Interpretation of Lyme disease Serology – Present and Future

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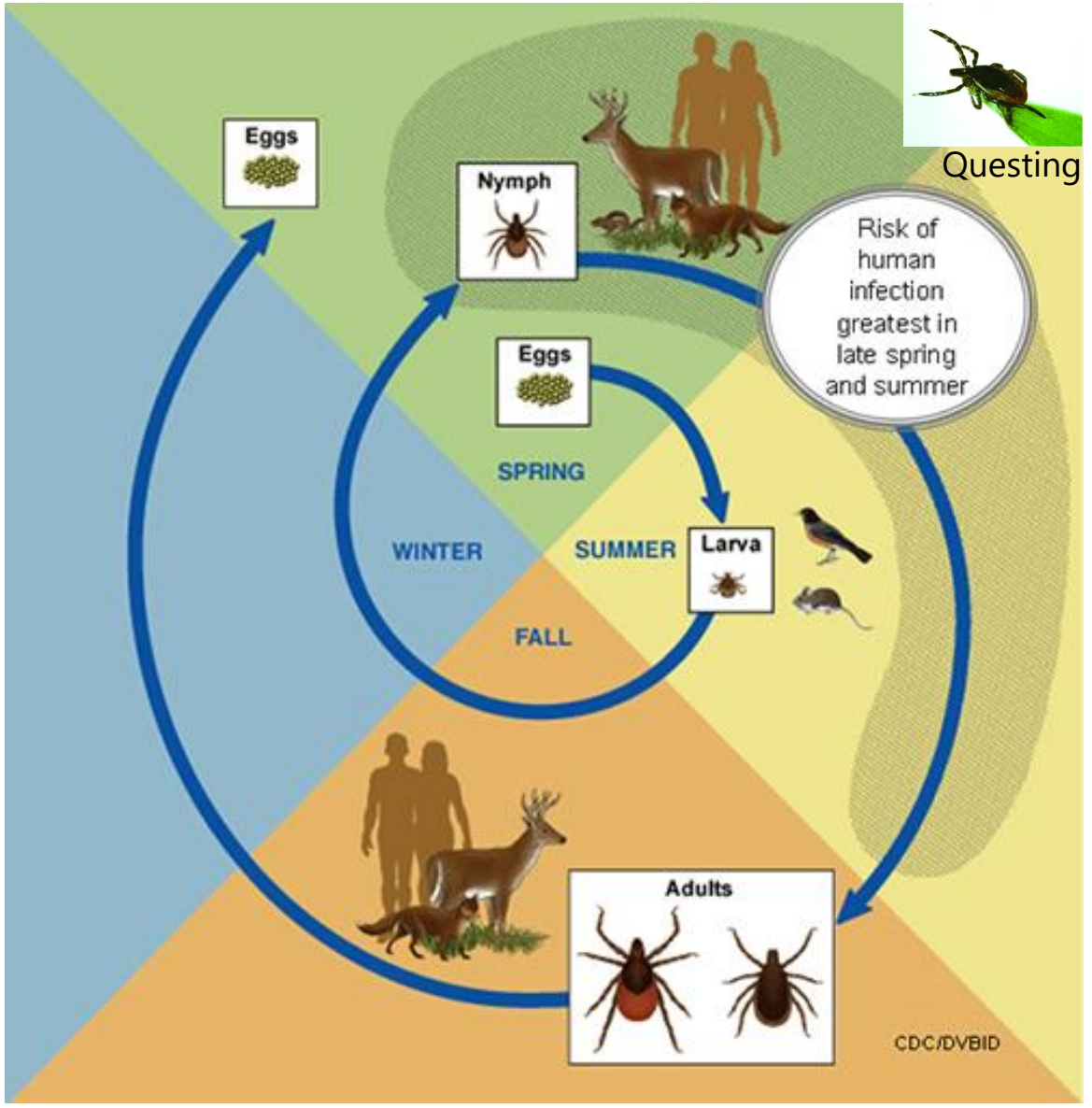
NBIMU

April 23, 2021

Lyme Disease

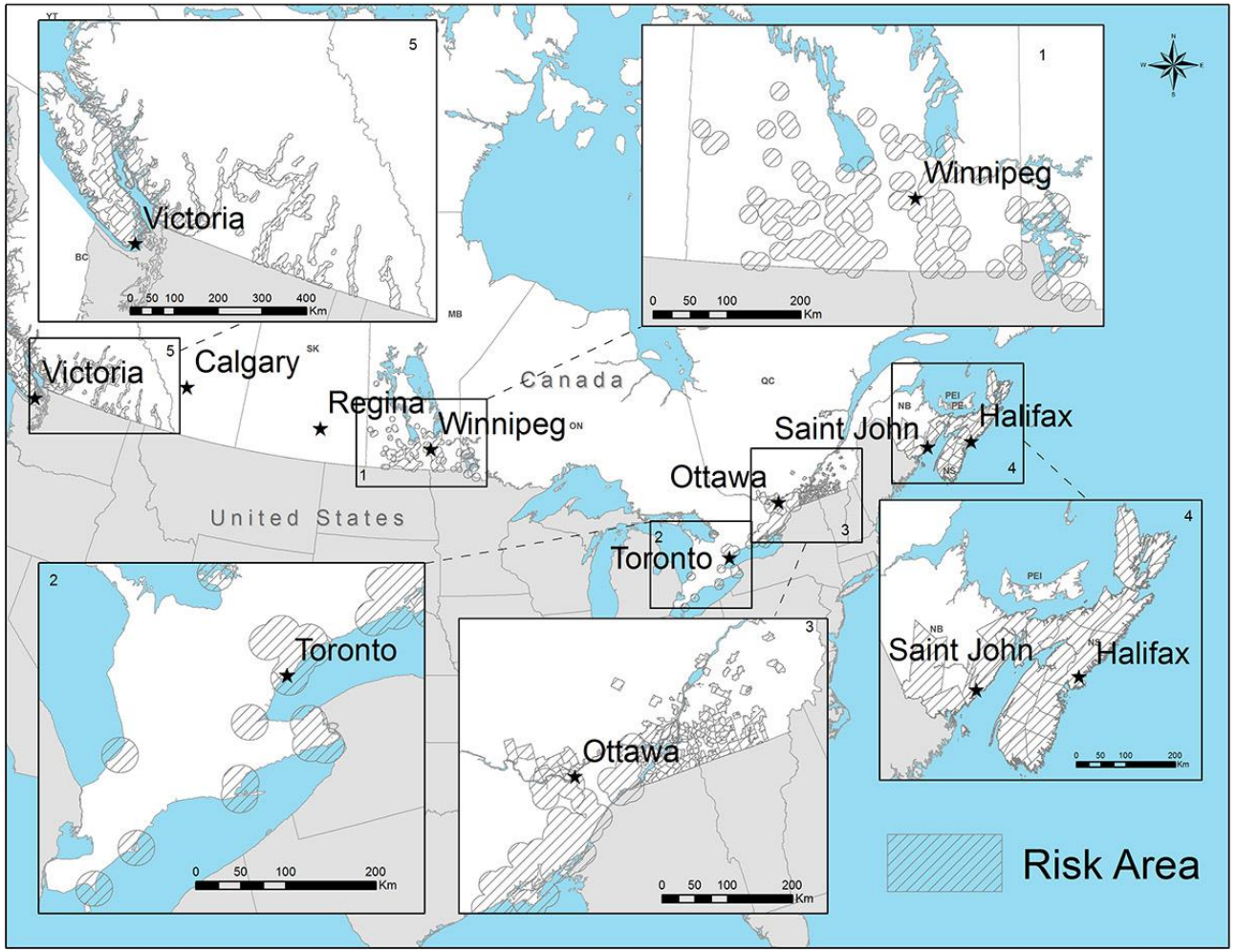
Blacklegged tick (*Ixodes scapularis)*



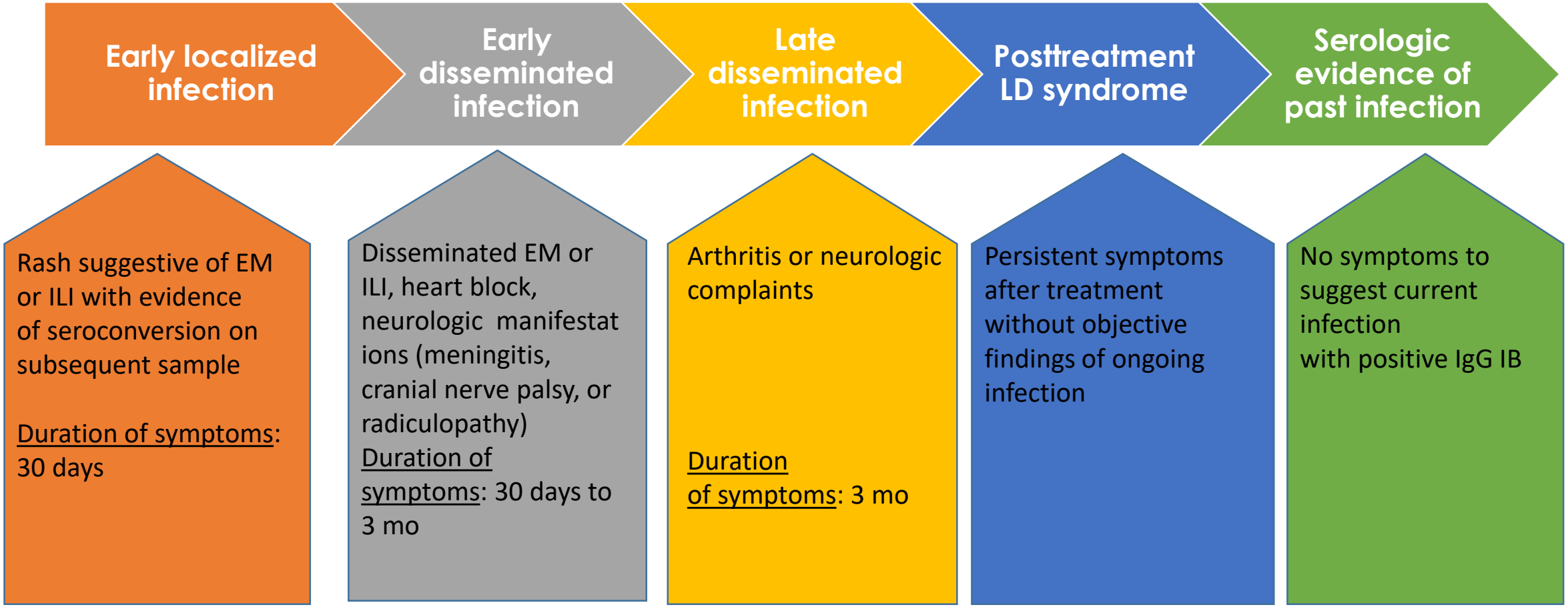
Questing

Lyme Disease is found in many areas in Canada

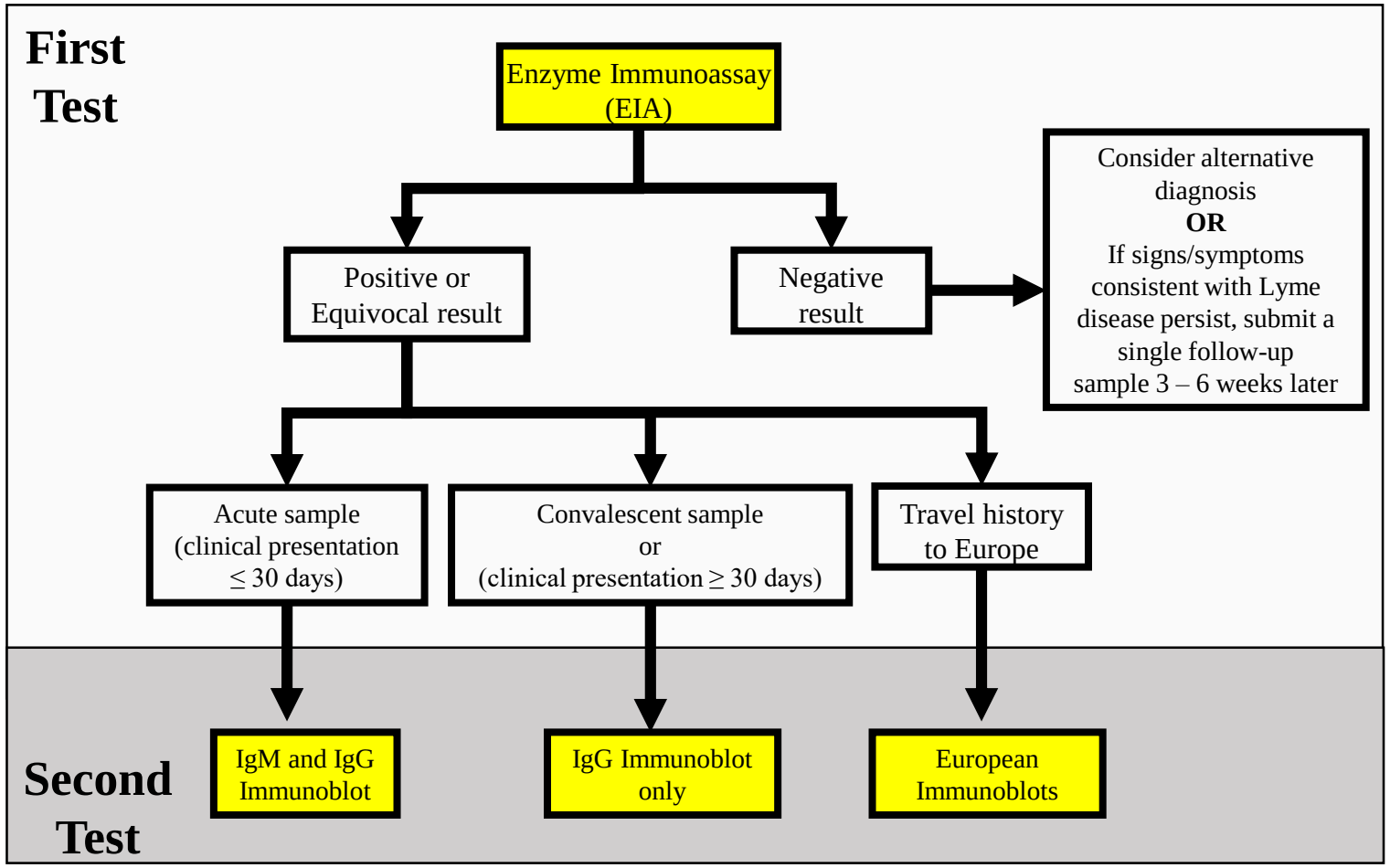
https://www2.gnb.ca/content/gnb/en/departments/ocmoh/cdc/content/vectorborne_andzoonotic/Tick-Borne_Diseases/risk.html



Lyme Disease – Clinical Stages



Current Lyme Disease Diagnostics "Standard Two Tier Testing Algorithm"



- Performance of testing depends on the stage of illness
  - early-poor ; late-good
- Diagnosis of earl localized infection is a predominantly clinical
- Western blots can not be used independent of EIAs
  - EIAs are quantitative, blots are subjective
  - IgM western blots have poor specificity. Only diagnostic if used in first 6 weeks of infection
  - European species can be falsely negative on NA WB – **Travel history is important**

Performance of Serology Depends on the Stage of Infection

Sample category (n^a)	No. (%) positive by method					
		WB[c]		Two-tiered testing		
	WCS EIA (Vidas)[b]	IgM	IgG	Vidas-WB IgM[d]	Vidas-WB IgG	Standard[e]
Lyme disease, total patients = 86						
Early Lyme disease with EM						
Acute phase (40)	27 (68)	14 (35)	8 (20)	12 (30)	8 (20)	16 (40)
Convalescent phase (38)	34 (89)	20 (53)	14 (37)	20 (53)	13 (34)	23 (61)
Early Lyme disease with Lyme neuroborreliosis or Lyme carditis						
Lyme neuroborreliosis (10)	9 (90)	10 (100)	3 (30)	9 (90.0)	3 (30)	9 (90)
Lyme carditis (7)	7 (100)	4 (57)	4 (57)	4 (57)	4 (57)	6 (86)
Late Lyme diseases						
Lyme arthritis (29)						29 (100)
Look-alike diseases, total patients = 144						
Fibromyalgia (31)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)
Severe periodontitis (20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Rheumatoid arthritis (21)	2 (10)	1 (5)	0 (0)	1 (5)	0 (0)	0 (0)
Syphilis (20)	17 (85)	2 (10)	0 (0)	2 (10)	0 (0)	1 (5)
Multiple sclerosis (22)	4 (18)	2 (9)	0 (0)	1 (5)	0 (0)	0 (0)
Infectious mononucleosis (30)	16 (53)	5 (17)	0 (0)	3 (10)	0 (0)	3 (10)
Healthy controls, total donors = 203						
Area of disease endemicity (101)	9 (9)	2 (2)	2 (2)	1 (1)	2 (2)	2 (2)
Area of disease nonendemicity (102)	5 (5)	2 (2)	0 (0)	0 (0)	0 (0)	0 (0)

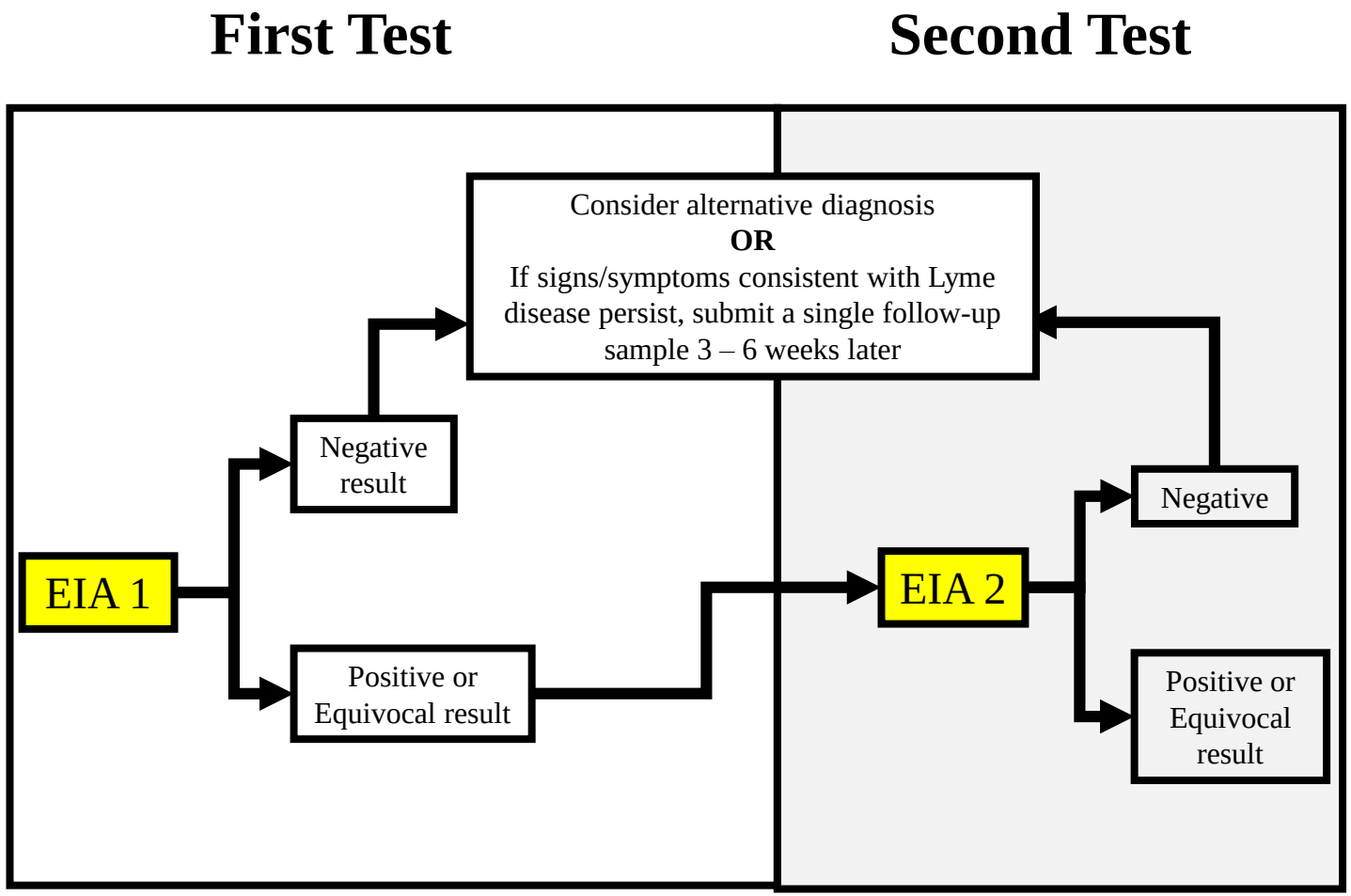
Poor sensitivity for early Lyme diagnosis

BUT……

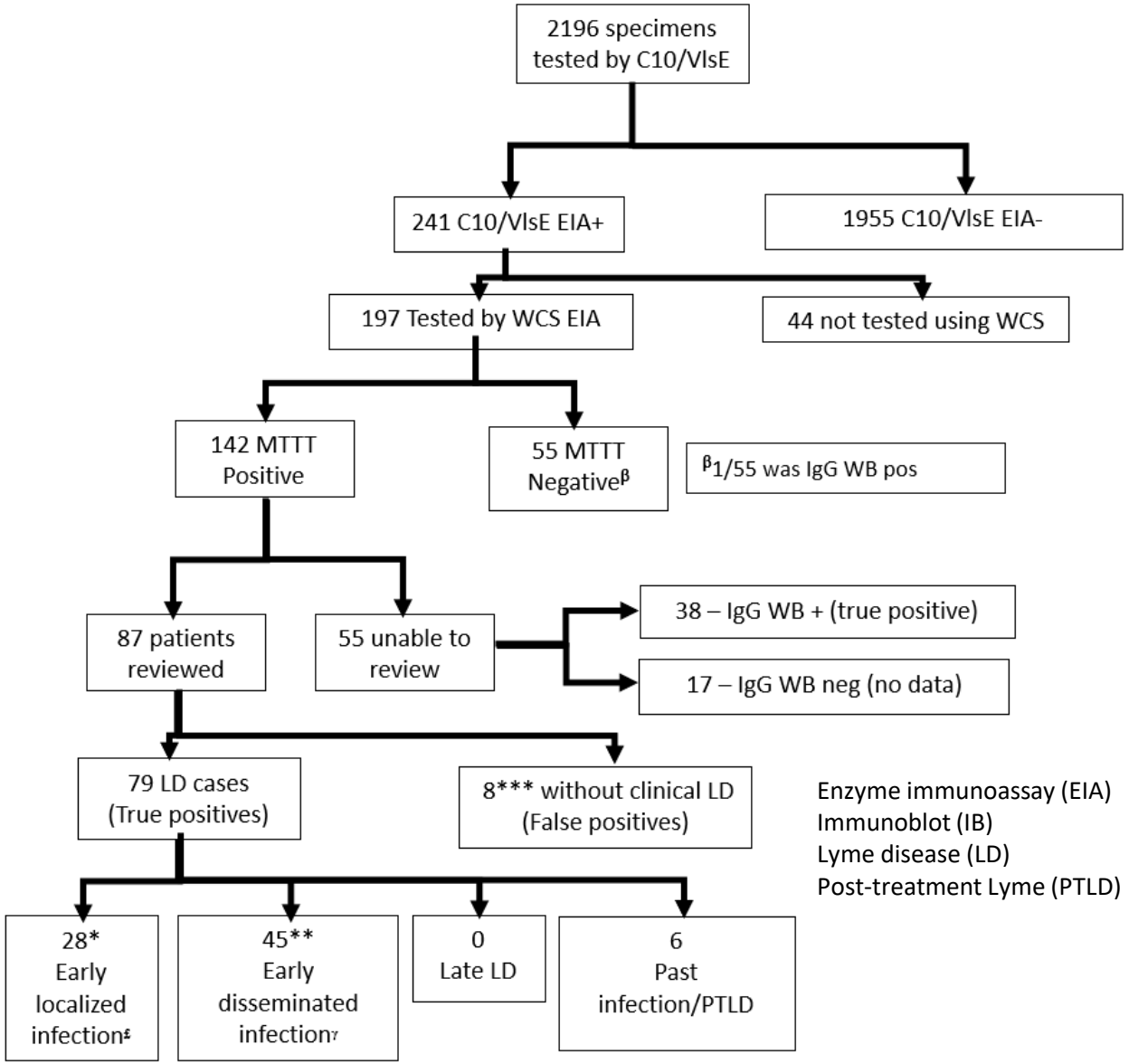
EXCELLENT SENSITIVITY FOR LATE DISEASE

Molins et al 2014 JCM 52:10

Enhancements to serologic testing
Modified Two Tier Testing (MTTT)



- US Data
- Increases sensitivity for early infection
  - 20-25%
- NS Validation Data
- Increases sensitivity for early infection
  - 20-25%
- Specificity – 99.6



- NS MTTT Validation Data
- increased sensitivity of 28% compared to the STTT algorithm
  - 20 of the 73 patients with clinical manifestations of early localized or early disseminated infection did not have a positive immunoblot (either IgM or IgG)
- specificity is 99.6% (99.2%-99.8%)
  - 8 of 2196 patients would be considered a false positive test

MTTT or STTT that is the question

Advantages

- Faster TAT
  - 2 EIAs could be done in-house rather than referral of IB to NML
  - May facilitate acute and convalescent testing for non-EM early localized LD
- Improved sensitivity in early disease (25% more early infections detected)
- US data suggests cost savings

Disadvantages

- Still need to treat patients with acute LD (EM rash) empirically as sensitivity of MTTT still well below 100% (like STTT)
- Can not differentiate between recent and past infections (like STTT)
- Impacts of MTTT on specificity in areas of low prevalence unclear
- Reduced specificity in patients with Lyme arthritis, may still need STTT for this cohort (as recommended in draft IDSA guidelines)

Alternative Methods can Lead to Spurious Results

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.

57% false positives

- Fallon et al., 2014. Clin Infect Dis 59(12):1705–10
- In-house laboratory criteria for a positive IgM WB at Specialty Laboratory B were ≥2 of the following bands: 23–25, 31, 34, 39, 41, 83/93. Criteria for a positive IgG WB were ≥2 of the following bands: 23–25, 31, 34, 39, 41, 83/93.

Lyme Disease - Diagnostic Challenges

- Poor performance of serology in early infection
- Seroconversion may not occur with early treatment
- No test of cure –
  - serology can persist for a decade
- Diagnosis of re-infection is a challenge
- Influence of biodiversity needs to be explored further
- No current diagnostic testing for PLDS

Questions?