Diagnostics for *B. burgdorferi* Infection and Other Tick-Borne Illnesses

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Isabella Martin, MD

Medical Director of Microbiology, Dartmouth-Hitchcock Medical Center

Assistant Professor of Pathology, Geisel School of Medicine

Financial Conflict of Interest

- Nothing to Disclose

Testing Regulatory Framework

↓

Commercial *In Vitro* Diagnostic Tests (IVD)

Clinical Laboratory Improvement Act (CLIA)

↓

Academic & Commercial Laboratories

1) adopt IVDs
2) create Lab-Developed Tests (LDT)

Regardless of method, laboratories must do proficiency & competency testing

No Test is Perfect

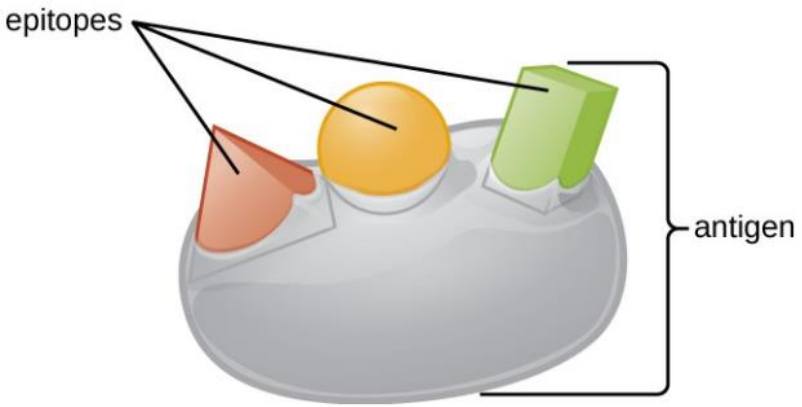
- Positive predictive value of a test depends on disease prevalence
- Assuming performance characteristics of test X for disease Y:
  - Sensitivity: 95%
  - Specificity: 95%
- Assuming disease Y has a prevalence of 1% in the population
  - Only 1 in 6 positive tests are true positives

Testing Paradigm for Lyme Disease

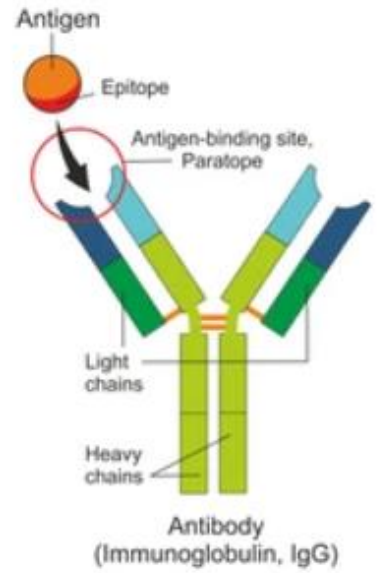
- Direct detection of the organism itself is not useful
- Detect antibodies made by body in response to infection
- Antibodies:
  - Can take several weeks to develop
  - Can persist for months or years after the infection is gone
- Other diseases/infections can cause false positive test results
  - Infectious mononucleosis
  - Syphilis
  - Bacterial endocarditis
  - Rheumatoid arthritis
  - Others

Antigens & Antibodies

- Antigen
  - Substance our body sees as foreign
    - Generates immune response
  - High molecular weight protein or polysaccharide
  - Chemically complex – many epitopes
- Antibodies
  - Water-soluble immunoglobulin proteins
  - Produced by B-lymphocytes
  - Unique configuration specifically targets surface of an antigen
  - Epitope = region of target binding

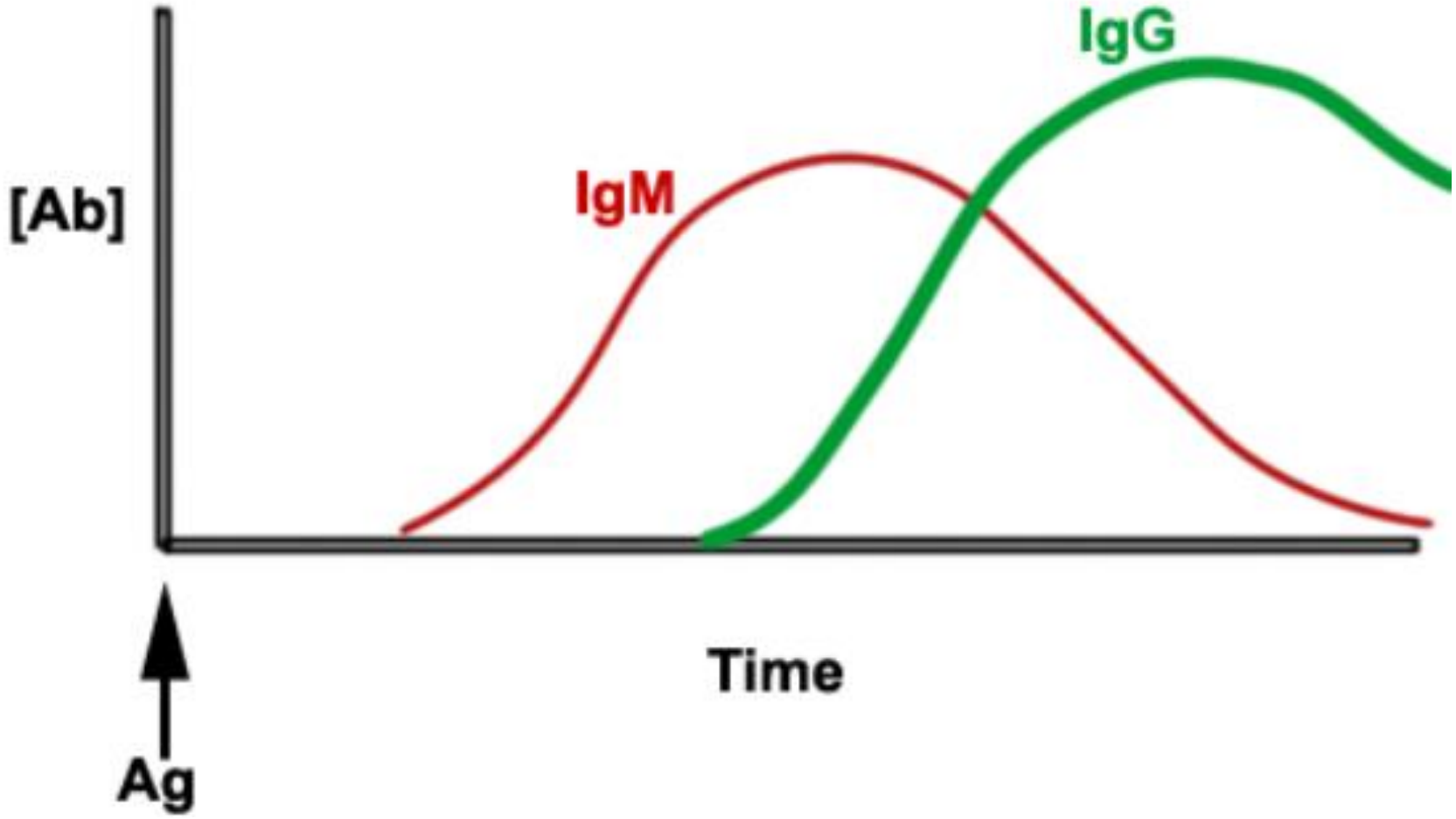


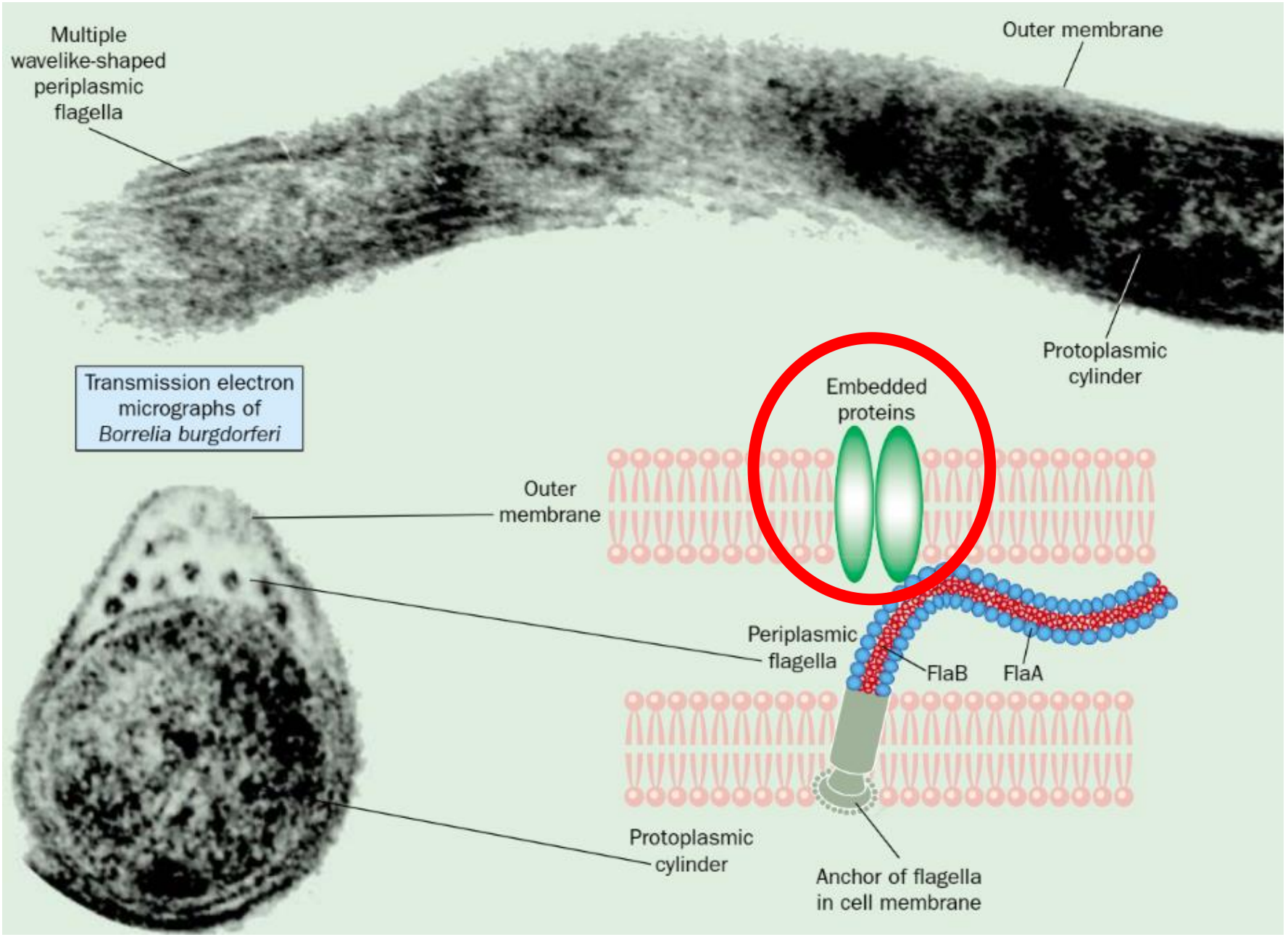
https://courses.lumenlearning.com/microbiology/chapter/overview-of-specific-adaptive-immunity/



https://www.shutterstock.com/search/epitope

Immune Response 101





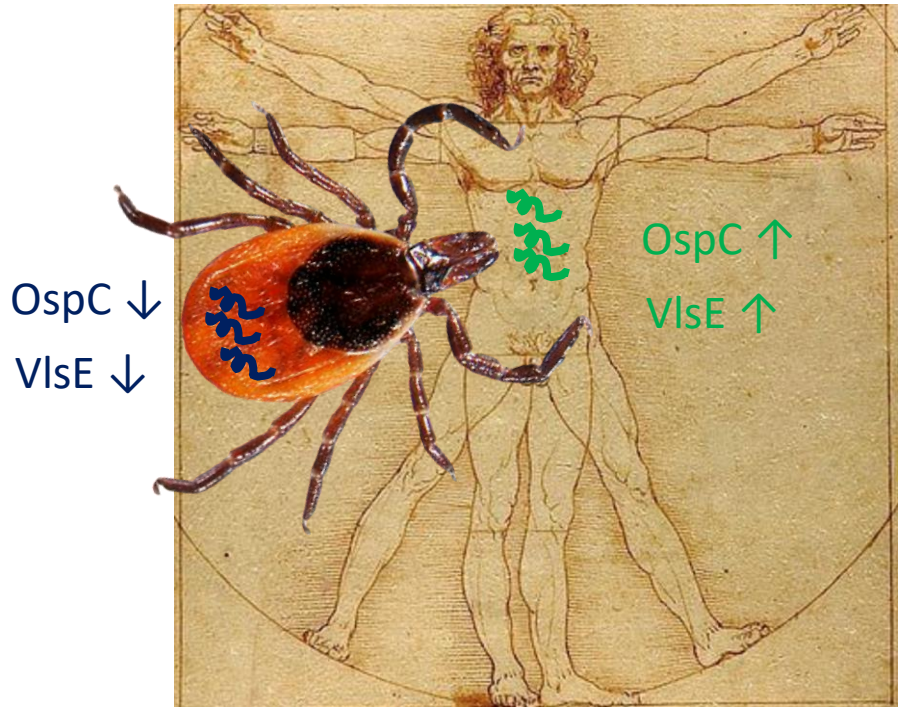
Singh SK and Girschick HJ. *Lancet*. 2004;4:575-583

Test Performance Depends on Many Factors

- Type of test used
  - Antigens used within test
- Stage of disease when patient is tested
- Prevalence of given disease in the region
- Stand-alone test versus a two-step algorithm

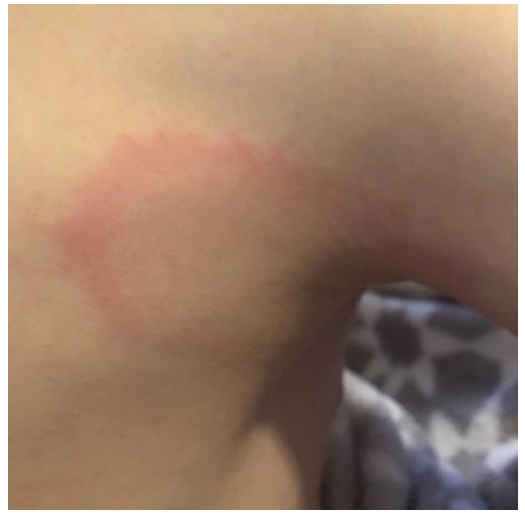
Key *Borrelia* Proteins/Antigens

- Whole Cell Sonicate (WCS)
  - Use of cultured *B. burgdorferi* is problematic
- OspC – Outer Surface Protein C
  - ~25 serotypes
  - Conserved epitopes: OspC1 and pepC10
- VlsE – Vmp-like sequence-expressed protein
  - Antigenic variation and immune evasion
  - Expressed after host infection
  - Invariant region (IR_6 or C6) elicits earlier IgG response



Jacek E, et al. *J Immunol*. 2016; 196:1036-1043
Singh SK and Girschick HJ. *Lancet*. 2004;4:575-583

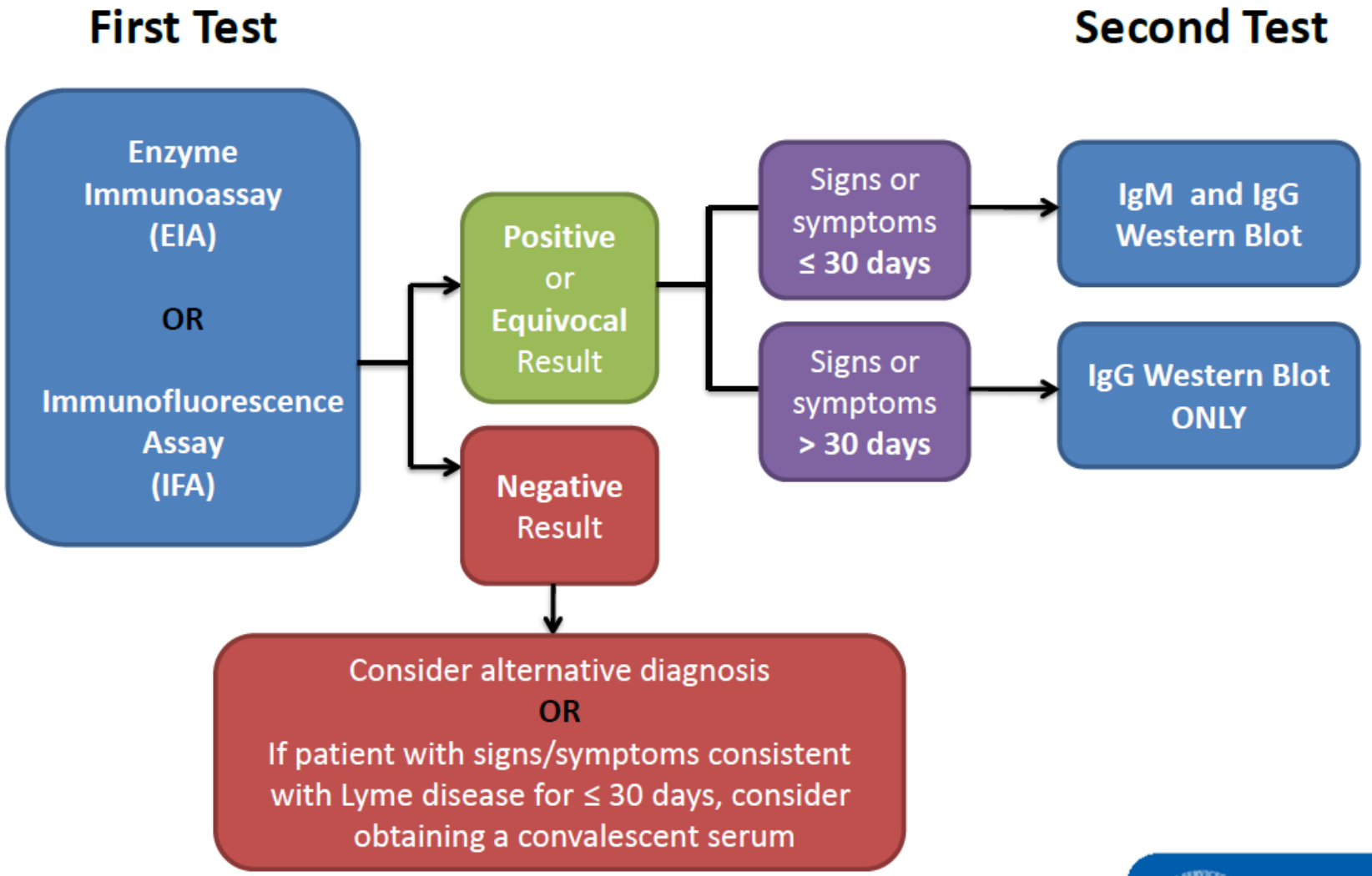
Lyme Disease (LD): Stages of Disease



- Natural course if untreated: variable
- Most cases: self-limited
- Early localized LD (Stage 1, 7-14 days after tick bite)
  - Flu-like illness (fever, myalgias, fatigue)
  - Erythema migrans rash (70-80%)
- Early disseminated LD (Stage 2, weeks to months after bite)
  - Neurologic manifestations (facial nerve palsy, meningitis, radiculoneuropathy)
    - Occur in 15-20% of untreated
  - Carditis (rare)
- Late LD (Stage 3, usually months after tick bite)
  - Arthritis (usually large joint[s])

Two-Tiered Testing for Lyme Disease

STTTA

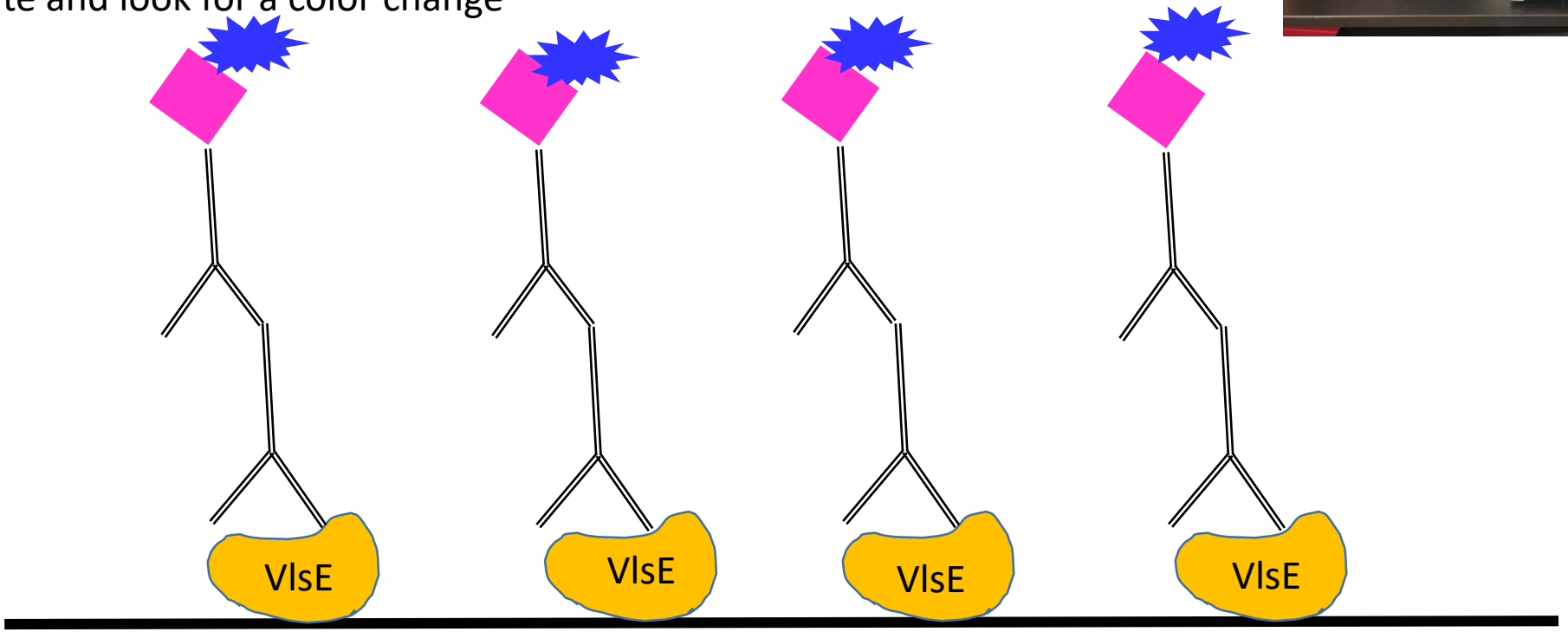


Since 1995

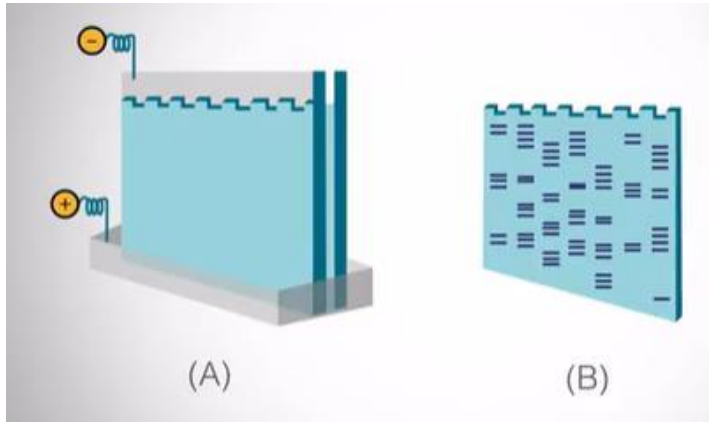
National Center for Emerging and Zoonotic Infectious Diseases
Division of Vector Borne Diseases | Bacterial Diseases Branch

Enzyme-Linked Immunosorbent Assay (ELISA) (a type of EIA)

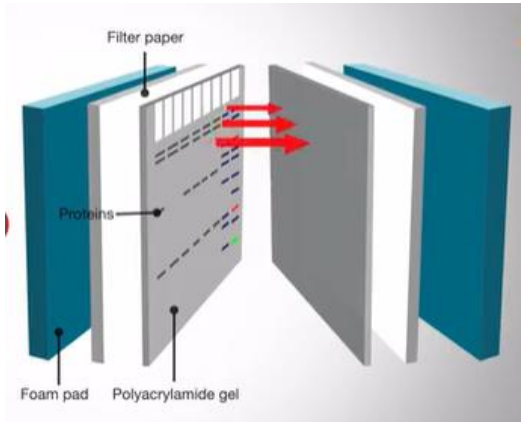
1. Add patient serum to test well that is pre-coated with *B. burgdorferi* antigen(s)
2. If anti-*B. burgdorferi* antibody is present, it will bind to antigen
3. Wash
4. Add an animal-derived anti-human antibody conjugated with peroxidase
5. Wash
6. Add a substrate and look for a color change



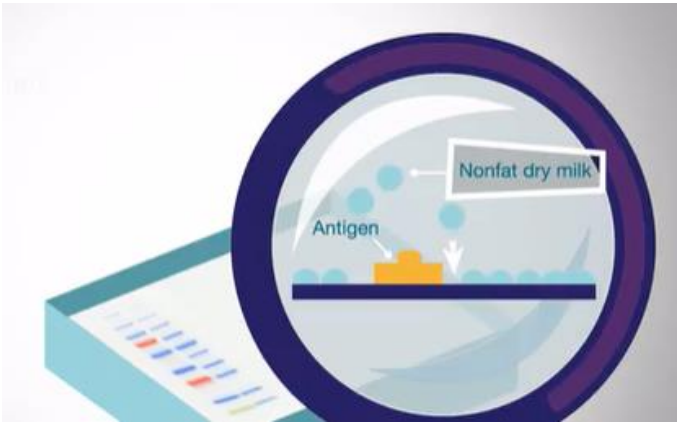
Western Blot



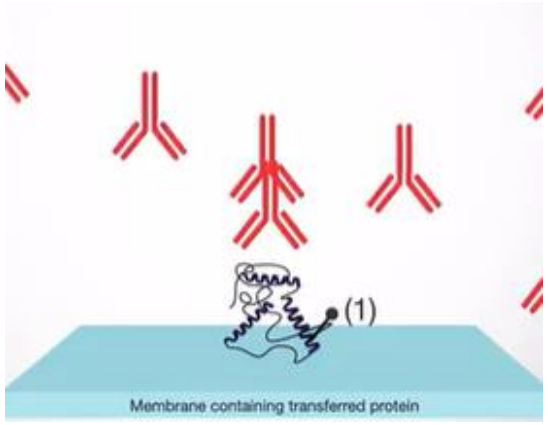
1. Start with whole cell sonicate: separate proteins with electropheresis



2. Transfer to nitrocellulose membrane

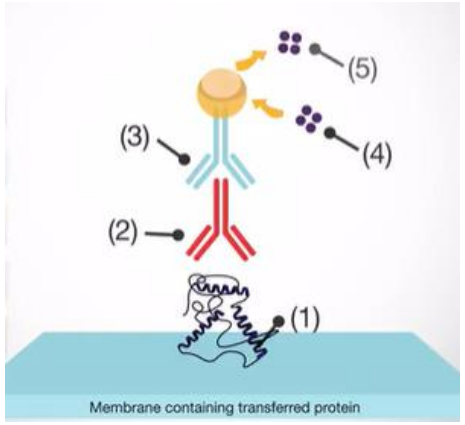


3. Block areas around antigens



4. Add patient serum

5. Wash

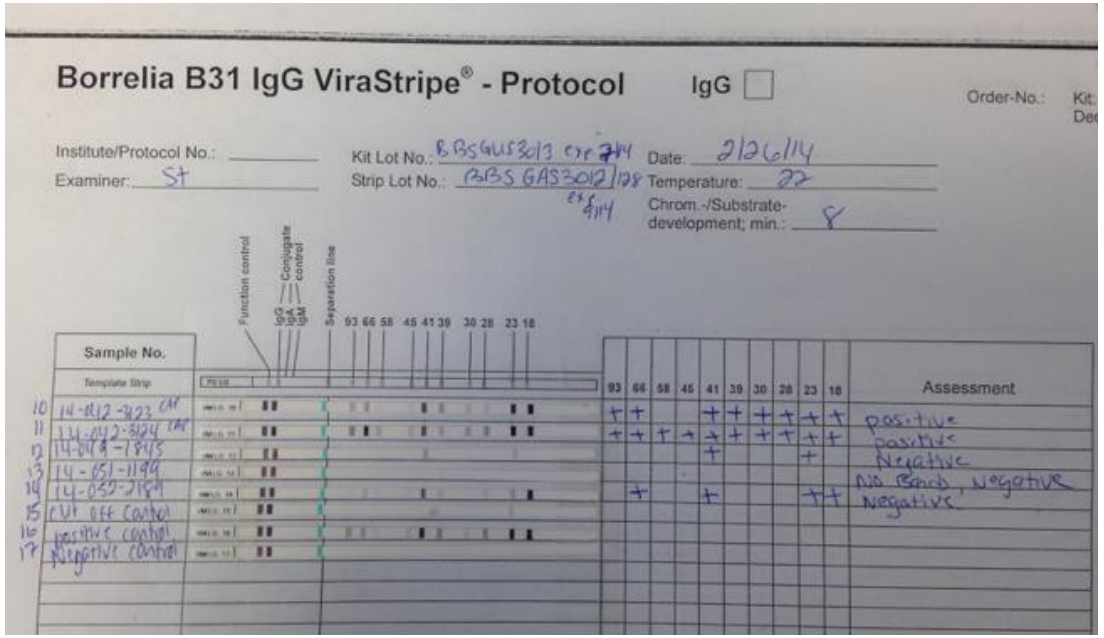


5. Add capture antibody

Images: www.thermofischer.com

Western Blot

- Current CDC criteria to interpret as positive:
  - ≥2 of 3 diagnostic bands for IgM
  - ≥5 of 10 diagnostic bands for IgG
- Do not perform WB if screening ELISA is negative



Issues with the Western Blot

- Use of whole cell sonicate
  - Protein expression of lab-grown Borrelia different from that in host
- Subjective interpretation
  - Potential for over-reading
- Use of alternative interpretive criteria
- Use as stand-alone test
- Confusing to patients & providers

Standard Two-Tiered Testing Algorithm (STTTA)

- Limitations of STTTA
  - Low sensitivity (~29-60%) during acute disease
    - Limited immunoblot sensitivity
  - Challenges with immunoblot result interpretation
    - Banding patterns and specificity concerns
  - Cannot differentiate between present or past infection
  - Tests in the USA are specific for *B. burgdorferi* only

Theel E. *J Clin Micro*. 2016; 54:1191-1196

ELISA Limitations

	Sensitivity %		Specificity %	
Assay	**Early (stage 1) N=403** (of these, 105 convalescent)	**Late (stages 2 & 3) N=166**	**Healthy Donors N=1800**	**Patients with non-LD infections or conditions N=399**
WCS ELISA	74.9	97.7, 98.4	96.4	89.3

Wormser et al. *Diag Micro Infect Dis*. 2013;75:9-15

STTTA Limitations

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WCS ELISA & WB	35.2	77.3, 95.9	99.5	99.2

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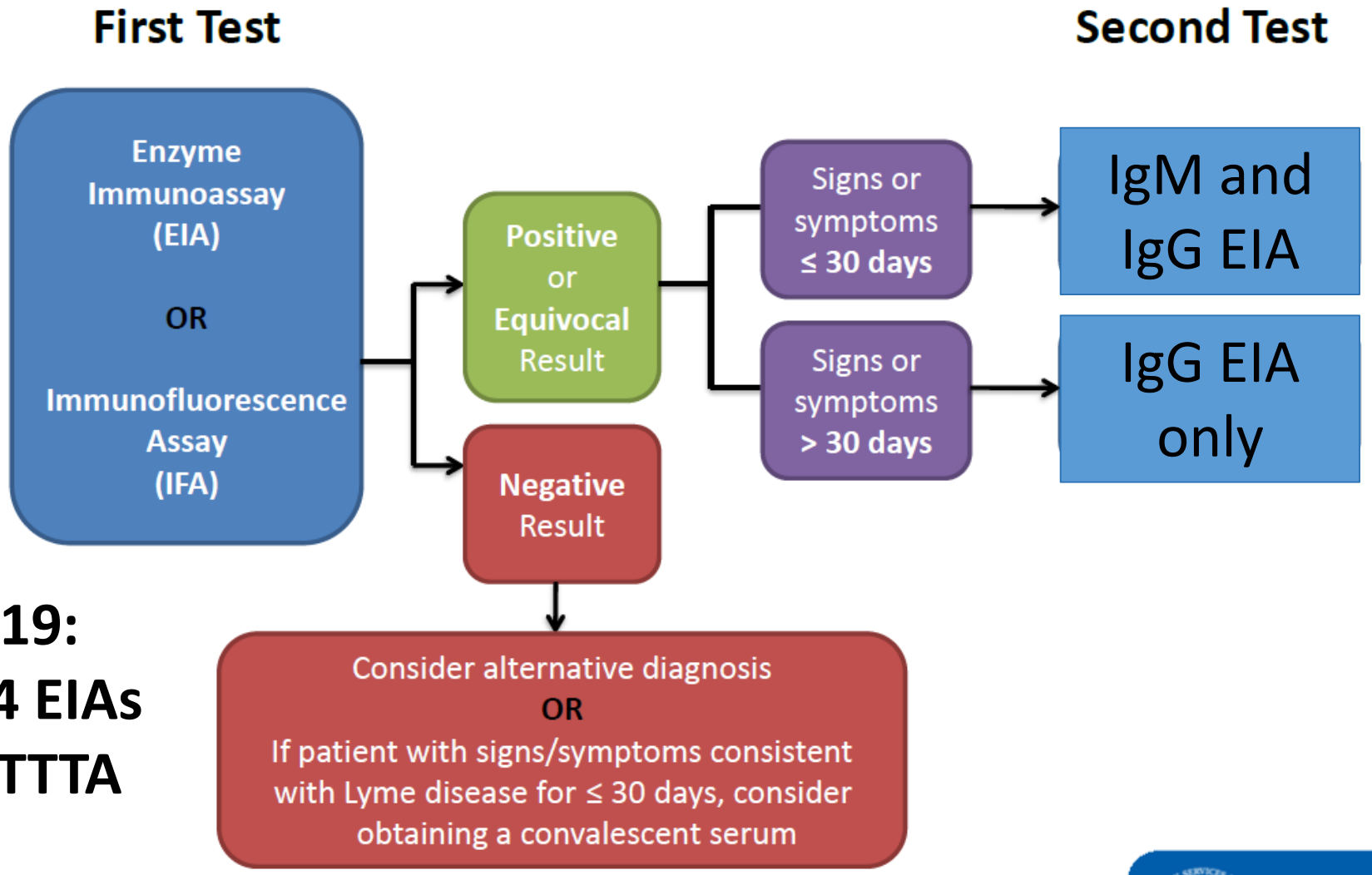
WCS ELISA	74.9	97.7, 98.4	96.4	89.3
WCS ELISA & WB	35.2	77.3, 95.9	99.5	99.2
C6 ELISA	66.5	88.6, 98.4	98.8	99.5
C6 ELISA & WB	34.5	75, 95.1	99.5	99.5

Wormser et al. *Diag Micro Infect Dis*. 2013;75:9-15

MODIFIED **Two-Tiered Testing for Lyme Disease**

MTTTA



July 30, 2019:
FDA cleared 4 EIAs for use in MTTTA

National Center for Emerging and Zoonotic Infectious Diseases
Division of Vector Borne Diseases | Bacterial Diseases Branch

EIA/antigen Combination in MTTA

	Stand-alone EIA % Positive			Modified Two-Tier Algorithm % Positive			Standard Two-Tier Algorithm % Positive
Sample Type (N)	**VlsE**	**C6**	**WCS**	**VlsE/C6**	**WCS/C6**	**WCS/VlsE**	**WCS/Blots**
Early LD w/ EM (40)	58%	58%	73%	50%	55%	58%	50%

Sens

Conclusions:

-Overall higher sensitivity of standalone EIAs and MTTTA vs. STTTA for early infection

Pegalajar-Jurado A et al. *J Clin Micro*. 2018;56(8)

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	Late (Stage 2 & 3) LD (46)	96%	98%	100%	96%	98%	96%	96%

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Sens	**Early LD w/ EM (40)**	58%	58%	73%	50%	55%	58%	50%
	Late (Stage 2 & 3) LD (46)	96%	98%	100%	96%	98%	96%	96%
Spec	**Other Disease Cont. (144)**	98%	95%	56%	100%	97%	98%	94%
	Healthy Control (203)	98%	98%	77%	99%	99%	100%	97%

Conclusions:

-Overall higher sensitivity of standalone EIAs and MTTTA vs. STTTA for early infection

-Lower specificity of standalone EIAs, especially WCS EIAs, vs. MTTTA/STTTA

-No statistical difference in overall accuracy between the MTTTA EIA combinations

Pegalajar-Jurado A et al. *J Clin Micro*. 2018;56(8)

Advantages and Disadvantages of the MTTTA

Advantages

- ↑ sensitivity in early LD
- Similar specificity to STTTA
- Less confusing results
- Smaller laboratories can perform
  - Better turnaround time

Disadvantages

- Sensitivity during early LD still a problem
  - Negative result does not rule out
- Inability to follow IgG antibody expansion
- Similar to STTTA:
  - Cannot monitor response to therapy
  - Challenging to diagnose re-infection

Table 2. Number and Percentage of False-Positive Serologic Test Results and Discordant Pairs for 40 Medically Healthy Controls (University Reference Laboratory Versus Commercial and Lyme Specialty Laboratories)

	University Reference Laboratory	Commercial Laboratory			Specialty Laboratory A			Specialty Laboratory B		
Test	No. Positive[a] (%)	No. Positive[a] (%)	*P* Value	Disc Pairs	No. Positive[a] (%)	*P* Value	Disc Pairs	No. Positive[a] (%)	*P* Value	Disc Pairs
?/+ ELISA	5 (12.5)	3 (7.5)	.683	6	1 (2.5)	.125	4	3 (7.5)	.683	6
C6 ELISA	. . .	. . .	. . .	. . .	0	. . .	. . .	0	. . .	. . .
WB IgM (CDC)	5 (12.5)	0	.074	5	1 (2.5)	.125	4	8 (20.0)	.505	9
WB IgM (laboratory)	. . .	. . .	. . .	. . .	1 (2.5)	.125[b]	4	15 (37.5)	.024	16[b]
WB IgG (CDC)	1 (2.5)	0	1.00	1	0	1.00	1	3 (7.5)	.480	2
WB IgG (laboratory)	. . .	. . .	. . .	. . .	0	1.00[b]	1	11 (27.5)	.004	10[b]
2-tier: ?/+ ELISA & WB IgG	0	0	. . .	0	0	. . .	0	1 (2.5)	1.000	1
2-tier: C6 ELISA & WB IgG	. . .	. . .	. . .	. . .	0	. . .	. . .	0	. . .	. . .
2-tier: ?/+ ELISA & C6 ELISA	. . .	. . .	. . .	. . .	0	. . .	. . .	0	. . .	. . .
+ WB IgM or IgG (CDC)	5 (12.5)	0	.074	5	1 (2.5)	.133	4	10 (25.0)	.182	9
+WB IgM or IgG (laboratory)					1 (2.5)	.133	4	23 (57.5)	<.001	22

Abbreviations: ?/+, indeterminate/positive; CDC, Centers for Disease Control and Prevention; Disc pairs, discordant pairs; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; WB, Western blot.

[a] Criteria for a positive test are given in Table 1.

[b] Results using in-house criteria at Specialty Laboratories A and B were compared with results using CDC criteria at the university-based reference laboratory.

False positive results as Lyme specialty lab for healthy controls

Fallon et al. *Clin Infect Dis*. 2014:59 (1705-1710)

STATE OF NEW HAMPSHIRE

In the Year of Our Lord Two Thousand Nineteen

AN ACT relative to serologic testing including Lyme disease.

Be it Enacted by the Senate and House of Representatives in General Court convened:

1 Statement of Intent.

I. The general court hereby finds that:

"Many serologic tests only test for the present of antibodies to antigens, and not for specific diseases"

151, including serologic tests confirming the diagnosis of Lyme disease, to reflect that many serologic tests only test for the presence of antibodies to antigens, and not for specific diseases. When such rules are written or changed, the commissioner shall use reasonable means to educate the public and health care providers to minimize confusion regarding Lyme disease, methods of diagnosis, and testing affected.

3 Effective Date. This act shall take effect upon its passage.

Other Diseases Diagnosed by Serology

- Syphilis
- Arboviruses (e.g. West Nile Virus, Zika virus, Powassan virus)
- HIV
- Congenital cytomegalovirus (prenatal diagnosis)
- Hepatitis B
- Hepatitis C

Current Reporting Interpretive Comments

- **IgG & IgM confirmation both positive (OR equivocal):** Consistent with active or previous infection with *B. burgdorferi*. IgM antibody testing is of diagnostic utility only during the first 4 weeks of early Lyme disease. Results of IgM antibody testing should be disregarded in patients with ≥30 days of symptoms.

Non-Lyme Tick-Borne Illnesses in NH

- *Anaplasma phagocytophilum*
- *Babesia microti*
- *Borrelia miyamotoi* (rare, emerging)
- Powassan virus (extremely rare in NH)

Symptoms & Laboratory Abnormalities

Less common:
-GI
-Respiratory

Anaplasmosis

Low white blood cell count

Borrelia miyamotoi disease

Uncommon: Meningoencephalitis in immunocompromised

Low white blood cell count

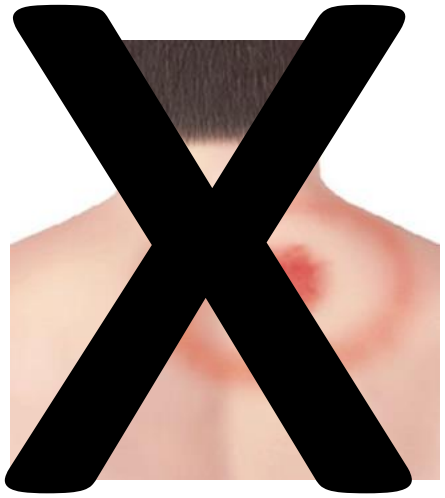
Fever

Chills
Headache
Myalgia

Low platelets
Elevated liver tests

Babesiosis

Low red blood cell count



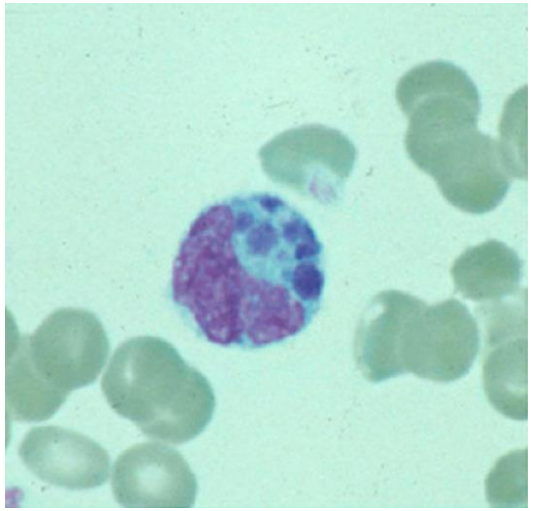
***Erythema migrans rash uncommon!

Timeframe of symptom onset after tick bite:
Anaplasma: 1-2 weeks
Babesia: 1-8 weeks

Testing Methods

Anaplasmosis

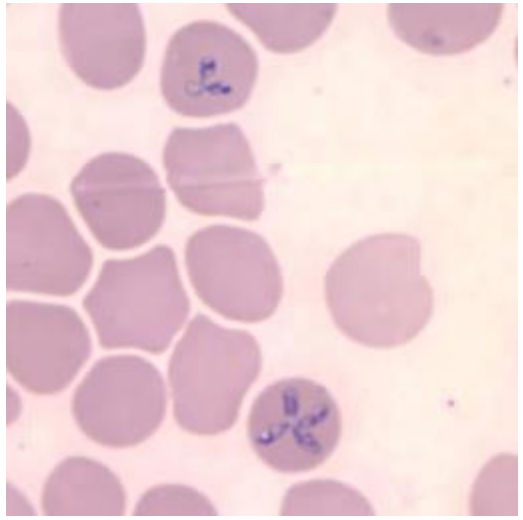
NAAT (<14 days symptoms)
(Antibody)



https://doi.org/10.1016/j.disamonth.2018.01.005

Babesiosis

NAAT
Blood parasite examination
(Antibody)

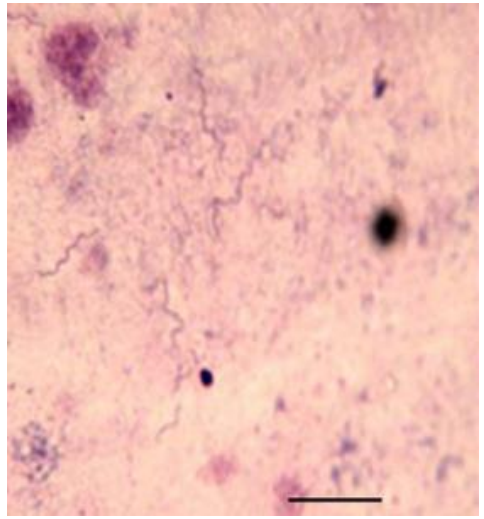


https://www.cdc.gov/dpdx/babesiosis/index.html

NAAT panel testing available

Borrelia miyamotoi disease

NAAT
(Antibody)



Telford et al. J Clin Microbiol 2019

Summary Points

- Performance of Lyme diagnostic tests is highly dependent on stage of disease
  - Timing of immune response likely responsible for low sensitivity in early LD
- Current methods are evolving: STTTA -> MTTTA
- Confusion on the part of patients and clinicians persists
- Legislation mandating a vague (albeit true) comment to accompany test results not helpful