

Vectorborne Disease Laboratory Tests

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Objectives

- Understand the role of diagnostic testing for surveillance of TBRD, Lyme, arboviral (LAC) encephalitis
- Gain familiarity with common diagnostic tests
- Differentiate clinical diagnosis from surveillance case definition
- Understand limitations of diagnostic tests used for surveillance

RMSF / SFR

- Most common vector-borne disease in NC
- Nationally, 46% of all RMSF cases come from NC¹
- However ≈ 95% of cases from NC and nationally are probable, based on one positive serologic test
- Additionally, NC cases tend to be much less severe¹
 - Due to enhanced detection and prompt treatment by NC HCPs?
 - Illness due to milder strains of rickettsiae which are detected by RMSF serologic tests?
 - Both?

RMSF / SFR

- Surveillance based on
 - **Clinical:** Any reported fever and one or more of the following: rash, eschar, headache, myalgia, anemia, thrombocytopenia, or any hepatic transaminase elevation
 - **Laboratory:** PCR, IHC, Serology (preferably PAIRED)
- A confirmed diagnosis cannot be made without supporting laboratory results. Cases diagnosed without laboratory findings do not meet the national surveillance case definition.

Lab Criteria for Diagnosis

- To enhance surveillance we need to receive more laboratory information
- Barriers to this include:
 - Lab information not essential to clinical management; **Treatment should proceed on clinical suspicion without waiting for lab results**
 - Convalescent serology not ordered once patient improves clinically

Laboratory Methods for RMSF Surveillance

- PCR and IHC – only useful if patients have rash and have not begun antibiotic therapy
- These methods **directly** identify the presence of the organism in affected tissues
- *Rickettsia rickettsii* infects endothelial cells, which leads to rash
- These tests can be run on a punch biopsy specimen collected from the rash site

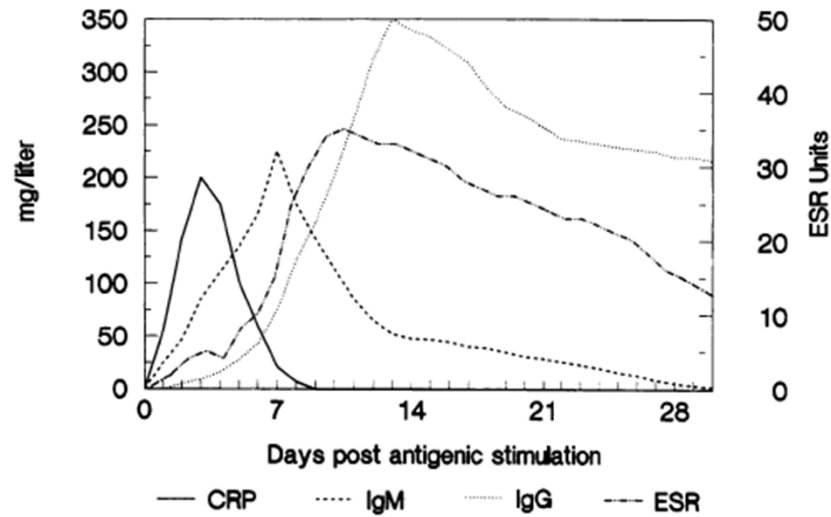
✓ **Treatment should be initiated on clinical suspicion;
do not wait for the results of diagnostic tests.**



Serologic Methods for RMSF Surveillance

- Immunofluorescent Assay (IFA) – **Indirect** measurement of presence of an organism
- Gold standard and preferred method for surveillance
- IgG IFA testing on paired serum specimens collected 2-4 weeks apart is ideal
- Fourfold rise in titer is confirmatory
 - e.g., 1:64 → 1:256

Nonspecific and specific immune responses²



INDIRECT IFA



Interpreting Rickettsia IgG IFA Results³

≥ 1:256	IgG serum endpoint titers of 1:256 and greater are considered presumptive evidence of recent or current infection by organisms of the appropriate Rickettsial antigen group
< 1:256 and ≥ 1:64	Single IgG serum endpoint titers ≥ 1:64 and < 1:256 are suggestive of infection at an undetermined time and may be indicative of either past infection or early response to a recent infection. The best serologic evidence of recent Rickettsial infection is a 4-fold or greater increase in IgG titer between 2 serum samples drawn 1 to 2 weeks apart and tested in parallel.
< 1:64	No antibody detected.

Group Specificity

Antibody reactivity to the *R. rickettsii* antigen should be considered Spotted Fever group reactive. Other organisms within the group include *R. akari*, *R. conorii*, *R. australis*, and *R. sibirica*. Infections by any of these species will induce the production of antibody reactive with *R. rickettsii*.

What about Rickettsia ELISA and IgM IFA?

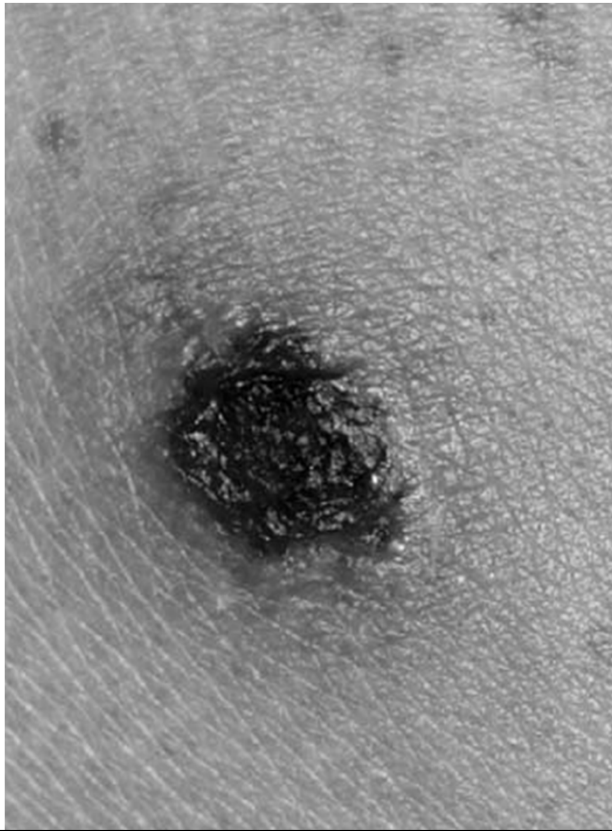
- Current commercially available ELISA tests are not quantitative, cannot be used to evaluate changes in antibody titer, and hence are not useful for serological confirmation.
- IgM tests are not strongly supported for use in serodiagnosis of acute disease, as the response may not be specific for the agent (resulting in false positives) and the IgM response may be persistent⁴

IFA Cross-Reactivity with other Rickettsia species

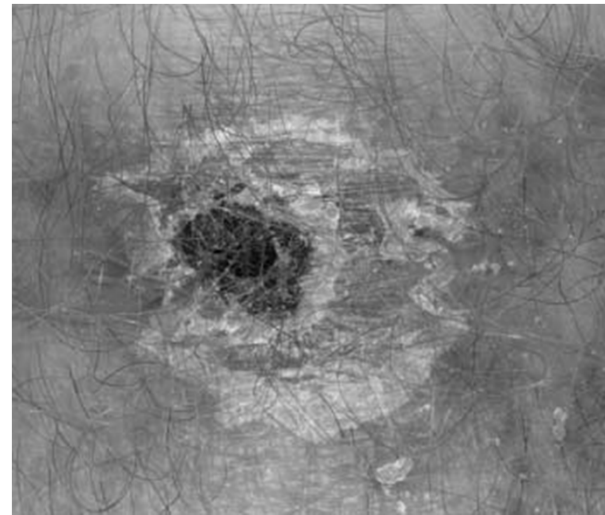
- Infection with other species, such as *R. parkeri* and *R. amblyomii*, appear to be less pathogenic, but present with generally similar symptoms.
- IFA serology cannot be reliably used for specific diagnosis due to cross-reactivity of rickettsial antigens resulting in group-specific rather than species-specific antibody production
- For this reason, the case definition for “RMSF” was changed to “SFR” in 2010.⁵

R. parkerii Illness⁶

Eschar following Lone Star Tick bite Texas patient. Punch biopsy IHC +, PCR -



Eschar following Lone Star Tick bite Texas patient. Punch biopsy IHC +, PCR +



Surveillance for Eschar-Associated SFR⁶

Assay	Optimal Specimen	Advantages	Limitations
IFA assay	Approximately 1 mL of serum or plasma, shipped at -20°C or 4°C	Commercial IFA assays widely available; results often more rapid relative to other diagnostic assays	Specimens obtained during the first 7-10 d of illness are often negative, paired samples (eg, to include a second sample collected ≥ 4 wk after acute illness) may be needed to confirm infection; IFA results indicate only infection with SFGR, and are not species specific
IHC testing ^b	0.4-cm punch biopsy specimen from central aspect of eschar, fixed in 10% formalin, or embedded in paraffin, shipped at room temperature	Demonstrates SFGR in context with the histopathologic features to confirm active infection; can be used to make a retrospective diagnosis from paraffin-embedded tissue months to years after active infection; results generally available more quickly than PCR or culture	Assay limited to specialized infectious disease laboratories, including CDC ^a ; IHC results indicate only infection with SFGR, and are not species specific
PCR assay ^b	0.4-cm punch biopsy specimen from central aspect of eschar, preferably shipped fresh, in sterile saline-moistened gauze at 4°C or -20°C; can also be attempted from paraffin-embedded tissue	Provides species-specific identity of <i>Rickettsia</i> species responsible for infection	Assay limited to specialized infectious disease laboratories, including CDC ^a ; results may take several weeks
Cell culture isolation ^b	0.4-cm punch biopsy specimen from central aspect of eschar, shipped fresh, in sterile saline-moistened gauze at 4°C	Criterion standard of diagnosis; provides species-specific identity of <i>Rickettsia</i> species responsible for infection	Assay limited to specialized infectious disease laboratories, including CDC ^a ; results may take several weeks

Ehrlichiosis and Anaplasmosis

- Surveillance requires laboratory and clinical components
- IFA IgG analysis is the most common laboratory method employed
- Four-fold rise in titer between acute and convalescent sera allows for confirmation of case with clinically compatible illness

Lyme Disease

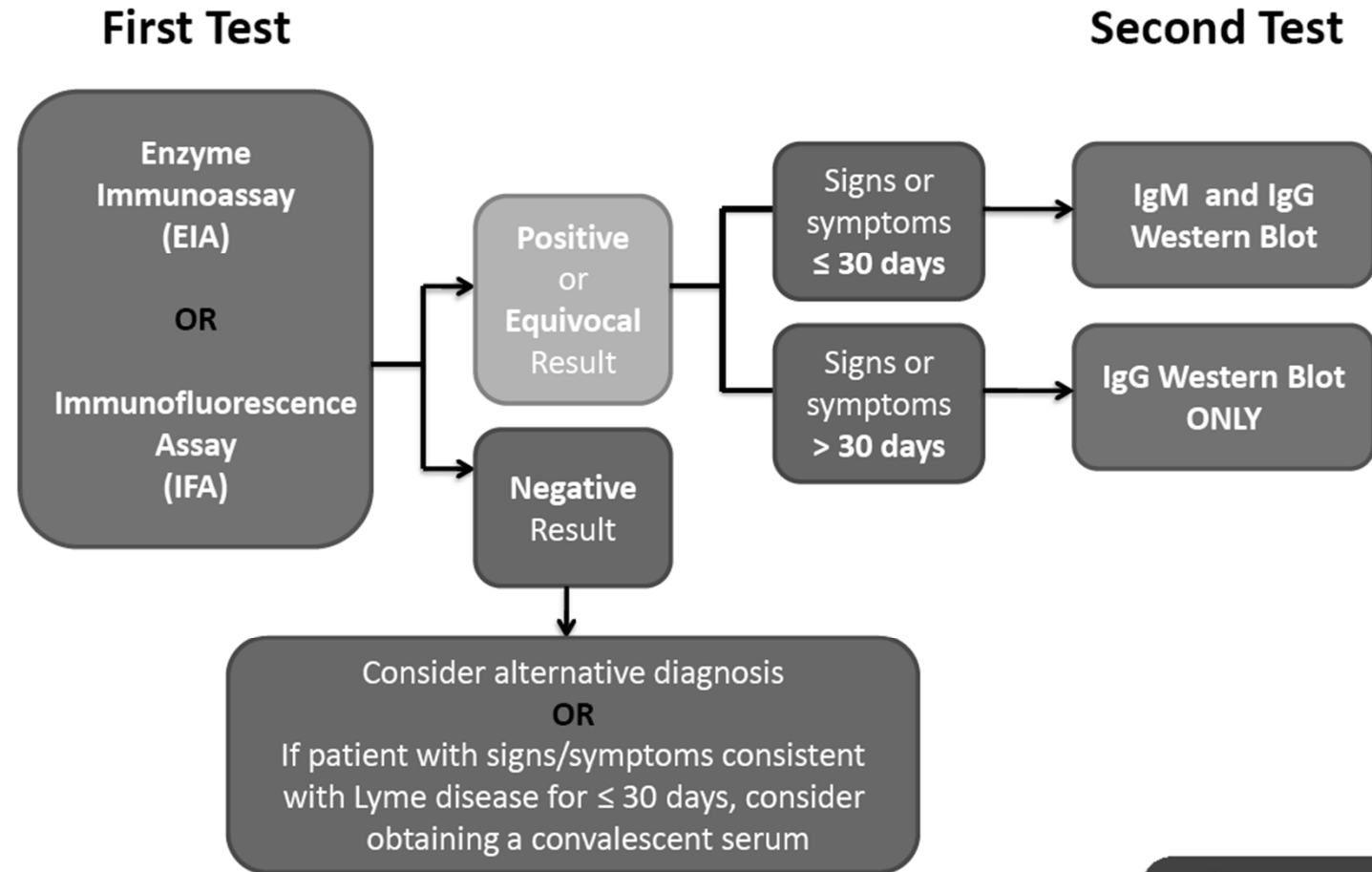
- The most common vector-borne disease in the U.S.
- Surveillance is complicated; similar to SFR: a combination of clinical and laboratory elements are required for confirmation of a case

Lyme Disease Surveillance

- Clinical Components:
 - EM skin lesion, or
 - Late Manifestations
 - Musculoskeletal
 - Cardiac
 - Nervous
- Laboratory Components:
 - Positive Culture for *B. burgdorferi*, or
 - Two-tier testing interpreted using established criteria , where:
 - Positive **IgM** is sufficient only when ≤ 30 days from symptom onset
 - Positive **IgG** is sufficient at any point during illness
 - Single-tier IgG immunoblot seropositivity using established criteria



Two-Tiered Testing for Lyme Disease



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



Lyme disease EIA → Tier One

www.youtube.com/watch?v=RRbuz3VQ100

Lyme disease WB → Tier Two

IgM: 2 of the 3 following bands must be present to be considered positive

24 kDa (OspC)

39 kDa (BmpA)

41 kDa (Fla)

A positive IgM immunoblot is only meaningful during the first 4 weeks of illness

By 4 - 6 weeks post-infection, the IgG WB is virtually always positive

IgG: 5 of the following 10 bands must be present to be considered positive

18 kDa

21 kDa (OspC)

28 kDa

30 kDa

39 kDa (BmpA)

41 kDa (Fla)

45 kDa

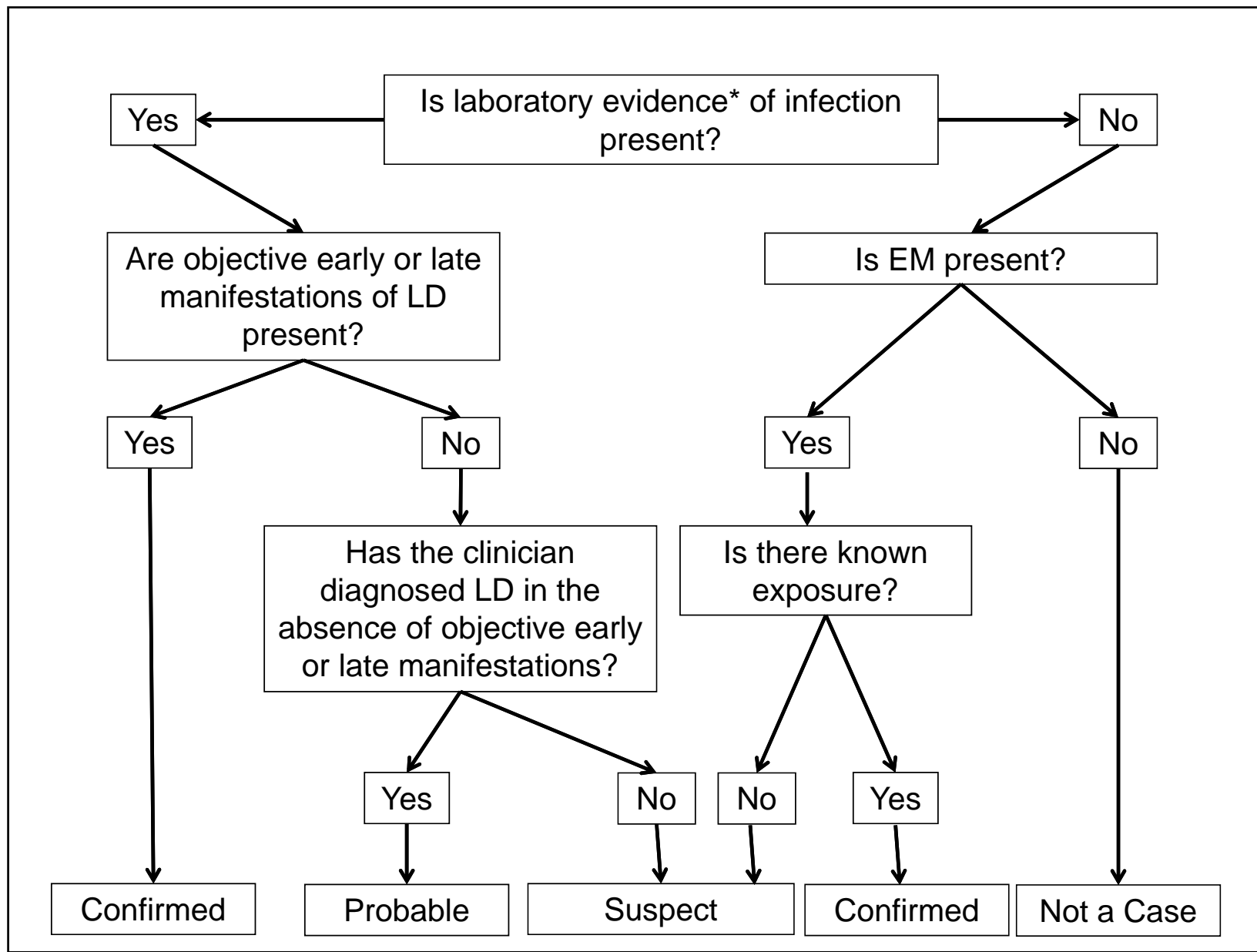
58 kDa (not GroEL)

66 kDa

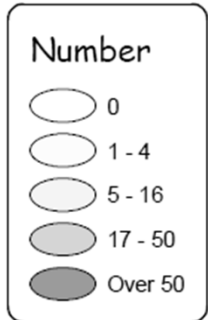
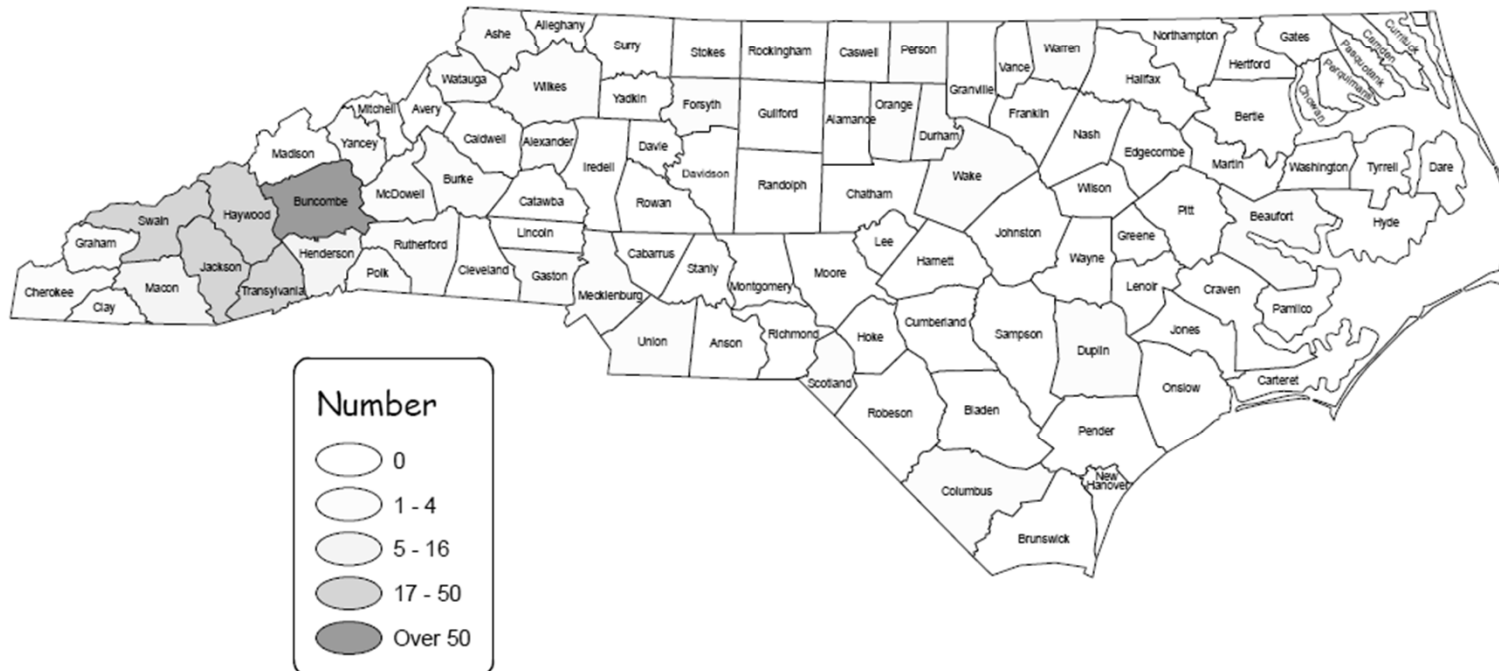
93 kDa

Note: PCR is NOT an Accepted Test for LD Surveillance

- *B. burgdorferi* initially disseminates from the site of an infected tick bite via the blood, but the bloodborne phase is relatively brief and the concentration of spirochetes is quite low.
- This test is not clinically useful for LD diagnosis.
- There are no PCR-based assays for the diagnosis of Lyme disease cleared by the US FDA.
- Two-tiered serology remains the mainstay of laboratory testing for Lyme disease
- See: www.medscape.com/viewarticle/764501 for more information



North Carolina Confirmed and Probable LaCrosse Encephalitis Cases 1998 - 2011



N = 212



LAC / Arboviral Encephalitis

Surveillance and Reporting

- Reporting should be etiology-specific (these six diseases are nationally notifiable to CDC):
 - St. Louis encephalitis
 - West Nile virus
 - Powassan virus
 - Eastern equine encephalitis
 - Western equine encephalitis
 - California serogroup virus
 - (includes infections with the following viruses: California encephalitis, Jamestown Canyon, Keystone, La Crosse, snowshoe hare, and trivittatus)

LAC / Arboviral Encephalitis

Surveillance and Reporting

- In N.C., only Neuroinvasive disease is reportable
- A combination of clinical and lab evidence is required
- EEE and WNV occur in N.C.
- LaCrosse Encephalitis is the most common arboviral infection in N.C.

LAC Encephalitis

Surveillance and Reporting

- **Clinical criteria**
 - **Fever** ($\geq 100.4^{\circ}\text{F}$ or 38°C) as reported by the patient or a health-care provider, AND
 - **Meningitis, encephalitis, acute flaccid paralysis**, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, AND
 - **Absence of a more likely clinical explanation**
- **Laboratory criteria**
 - Virus isolation or PCR
 - IFA: serum, four fold rise in titer
 - IgM ELISA: serum
 - PRNT for confirmation
 - IgM ELISA: CSF
 - EEE & WNV Negative for confirmation
 - Specific IgM does not cross the blood brain barrier and its presence in CSF is indicative of virus replication

Surveillance and Diagnosis

- Serology plays an important part in arbovirus diagnosis
 - IgM antibody capture EIA (MAC EIA) is the test of choice for rapid serodiagnosis based on a single serum sample and is of greater or equal value to HI and CF tests using paired acute and convalescent samples¹²

MAC ELISA is Sensitive

- The sensitivity of the MAC ELISA and the rapidity with which it can be performed appear to provide a powerful tool for the clinically relevant serodiagnosis of LAC virus infections in humans
- Study involving 20 patients confirmed to have LAC
 - 7/8 patients had positive specimens when sample collected on day of illness onset
 - 12/12 patients had positive specimens when sample collected one or more days after illness onset¹²

For Rickettsial Diseases we prefer IgG based tests
For Arboviral Diseases we prefer IgM based tests...

Why?

- Finding virus-specific IgM antibodies in CSF or a fourfold or greater change in virus-specific antibody titers between acute- and convalescent-phase serum specimens provides additional laboratory evidence that the arbovirus was the likely cause of the patient's recent illness
- Arboviral IgG antibodies can persist for many years following a symptomatic or asymptomatic infection. Therefore, the presence of these antibodies alone is only evidence of previous infection and clinically compatible cases with the presence of IgG, but not IgM, should be evaluated for other etiologic agents.

Early Diagnosis can Reduce Unnecessary Treatment¹³

The ability to diagnose La Crosse encephalitis helped to reduce the duration of treatment of presumed herpes simplex encephalitis with acyclovir or presumed bacterial meningitis with antibiotics

Common Limitations of Tests used for Surveillance & Diagnosis

- Poor sensitivity when IFA or EIA testing is ordered in the first 10 days – 3 weeks of symptom onset
 - Antibodies often not present at the time of clinical presentation
 - Sensitivity of IFA testing for RMSF increases to over 94% when ordered 14 – 21 days post infection⁷
 - Sensitivity of Two-Tier testing for Lyme increases significantly when ordered 14 – 28 days post infection⁸

continued...

Common Limitations of Tests used for Surveillance & Diagnosis *(continued)*

- Prompt antibiotic therapy, while necessary and appropriate for tick-borne infections, may suppress antibody production, at least with Lyme disease and RMSF^{9,10}
- Prompt therapy should NEVER be delayed even if it means surveillance may be negatively impacted
 - Patients with RMSF who received anti-rickettsial therapy within 5 days of the onset of symptoms were significantly less likely to die than were those who received treatment after the 5th day of illness (6.5% vs. 22.9%)¹¹

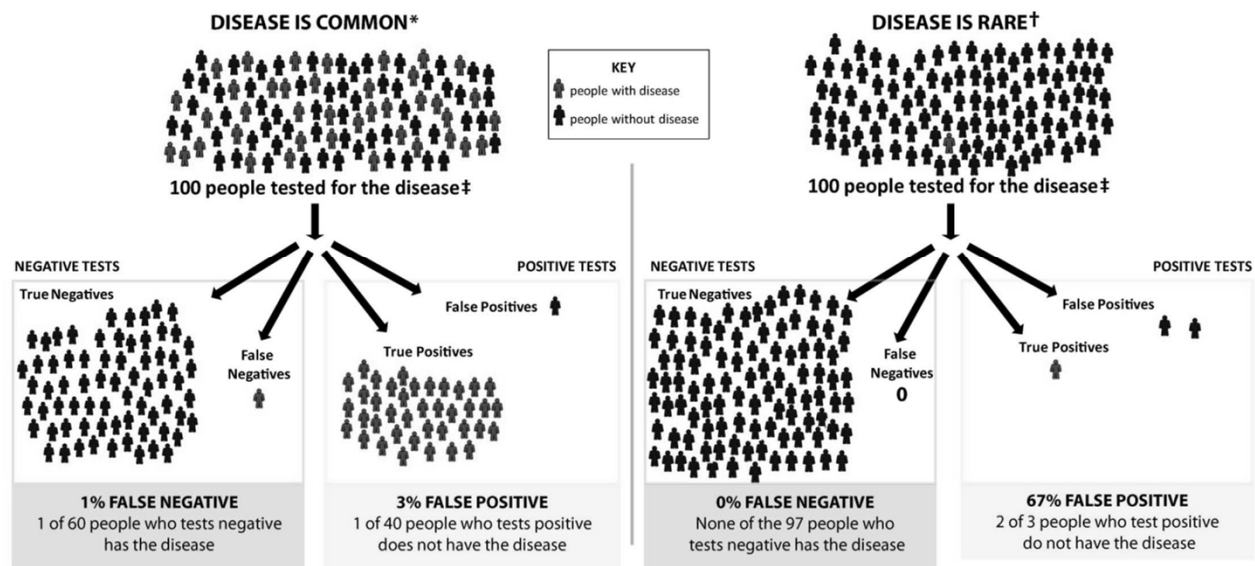
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Common Limitations of Tests used for Surveillance & Diagnosis *(continued)*

Understanding Test Results for Infectious Diseases
 Consider the likelihood of disease *before* performing laboratory testing

The likelihood that a patient has a disease depends on many factors:

- Has the patient been in an area where the disease is found?
- Does the patient have signs and symptoms typical of the disease?
- Does the patient have risk factors for contracting or developing the disease?



* 40 out of 100 patients in this area have the disease
 † 1 out of 100 patients in this area have the disease
 ‡ Test specificity = 98% (high) and test sensitivity = 98% (high)



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Additional LD Material

- Understanding the relationship of
 - Clinical manifestations
 - Diagnostic testing
 - Treatment

