Tick-Borne Diseases (TBD)

Philip Molloy, MD

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Disclosure

Dr. Molloy is Medical Director of Tick-Borne Diseases at Imugen, a Division of Oxford Immunotec, Inc.

Assistant Clinical Professor of Medicine, Tufts Medical School, Boston, MA

Staff physician BID Plymouth and Nantucket Cottage Hospital

Objectives

- Describe epidemiology & clinical presentations of tick-borne diseases (TBD)
- Describe TBD diagnostic testing & result interpretation for both single and co-infected TBD patients

Take Home Message:
These infections are prevalent, often challenging to differentiate from one another, and co-infections are common

Pre-test: True or False?

Q1: If the whole blood Lyme PCR test is positive, and all the Serologies are negative, it is likely that the PCR is a false positive test result.

Q2: After treating a patient for Lyme disease, a follow-up Lyme test is recommended; if it is still positive, the patient should be re-treated.

Common Tick-Borne Diseases

Which one does not fit in with the others?

Lyme disease

Babesiosis

B. miyamotoi disease

HGA

HME

Transmitted by the Deer Tick (*Ixodes*)

Transmitted by the Lone Star Tick

Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed August 30. 2017.
*B. miyamoto*i. https://www.cdc.gov/ticks/miyamotoi.htm. Accessed August 30, 2017.

Methods for Diagnosis of Tick-Borne Disease

Clinical Evaluation

- Symptoms
  Lyme- Erythema migrans
  RMSF – Maculopapular rash

Direct Tests

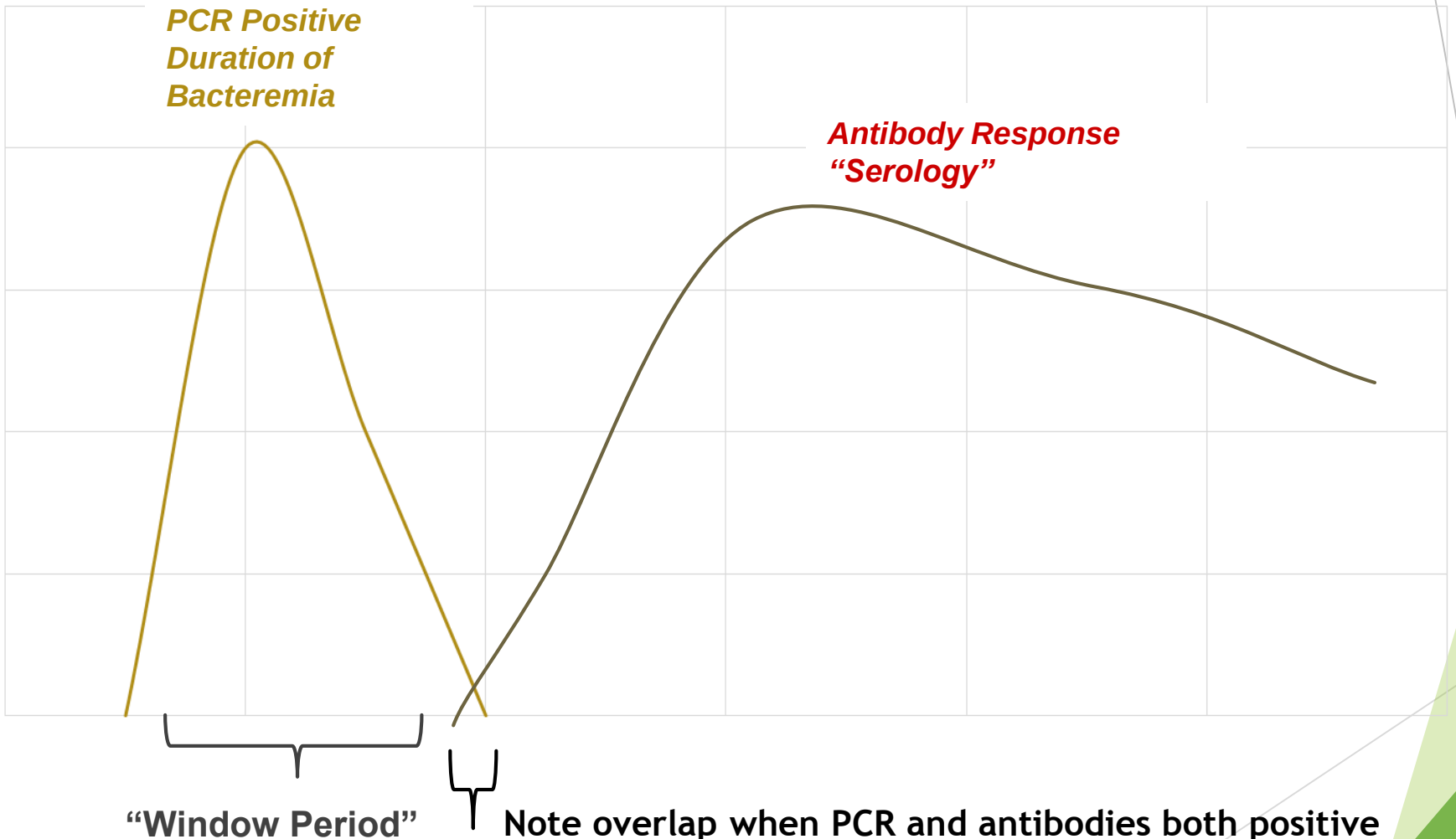
- Culture
- Direct smear microscopy
- PCR

Indirect Tests

- Serology

Aguero-Rosenfeld ME, et al. Diagnosis of Lyme Borreliosis. *Clinical Microbiology Reviews*. 2005;18(3):484-509.
Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed July17, 2017.

Diagnosis: General Pattern of Bacteremia in Relation to Antibody Response



Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed July17, 2017.
Schotthoefer AM, Meece JK, Ivacic LC, et al. Comparison of a Real-Time PCR Method with Serology and Blood Smear Analysis for Diagnosis of Human Anaplasmosis: Importance of Infection Time Course for Optimal Test Utilization. *Journal of Clinical Microbiology*. 2013;51(7):2147-2153. doi:10.1128/JCM.00347-13.

General Pattern of Bacteremia in Relation to Antibody Response

Example: Narrow bacteremia

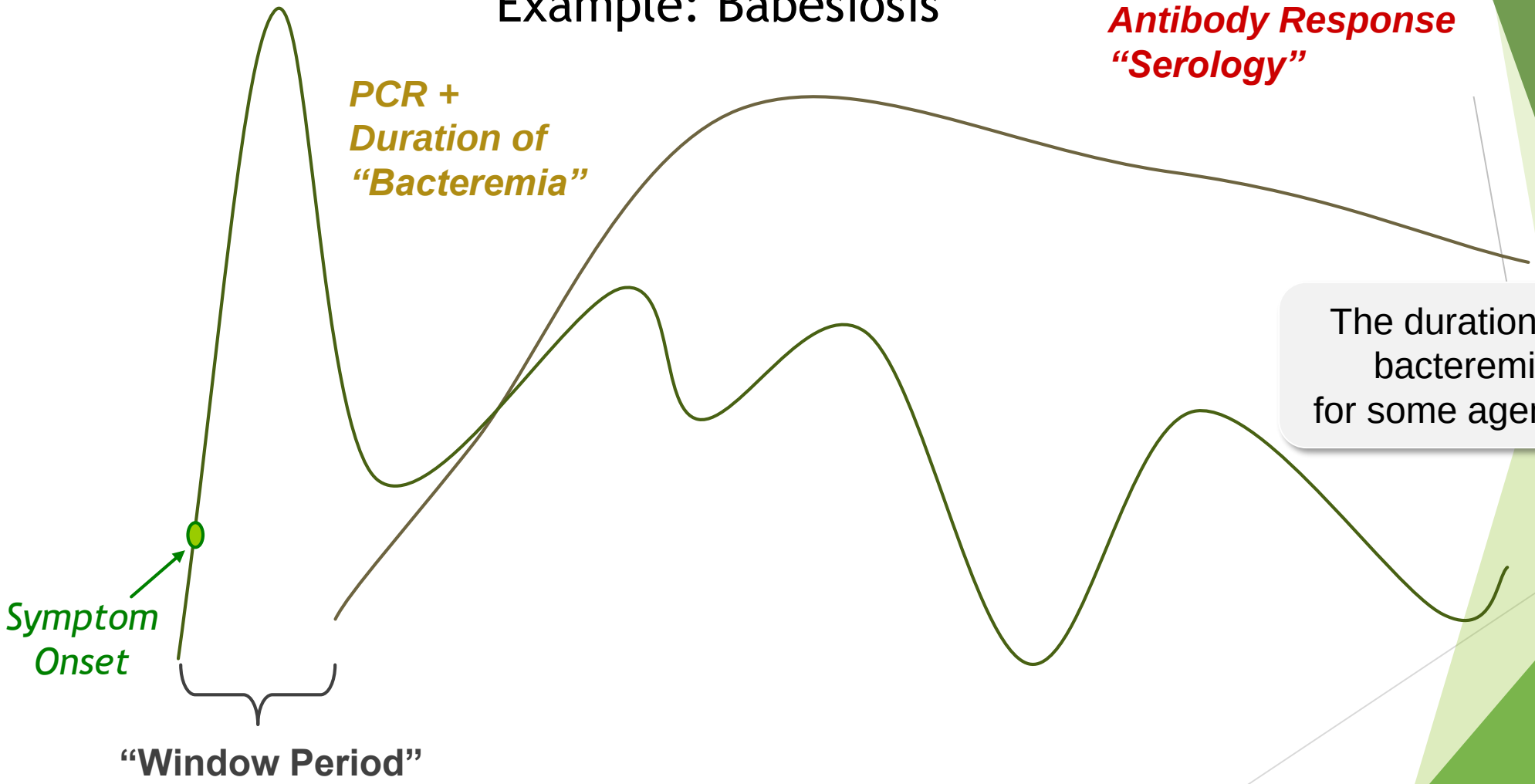
The duration of PCR positive bacteremia is very narrow for some agents (e.g., *B. burgdorferi* in Lyme)

Note overlap when PCR and antibodies may both be positive

Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed July17, 2017.
Schotthoefer AM, Meece JK, Ivacic LC, et al. Comparison of a Real-Time PCR Method with Serology and Blood Smear Analysis for Diagnosis of Human Anaplasmosis: Importance of Infection Time Course for Optimal Test Utilization. *Journal of Clinical Microbiology*. 2013;51(7):2147-2153.

General Pattern of Bacteremia in Relation to Antibody Response

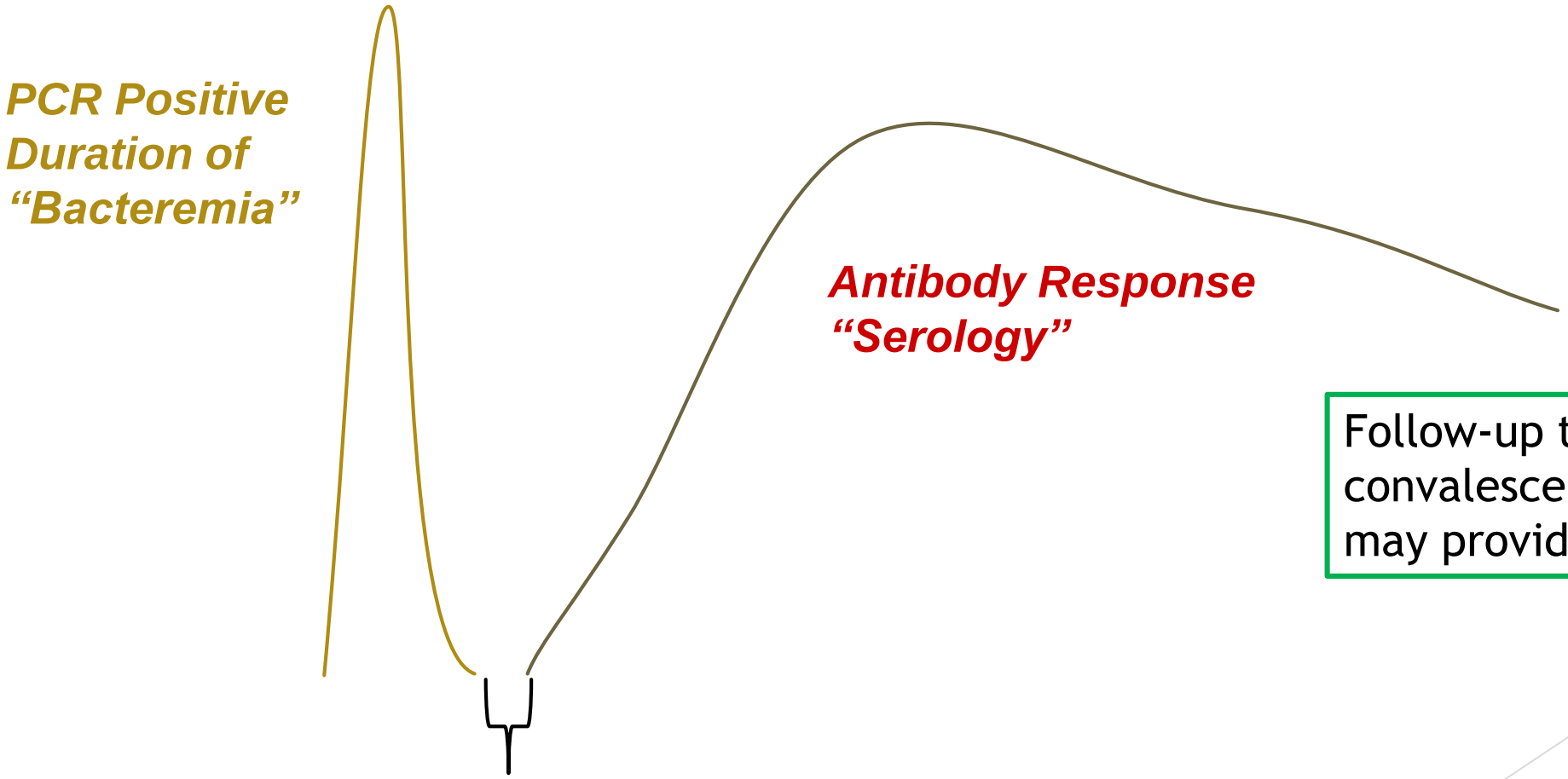
Example: Babesiosis



The duration of PCR positive bacteremia is very wide for some agents like *B. microti*.

Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed July17, 2017.
Schotthoefer AM, Meece JK, Ivacic LC, et al. Comparison of a Real-Time PCR Method with Serology and Blood Smear Analysis for Diagnosis of Human Anaplasmosis: Importance of Infection Time Course for Optimal Test Utilization. *Journal of Clinical Microbiology*. 2013;51(7):2147-2153.

Diagnosis: General Pattern of Negative Tests in Symptomatic Patients



Follow-up testing on convalescent samples may provide insight

Possibility of a "window" when both PCR and serology are negative

Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed July17, 2017.
Schotthoefer AM, Meece JK, Ivacic LC, et al. Comparison of a Real-Time PCR Method with Serology and Blood Smear Analysis for Diagnosis of Human Anaplasmosis: Importance of Infection Time Course for Optimal Test Utilization. *Journal of Clinical Microbiology*. 2013;51(7):2147-2153.

Lyme Serologic Tests

Many ELISA formats and commercial test kits available

- Whole Cell Sonicate as antigen
- Recombinant peptide antigens e.g. C6
- Antibody Capture Immunoassays
  - IgM, IgG, IgA detection
  - Designed to optimize the identification of the early serologic response

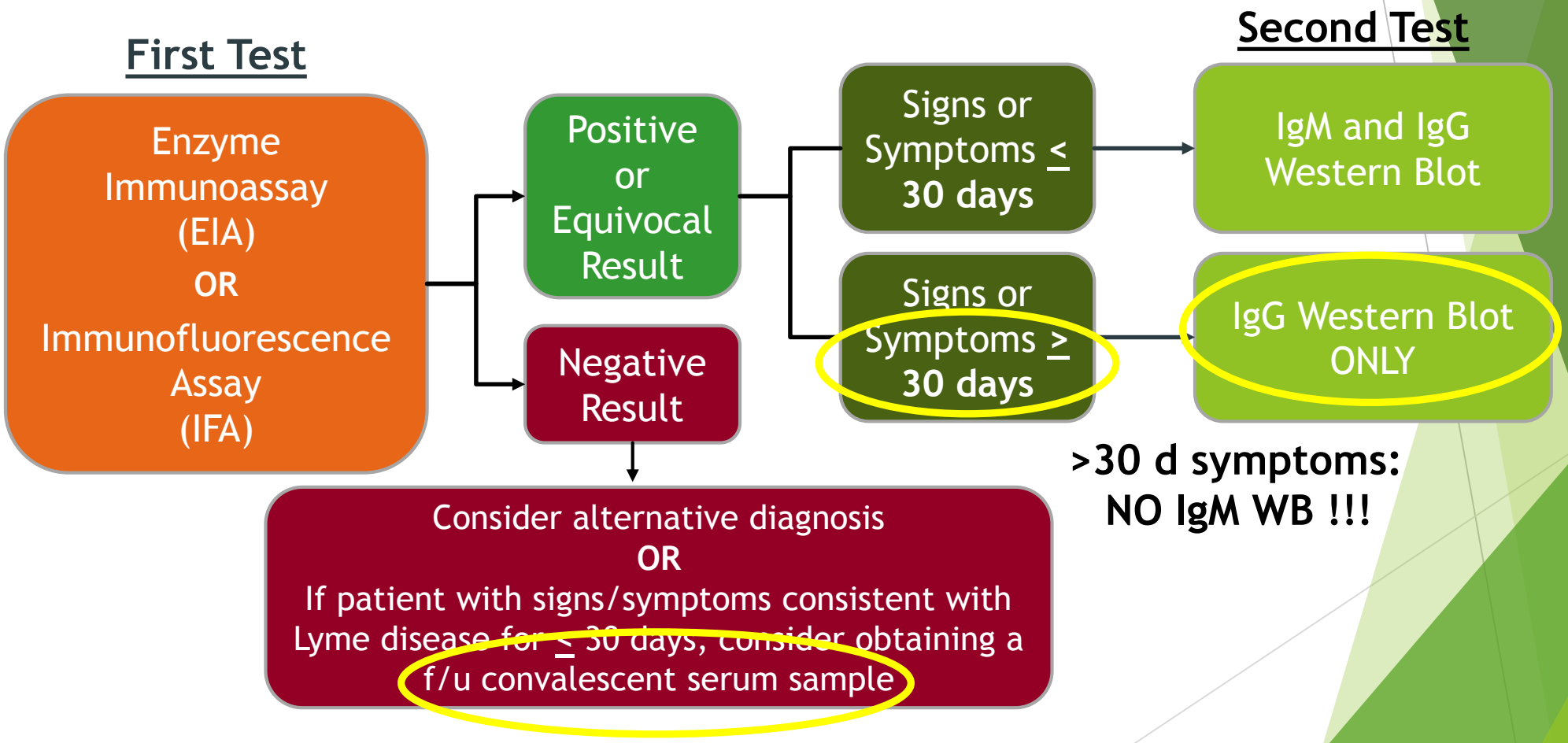
Western Blots (IgG)

- May utilize “wild” and OspA-free mutant *Bb* strains
- Designed to characterize later, more evolved immune response, for specificity and “staging”
- May miss the earliest part of the serologic response

Berardi VP, Weeks KE, Steere AC. Serodiagnosis of early Lyme disease; evaluation of IgM and IgG antibody responses by using an antibody capture enzyme immunoassay. J Infect Dis.1988;158:754-760.
Aguero-Rosenfeld ME, et al. Diagnosis of Lyme Borreliosis. *Clinical Microbiology Reviews*. 2005;18(3):484-509
Molloy, PJ. Berardi, VP., Persing, DH., Sigal, LH.; Detection of Multiple Reactive Protein Species by Immunoblotting after Recombinant Outer Surface Protein A Lyme Disease Vaccination, *Clinical Infectious Diseases*, Volume 31, Issue 1, 1 July 2000, Pages 42–47,

Current CDC Guidelines for Laboratory Diagnosis of Lyme Disease

Two-tiered testing for Lyme disease



CDC. Lyme Disease Two-step Laboratory Testing Process. www.cdc.gov/lyme/diagnosistesting/labtest/twostep/index.html.

Lyme Disease: Direct Detection of *B. burgdorferi*

- **Culture**
  - Useful for research and understanding this infection
  - Can culture skin lesions, blood, occasionally other tissues
  - Requires large volumes of blood and weeks of incubation

- **Polymerase Chain Reaction (PCR)**
  - Detects the organism's specific DNA sequences
  - May employ different probes/primers
  - Useful in Synovial Fluid (SF)
  - Also utilized in blood (brief window in early infection), skin, CSF samples
  - Capable of detecting small numbers of DNA copies
  - Rapid turnaround times

Aguero-Rosenfeld ME, et al. Diagnosis of Lyme Borreliosis. *Clinical Microbiology Reviews*. 2005;18(3):484-509
Oxford Immunotec, Inc. *Borrelia* Species PCR Blood Test Validation Summary. Document Number PRD-VAL-1. 2017.

Lyme Disease Diagnosis – What's New?

- Appreciation that there is an early window of bacteremia, potentially detectable by advanced DNA extraction methodologies.

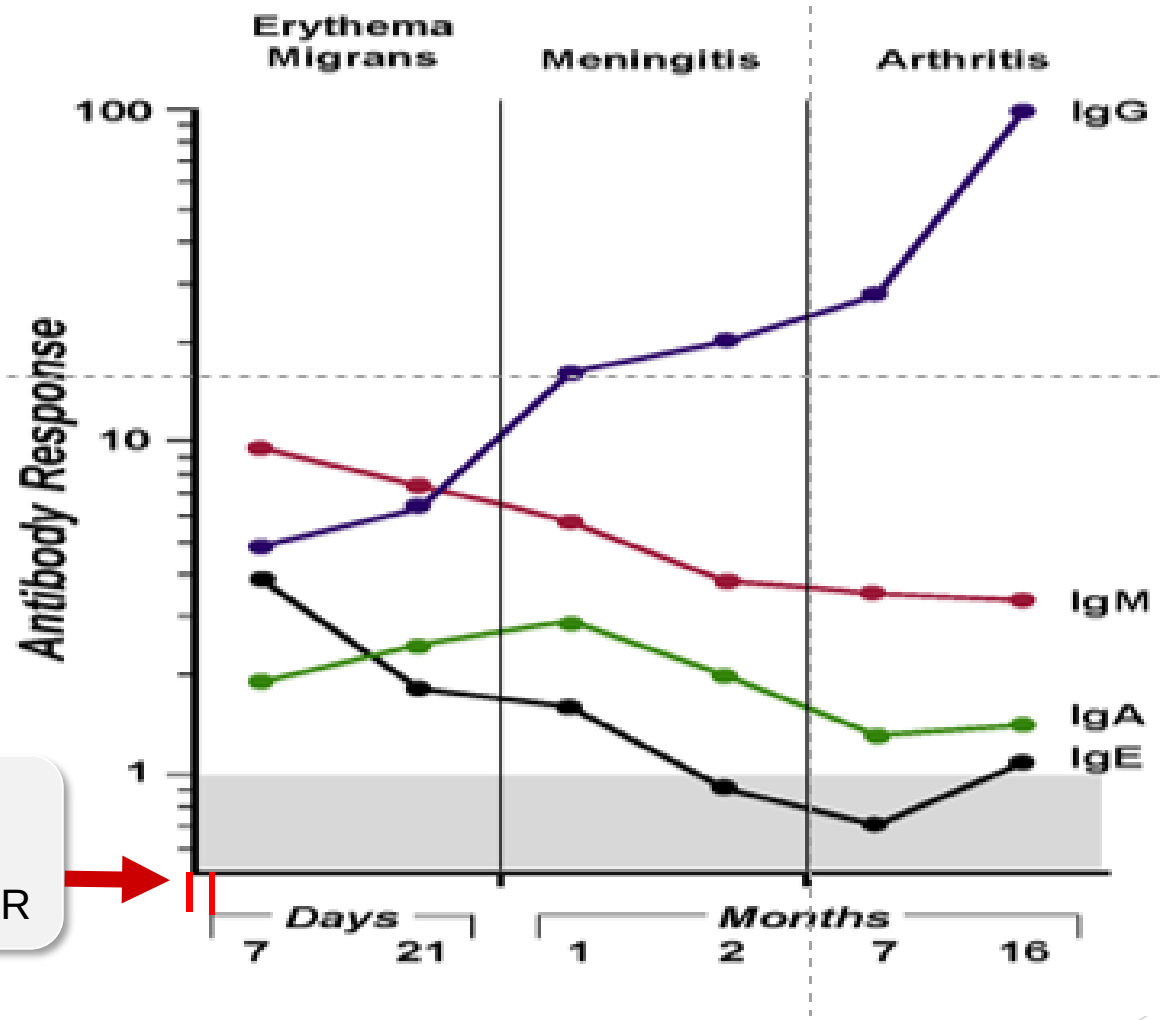
- Acutely ill patients with early Lyme may be briefly whole blood PCR positive prior to the development of detectable antibodies. **("window period")**

- By the time Lyme antibodies are present, the whole blood PCR is typically becoming negative.

Goodman JL, Bradley JF, Ross AE, et al. Bloodstream invasion in early Lyme disease: results from a prospective, controlled, blinded study using the polymerase chain reaction. *The American journal of medicine*. 1995;99(1):6–12.
Oxford Immunotec, Inc. *Borrelia burgdorferi* Lyme Antibody Analysis Test Validation Summary. Document Number PRD-VAL-2 REV 1.0. 2017.
Aguero-Rosenfeld ME, et al. Diagnosis of Lyme Borreliosis. *Clinical Microbiology Reviews*. 2005;18(3):484-509

Antibody Capture EIA: Antibody Response to *B. burgdorferi*

Geometric mean antibody responses to *Borrelia burgdorferi* in sixty serum samples from ten patients with Lyme disease. Antibody responses determined by capture EIA.



Note high robust serologies in Lyme arthritis

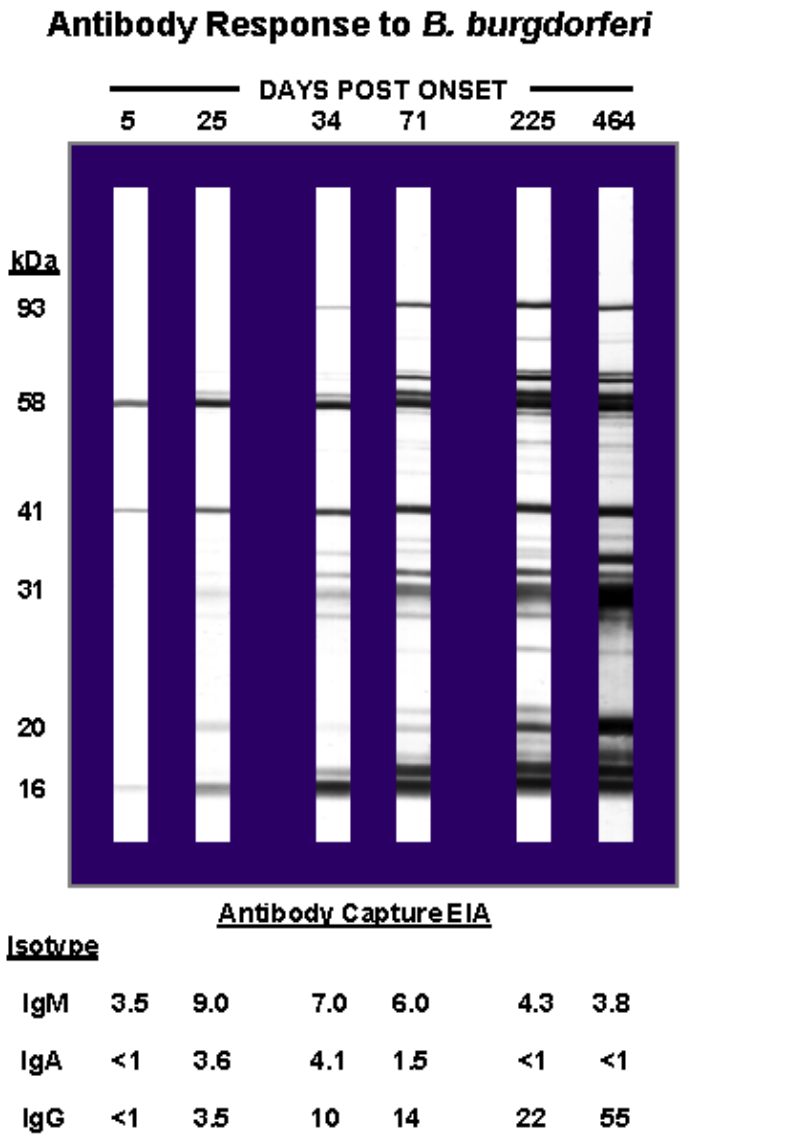
And IgM is persisting

Brief early window of spirochetemia with positive whole blood PCR

Sigler SJ. Lyme Disease Antibody Response A Handbook for Physicians. Presentation at the South Hampton Hospital Tick-Borne Disease Testing Program. 1995.

Lyme Serologies *B.* Over Time: Untreated

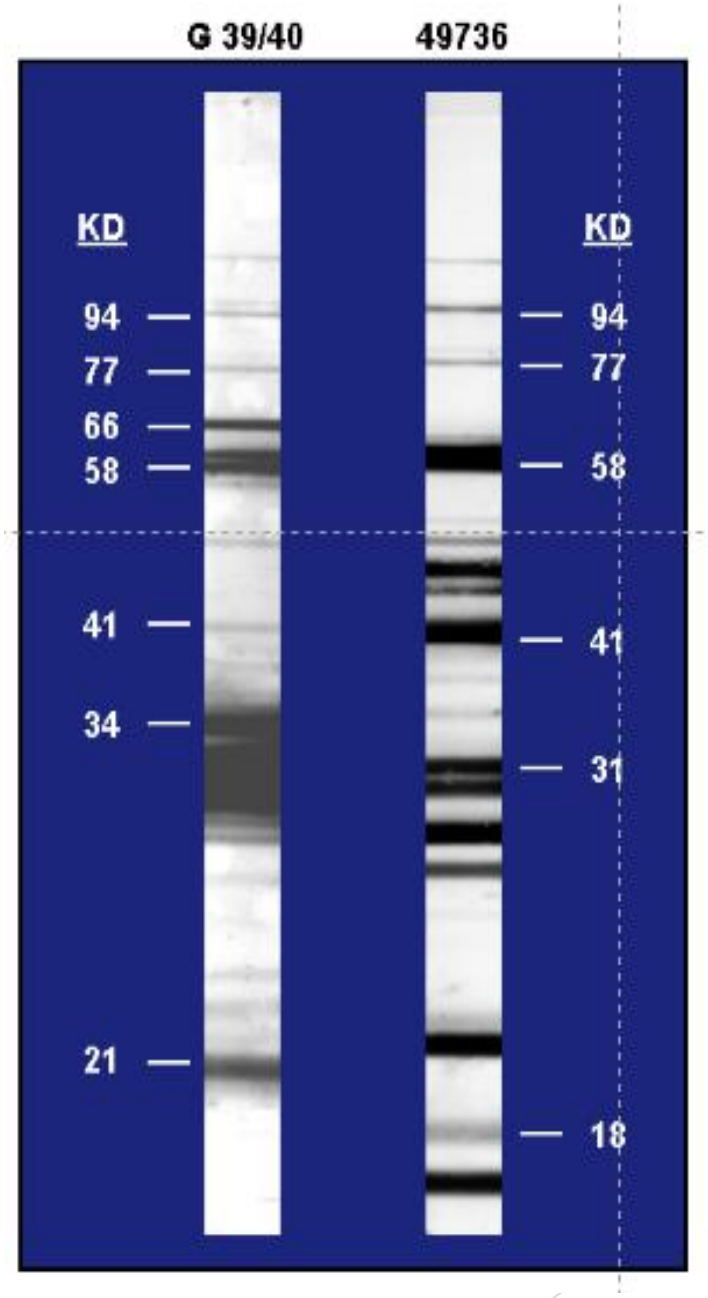
IgG Western blot and Antibody capture EIA responses to *Borrelia burgdorferi* in six serial serum specimens from one patient with untreated Lyme disease.



Western blot IgG and antibody capture IgM, IgA and IgG EIA responses to *B. burgdorferi* in 6 serial serum specimens from 1 untreated patient with Lyme disease. The patient presented initially with erythema migrans (EM) (5 days) and subsequently developed meningitis (day 34) and later developed synovitis (day 225). The patient had a second episode of Lyme arthritis on day 464. Days post onset are the time points of the collection of each sample following the appearence of EM.

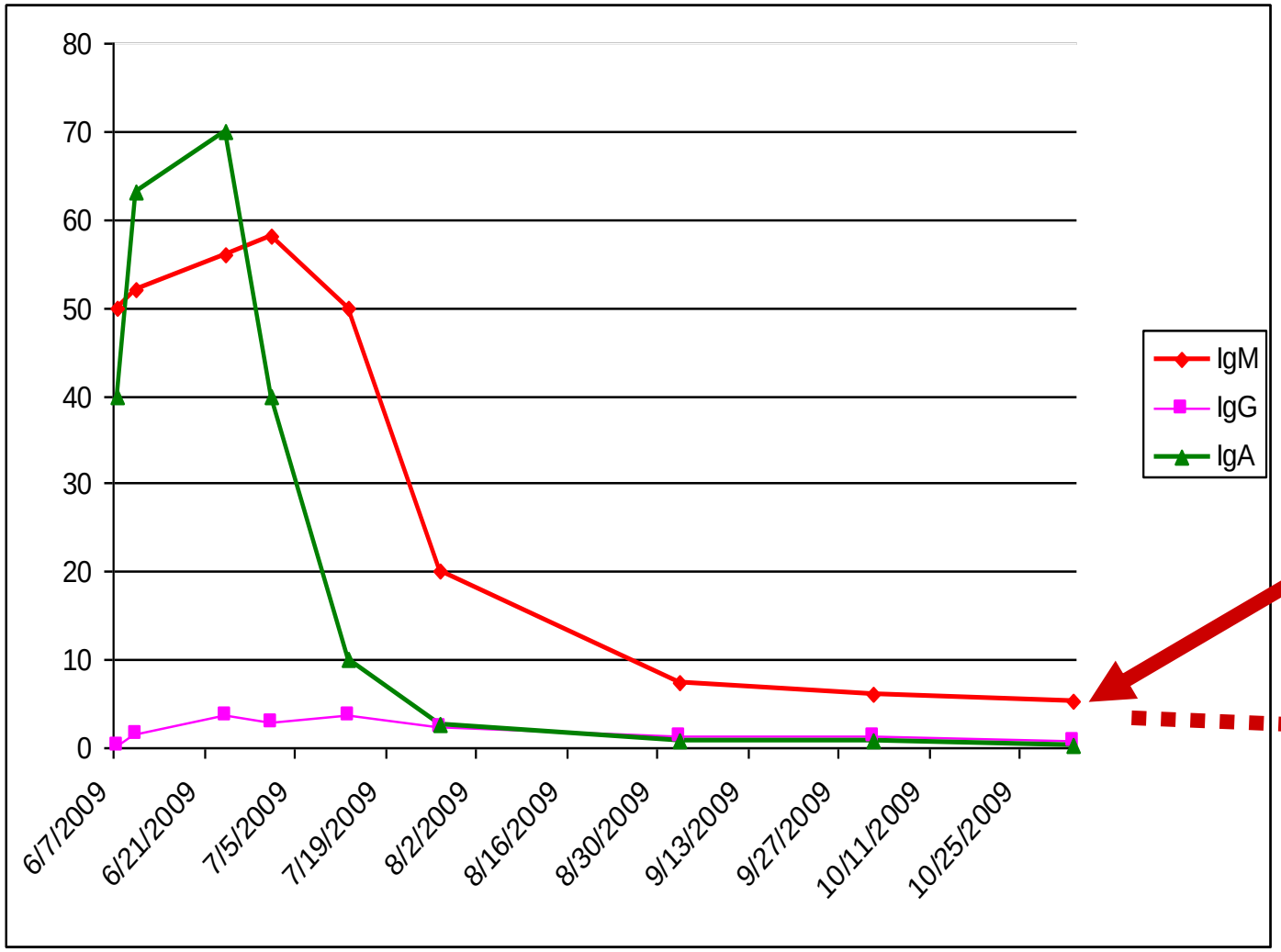
Sigler SJ. Lyme Disease Antibody Response A Handbook for Physicians. Presentation at the 1995 South Hampton Hospital Tick-Borne Disease Testing Program. Oxford Immunotec, Inc. *Borrelia burgdorferi* Lyme Antibody Analysis Test Validation Summary. Document Number PRD-VAL-2 REV 1.0. 2017.

WB Reactivity ("bands") vary with different B.b strains and in different labs



Imugen Internal Data
Oxford Immunotec, Inc. *Borrelia* Species PCR Blood Test Validation Summary. Document Number PRD-VAL-1 REV 1.0. 2017.
Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme Borreliosis.
Clinical Microbiology Reviews. 2005;18(3):484-509. doi:10.1128/CMR.18.3.484-509.2005.

Lyme Antibody Capture EIA: Isotype Response Over Time (treated)



Note: IgM persistence

May remain for many years

Case study: Imugen Internal Data

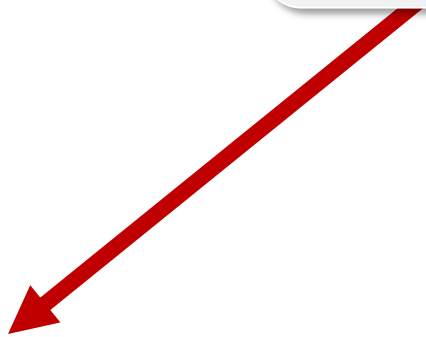
Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme Borreliosis. *Clinical Microbiology Reviews*. 2005;18(3):484-509.

Illustrative Case: Common dilemma

- How to interpret an isolated low IgM antibody capture EIA result
  - IgM = 1-2 range
  - IgG < 1 and negative IgG WB's
  - IgA < 1

- What could it be?
  - Cross-reactive, not Lyme related
  - Recent new Lyme infection, early immune response
  - Residual left-over AB from remote infection

The possibility we do **not** want to miss



Illustrative Case: The isolated low IgM dilemma

"Recent new Lyme infection, early immune response"

How can you resolve it?

- IgM WB frequently does not help
- If true recent exposure, the titer will rise with time, promptly and usually dramatically
- So if you clinically suspect a recent Lyme event, repeat the test in 1-2 weeks

Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. Diagnosis of Lyme Borreliosis. *Clinical Microbiology Reviews*. 2005;18(3):484-509. doi:10.1128/CMR.18.3.484-509.2005.
Oxford Immunotec, Inc. *Borrelia burgdorferi* Lyme Antibody Analysis Test Validation Summary. Document Number PRD-VAL-2 REV 1.0. 2017.

Illustrative Case: The isolated low IgM dilemma

If it is a recent Lyme infection you would expect an *increased* IgM antibody capture result 1 to 2 weeks later

Index Test		1-2 Weeks Later
IgM	2.0	4 - 20 +
IgG	< 1	< 1
IgA	< 1	Variably +
IgG	Neg	Neg

Treated or Untreated

PCR Combined with Serology Increases the Window of Detection in Early Lyme

Clinical testing of over 20,000 patients for Lyme disease utilizing serology and PCR determined:

- **1,569 cases of Lyme disease were laboratory confirmed**
  - **1,310 cases were serology positive, PCR negative**
  - **79 cases were positive by both serology and PCR**
  - **180 cases were serology negative, PCR positive**
- 11.5% (180/1,569) of early *Borrelia* positive specimens were *Borrelia* PCR positive and Lyme serology negative (window period)

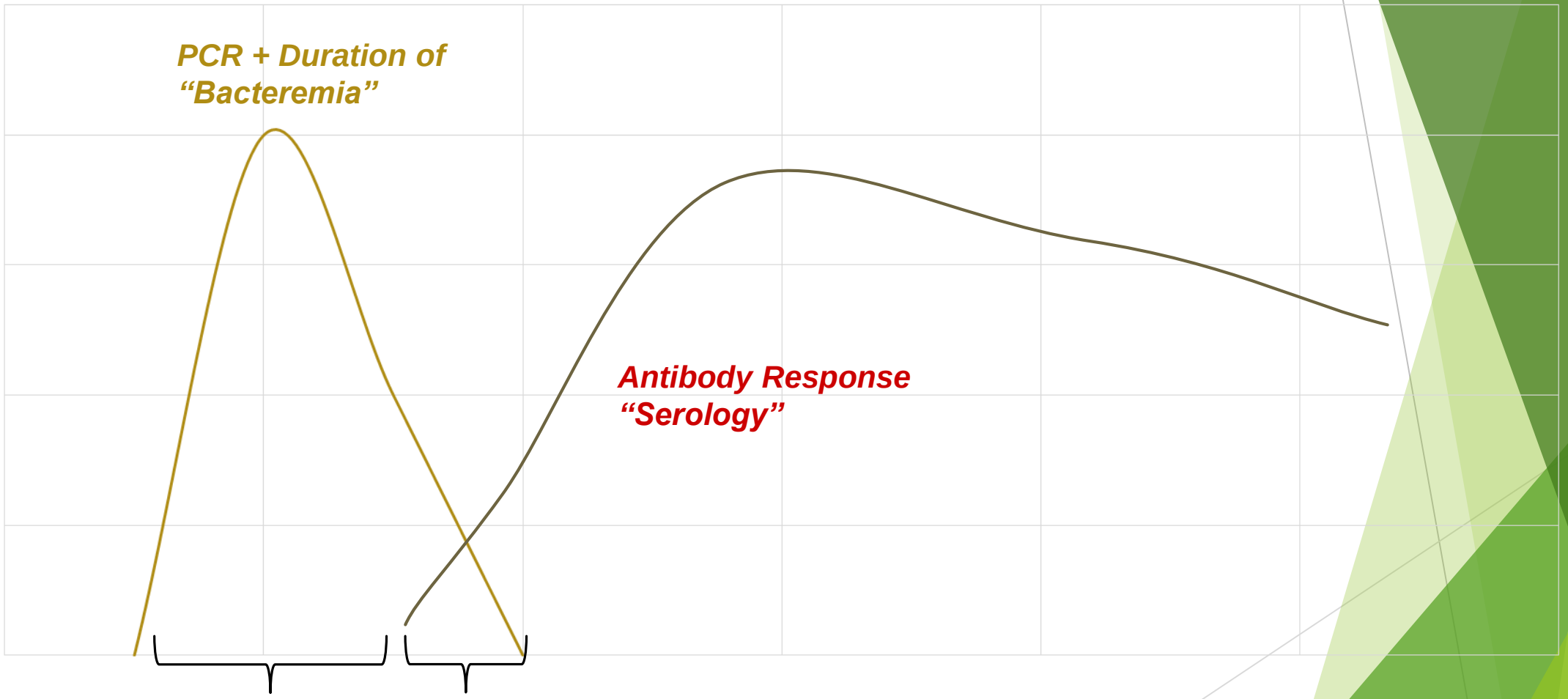
These 180 patients would have been initially missed with serology alone.

Pomerantz, K. and Pieken, W. *Report On 2016 Data Mining Of Imugen Submissions*. Norwood, MA: Imugen, a division of Oxford Immunotec, Inc.; 2017. Internal Report DOC-PRT-138 Rev 1.0.

Illustrative Case

- 60 year old patient from Long Island presented on a Saturday with 2-3 days of non-specific not feeling well, sweats, clammy, feeling feverish, no rash
- MD suspected tick-borne infection, tested and treated "If you're not getting better, come back"
- Courier did not pick up specimens that day
- Patient returned Sunday, different physician, re-tested

REMEMBER THIS….



Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed July17, 2017.
Schotthoefer AM, Meece JK, Ivacic LC, et al. Comparison of a Real-Time PCR Method with Serology and Blood Smear Analysis for Diagnosis of Human Anaplasmosis: Importance of Infection Time Course for Optimal Test Utilization. *Journal of Clinical Microbiology*. 2013;51(7):2147-2153. doi:10.1128/JCM.00347-13.

Illustrative Case:
Tests collected 24 hours apart

Both specimens show up in lab Monday morning

	Saturday Specimen	
Lyme PCR	Positive	
Lyme IgM	3.9	
Other Lyme Ab's	Negative	

Illustrative Case:
Tests collected 24 hours apart

Both specimens show up in lab Monday morning

	Saturday Specimen	Sunday Specimen
Lyme PCR	Positive	Negative
Lyme IgM	3.9	8.9
Other Lyme Ab's	Negative	Negative

The IgM WB on this patient was negative!

Laboratory Diagnosis of *B. microti* Infection

- **Direct Demonstration of Organism**
  - Thick Smear Microscopy
  - Detection of DNA by Polymerase Chain Reaction
- **Indirect Immune Responses (Serology)**
  - Immunofluorescence
  - Immunoblot
  - ELISA (Peptide)

https://www.uptodate.com/contents/clinical-manifestations-diagnosis-treatment-and-prevention-of-babesiosis. Accessed 8/31/17
Supplement to: Moritz ED, Winton CS, Tonnetti L, et al. Screening for Babesia microti in the U.S. blood supply. N Engl J Med 2016;375:2236-45.
Levin AE, et al. Determination of *Babesia microti* seroprevalence in blood donor populations using an investigational enzyme immunoassay. *Transfusion*. 2014;54(9):2237-2244.

PCR vs. Blood Smear Test Performance

- Both directly demonstrate the presence of Babesia organisms
- **SMEAR:** to be positive requires about 1/4,000 RBC's infected, or about 250,000 organisms/ml
- **PCR:** to be positive requires about 1/40,000,000 RBC's infected, or about 10 organisms/ml

https://www.uptodate.com/contents/clinical-manifestations-diagnosis-treatment-and-prevention-of-babesiosis. Accessed August 31, 2017
www.marshfieldlabs.org/sites/ltrm/Human/Newsletter%20Document%20Library/Laboratory%20News%20Vol%2036%20No%2007%20May%2017%202013[1].pdf. Accessed August 31, 2017

Laboratory Diagnosis of Human Granulocytic Anaplasmosis (HGA) Infection

- **Direct**
  - Visualization of Organism
    - Thick Smear Microscopy
  - Detection of DNA
    - Polymerase Chain Reaction
- **Indirect**
  - Immune Responses (Serology)
    - Immunofluorescence
    - ELISA (Native or Recombinant)

https://www.uptodate.com/contents/human-ehrlichiosis-and-anaplasmosis. Accessed August 31, 2017.
Yu Q, et al. Expression and Immunogenicity of Recombinant Immunoreactive Surface Protein 2 of Anaplasma phagocytophilum. *Clinical and Vaccine Immunology : CVI*. 2012;19(6):919-923.

Illustrative Case: 82 year old patient from CT

- Presented to ER acutely/critically ill, febrile, non-specific lab abnormalities, adm to ICU;
- Physician considered HGA, ordered serology

	Initial Visit	1 Month Later
HGA IgM	< 1	
HGA IgG	< 1	
HGA PCR	ND*	

ACUTELY ILL

Presumably would have been PCR positive on the initial visit had it been ordered then.
PCR, not serology, is the test of choice for acutely ill patients suspected of having HGA.

Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed August 30, 2017

Illustrative Case: 82 year old patient from CT

- Presented to ER acutely/critically ill, febrile, non-specific lab abnormalities, adm to ICU;
- Physician considered HGA, ordered serology

	Initial Visit	1 Month Later
HGA IgM	< 1	> 48
HGA IgG	< 1	> 22
HGA PCR	ND*	Positive

ACUTELY ILL

Presumably would have been PCR positive on the initial visit had it been ordered then.
PCR, not serology, is the test of choice for acutely ill patients suspected of having HGA.

Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed August 30, 2017

Laboratory Diagnosis of Human Monocytic Ehrlichiosis (HME) Infection

- **DIRECT**
  - VISUALIZATION OF ORGANISM
    - Thick Smear Microscopy
  - DETECTION OF DNA
    - Polymerase Chain Reaction

- **INDIRECT**
  - IMMUNE RESPONSES (SEROLOGY)
    - Immunofluorescence
    - ELISA (Native or Recombinant)

https://www.uptodate.com/contents/human-ehrlichiosis-and-anaplasmosis. Accessed September 11, 2017.

Borrelia miyamotoi

- *Borrelia* are well-known veterinary pathogens
- Related to relapsing fever *Borrelia* species
- Same deer tick vector, *Ixodes scapularis*
- Japan (in ticks,1995), Russia (humans, 2011)
- 1st cases in North America identified in IMUGEN lab in 2012 (NJ & MA patients)
  - Gugliotta JL, Goethert HK, Berardi VP, Telford SR. Meningoencephalitis from *Borrelia miyamotoi* in an Immunocompromised Patient. *The New England journal of medicine*. 2013;368(3):240-245. doi:10.1056/NEJMoa1209039.
- Since then, other cases identified
  - Molloy PJ, Telford SR, Chowdri HR, Lepore TJ, Gugliotta JL, Weeks KE, et al. *Borrelia miyamotoi* Disease in the Northeastern United States: A Case Series. Ann Intern Med. 2015;163:91–98. doi: 10.7326/M15-0333

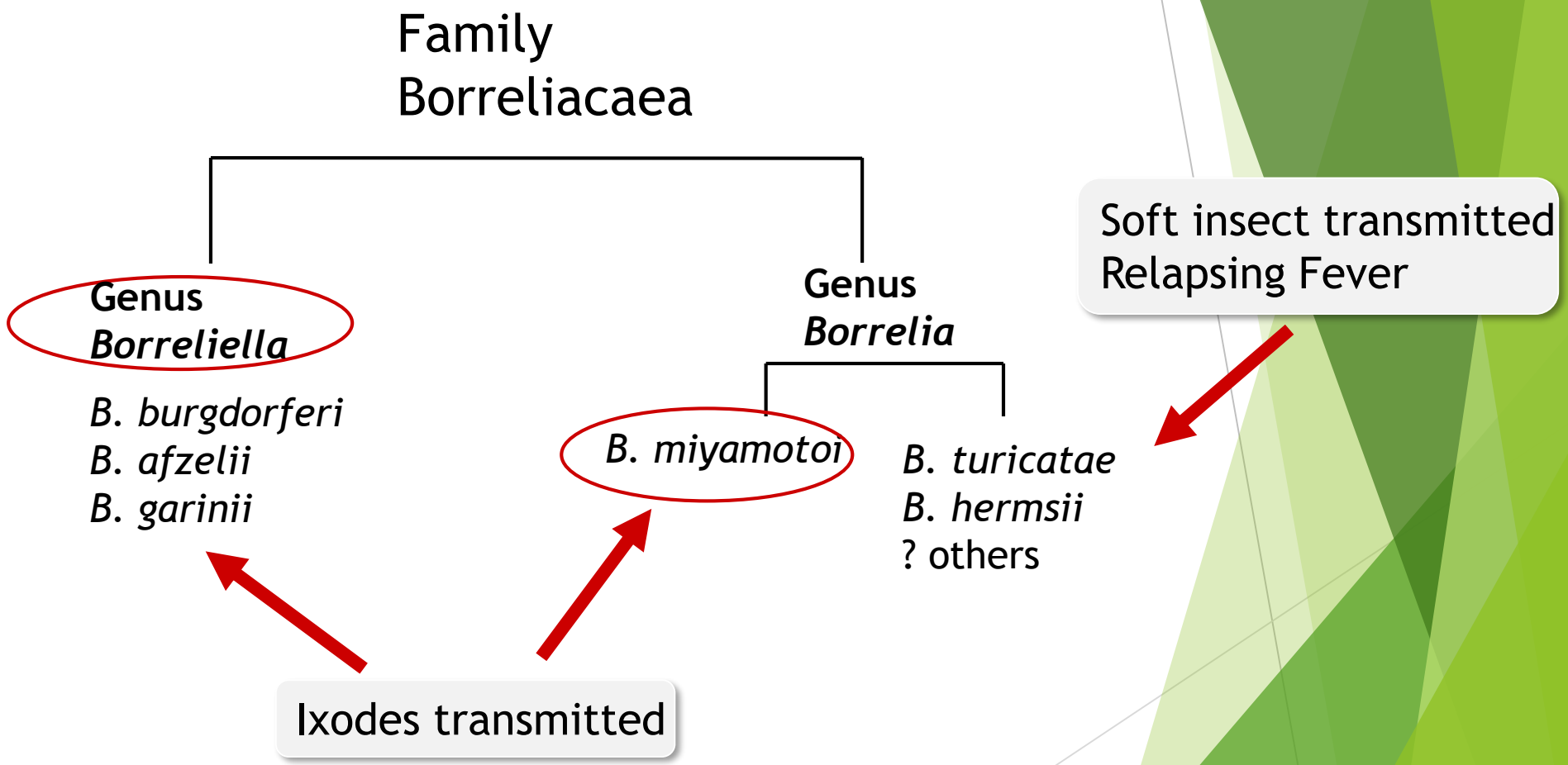
Laboratory Diagnosis of *Borrelia miyamotoi* Infection

- **DIRECT**
  - Difficult to culture at present
  - DETECTION OF DNA
    - Polymerase Chain Reaction

- **INDIRECT**
  - IMMUNE RESPONSES (SEROLOGY)
    - IgM & IgG specific recombinant antigen ELISA
      - Useful for convalescent seroconversion

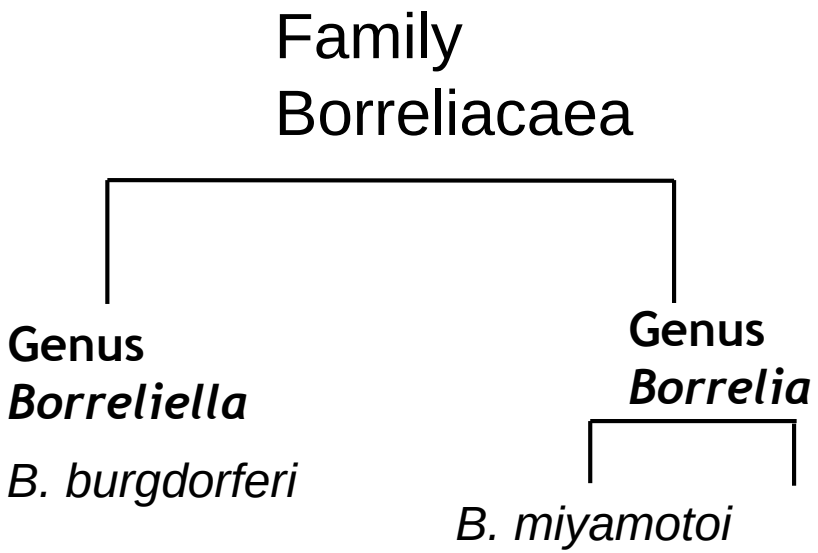
Krause PJ, et al. *Borrelia miyamotoi* Infection in Nature and in Humans. *Clinical microbiology and infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2015;21(7):631-639.

Family Spirochaetaceae



Barbour et.al. Int J Syst Evol. Microbiol 2017:67:2058-2067.
Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus Borrelia into two genera: the emended genus Borrelia containing only the members of the relapsing fever Borrelia, and the genus Borreliella gen. nov. containing the members of the Lyme disease Borrelia (Borrelia burgdorferi sensu lato complex). *Antonie van Leeuwenhoek*. 2014;105(6):1049-1072.

Implications for Diagnosis (Serology)



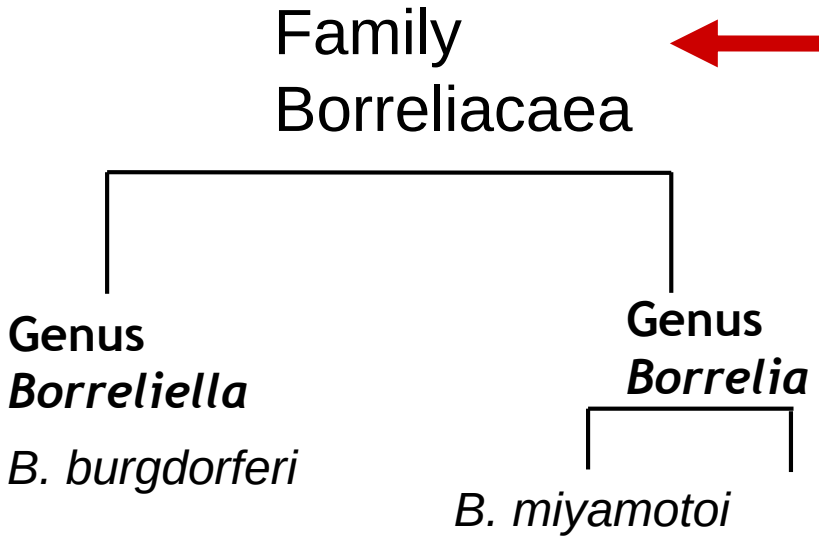
- Antigen preparations using whole cell sonicates of *B.burgdorferi* are reactive with *B. miyamotoi* patients **(i.e., *miyamotoi* patients cross-react on Lyme testing)**

- Specific recombinant *B. miyamotoi* peptide serology also is available **(Lyme patients do not cross-react on this *B. miyamotoi* testing)**

Acutely, B. *miyamotoi* infected patients are typically seronegative and PCR positive.

Molloy PJ, Telford SR, Chowdri HR, Lepore TJ, Gugliotta JL, Weeks KE, et al. Borrelia miyamotoi Disease in the Northeastern United States: A Case Series. Ann Intern Med. 2015;163:91–98. Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus Borrelia into two genera: the emended genus Borrelia containing only the members of the relapsing fever Borrelia, and the genus Borreliella gen. nov. containing the members of the Lyme disease Borrelia (Borrelia burgdorferi sensu lato complex). *Antonie van Leeuwenhoek*. 2014;105(6):1049-1072.

Implications for Diagnosis (PCR)



Broad range *Borrelia* primers are first utilized and react with both agents (any Borrelia)

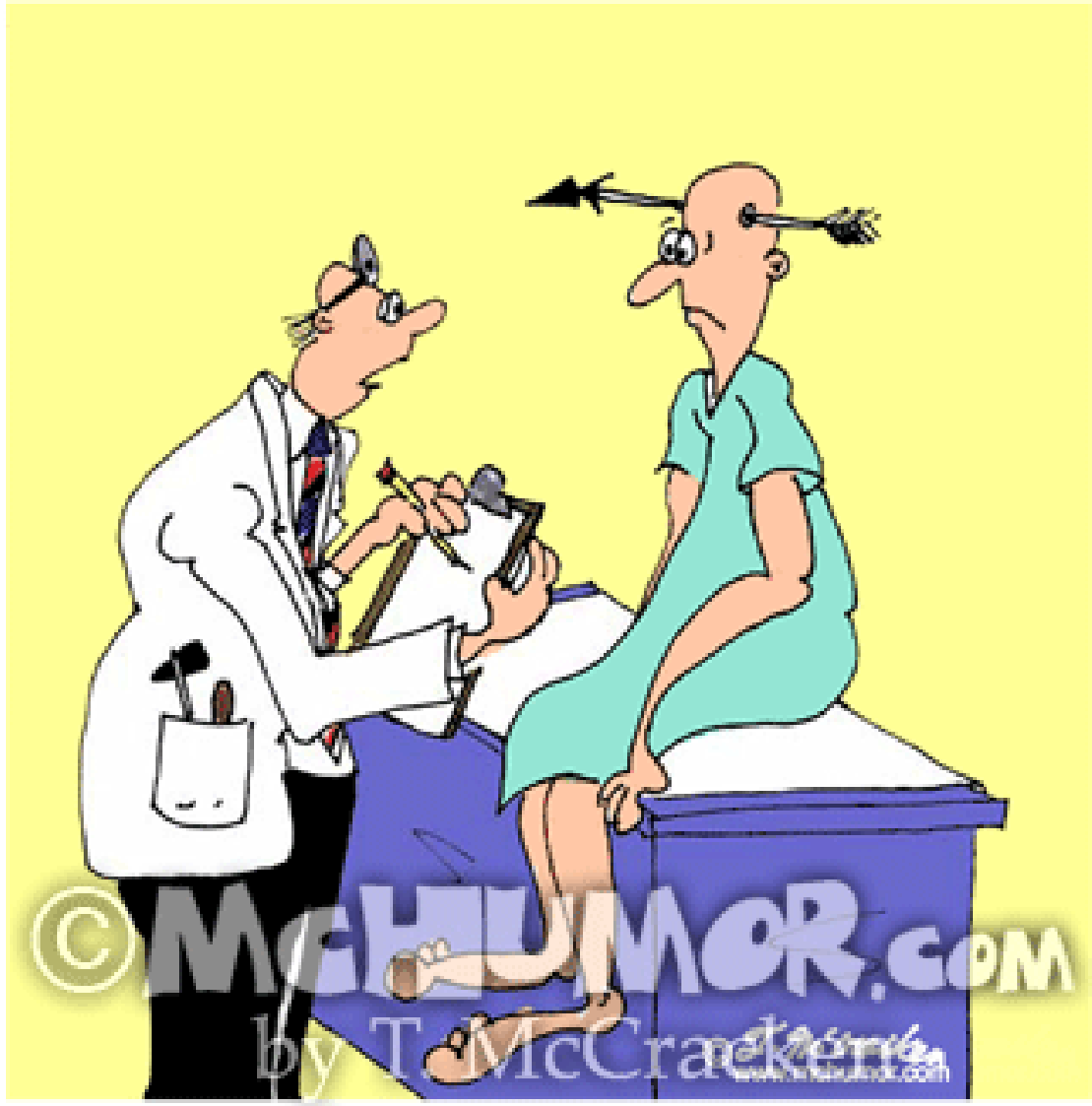
If positive reactivity, species- specific *B. burgdorferi* and *B. miyamotoi* primers are then employed

Case finding for acute *B. miyamotoi* predominantly relies on clinical presentation and PCR.

Molloy PJ, Telford SR, Chowdri HR, Lepore TJ, Gugliotta JL, Weeks KE, et al. Borrelia miyamotoi Disease in the Northeastern United States: A Case Series. Ann Intern Med. 2015;163:91–98.
Adeolu M, Gupta RS. A phylogenomic and molecular marker based proposal for the division of the genus Borrelia into two genera: the emended genus Borrelia containing only the members of the relapsing fever Borrelia, and the genus Borreliella gen. nov. containing the members of the Lyme disease Borrelia (Borrelia burgdorferi sensu lato complex). *Antonie van Leeuwenhoek*. 2014;105(6):1049-1072.

Patient with classic EM – Should you order a lab test ??

065-003 (BL) 24Aug17



"OFF HAND, I'D SAY YOU'RE SUFFERING FROM AN ARROW THROUGH YOUR HEAD, BUT JUST TO PLAY IT SAFE, I'M ORDERING A BUNCH OF TESTS."

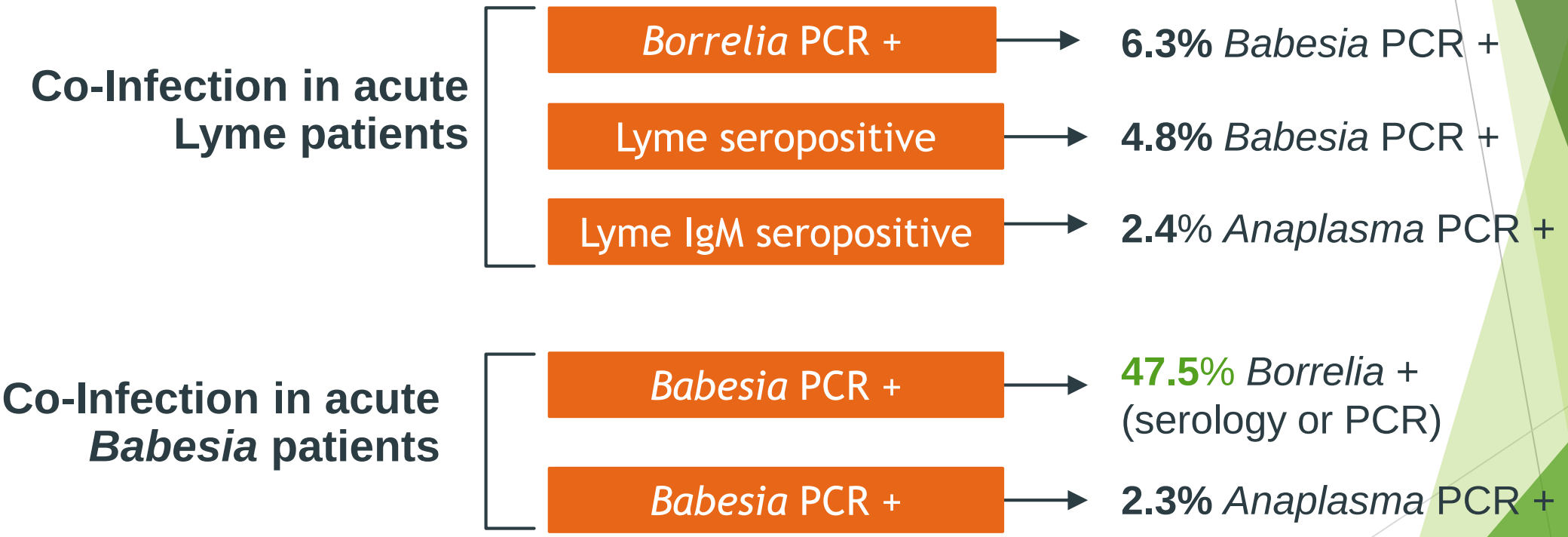
Why Look for Co-infections?

- Possible co-infection should be considered
  - There are many publications demonstrating both co-infection of ticks and of humans
  - Analysis of a large clinical data set demonstrated many co-infection combinations
- Presenting symptoms are frequently non-specific (fever, headaches, myalgias, etc.), and it often is not possible to distinguish one from another clinically
- Even with “pathognomonic” presentations (EM rash, Bell’s palsy) you haven’t ruled out co-infections with another tick-borne infectious agent

Tickborne Diseases of the United States. CDC. https://www.cdc.gov/lyme/resources/tickbornediseases.pdf. Accessed March 17, 2017.
Pomerantz, K. and Pieken, W. *Report On 2016 Data Mining Of Imugen Submissions*. Norwood, MA: Imugen, a division of Oxford Immunotec, Inc.; 2017. Internal Report DOC-PRT-138 Rev 1.0.

Co-Infection Determination in Patient Samples

Analysis of Imugen 2016 clinical testing data set demonstrated



Pomerantz K, Pieken W. Report On 2016 Data Mining of Imugen Submissions. Norwood, MA: Imugen, a division of Oxford Immunotec, Inc.; 2017. Internal Report DOC-PRT-138 Rev 1.0.

Core Technologies for Acute Illness

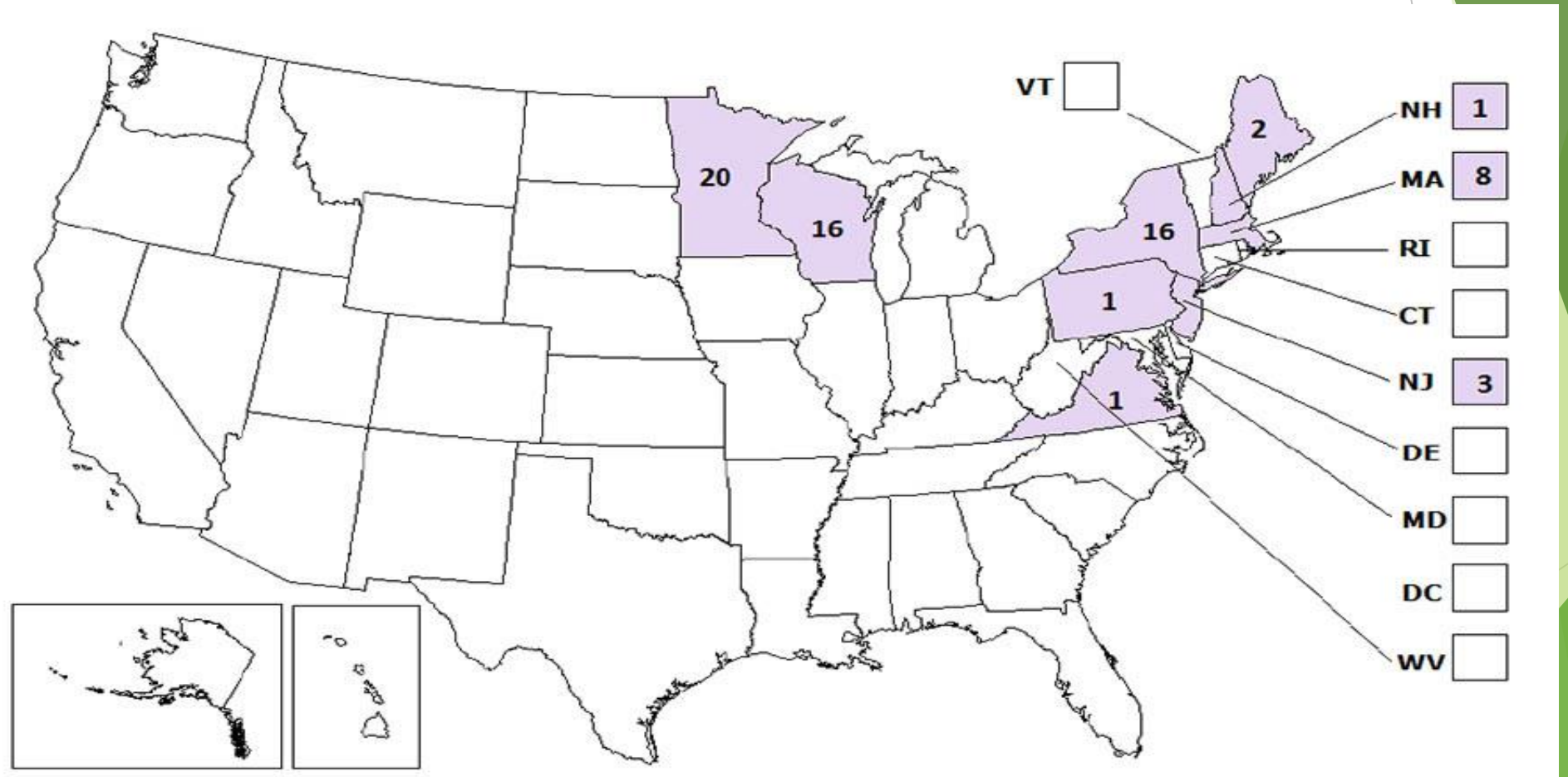
- ***B. burgdorferi***
  - Antibody Detection - Antibody Capture EIA
- ***B. burgdorferi***
  - PCR (Polymerase Chain Reaction; DNA Detection)
- ***B. miyamotoi***
  - PCR (Polymerase Chain Reaction; DNA Detection)
- ***B. microti***
  - PCR (Polymerase Chain Reaction; DNA Detection)
- ***A. phagocytophilum***
  - PCR (Polymerase Chain Reaction; DNA Detection)

Powassan virus

- Flavivirus, not unlike mosquito-borne encephalitides
  - Lineage 1 traditional POW
  - Lineage 2 Deer Tick Virus
    (found in 2% of adult ticks in NY State)
- 75 US cases 2006-2015 (esp. upper Midwest)
- Symptoms: fever, headache, vomiting, and generalized weakness, progressing to meningoencephalitis, (meningeal signs, altered mental status, seizures, aphasia, paresis, movement disorders, or cranial nerve palsies)
- Pleocytosis; difficult to isolate virus directly
- Serology is the mainstay for diagnosis

https://www.cdc.gov/powassan/index.html. Accessed September 14,2017.
https://www.cdc.gov/ticks/tickbornediseases/powassan.html.Accessed September 14, 2017.

Powassan virus neuroinvasive disease cases reported by state, 2006–2015



Source: ArboNET, Arboviral Diseases Branch, Centers for Disease Control and Prevention
https://www.cdc.gov/powassan/statistics.html. Accessed September 6, 2017