AVOID THOSE TICK BITES!

WCLN Network Presentation 5/3/2023

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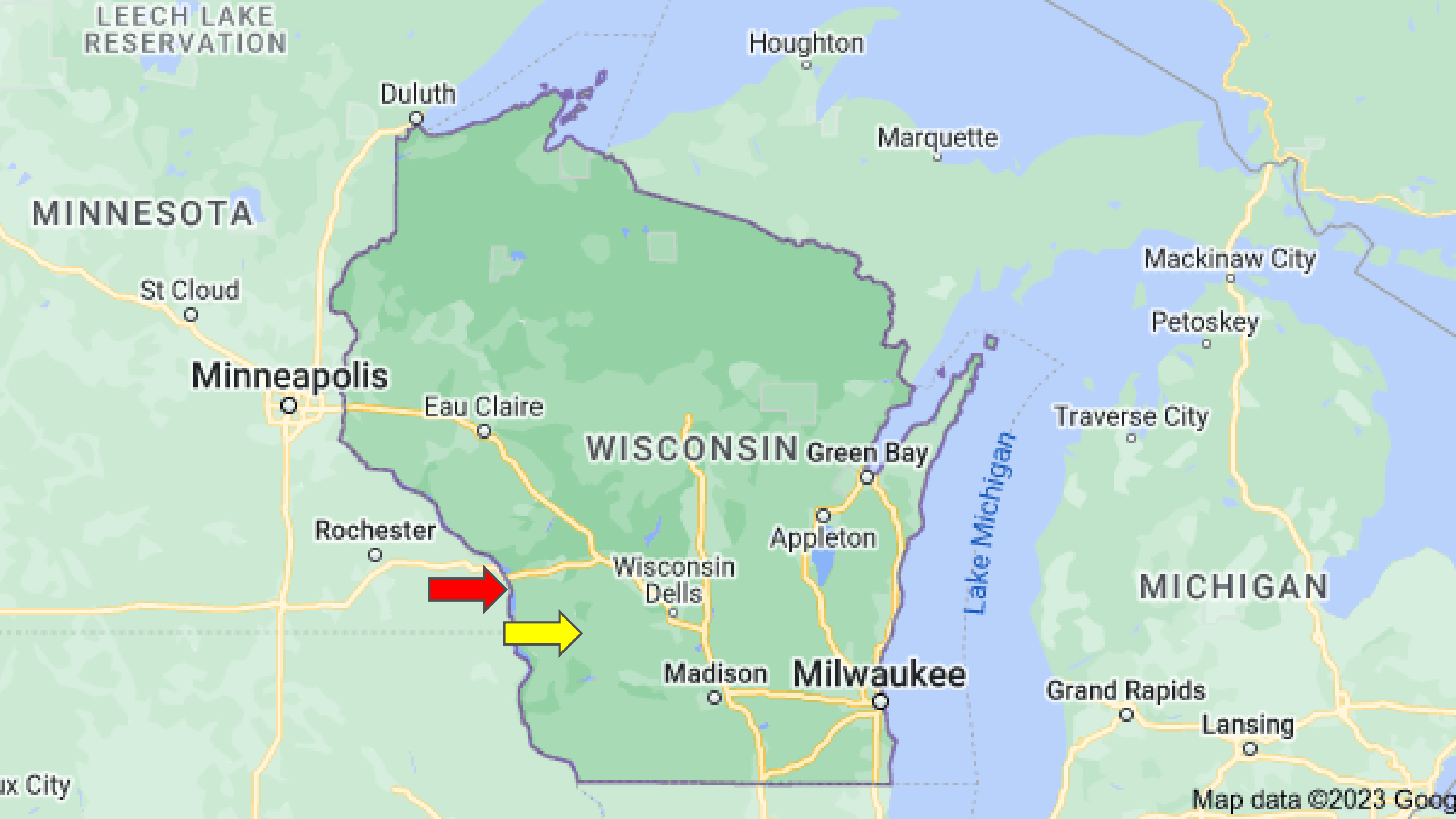
DISCLOSURES

- I HAVE NO FINANCIAL, COMMERCIAL, OR INDUSTRY DISCLOSURES
- I PARTICIPATE AS A NON-COMPENSATED INVESTIGATOR IN INDUSTRY-FUNDED RESEARCH ON ZOONOTIC AND EMERGING INFECTIOUS DISEASES

Objectives du jour:

- Discuss the Relevant Tick-borne diseases in Wisconsin, and when they should be considered in a diagnostic workup
- Review which tests to order for various zoonotic illnesses
- Discuss how the MTTT vs STTT paradigm has changed Lyme Disease diagnostics (at least in my local area)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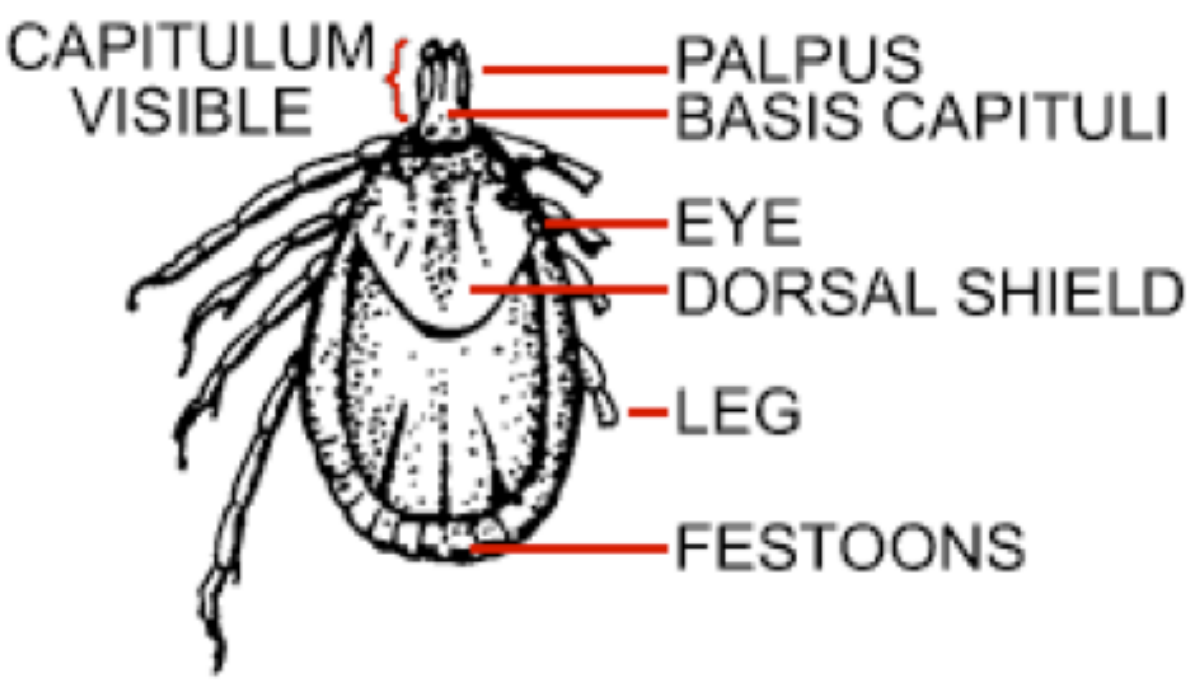
WHAT IS A TICK?

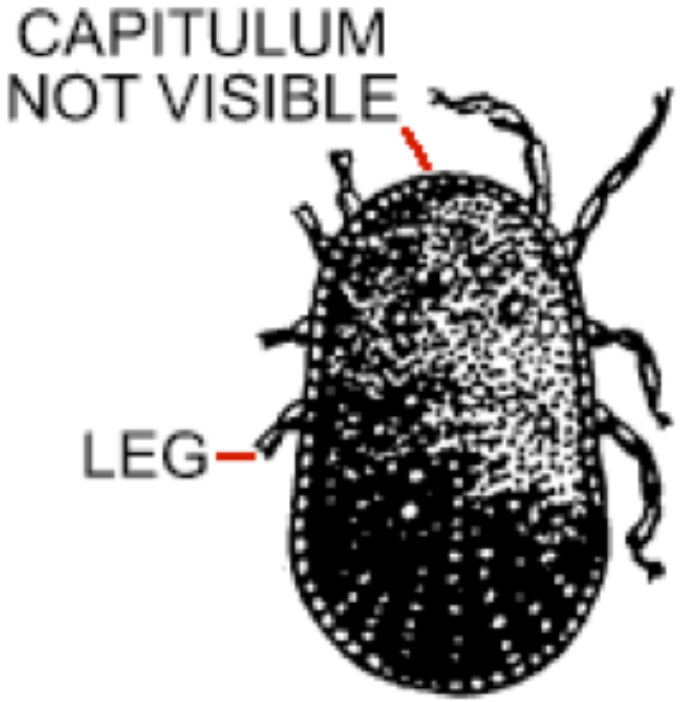
- 8 LEGGED ARTHROPOD (ARACHNID)
- TWO BODY SEGMENTS
- TWO LIFE STAGES (NYMPH, ADULT)
  - REQUIRES BLOOD MEAL AT EACH PHASE
- HALLER'S ORGANS (SENSE CO_2)
  - ABSENT IN MITES

Source: my desk
(previously, the woods)

Anatomy of Hard and Soft Ticks



Hard Tick
(Family Ixodidae)

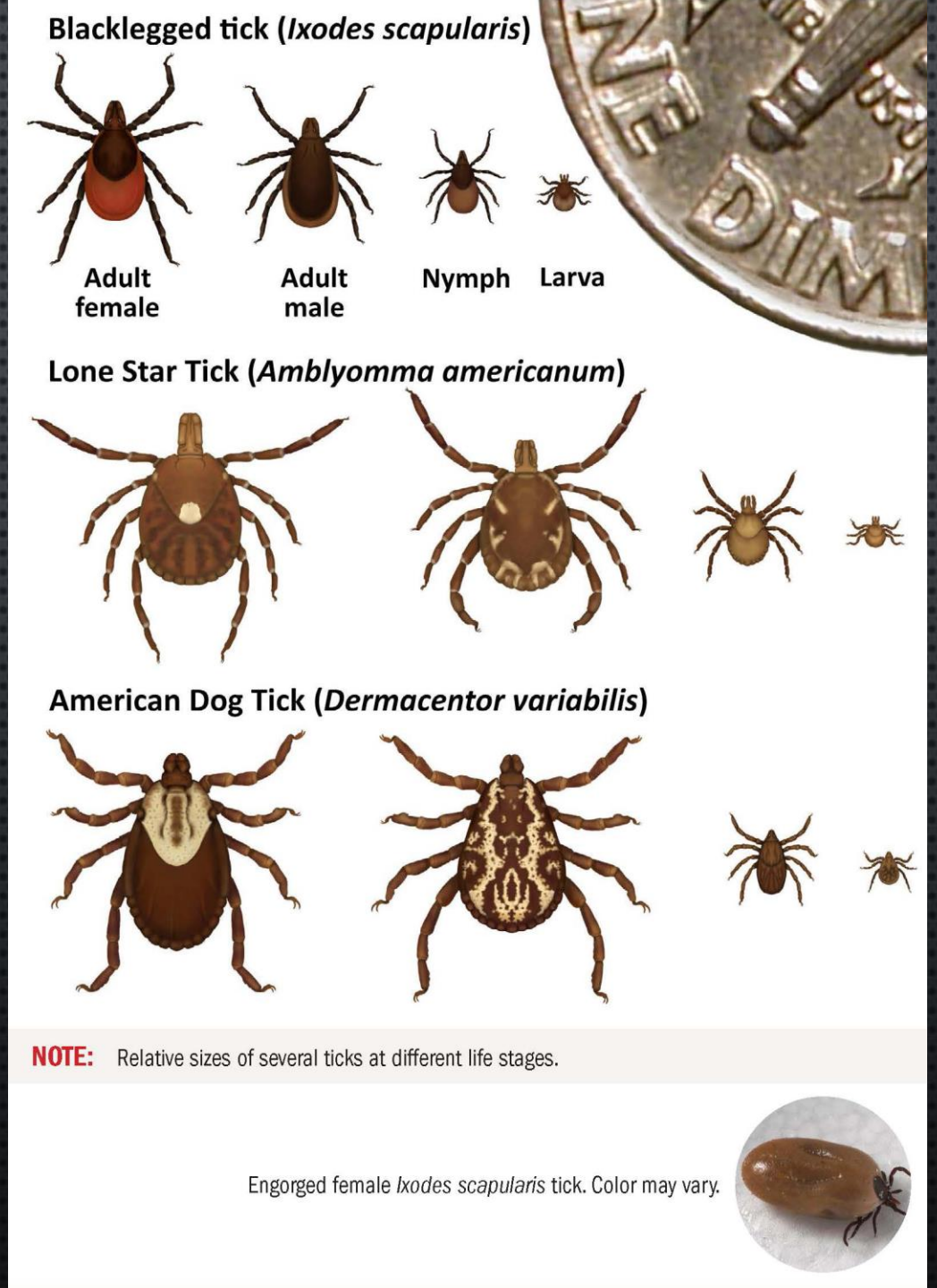


Soft Tick

Source: http://www.ticktexas.org/ticks/ticks101_anatomy.htm

Wisconsin Ticks

- 16 species
  - Only a few known to feed deliberately on humans
- The Big Three Ticks:
  - Deer Ticks (*Ixodes scapularis, Ixodes cookei*)
  - Wood Ticks (*Dermacentor variabilis, Rhipicephalus sanguineus*)
  - Lone Star Ticks (*Amblyomma Americanum*)


Blacklegged tick (*Ixodes scapularis*)
Adult female
Adult male
Nymph
Larva
Lone Star Tick (*Amblyomma americanum*)
American Dog Tick (*Dermacentor variabilis*)
NOTE: Relative sizes of several ticks at different life stages.
Engorged female *Ixodes scapularis* tick. Color may vary.

Source: CDC

When are the ticks "out"

- Activity depends on temperature
  - Active/biting as low as 40 deg Fahrenheit!
    - Yes, we see real Lyme cases in February, March, etc…
  - Nymphs are most active in June/July

THE BIG LIST

What are the Zoonotic Tick-Borne Diseases in Wisconsin?

WISCONSIN TICK-BORNE DISEASES:

- Lyme Disease (*Borrelia burgdorferi SS, Borrelia mayonii*)
- *Borrelia miyamotoi*
- HGA (Anaplasmosis)
- Babesiosis
- HME (Ehrlichiosis)
- Powassan Virus
- Spotted Fever Rickettsiosis (RMSF)
- Tularemia
- Emerging Infectious Diseases:
  - Bourbon Virus, Heartland Virus, STARI (?)

When consider a tick-borne disease?

- Three Variables:
  - Exposure to ticks
  - Absence of Protective Measures for Ticks
  - Classic Cadre of Symptoms

When consider a tick-borne disease?

- Three Variables:
  - Exposure to ticks
    - Chronology – time of year?
    - Geography – hotspots in the state?
    - Hunting
    - Hiking, Trail Running, Bicycling
    - Agriculture
    - Occupational – Veterinary, rural workers
    - Attached Ticks (> 24 hours)

1%

When consider a tick-borne disease?

- Three Variables:
  - Absence of Protective Measures for Ticks
    - Protective clothing
      - Long socks/pants, "blousers", hats, etc.
    - DEET/Permethrin
    - Did they check for ticks?

Ready for ticks…and to paint a barn or two

When Consider a Tick-Borne Disease?

- Three Variables:
  - Classic Cadre of Symptoms
    - Fever + Rash (erythema migrans)
    - Fever + Joint Pain
    - Joint Effusions
    - Palsy (Classic Bell's Palsy)
    - "Flu-like" Illness in hunter, hiker, camper, etc.

So, which test(s) do I order?

The age-old question occasionally posed to the lab…

International Journal of
Environmental Research and Public Health

MDPI

Article

Spatio-Temporal Dynamics of Tick-Borne Diseases in North-Central Wisconsin from 2000–2016

Austin Rau [1], Claudia Munoz-Zanzi [1], Anna M. Schotthoefer [2], Jonathan D. Oliver [1] and Jesse D. Berman [1,*]

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Lyme disease (*Borrelia burgdorferi*)

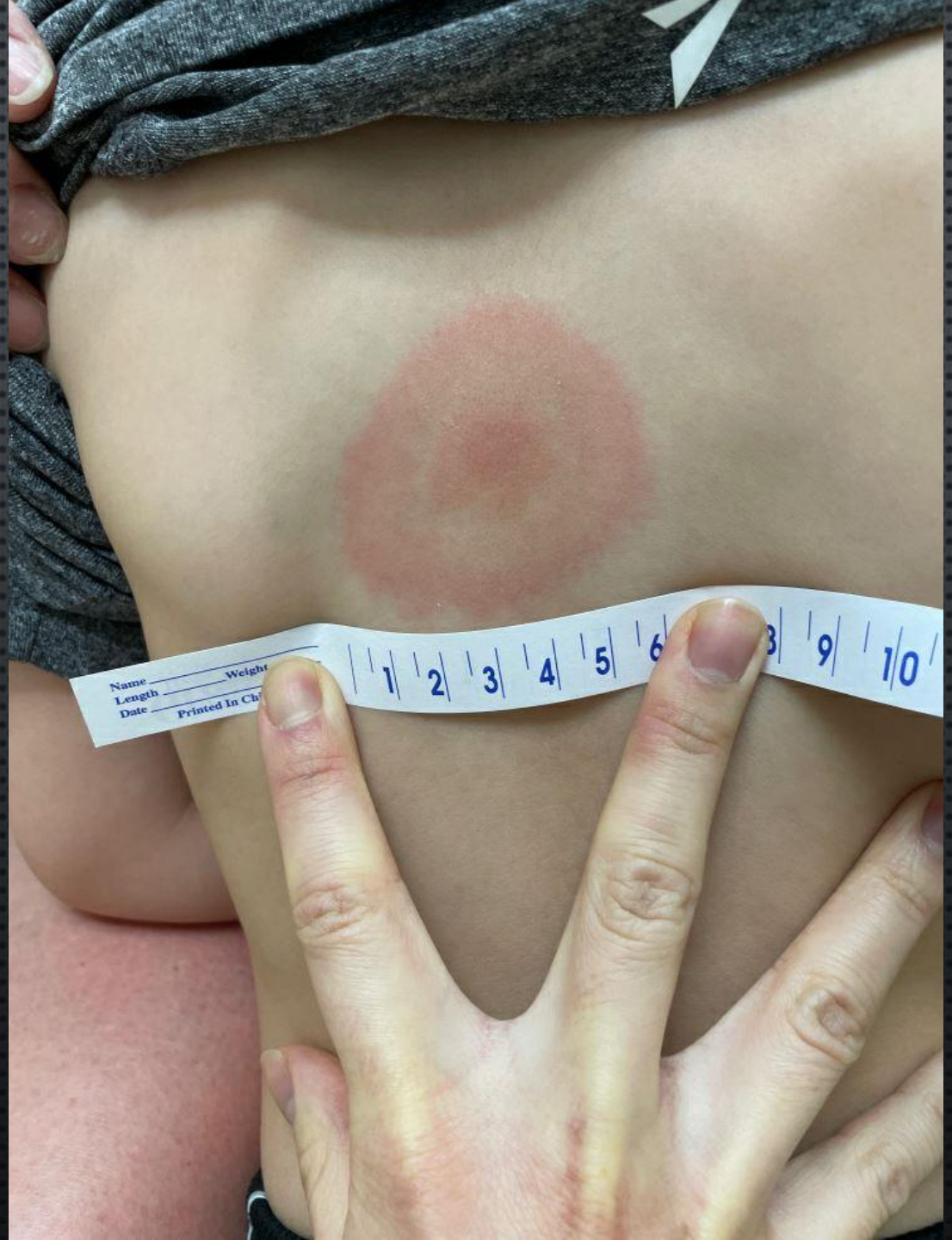
- Consider ordering no testing whatsoever
  - No testing is needed to "confirm" an ECM in the right clnical setting
- Lyme Serology (STTT vs MTTT)
  - IgM, IgG
  - Western Blot
- CSF Antibody Index
- PCR
  - Skin biopsy???

Clinical Infectious Diseases

IDSA FEATURES

Clinical Practice Guidelines by the Infectious Diseases Society of America (IDSA), American Academy of Neurology (AAN), and American College of Rheumatology (ACR): 2020 Guidelines for the Prevention, Diagnosis and Treatment of Lyme Disease

Paul M. Lantos,[1] Jeffrey Rumbaugh,[2] Linda K. Bockenstedt,[3] Yngve T. Falck-Ytter,[4] Maria E. Aguero-Rosenfeld,[5] Paul G. Auwaerter,[6] Kelly Baldwin,[7] Raveendhara R. Bannuru,[8] Kiran K. Belani,[9] William R. Bowie,[10] John A. Branda,[11] David B. Clifford,[12] Francis J. DiMario Jr,[13] John J. Halperin,[14] Peter J. Krause,[15] Valery Lavergne,[16] Matthew H. Liang,[17] H. Cody Meissner,[8] Lise E. Nigrovic,[18] James (Jay) J. Nocton,[19] Mikala C. Osani,[8] Amy A. Pruitt,[20] Jane Rips,[21] Lynda E. Rosenfeld,[3] Margot L. Savoy,[22] Sunil K. Sood,[23] Allen C. Steere,[11] Franc Strle,[24] Robert Sundel,[18] Jean Tsao,[25] Elizaveta E. Vaysbrot,[8] Gary P. Wormser,[26] and Lawrence S. Zemel[13]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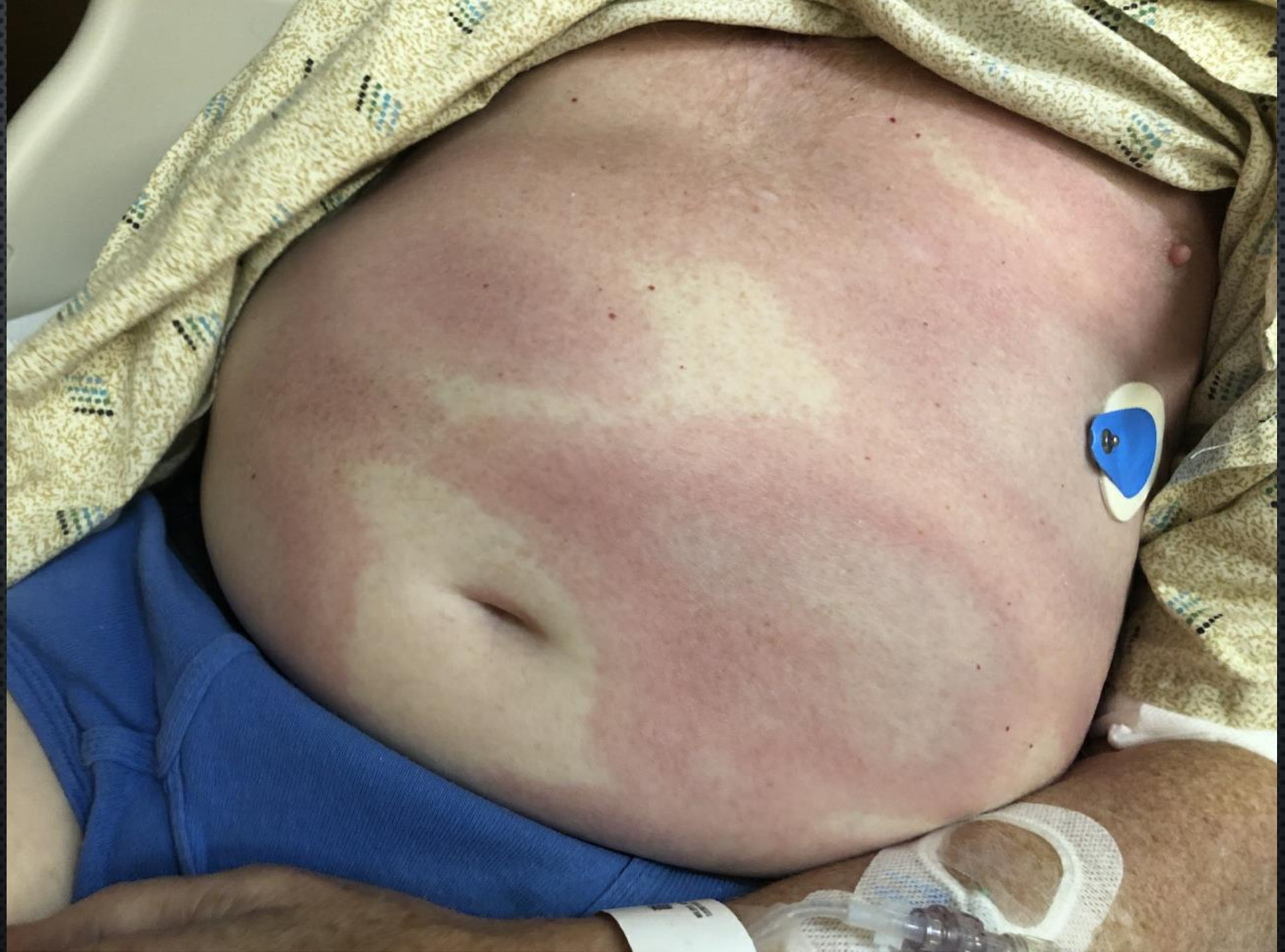

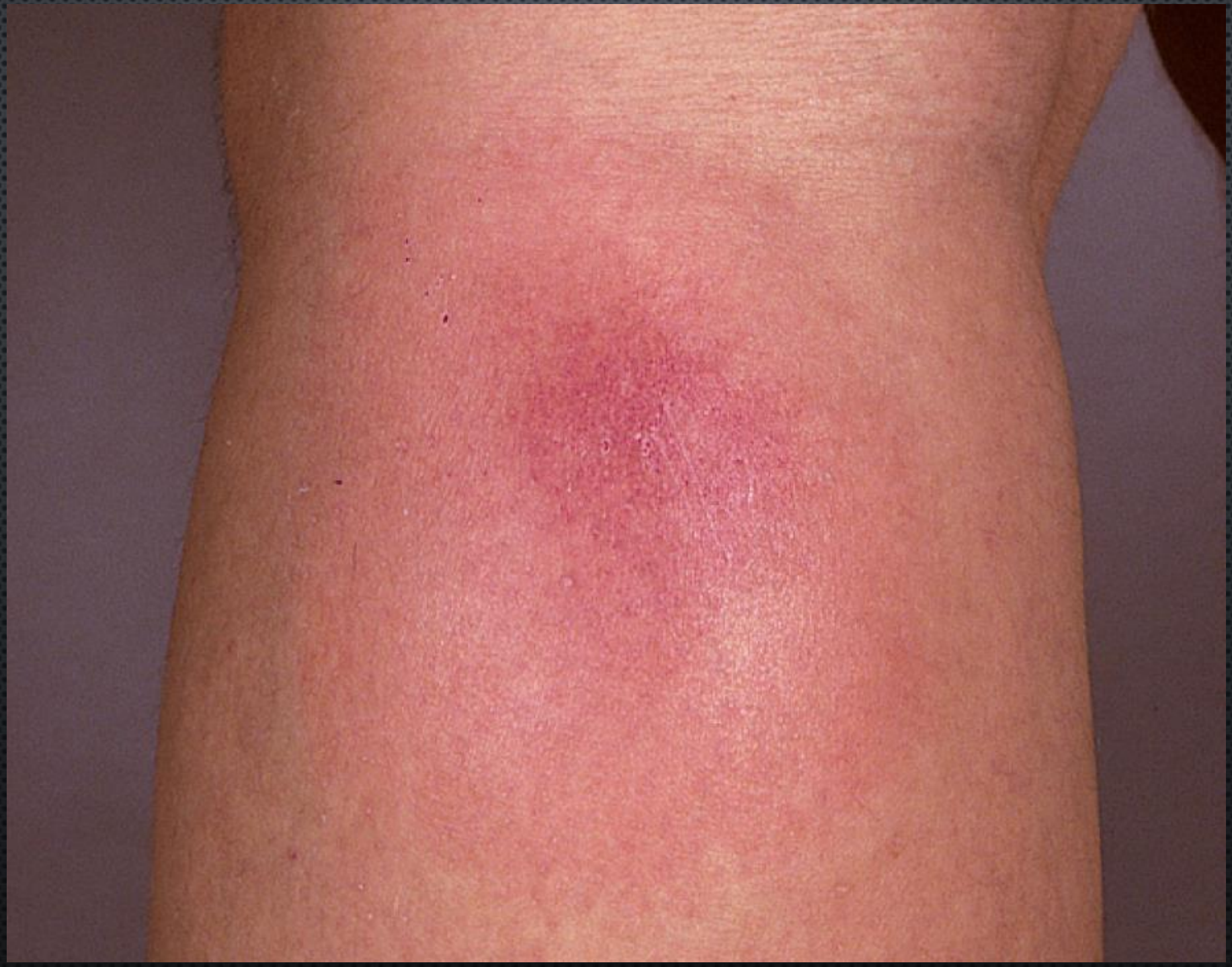


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IMMUNOASSAYS

Multicenter Clinical Evaluation of Modified Two-Tiered Testing Algorithms for Lyme Disease Using Zeus Scientific Commercial Assays

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[b]Marshfield Clinic Health System, Marshfield, Wisconsin, USA

[c]Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA

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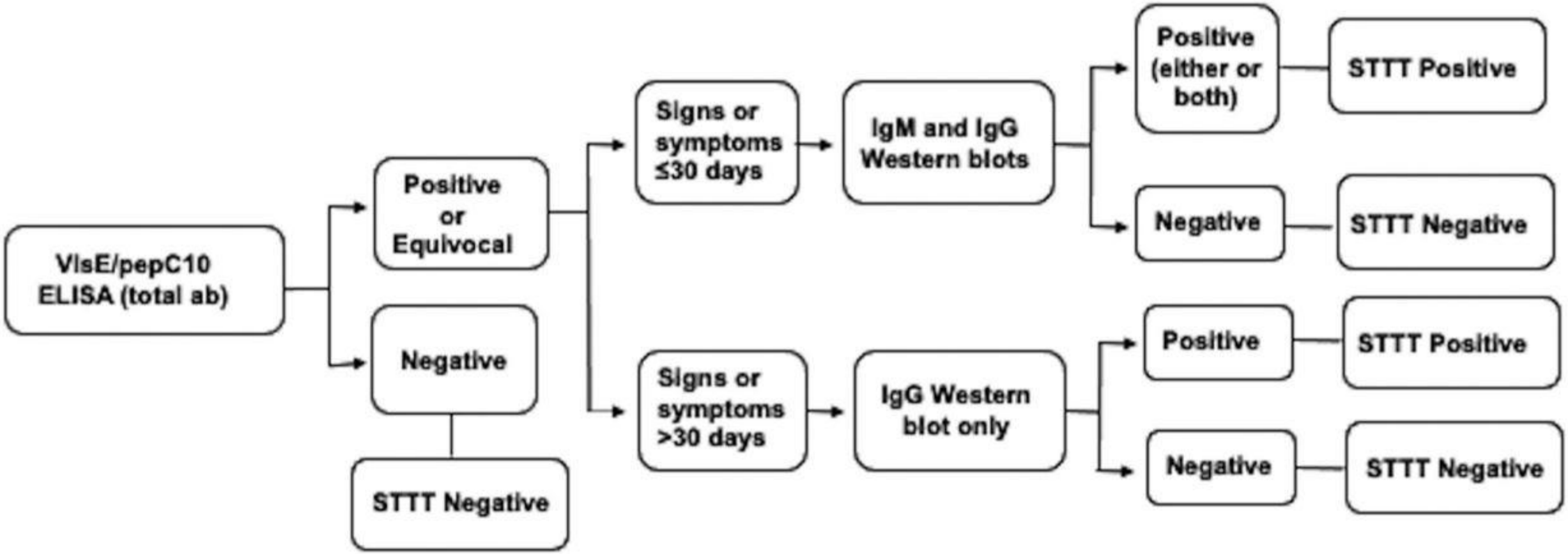
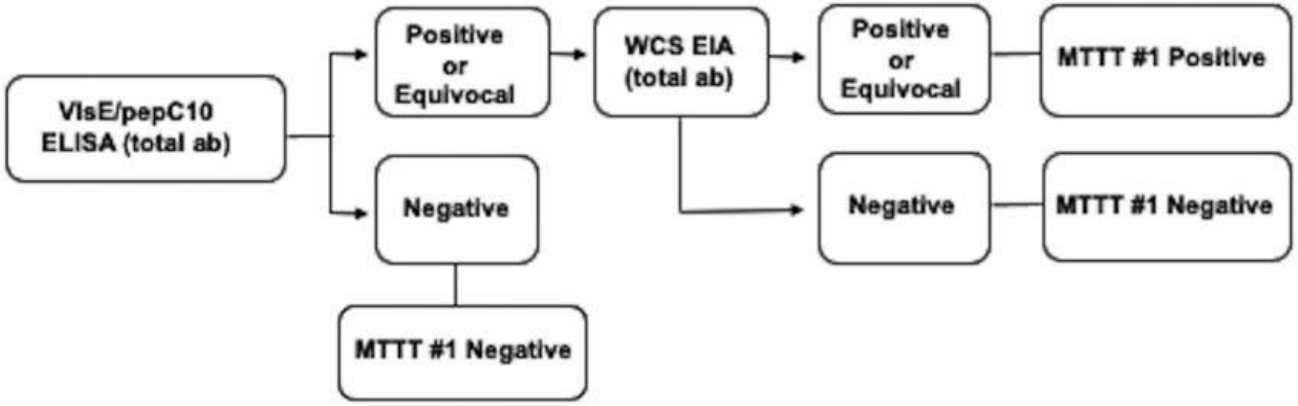
Maroun M. Sfeir and Jennifer K. Meece contributed equally to this article. Author order was determined by drawing straws.

Standard Two-tiered Testing (STTT) Algorithm



Modified Two-tiered Testing (MTTT) Algorithm #1



Modified Two-tiered Testing (MTTT) Algorithm #2

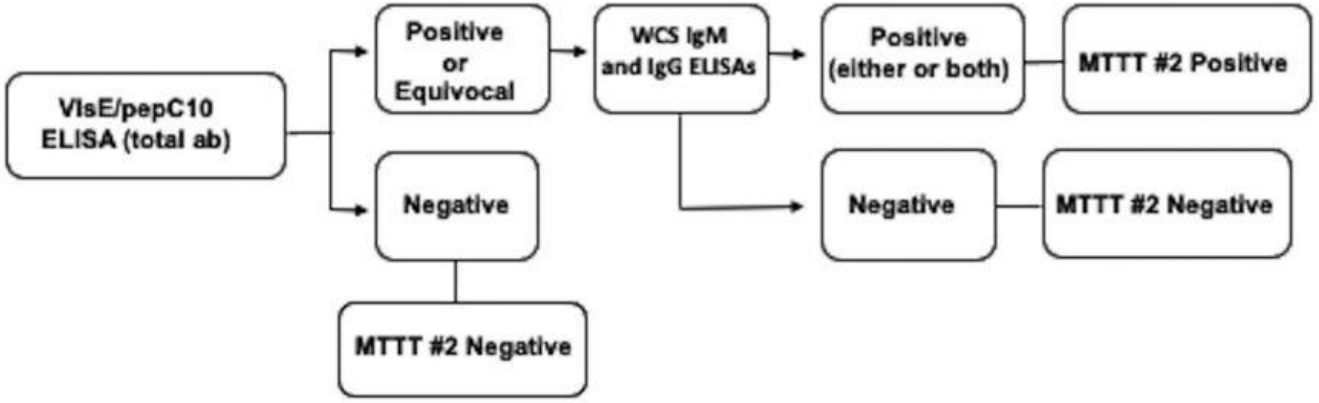


TABLE 3 Specificity of individual EIAs compared with MTTT algorithms[a]

MTTT algorithm	First-tier test		Second-tier test(s)		MTTT algorithm specificity (%, 95% CI)
	Specificity (%)	*P*[b]	Specificity (%)	*P*[b]	
1 (VlsE1/pepC10 IgG/IgM EIA f/b WCS IgG/IgM EIA)	180/190 (95)	0.01	177/190 (93)	0.003	188/190 (99, 96–100)
2 (VlsE1/pepC10 IgG/IgM EIA f/b WCS IgM and IgG EIAs)	180/190 (95)	0.01	161/190 (85)	0.0001	188/190 (99, 96–100)

[a]MTTT, modified two-tiered testing; CI, confidence interval; f/b, followed by; WCS, whole-cell sonicate; EIA, enzyme immunoassay.
[b]*P* values refer to the comparison between the individual EIA and the relevant MTTT algorithm.

TABLE 4 Sensitivity of two-tiered testing algorithms for Lyme disease[a]

Algorithm	Test 1	Test 2	No. positive results (sensitivity [%]; 95% CI)					
			Paired samples from CDC repository		Acute EM with hematogenous dissemination ($n = 31$)	All acute EM combined ($n = 61$)	Neuritis or carditis ($n = 25$)	Lyme arthritis ($n = 50$)
			EM acute phase ($n = 30$)	EM conv. phase ($n = 30$)				
STTT	VlsE1/pepC10 IgG/IgM EIA	IgM and IgG immunoblots	15 (50; 33–67)	23 (77; 59–88)	10 (32, 18–50)	25 (41, 30–54)	24[b] (96, 79–100)	50 (100, 91–100)
MTTT 1	VlsE1/pepC10 IgG/IgM EIA	WCS EIA (IgG/IgM)	22 (73, 55–86), $P = 0.02$[c]	25 (83, 66–93), $P = 0.48$	12 (39, 24–56), $P = 0.68$	34 (56, 43–68), $P = 0.03$	25 (100, 84–100), $P = 1.0$	50 (100, 91–100), $P = 1.0$
MTTT 2	VlsE1/pepC10 IgG/IgM EIA	WCS IgM and IgG EIAs	23 (77, 59–88), $P = 0.01$	27 (90, 74–97), $P = 0.13$	22 (71, 53–84), $P = 0.002$	45 (74, 61–83), $P = 0.0001$	25 (100, 84–100), $P = 1.0$	50 (100, 91–100), $P = 1.0$

[a]CI, confidence interval; EM, erythema migrans; conv., convalescent; STTT, standard two-tiered testing; MTTT, modified two-tiered testing algorithm; EIA, enzyme immunoassay; WCS, whole-cell sonicate.
[b]One sample was negative by IgM immunoblot (strong 23-kd band, weak 41-kd band, absent 39-kd band) and IgG immunoblot (only 41-kd and 45-kd bands present).
[c]All *P* values refer to comparison with standard two-tiered testing (VlsE1/pepC10 polyvalent EIA followed by IgM and IgG immunoblots).

Diagnostic Microbiology and Infectious Disease
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Clinical evaluation of a *Borrelia* modified two-tiered testing (MTTT) shows increased early sensitivity for *Borrelia burgdorferi* but not other endemic *Borrelia* species in a high incidence region for Lyme disease in Wisconsin

Arick P. Sabin [a b], Brooklynn P. Scholze [b], Steven D. Lovrich [b], Steven M. Callister [b]

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Advance Access Publication Date: 4 June 2021
Short Communication

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Vector-Borne Diseases, Surveillance, Prevention

High Prevalence of *Borrelia mayonii* (Spirochaetales: Spirochaetaceae) in Field-Caught *Tamias striatus* (Rodentia: Sciuridae) From Northern Wisconsin

Patricia N. Siy,[1] Ryan T. Larson,[1,2] Tela E. Zembsch,[1,3] Xia Lee,[1] and Susan M. Paskewitz[1,4]

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Subject Editor: Sarah Hamer

Received 16 March 2021; Editorial decision 9 May 2021

Open Forum Infectious Diseases

MAJOR ARTICLE

Multiplex High-Definition Polymerase Chain Reaction Assay for the Diagnosis of Tick-borne Infections in Children

Lise E. Nigrovic,[1] Desiree N. Neville,[2] Laura Chapman,[3] Fran Balamuth,[4] Michael N. Levas,[5] Amy D. Thompson,[6] Anupam B. Kharbanda,[7] Derek Gerstbrein,[8] John A. Branda,[9] and Blake W. Buchan,[8] for Pedi Lyme Net

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Background. *Ixodes scapularis* ticks can carry *Borrelia* species as well as other pathogens that cause human disease. The frequency of tick-borne infections and coinfections in children with suspected Lyme disease is unknown, creating clinical uncertainty about the optimal approach to diagnosis.

Methods. We enrolled children aged 1–21 years presenting to 1 of 8 Pedi Lyme Net emergency departments for evaluation of Lyme disease. We selected cases with serologically or clinically diagnosed Lyme disease (erythema migrans or early neurologic disease) matched by symptoms, age, gender, and center to control subjects without Lyme disease. We tested whole blood samples collected at the time of diagnosis using a multiplex high-definition polymerase chain reaction (HDPCR) panel to identify 9 bacterial or protozoan pathogens associated with human disease. We compared the frequency of tick-borne coinfections in children with Lyme disease to matched controls.

Results. Of the 612 selected samples, 594 (97.1%) had an interpretable multiplex HDPCR result. We identified the following non-*Borrelia* tick-borne infections: *Anaplasma phagocytophilum* (2), *Ehrlichia chaffeensis* (1), and *Babesia microti* (12). Children with Lyme disease were more likely to have another tick-borne pathogen identified than matched controls (15/297 [5.1%] Lyme cases vs 0/297 [0%]; difference, 5.1% [95% confidence interval, 2.7%–8.2%]).

Conclusions. Although a substantial minority of children with Lyme disease had another tick-borne pathogen identified, either first-line Lyme disease antibiotics provided adequate treatment or the coinfection was subclinical and did not require specific treatment. Further studies are needed to establish the optimal approach to testing for tick-borne coinfections in children.

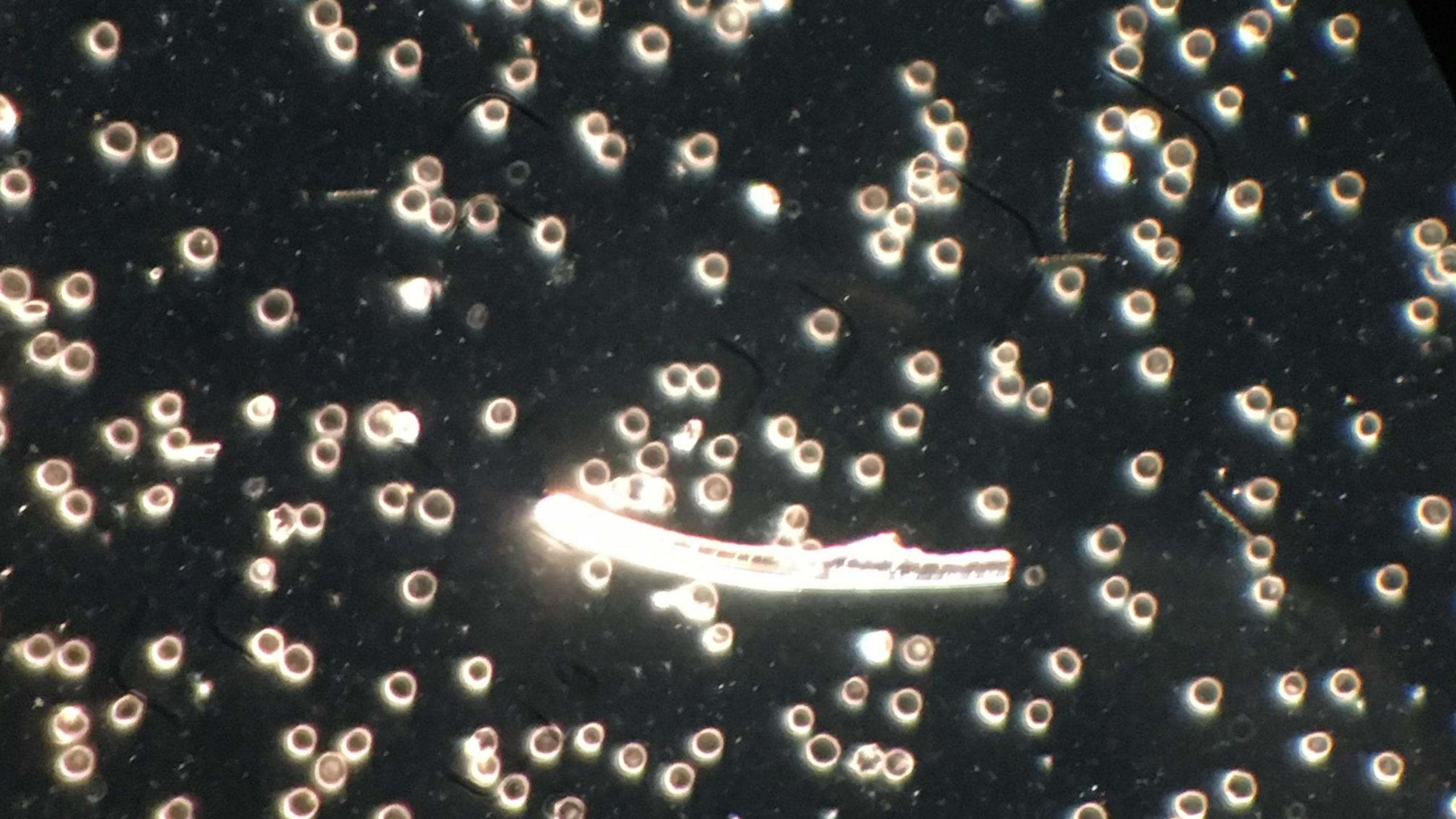
Keywords. children; coinfections; Lyme disease; tick-borne infection.

Should we PCR this tick someone submitted?

- No!
  - Totally useless in routine clinical practice
  - Discourage this practice among patients/providers!
    - "Should we test it for Co-infections?"
      - No. Absolutely not.

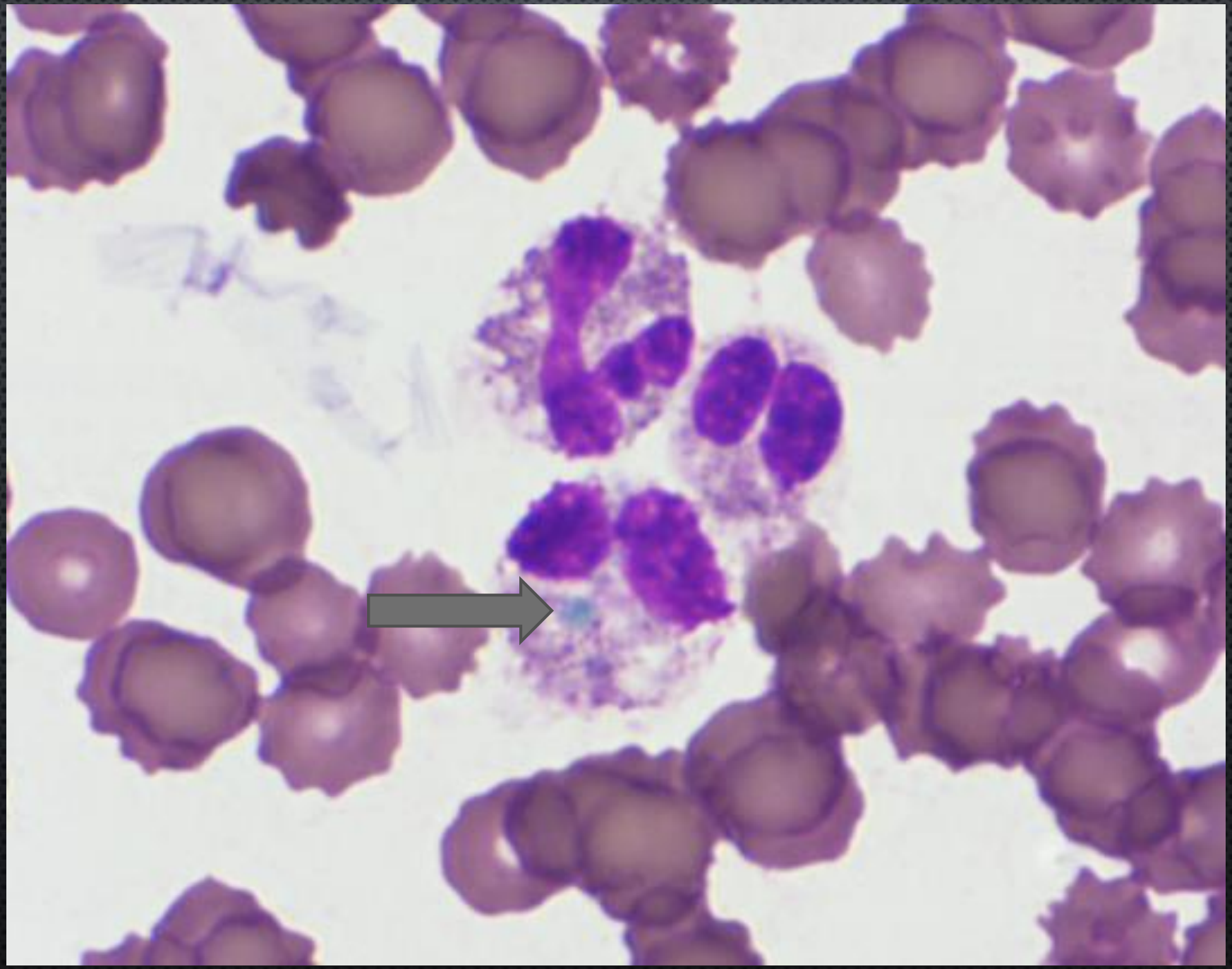
Borrelia miyamotoi

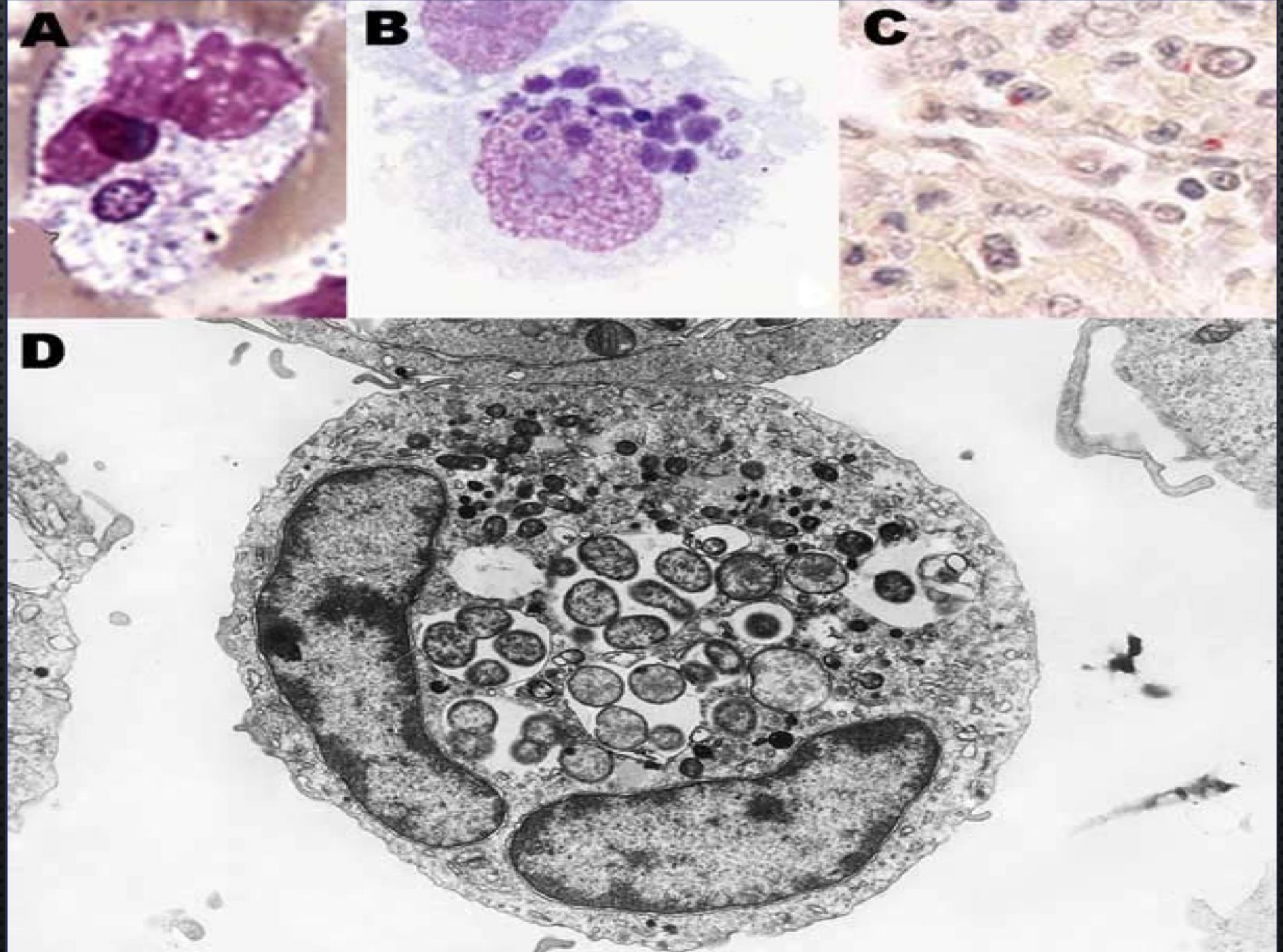
- Fundamentally different than *Borrelia burgdorferi*
  - Think of it like a relapsing fever spirochete
  - Prominent bloodstream phase, eliciting fever
    - PCR



Anaplasma

- Serology (IFA)
  - Paired IgM/IgG 3-4 weeks apart
- Morulae in Giemsa blood smear
  - (backup to second method)
- PCR (days 1-7)
  - HE1/HE3 target
- Culture (CSF, Blood)…rarely done

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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

A. M. Schotthoefer,[a] J. K. Meece,[a] L. C. Ivacic,[a] P. D. Bertz,[a] K. Zhang,[a] T. Weiler,[b] T. S. Uphoff,[b] T. R. Fritsche[b,c]

Marshfield Clinic Research Foundation, Marshfield, Wisconsin, USA[a]; Marshfield Clinic, Marshfield, Wisconsin, USA[b]; University of Wisconsin, La Crosse, Wisconsin, USA[c]

Anaplasmosis and ehrlichiosis are emerging tick-borne diseases with clinically similar presentations caused by closely related pathogens. Currently, laboratories rely predominantly on blood smear analysis (for the detection of intracellular morulae) and on serologic tests, both of which have recognized limitations, for diagnostic purposes. We compared the performance of a published real-time PCR assay that incorporates melt curve analysis to differentiate *Anaplasma* and *Ehrlichia* species with blood smear and serologic methods in an upper Midwest population. Overall, 38.5% of the specimens selected for evaluation had one or more tests that were positive for anaplasmosis. The PCR positivity for all specimens was maximal (21.2%; 29/137) during the early acute phase of illness (0 to 4 days since illness onset) and significantly less frequent (11.5%; 20/174) during later phases (>4 days since illness onset). All positive specimens were *Anaplasma phagocytophilum*; no *Ehrlichia* species were identified. The real-time PCR detected 100% of infections that were detected by blood smear analysis (14/14) and broadened the detection window from a maximum of 14 days for smear positivity to 30 days for PCR. Additional infections were detected by real-time PCR in 12.9% (11/85) of smear-negative patients. There was poor agreement between the real-time PCR assay and serologic test results: 19.8% (19/96) and 13.7% (29/212) of seropositive and -negative patients, respectively, were PCR positive. Seropositivity increased with increasing days of illness, demonstrating that serologic detection methods are best utilized during presumed convalescence. Our results indicate that the optimal performance and utilization of laboratory tests for the diagnosis of anaplasmosis require knowledge regarding time of symptom onset or days of illness.

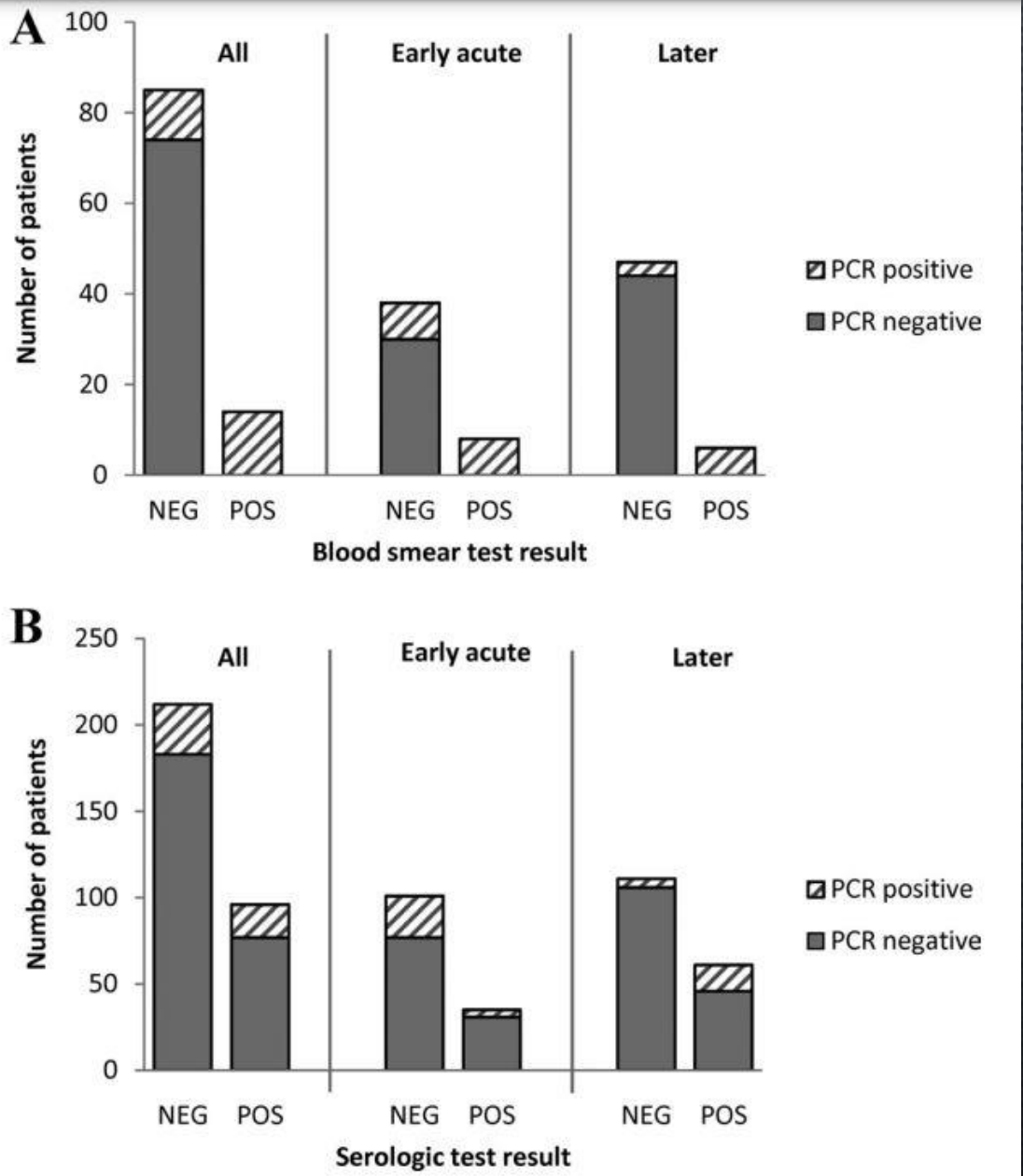


FIG 2 Comparisons of positive and negative blood smear (A) and serologic (B) tests with that of the real-time PCR assay for detecting *A. phagocytophilum* infection in all patients, and for those in the early acute (0 to 4 days since illness onset) and later (>4 days since illness onset) phases of infection.

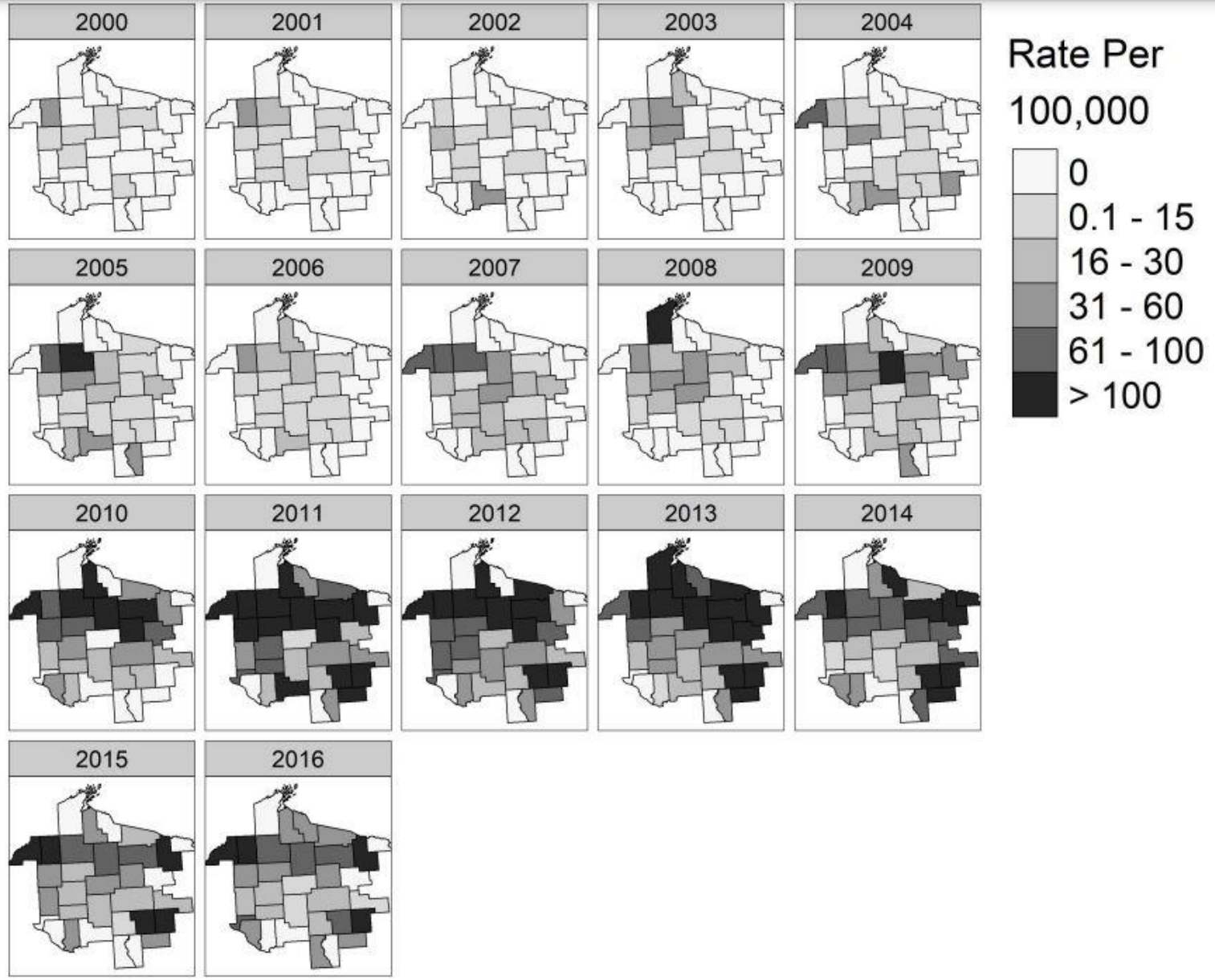
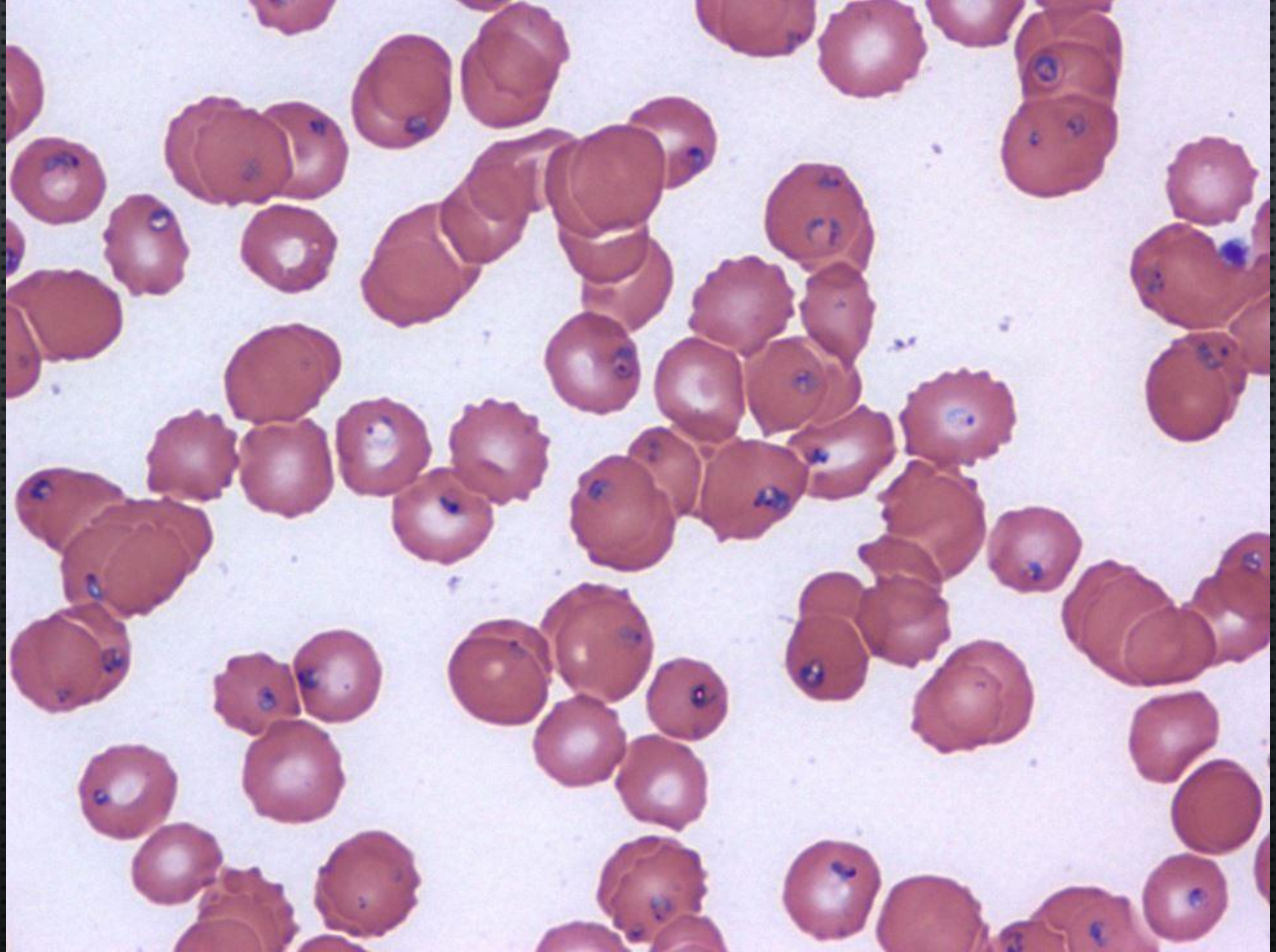


Figure 4. Annual county level incidence rate for positive laboratory cases of combined tick-borne diseases caused by human granulocytic anaplasmosis, babesiosis, ehrlichiosis per 100,000 patients, 2000–2016 for the Marshfield Clinic Health System.

Babesia

- Blood Smear (Histopathology)
  - Giemsa staining
    - Occasionally seen in urine (hematuria)
- IFA
- PCR

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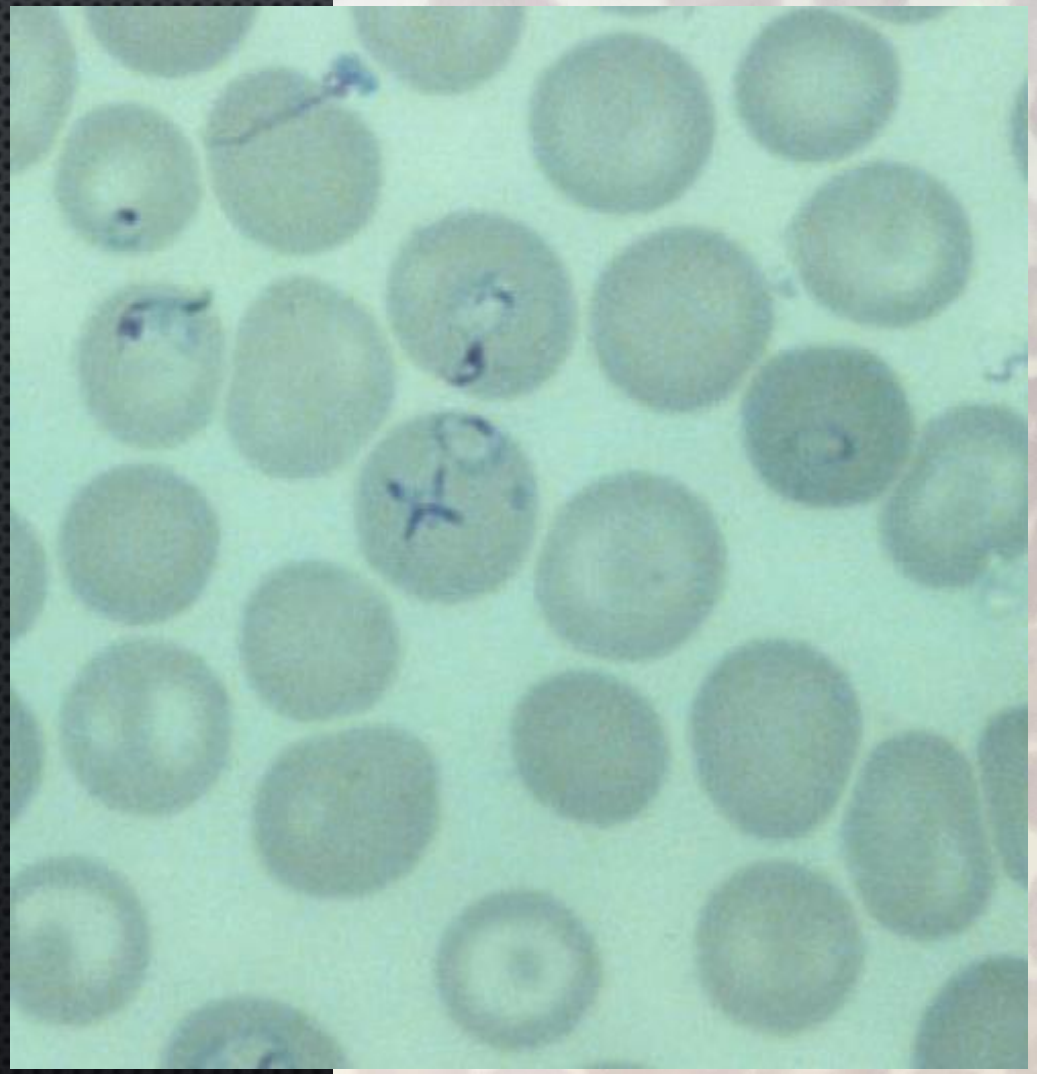
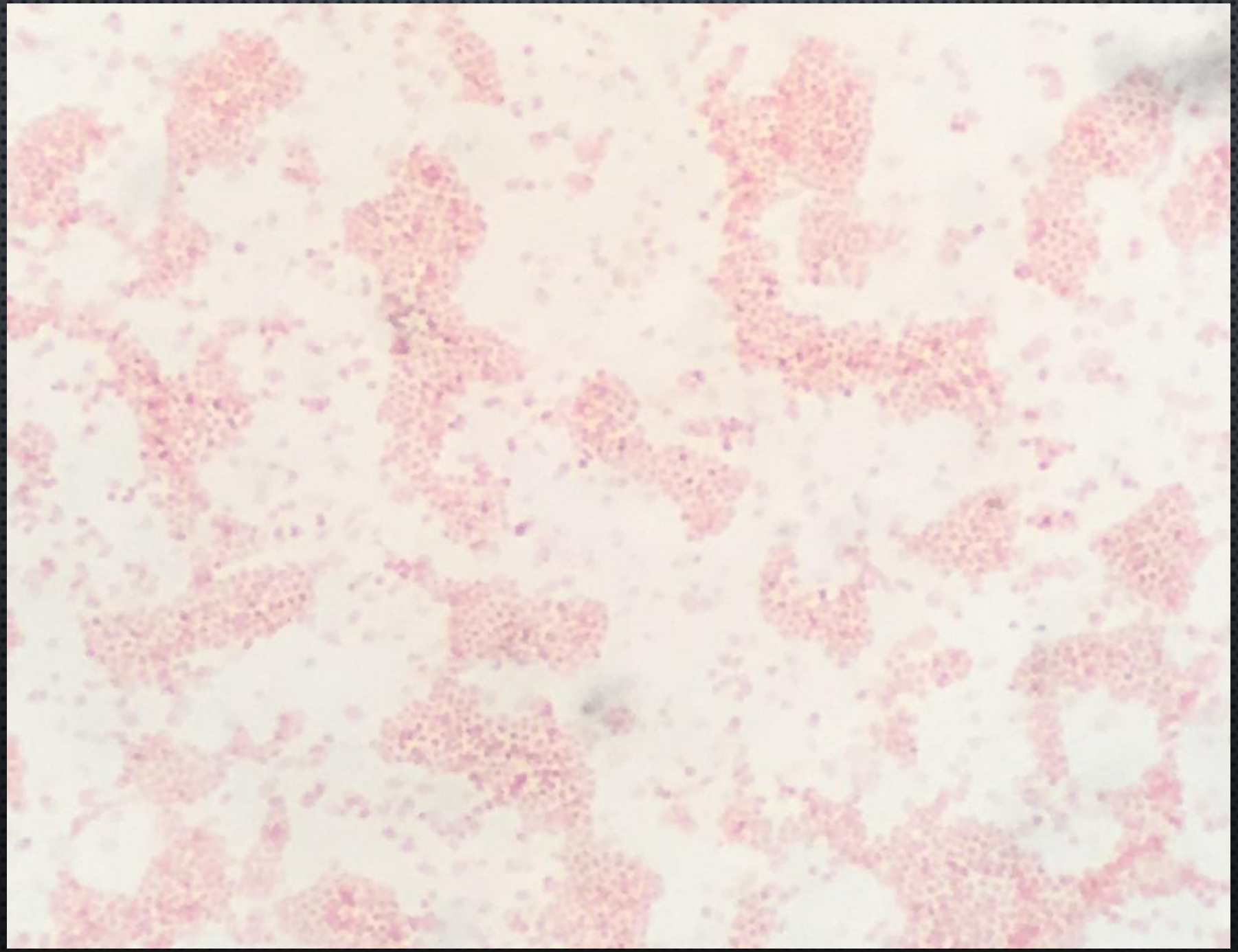


Ehrlichia

- Testing not widely available, or needs to be sent out
  - Treatment for Lyme/*Anaplasma* often presumes coverage for *Ehrlichia* spp.
  - IFA (4x rise over 3-4 weeks)
- Specialized PCRs
  - Organism-specific
  - Genus-specific

Tularemia (*Francisella tularensis*)

- Recovery from culture
  - Laboratory hazard!
    - Appropriate shipping considerations if sending out for ID
  - Serology (too slow…)
- PCR?



POWASSAN VIRUS

- PCR
  - Brain tissue; followed by plaque reduction assay
- Serology
  - CSF IgM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Questions?

- Thank you for inviting me to speak!