Borne diseases.

- But it's challenging.
  - Technical limitations, because TBDs are adapted to thrive in living organisms, not artificial culture media
  - Even in active infection, immune and other factors in host blood inhibit pathogen replication *in vitro*
  - Highly likely that these organisms are in a persister state- hibernation precludes growth in the laboratory setting
  - Even planktonic forms of the TBDs grow very slowly, so culturing may take weeks
  - Other pathogens which may be present can overgrow and spoil the culture
  - Once cultured, how do you confirm identity of what has grown?

Culture-Enhanced PCR (cePCR)

IGeneX introduced cePCR™ (Culture Enhanced PCR), available for all of the major tick-borne infections, in February 2023

- Basically, needed to duplicate *in vitro* the unique growing conditions found in living organisms
- Took over two years of research and development!
- Positive cultures are confirmed with a unique, highly sensitive PCR

Available individually for Lyme, TBRF, Bartonella, Babesia, Rickettsia, Ehrlichia and Anaplasma

(each test needs to be ordered separately, but test panel combinations are available)

Culture-Enhanced PCR (cePCR) Validation

PCRs needed to be optimized and validated

- PCR inhibitors in peripheral blood had to be removed or neutralized
- PCR process had to be rigidly controlled and standardized

- During development, **all positive samples were sent to an outside lab for sequencing** to confirm identity and to validate the PCR
- In addition, to further confirm the pathogen was really present, **reverse western blots using recombinant technology were performed on all samples**

cePCR Validation and Performance

IGeneX cePCR Validation Results

Specificity

- All sequencing results matched initial PCR determination - **100% specificity**
- Reverse western blots demonstrated that tick-borne pathogens grow in IGeneX proprietary culture medium.

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

cePCR Validation- Limit of Detection

Comparison of Blood samples spiked with either Borrelia or Bartonella at day 0 and after culturing in IGeneX culture medium for 14 days, by q-PCR

Borrelia burgdorferi				
BB B31 Spiked into blood	**Day 0**		**Day 14**	
	Cq values	**Result**	**Cq values**	**Result**
10^{-4}	20.4	Pos	19.03	Pos
10^{-5}	22.9	Pos	18.41	Pos
10^{-6}	26.4	Pos	20.08	Pos
10^{-7}	28.32	Pos	21.14	Pos
10^{-8}	30.41	Pos	18.37	Pos
10^{-9}	31.81	Pos	18.28	Pos
10^{-10}	33.53	Pos	20.96	Pos
10^{-11}	Neg	Neg	21.32	Pos

Bartonella				
B. henselae Spiked into blood	**Day 0**		**Day 14**	
	Cq values	**Result**	**Cq values**	**Result**
10^{-4}	29.81	Pos	10.83	Pos
10^{-5}	34.3	Pos	12.03	Pos
10^{-6}	36.99	Pos	27.74	Pos
10^{-7}	37.72	Pos	11.64	Pos
10^{-8}	38.17	Pos	12.05	Pos
10^{-9}	37.81	Pos	12.36	Pos
10^{-10}	40.06	Pos	10.88	Pos
10^{-11}	Neg	Neg	20.23	Pos

Limit of detection- <10 organisms per ml !!

cePCR- Broad species coverage

Genus-level reporting: broadens test sensitivity and improves test accuracy

Early series

Lyme Borrelia	**Bartonella**
B. burgdorferi (17)	B. henselae (5)
B. garinii (2)	B. elizabethae (1)
B. mayonii (4)	B. tribocorum (1)
Tick-Borne RF	**Anaplasma**
B. miyamotoi (4)	A. phagocytophilum (1)
B. hermsii (1)	A. platys (1)
Babesia	
B. microti (12)	
B. duncani (7)	
Babesia species (1)	

More recent series

Lyme Borrelia
B. burgdorferi
B. mayonii
B. garinii
B. valaisiana
Babesia
B. microti
B. duncani
Babesia species
Anaplasma
A. phagocytophilum

cePCR Validation Produced Interesting Findings!

Some of the species that were detected are rarely found in the US, and likely would not be detected with traditional PCR

- **Anaplasma platys** (formerly Ehrlichia platys), a known canine pathogen
  - Only four reported cases of human infection
- **Colpodella species**
  - Are free-living pond and soil-dwellers that are related to apicomplexans (Malaria, Babesia)
  - Identified this in a CFS patient!
  - Just two reported cases of human infection

These could not be contaminants, because these species are not kept in the laboratory!!

Advantages of Culture-Enhanced PCR

- Provides higher sensitivity than standard PCR testing
- A proven 100% specific method for identification of a tick-borne pathogen
- Unlike a standard PCR, if a pathogen grows in culture, it is guaranteed to be active and not a remnant of a prior exposure or past infection

Lyme: Positive Cultures, Compared to Other Lyme Tests

Nine patients who were positive with a Lyme culture were also tested using other methods. Below are the results of those other tests. In most cases, the non-culture test methods were negative.

Lyme cePCR Culture Positive Patients							
Patient	Culture	IB IgM	IB IgG	Std PCR	LSA serol	DotBlot	IFA
A	Pos	Neg	Pos				
B	Pos	Neg	Neg				Equivocal
C	Pos		Neg	Neg	Neg		
D	Pos	Pos	Neg				
E	Pos	Neg	Neg				
F	Pos	Neg	Neg				Pos
G	Pos	Neg	Neg	Pos			
H	Pos			Neg		Neg	
I	Pos	Neg	Neg				

Positive culture but negative antibody testing
- Very early
- Immune deficient
- Very high pathogen load

Positive culture, pos IgM, neg IgG
- Early disease
- Late highly active infection

Positive culture, neg IgM, pos IgG
- Less active infection
- Later in the course of treatment

TBRF: Positive Culture Compared to TBRF ImmunoBlots

One patient who was positive with a TBRF culture was also tested using ImmunoBlots. Both ImmunoBlots were negative.

TBRF cePCR Culture Positive		
Culture	**IB IgM**	**IB IgG**
Pos	Neg	Neg

Positive culture but negative serology

- Early disease
- Immune deficiency
- Very high pathogen load with immune complex formation

Babesia: Positive Culture Compared to Babesia ImmunoBlots

One patient who was positive with a Babesia culture was also tested using ImmunoBlots. ImmunoBlot IgM was positive but the IgG ImmunoBlot was negative

Babesia cePCR Culture Positive		
Culture	**IB IgM**	**IB IgG**
Pos	Pos	Neg

Positive culture, pos IgM, neg IgG

- Early infection
- Lyme co-infection with large infection load

Anaplasma: Positive Culture Compared to Anaplasma IFA

One patient who was positive with an Anaplasma culture was also tested using IFA. The IgM IFAs was negative but the IgG IFA was positive.

Anaplasma cePCR Culture Positive Patient		
Culture	**IFA IgM**	**IFA IgG**
Pos	Neg	Pos

Positive culture, neg IgM, pos IgG

- Later infection
- Reasonably intact immunity

Rickettsia: Positive Cultures Compared to Rickettsia IFAs

Two patients who were positive with a Rickettsia culture were also tested using IFA. Both IFAs were negative.

Rickettsia cePCR Culture Positive		
Patient	**Culture**	**IFA**
A	Pos	Neg
B	Pos	Neg

Positive culture, negative IFA

- Early disease
- Immune Deficiency
- Poor sensitivity of the IFA

More Patient Examples- Unexpected Results!

The following test results are from actual IGeneX patients. The tests were performed using the same sample and performed on the same date.

Patient #1 – Positive Lyme Culture, Negative Lyme ImmunoBlot

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Laboratory Director: Jyotsna S. Shah, Ph.D.

CLIA: 05D0643914
CALIFORNIA: CLF 4033
NPI: 1396837605

REFERRING PHYSICIAN

[redacted]

[redacted]

Collected: 04/12/2023 UNK
Received: 04/14/2023 12:13
Reported: 05/25/2023 20:44
Reprinted: 06/19/2023 10:28
Amended:
Corrected:

BORRELIOSIS - Lyme Disease

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
BLOOD CULTURE				
Blood Culture	CULTURE	**Positive**	Negative	
LYME cePCR				
Borrelia-genomic*	CULTURE	**Positive**	Negative	
Borrelia -plasmid	CULTURE	Negative	Negative	

** detects Borrelia specific DNA including but not limited to Borrelia burgdorferi sensu lato and Relapsing Fever Borrelia.*

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REFERRING PHYSICIAN

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[redacted]

Collected: 04/12/2023 UNK
Received: 04/14/2023 12:13
Reported: 04/25/2023 17:29
Reprinted: 06/19/2023 10:28
Amended: 04/26/2023 16:54
Corrected:

BORRELIOSIS - Lyme Disease

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
Lyme ImmunoBlot IgM	Serum			
IGX Criteria:		Negative		
CDC/NYS Criteria:		Negative		

[REVISED REPORT: EFFECTIVE APRIL 10, 2019]

Lyme ImmunoBlot IgM detects antibodies to B. burgdorferi strains and species

Band (kDa)	23*	31*	34*	39*	41*	93
Intensity	IND	-	++	-	IND	-

Band Intensity: Positive: + to ++++, Indeterminate: Ind, Negative: (-)

Patient #1 (cont.) – Negative Babesia Culture, Positive Babesia ImmunoBlot

BABESIOSIS

BLOOD CULTURE			
Blood Culture	CULTURE	Negative	Negative
BABESIA cePCR			
Babesia spp	CULTURE	Negative	Negative

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

Diagnosis should not be based on laboratory results alone. Results should be interpreted in conjunction with clinical symptoms and patient history.

NOTE: Western Blots, ImmunoBlots, Lyme Dot Blot, Epitope, PCR, IFA, FISH, C. pneumoniae IgG/IgA, CD57, IGXSpot, Broad Coverage Antibody, COVID-19 Test - These tests were developed and their performance characteristics determined by IGeneX, Inc. They have not been cleared or approved by the FDA. The FDA has determined that such approval is not necessary. These tests are used for clinical purposes and should not be regarded as investigational or for research. IGeneX, Inc. is licensed by CMS and NYS to perform high complexity clinical laboratory testing.

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Received: 04/14/2023 12:13
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Reprinted: 06/19/2023 10:28
Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
B. microti	CULTURE	Negative	Negative	
B.duncani	CULTURE	Negative	Negative	

[redacted]

Collected: 04/12/2023 UNK
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Reprinted: 06/19/2023 10:28
Amended: 04/26/2023 16:54
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
BABESIA IMMUNOBLOT IGM				
Babesia ImmunoBlot IgM(genus)	Serum	**Positive**	Positive: Presence of 2 or more Babesia specific antibodies. Negative: Presence of only 1 or no Babesia specific antibody.	

** Babesia ImmunoBlot test detects specific IgM antibodies to Babesia species, including but not limited to B. microti, B. duncani and B. divergens.*

B. microti ImmunoBlot IgM	Serum	Negative
B. duncani ImmunoBlot IgM	Serum	Negative
Babesia spp.	Serum	**Positive**

Limitations:
Negative test result does not exclude possibility of Babesia infection, may retest in 6-8 weeks.
Results should be interpreted in conjunction with clinical symptoms and other laboratory findings.

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
BABESIA IMMUNOBLOT IGG				
Babesia ImmunoBlot IgG(genus)	Serum	Negative	Positive: Presence of 2 or more Babesia specific antibodies. Negative: Presence of only 1 or no Babesia specific antibody.	

Patient #1 Summary

This patient had a positive Lyme culture but a negative Lyme ImmunoBlot. The Babesia culture was negative, but the Babesia ImmunoBlot IgM was positive with a negative IgG ImmunoBlot

Patient 1				
Lyme Culture	**Lyme IB**	**Babesia Culture**	**Babesia IB IgM**	**Babesia IB IgG**
Pos	Neg	Neg	Pos	Neg

Mixed results-
Results are not consistent!!

- Early disease
- Perhaps reflects differing treatment sequences

Clear example why it is important to use more than one testing method

Patient #2 – Positive Lyme Culture, Negative Lyme ImmunoBlot

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Collected: 03/28/2023 UNK
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Reported: 04/25/2023 14:12
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BORRELIOSIS - Lyme Disease

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
BLOOD CULTURE				
Blood Culture	CULTURE	**Positive**	Negative	
LYME cePCR				
Borrelia-genomic*	CULTURE	**Positive**	Negative	
Borrelia -plasmid	CULTURE	Negative	Negative	

** detects Borrelia specific DNA including but not limited to Borrelia burgdorferi sensu lato and Relapsing Fever Borrelia.*

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Collected: 03/28/2023 UNK
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Amended:
Corrected:

TEST | SPECIMEN | RESULT | REFERENCE RANGE | UNITS

BORRELIOSIS - Lyme Disease

Lyme ImmunoBlot IgM — Serum
IGX Criteria: Negative
CDC/NYS Criteria: Negative
***

[REVISED REPORT: EFFECTIVE APRIL 10, 2019]

Lyme ImmunoBlot IgM detects antibodies to B. burgdorferi strains and species

Band (kDa)	23*	31*	34*	39*	41*	93
Intensity	IND	-	+	-	-	-

Band Intensity: Positive: + to ++++, Indeterminate: Ind, Negative: (-)

Patient #2 Summary

This patient had a positive Lyme Borrelia culture but negative Lyme ImmunoBlots

Patient 2		
Lyme Culture	**Lyme IB IgM**	**Lyme IB IgG**
Pos	Neg?	Neg

The IgM ImmunoBlot was read as negative, but there was an equivocal band 23 and positive band 34.

- While technically negative, this suggests very early disease, or…
- ? Virus
- ? Autoimmunity

Whenever you see an equivocal serologic test, good to go further with additional testing using a different methodology

Patient #3 – Positive Lyme Culture, Negative Lyme ImmunoBlot

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NPI: 1396837605

REFERRING PHYSICIAN

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Collected: 03/13/2023 UNK
Received: 03/15/2023 12:45
Reported: 04/20/2023 15:45
Reprinted: 05/25/2023 11:22
Amended:
Corrected:

BORRELIOSIS - Lyme Disease

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
BLOOD CULTURE				
Blood Culture	CULTURE	**Positive**	Negative	
LYME cePCR				
Borrelia-genomic*	CULTURE	**Positive**	Negative	
Borrelia -plasmid	CULTURE	Negative	Negative	

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Collected: 03/13/2023 UNK
Received: 03/15/2023 12:43
Reported: 03/23/2023 16:16
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TEST SPECIMEN RESULT REFERENCE RANGE UNITS

BORRELIOSIS - Lyme Disease

Lyme ImmunoBlot IgM	Serum		
IGX Criteria:		Negative	
CDC/NYS Criteria:		Negative	

***

[REVISED REPORT: EFFECTIVE APRIL 10, 2019]

Lyme ImmunoBlot IgM detects antibodies to B. burgdorferi strains and species

Band (kDa)	23*	31*	34*	39*	41*	93
Intensity	IND	-	-	-	-	-

Band Intensity: Positive: + to ++++, Indeterminate: Ind, Negative: (-)

Patient #3 (cont.) – Negative Babesia Culture, Positive Babesia ImmunoBlot

BABESIOSIS

BLOOD CULTURE			
Blood Culture	CULTURE	Negative	Negative
BABESIA cePCR			
Babesia spp	CULTURE	Negative	Negative

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

Diagnosis should not be based on laboratory results alone. Results should be interpreted in conjunction with clinical symptoms and patient history.

NOTE: Western Blots, ImmunoBlots, Lyme Dot Blot, Epitope, PCR, IFA, FISH, C. pneumoniae IgG/IgA, CD57, IGXSpot, Broad Coverage Antibody, COVID-19 Test - These tests were developed and their performance characteristics determined by IGeneX, Inc. They have not been cleared or approved by the FDA. The FDA has determined that such approval is not necessary. These tests are used for clinical purposes and should not be regarded as investigational or for research. IGeneX, Inc. is licensed by CMS and NYS to perform high complexity clinical laboratory testing.

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Reported: 04/20/2023 15:45
Reprinted: 05/25/2023 11:22
Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
B. microti	CULTURE	Negative	Negative	
B.duncani	CULTURE	Negative	Negative	

BABESIOSIS

Babesia FISH	W blood	Negative	

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

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Collected: 03/13/2023 UNK
Received: 03/15/2023 12:43
Reported: 03/23/2023 16:16
Reprinted: 05/25/2023 11:22
Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
BABESIA IMMUNOBLOT IGM				
Babesia ImmunoBlot IgM(genus)	Serum	**Positive**	Positive: Presence of 2 or more Babesia specific antibodies. Negative: Presence of only 1 or no Babesia specific antibody.	

** Babesia ImmunoBlot test detects specific IgM antibodies to Babesia species, including but not limited to B. microti, B. duncani and B. divergens.*

B. microti ImmunoBlot IgM	Serum	Negative
B. duncani ImmunoBlot IgM	Serum	Negative
Babesia spp.	Serum	**Positive**

Limitations:

Negative test result does not exclude possibility of Babesia infection, may retest in 6-8 weeks.

Results should be interpreted in conjunction with clinical symptoms

Patient #3 Summary

This patient had a positive Lyme Borrelia culture but negative Lyme ImmunoBlots.

They also had a negative Babesia culture and a positive IgM ImmunoBlot

Patient 3				
Lyme Culture	**Lyme IB**	**Babesia Culture**	**Babesia IB IgM**	**Babesia IB IgG**
Pos	Neg	Neg	Pos	Neg

Discordant results:

- **Very early disease?**
- **Differing pathogen load?**
- **Partial treatment?**
- **Acquired the two infections at different time points?**

Example of the value of combining a direct test with an indirect test to increase yield

Patient #4 – Positive Anaplasma Culture, Indeterminate Anaplasma IFA

[redacted]

Collected: 04/18/2023 UNK
Received: 04/20/2023 12:30
Reported: 05/23/2023 15:39
Reprinted: 05/25/2023 11:18
Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE
B. microti	CULTURE	Negative	Negative
B.duncani	CULTURE	Negative	Negative
EHRLICHIOSIS			
BLOOD CULTURE			
Blood Culture	CULTURE	**Positive**	Negative
EHRLICHIA cePCR			
Human Monocytic Ehrlichia	CULTURE	Negative	Negative
Human Granulocytic Anaplasma	CULTURE	**Positive**	Negative

[redacted]

Collected: 04/18/2023 UNK
Received: 04/20/2023 12:28
Reported: 05/10/2023 08:46
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Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
			>=160 : Indicates active infection	
ANAPLASMOSIS				
HGA IFA - IgM	Serum	<20	< 20 : Negative = 20 : May or may not indicate active infection >=40 : Indicates active infection	Titer
HGA IFA - IgG	Serum	80	< 40 : Negative < 160 : May or may not suggest active infection >=160 : Indicates active infection	Titer

Patient #4 (cont.) – Negative Bartonella Culture, Positive Bartonella ImmunoBlot

[redacted]

Collected: 04/18/2023 UNK
Received: 04/20/2023 12:30
Reported: 05/23/2023 15:39
Reprinted: 05/25/2023 11:18
Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
B. microti	CULTURE	Negative	Negative	
B.duncani	CULTURE	Negative	Negative	
EHRLICHIOSIS				
BLOOD CULTURE Blood Culture	CULTURE	**Positive**	Negative	
EHRLICHIA cePCR Human Monocytic Ehrlichia	CULTURE	Negative	Negative	
Human Granulocytic Anaplasma	CULTURE	**Positive**	Negative	
BARTONELLOSIS				
BLOOD CULTURE Blood Culture	CULTURE	Negative	Negative	
BARTONELLA cePCR Bartonella genus	CULTURE	Negative	Negative	

[redacted]

Collected: 04/18/2023 UNK
Received: 04/20/2023 12:28
Reported: 05/10/2023 08:46
Reprinted: 05/25/2023 11:18
Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
Bartonella species, including but not limited to B.elizabethae, B. vinsonii, B.henselae, B.quintana, B.bacilliformis, B.clarridgeiae, B. grahamii, B.koehlerae, B.washoensis and B.rochalimae.				
BARTONELLA IMMUNOBLOT IGG Bartonella genus	Serum	**Positive**	Positive: Detected 2 or more Bartonella genus or species-specific antibody. Indeterminate: Detected only 1 Bartonella genus c species- specific antibody. Negative: No Bartonella specific antibody detecte	
B. elizabethae	Serum	Negative		
B. vinsonii	Serum	Negative		
B. henselae	Serum	Negative		
B. quintana	Serum	Negative		
Bartonella species	Serum	**Positive**		
Report revision: Effective 11-15-2022 *Bartonella ImmunoBlot test detects specific antibodies to Bartonella species, including but not limited to B.elizabethae, B. vinsonii, B.henselae, B.quintana, B.bacilliformis, B.clarridgeiae, B. grahamii, B.koehlerae, B.washoensis and B.rochalimae.*				

Patient #4 Summary

This patient had a positive Anaplasma culture but weakly reactive IFA; also had a negative Bartonella culture but positive Bartonella IgG ImmunoBlot

Patient 4					
Anaplasma Culture	**Anaplasma IFA IgM**	**Anaplasma IFA IgG**	**Bartonella Culture**	**Bartonella IB IgM**	**Bartonella IB IgG**
Pos	Neg	Equivocal	Neg	Neg	Pos

Discordant results:

- **Was this patient partially treated?**
- **Did they acquire the infections at different times?**

Example of the value of combining a direct test with an indirect test to increase yield, especially with organisms known to be difficult to detect by lab testing

Patient #5 – Positive Rickettsia Culture, Negative Rickettsia IFA

RICKETTSIOSIS

BLOOD CULTURE			
Blood Culture	CULTURE	**Positive**	Negative

RICKETTSIA GENUS POSITIVE PER DR. SHAH.

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

Diagnosis should not be based on laboratory results alone. Results should be interpreted in conjunction with clinical symptoms and patient history.

NOTE: Western Blots, ImmunoBlots, Lyme Dot Blot, Epitope, PCR, IFA, FISH, C. pneumoniae IgG/IgA, CD57, IGXSpot, Broad Coverage Antibody, COVID-19 Test - developed and their performance characteristics determined by IGeneX, Inc. They have not been cleared or approved by the FDA. The FDA has determined that such approval is not necessary. These tests are used for clinical purposes and should not be regarded as investigational or for research. IGeneX, Inc. is licensed by CMS and NYS to perform high complexity clinical laboratory testing.

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Collected: 03/13/2023 UNK
Received: 03/15/2023 12:59
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Amended:
Corrected:

RICKETTSIOSIS

R. rickettsii IFA - IgG	Serum	<40	< 40 : Negative < 160 : May or may not suggest	Titer

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

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TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
			active infection >=160 : Indicates active infection	

Patient #5 (cont.) – Negative TBRF and Babesia Cultures, Positive TBRF and Babesia ImmunoBlots

BORRELIOSIS- Relapsing Fever Borrelia

BLOOD CULTURE			
Blood Culture	CULTURE	Negative	Negative
RFBORRELIA & B.BURGDORFERI sl cePCR			
Relapsing Fever Borrelia Genus*	CULTURE	Negative	Negative

** detects RF Borrelia species specific DNA, including but not limited to B. hermsii, B.turicatae, B. miyamotoi, B. parkeri, B. coriaceae, B. turcica and B. recurrentis*

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

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NOTE: Western Blots, ImmunoBlots, Lyme Dot Blot, Epitope, PCR, IFA, FISH, C. pneumoniae IgG/IgA, CD57, IGXSpot, Broad Coverage Antibody, COVID-19 Test - These tests wer developed and their performance characteristics determined by IGeneX, Inc. They have not been cleared or approved by the FDA. The FDA has determined that such approval is not necessary. These tests are used for clinical purposes and should not be regarded as investigational or for research. IGeneX, Inc. is licensed by CMS and NYS to perform high complexity clinical laboratory testing.

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BABESIOSIS

BLOOD CULTURE			
Blood Culture	CULTURE	Negative	Negative
BABESIA cePCR			
Babesia spp	CULTURE	Negative	Negative

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

Diagnosis should not be based on laboratory results alone. Results should be interpreted in conjunction with clinical symptoms and patient history.

NOTE: Western Blots, ImmunoBlots, Lyme Dot Blot, Epitope, PCR, IFA, FISH, C. pneumoniae IgG/IgA, CD57, IGXSpot, Broad Coverage Antibody, COVID-19 Test - These tests we developed and their performance characteristics determined by IGeneX, Inc. They have not been cleared or approved by the FDA. The FDA has determined that such approval is not necessary. These tests are used for clinical purposes and should not be regarded as investigational or for research. IGeneX, Inc. is licensed by CMS and NYS to perform high complexity clinical laboratory testing.

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Collected: 03/13/2023 UNK
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Reported: 03/23/2023 16:16
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Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
B. hermsii	Serum	Negative		
B. turicatae	Serum	Negative		
TBRF Borrelia spp	Serum	Negative		
TBRF Borrelia ImmunoBlot IgG				
TBRF Borrelia genus ImmunoBlot IgG	Serum	**Pos(TBRF)**		

Positive: Detected 2 or more TBRF Borrelia species-specific antibodies.
Indeterminate: Detected only 1 TBRF Borrelia species-specific antibody.
Negative: No TBRF Borrelia specific antibody detected.

** TBRF ImmunoBlot IgG detects antibodies to Borrelia hermsii, B. turicatae, B. miyamotoi, B. coriciae and TBRF Borrelia species.*

TBRF Borrelia ImmunoBlot IgG Species		
B. miyamotoi	Serum	Negative
B. hermsii	Serum	Negative
B. turicatae	Serum	Negative
TBRF Borrelia spp	Serum	**Positive**

BABESIOSIS

Babesia FISH	W blood	Negative

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TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
B. microti ImmunoBlot IgM	Serum	**Positive**		
B. duncani ImmunoBlot IgM	Serum	Negative		
Babesia spp.	Serum	Negative		

Limitations:
Negative test result does not exclude possibility of Babesia infection, may retest in 6-8 weeks.
Results should be interpreted in conjunction with clinical symptoms and other laboratory findings.

Patient #5 Summary

Rickettsia: Positive culture, negative IFA

TBRF: Negative culture, positive ImmunoBlot IgG

Babesia: Negative culture, positive ImmunoBlot IgM

Patient 5					
Rickettsia Culture	**Rickettsia IFA**	**TBRF Culture**	**TBRF IB IgG**	**Babesia Culture**	**Babesia IB IgM**
Pos	Neg	Neg	Pos	Neg	Pos

Why is one pos only for IgM and the other one only pos for IgG?

Note:
I encourage all of you to look for Rickettsias because they may be far more common in our patients than previously realized

Patient #6 – Positive Lyme Culture, Positive Lyme ImmunoBlot

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Collected: 05/02/2023 UNK
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BORRELIOSIS - Lyme Disease

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
BLOOD CULTURE				
Blood Culture	CULTURE	**Positive**	Negative	
LYME cePCR				
Borrelia-genomic*	CULTURE	**Positive**	Negative	
Borrelia -plasmid	CULTURE	Negative	Negative	

** detects Borrelia specific DNA including but not limited to Borrelia burgdorferi sensu lato and Relapsing Fever Borrelia.*

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Laboratory Director: Jyotsna S. Shah, Ph.D.
CLIA: 05D0643914
CALIFORNIA: CLF 4033
NPI: 1396837605

REFERRING PHYSICIAN

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Collected: 05/02/2023 UNK
Received: 05/04/2023 13:49
Reported: 05/15/2023 15:51
Reprinted: 06/13/2023 17:15
Amended:
Corrected:

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS

BORRELIOSIS - Lyme Disease

Lyme ImmunoBlot IgM	Serum	
IGX Criteria:		**Positive**
CDC/NYS Criteria:		**Positive**

***
[REVISED REPORT: EFFECTIVE APRIL 10, 2019]

Lyme ImmunoBlot IgM detects antibodies to B. burgdorferi strains and species

Band (kDa)	23*	31*	34*	39*	41*	93
Intensity	+	-	-	-	+	-

Band Intensity: Positive: + to ++++, Indeterminate: Ind, Negative: (-)

Patient #6 (cont.) – Negative Babesia and Bartonella Cultures, Positive Babesia and Bartonella ImmunoBlots

BABESIOSIS

BLOOD CULTURE Blood Culture	CULTURE	Negative	Negative
BABESIA cePCR Babesia spp	CULTURE	Negative	Negative

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

Diagnosis should not be based on laboratory results alone. Results should be interpreted in conjunction with clinical symptoms and patient history.

NOTE: Western Blots, ImmunoBlots, Lyme Dot Blot, Epitope, PCR, IFA, FISH, C. pneumoniae IgG/IgA, CD57, IGXSpot, Broad Coverage Antibody, COVID-19 Test - These tests developed and their performance characteristics determined by IGeneX, Inc. They have not been cleared or approved by the FDA. The FDA has determined that such approval is not necessary. These tests are used for clinical purposes and should not be regarded as investigational or for research. IGeneX, Inc. is licensed by CMS and NYS to perform high complexity clinical laboratory testing.

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Collected: 05/02/2023 UNK
Received: 05/04/2023 13:51
Reported: 06/02/2023 16:14

BARTONELLOSIS

BLOOD CULTURE Blood Culture	CULTURE	Negative	Negative
BARTONELLA cePCR Bartonella genus	CULTURE	Negative	Negative

BABESIOSIS

Babesia FISH	W blood	Negative	
BABESIA IMMUNOBLOT IGM Babesia ImmunoBlot IgM(genus)	Serum	**Positive**	Positive: Presence of 2 or more Babesia specific antibodies. Negative: Presence of only 1 or no Babesia specific antibody.

** Babesia ImmunoBlot test detects specific IgM antibodies to Babesia species, including but not limited to B. microti, B. duncani and B. divergens.*

BARTONELLOSIS

Bartonella FISH	W blood	Negative	
BARTONELLA IMMUNOBLOT IGM Bartonella genus	Serum	**Positive**	Positive: Detected 2 or more Bartonella genus or species-specific antibody. Indeterminate: Detected only 1 Bartonella genus or species- specific antibody. Negative: No Bartonella specific antibody detected.

Patient #6 Summary

Lyme: Culture pos, IB IgM pos

Babesia: Culture neg, FISH neg, IB IgM pos

Bartonella: Culture neg, FISH neg, IB IgM pos

Patient 6							
Lyme Culture	Lyme IB IgM	Babes Culture	Babes FISH	Babes IB IgM	Bart Culture	Bart FISH	Bart IB
Pos	Pos	Neg	Neg	Pos	Neg	Neg	Pos

Note:
Cultures, FISH and ImmunoBlots are very highly specific so do not disregard a positive result of any of these tests!

B. duncani- Positive Culture from East Coast Patient

BABESIA CULTURE	CULTURE	**Positive**	Negative
BABESIA cePCR			
Babesia spp	CULTURE	Negative	Negative
B. microti	CULTURE	Negative	Negative

Testing performed at IGeneX 556 Gibraltar Drive Milpitas CA 95035 (800) 832-3200

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Collected: 05/15/2023 UNK
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TEST	SPECIMEN	RESULT	REFERENCE RANGE	UN
B.duncani	CULTURE	**Positive**	Negative	

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REFERRING PHYSICIAN

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Reported: 05/23/2023 15:39
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BABESIOSIS

TEST	SPECIMEN	RESULT	REFERENCE RANGE	UNITS
BLOOD CULTURE				
Blood Culture	CULTURE	**Positive**	Negative	
BABESIA cePCR				
Babesia spp	CULTURE	Negative	Negative	
B. microti	CULTURE	Negative	Negative	
B.duncani	CULTURE	**Positive**	Negative	

Key Findings

- Clearly, the combination of the culture + ImmunoBlot results in a higher yield than either one alone
- Testing only for Lyme will miss many positive patients
- Basic idea- highest yield results from using a combination of the best direct tests, + the best indirect tests for all the most likely pathogens
- The IGeneX cePCR (culture), ImmunoBlots and FISH are all very highly specific, so if you get discordant results, trust the positive one if it clinically fits

Uh-Oh thought to ponder…

When I was preparing these slides, it dawned upon me that testing each pathogen using multiple methods can uncover completely unexpected positives.

What are the implications of this?

DOES THIS MEAN THAT WE ARE OBLIGATED TO USE COMBINATION TESTING ON ALL PATIENTS?

DOES THIS ALSO MEAN THAT WE NEED TO TEST FOR ALL THE MAJOR TBDs, EVEN IF THEY WERE NOT SUSPECTED PREVIOUSLY?

DO WE RISK LIABILITY IF WE DO NOT??

Available cePCR Test Panels

Culture tests can be purchased for each disease individually, or as a panel

- Panels provide a significant discount

Available cePCR Panels

- Borreliosis cePCR Test Panel
  - Includes two-week culture, plus PCR for Lyme and TBRF.
- Co-infection cePCR Test Panel
  - Includes two-week culture, plus PCR for Babesia, Bartonella, HME, HGA, and Rickettsia.
- Tick-Borne Disease cePCR Test Panel
  - Includes two-week culture, plus PCR for Lyme, TBRF, Babesia, Bartonella, HME, HGA, and Rickettsia.

Current Special IGeneX ImmunoBlot Test Panels

ImmunoBlots can be purchased for each disease individually, or as a panel

Special ImmunoBlot Panel Offers (available through summer 2023)

- ImmunoBlots for three diseases: $999
- ImmunoBlots for four diseases $1,299

Summary and Recommendations

- A positive culture confirms active disease
- Highest culture yield is expected during symptom flares, especially if not on treatment at time of testing
- Highest overall testing sensitivity occurs if multiple methods are combined
  - Direct + Indirect
  - Culture and/or FISH + ImmunoBlots
    - Add T-cell response assay (IGXSpot) if B-cell deficiency is present or suspected

Based upon the data presented today, consider deeply the implications of NOT doing combination testing!!

The IGeneX Resource

Additional information is readily available on the IGeneX website

- Please visit igenex.com
  - Test methodologies and interpretations
  - Webinars
  - Research papers
  - Price lists
- For customer support, please call 1-800-832-3200