Lyme Disease
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Agenda

- Lyme borreliosis
- Epidemiology
- Lyme disease in California
- Diagnosing Lyme disease
- Current state of testing
- Sales pitch
Lyme borreliosis

Lyme Disease (Lyme borreliosis)
Lyme Borreliosis (LB)

- Tick borne disease
- Spirochete bacteria from the genus *Borrelia*
  - *B. burgdorferi* s.s.
  - *B. afzelii*
  - *B. garinii*
  - *B. mayonii*

Quick History

- Willy Burgdorfer
  - Isolated the bacterium in 1982
  - *Borrelia burgdorferi* is named for Willy
  - It was discovered later that there is more than one LB-causing bacteria...
**Lyme Question #1**

True or False: *Borrelia burgdorferi* is the only Lyme disease-causing bacteria

**FALSE**

Although the disease-causing bacteria was originally named *Borrelia burgdorferi*, later research showed there are multiple Lyme disease-causing species of *Borrelia*

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**Borrelia burgdorferi sensu lato**

- sensu lato – “in the lax sense”
- *Borrelia burgdorferi* s.l.
  - spirochete group comprised of over 15 genospecies
  - 4 are known to cause Lyme disease

<table>
<thead>
<tr>
<th>Bacteria Name</th>
<th>Associated Clinical Manifestation</th>
<th>Geographic Region</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. burgdorferi</em> s.s.</td>
<td>Lyme arthritis, carditis</td>
<td>US and Western Europe</td>
</tr>
<tr>
<td><em>B. mayonii</em></td>
<td>Diffuse rash</td>
<td>US</td>
</tr>
<tr>
<td><em>B. afzelii</em></td>
<td>ACA</td>
<td>Europe</td>
</tr>
<tr>
<td><em>B. garinii</em></td>
<td>Neuroborreliosis</td>
<td>Europe</td>
</tr>
</tbody>
</table>
**Borrelia**

Not all *Borrelia* species cause Lyme disease – but are still pathogenic to humans

- Tick-borne relapsing fever
  - *Borrelia miyamotoi*
  - *Borrelia hermsii*
  - *Borrelia cuocidurae*
  - *Borrelia spp.*

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**Clinical Presentation of Untreated LB**

- **Stage 1**
  - Days-weeks
  - Early localized infection
  - *Erythema migrans*

- **Stage 2**
  - Weeks-months
  - Disseminated infection
  - Systemic symptoms
  - Lyme Neuroborreliosis
  - Acute neurological involvement

- **Stage 3**
  - Months-years
  - Localized infection, usually without systemic symptoms
  - Chronis arthritis
  - *Acinetobacter baumannii* infections in adults and elsewhere in children

**Disease timeline**

(if untreated)

North America/Europe
Treatment

- Lyme disease exists
- Treatable with antibiotics (2-4 wks)
- Post-Lyme disease Syndrome
  - ~10% of patients
  - No known effective treatment

True or False: Post Lyme disease Syndrome had the same symptoms of Lyme disease

**TRUE**

PLDS is characterized by a continuation of symptoms despite the clearance of infection and the absence of the Lyme disease-causing bacteria
Epidemiology

Clinical Background
Seasonality

Confirmed LB cases by month of disease onset – US, 2001-2006

Vector Distribution
Geography

Reported Cases of Lyme Disease–United States, 2016

Each tick box represents one case of Lyme disease not confirmed at time of submission. The presence of a tick in a state does not necessarily mean that Lyme disease was acquired in that state. Maps labeled between states, and the place of residence is sometimes different from the place where the patient became infected.

Endemic Areas

Tick Season in Maine

AH! The Glory of Nature!
The epidemiology of Lyme disease is affected by:
A) The life-cycle of the tick
B) The vector hosts
C) The weather
D) All of the above

D) All of the above
The life-cycle of the tick and its ability to reproduce and move are all factors that contribute to the distribution of Lyme disease.

Lyme is Spreading

- Lyme vector is detected in 50% more counties in 2015 than 1996
- Incidence
  - US: 2004-2016, nearly double
  - Canada: 2009-2015, six-fold increase
Climate Change

- Longer summers
- Warmer average temperatures
- More hospitable climates for vectors
- Changing migratory pattern of vectors
  - Birds
  - Deer
  - Mice


Lyme disease in California
Lyme Question #4

True or False: Lyme disease is endemic to California

FALSE
Lyme disease is not endemic to the state of California, but it is endemic in some counties in California

Public Service Announcement

• The black-legged tick is found in 56 of 58 counties in CA
• Endemic in:
  • Marin County
  • Santa Cruz County
  • Sonoma County
  • Mendocino County
  • Trinity County
  • Humboldt County
  • Mono County
  • Mariposa County
  • Nevada County
  • Amador County

Incidence of Lyme disease in 2011, source: CA Department of Public Health
Lyme Question #5

Nymph ticks are the size of a:
A) Small Pea  
B) Sunflower seed  
C) Marble  
D) Poppy seed

D) Poppy seed

Public Service Announcement

Ticks can be the size of a poppy seed. Can you spot all 5 ticks in this photo? Learn how to prevent tick bites.  
bit.ly/Zrjox6U
Diagnosing Lyme disease

Diagnosis: Symptoms + Risk Factors + Testing

- Symptoms:
  - Erythema migrans (EM) – Bull’s-eye rash
  - Fever, fatigue, malaise
  - Symptom checklists

- Risk Factors:
  - Hiking
  - Location
  - Season
  - Memory of a tick bite
Testing: Culture

- Basic research tool
- Labor intensive
- Expensive
- 12 week incubation time before considered negative
- Only useful for untreated patients

Testing: Molecular (PCR and NGS)

- Basic research tool
- Need a high initial bacterial DNA concentration
  - EM scraping
  - Inflamed joint fluid drawing
- Difficult to reproduce
- Inhibitors in samples
- No molecular tests have been FDA cleared
Testing: NGS

- Push to use NGS
- Benefits:
  - Screen for all tick-borne disease at one time
  - Epidemiological tool
- Limitations:
  - It is a molecular method
  - Expensive
  - All tick-borne disease are treated the same way

Testing: Serology

- Quick, cheap, easy
- Protein-based
- 94 FDA cleared tests (1987-2018)
- <20 routinely used today
- Screening: highly sensitive
- Western blot: highly specific
CDC Testing Algorithm

Two-Tiered Testing for Lyme Disease

First Test

- Enzyme Immunoassay (EIA)
- OR
- Immunofluorescence Assay (IFA)

Second Test

- Positive or Equivocal Result
- Signs or symptoms ≤ 30 days
- IgM and IgG Western Blot
- Signs or symptoms > 30 days
- IgG Western Blot ONLY

Consider alternative diagnosis OR
If patient with signs/symptoms consistent with Lyme disease for ≤ 30 days, consider obtaining a convalescent serum

Testing For Lyme disease
History: Lyme testing’s “bad rap”

- The first Lyme disease test was cleared by the FDA in 1987
  - IFA test for antibodies to *Borrelia burgdorferi* – Zeus Scientific
  - 18 other tests introduced by 2000
- Used whole cell lysates
  - High cross-reactivity
  - Low specificity
  - High sensitivity

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History: Western Blot

- Introduction of the Western blot algorithm shortly after
- Western blots:
  - 100% specificity
  - Low sensitivity
    - 62% Early (0-3 mons)
    - 81% Convalescent (3-12 mons)
    - 100% Late (>12 months)

Current State: Antigen testing

- 2007 first assay released using Lyme-specific antigens (VlsE)
- Most commonly used assays have Lyme-specific antigens
- Common antigens:
  - FlaB – flagellar protein
  - OspC – outer surface protein
  - VlsE – outer membrane protein
  - p58 – membrane protein
  - DbpA – membrane protein
- This increased specificity significantly

Current State: Problems

- Western blotting technology has not changed
  - Extremely low sensitivity
  - Early and Convalescent Lyme disease cases (0-12 mon)
- Movements to remove WBing from the algorithm are underway
Lyme Question #5

Using a whole cell lysate in a diagnostic test:
A) Increases specificity
B) Increases sensitivity
C) Decreases specificity
D) Increases specificity

C) Decreases specificity

Whole cell lysates are very non-specific and have good sensitivity, but high cross-reactivity leading to decreased specificity.
Assay Design

Beads coated with *B. burgdorferi* antigens

Bead 1: FVlsE (fusion of flagellar and modified VlsE peptide)
Bead 2: OspCB (recombinant outer surface protein)
Bead 3: p58 (recombinant membrane protein)

Lyme Total Multiplex Testing

Analyte beads are manufactured and calibrated individually

FVlsE (Fusion peptide FlaB & modified VlsE)

OspCB

Internal QC beads
- ISB
- SVB

Beads Combined into a Single “Bead Reagent” for Multiplex Analysis
Lyme Question #6

Which was the first recombinant protein that was added to an assay:
A) FlaB  
B) p58  
C) VlsE  
D) DpbA

C) VlsE

In 2007 the first assay that did not contain a whole cell lysate was released and contained VlsE

Intended Use Statement

The BioPlex 2200 Lyme Total kit is a multiplex flow immunoassay intended for the qualitative detection of total (IgM/IgG) antibodies to Borrelia burgdorferi in human serum or plasma (EDTA, heparin). This assay should be used to test patients with history and/or symptoms of infection with B. burgdorferi. The BioPlex 2200 Lyme Total assay is intended for use with the Bio-Rad BioPlex 2200 System. All reactive and equivocal specimens should be tested with a second tier test such as Western blot. Positive second tier results are supportive evidence of infection with B. burgdorferi. Diagnosis of Lyme borreliosis should be made based on the presence of B. burgdorferi antibodies, history, symptoms, and other laboratory data. Non-reactive first tier or negative second tier results should not be used to exclude borreliosis.
Review

**Sensitivity** (also called the true positive rate)
- measures the proportion of actual positives that are correctly identified as such
- e.g., the percentage of Lyme disease patients who are correctly identified as having LB

**Specificity** (also called the true negative rate)
- measures the proportion of that are correctly identified as such
- e.g., the percentage of LB negative patients who are correctly identified as not having Lyme disease

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**Lyme Question #7**

Statistically, which is likely to be the most sensitive algorithm?
A) 2 different serological assays
B) 1 serological assay
C) 1 serological assay + 1 WB
D) 2 different WBs

B) 1 serological assay

When testing with only 1 serological assay you will not have any disagreement between methods and you there is no probability of loosing a true positive
Statistically, which is likely to be the most specific algorithm?
A) 2 serological different assays
B) 1 serological assay + 1 WB
C) 1 WB
D) both B and C

D) Both B and C

Western blots should always be 100% specific, so your specificity shouldn’t be affected if you use it alone or after a serological assay.

### CDC LSR Premarketing Panel Summary

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<tr>
<th></th>
<th>N</th>
<th>Reactive</th>
<th>Equivocal</th>
<th>Non-Reactive</th>
<th>% Agreement with Clinical Diagnosis</th>
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<td>Acute</td>
<td>39</td>
<td>33</td>
<td>0</td>
<td>6</td>
<td>84.6%</td>
</tr>
<tr>
<td>Convalescent</td>
<td>31</td>
<td>29</td>
<td>0</td>
<td>2</td>
<td>93.5%</td>
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<tr>
<td>Late</td>
<td>20</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>100%</td>
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<tr>
<td>Look-alike Diseases</td>
<td>90</td>
<td>1</td>
<td>2</td>
<td>87</td>
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<tr>
<td>Healthy Controls</td>
<td>100</td>
<td>1</td>
<td>2</td>
<td>97</td>
<td>97.0%</td>
</tr>
</tbody>
</table>

Analysis: The CDC LSR Premarketing panel are the most highly characterized samples that were tested.

Each assay gets a slightly different panel with different compositions.
# CDC Panel: Head-to-Head Comparison

|        | N  | BioPlex 2200 | Immune
clicics C6 | Zeus Borrelia VlsE1/pepC10 IgG/IgM | VIDAS IgG+IgM | MarDx (Western Blot) |
<table>
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<td>39</td>
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<tr>
<td>Convalescent</td>
<td>31</td>
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<td>90.3%</td>
<td>90.3%</td>
<td>83.9%</td>
<td>80.6%</td>
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<tr>
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<td>20</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>NA*</td>
<td>100%</td>
</tr>
<tr>
<td>Total Sensitivity</td>
<td>90</td>
<td>91.1%</td>
<td>84.4%</td>
<td>88.9%</td>
<td>85.6%**</td>
<td>76.6%</td>
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<tr>
<td>Total Specificity</td>
<td>190</td>
<td>96.8%</td>
<td>97.9%</td>
<td>89.5%</td>
<td>84.7%</td>
<td>100%</td>
</tr>
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</table>

*Not enough sample for the late stage of disease

**Assumed 100% sensitivity for the Total calculation

*All tested with the same CDC panel. Zeus, VIDAS, and BioPlex 2200 in-house, Immunetics C6 from the CDC.*

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# Lyme Testing Agreement: CDC Panel

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*Overall Clinical Sensitivity & Specificity*
Questions?

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