



# Underutilization of Molecular Testing Contributes to Sub-Optimal Diagnosis of Ehrlichiosis and Poor Diagnostic Stewardship in a High-Incidence Area of North Carolina

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## Abstract

Cases of ehrlichiosis have been rapidly increasing in the United States. While serological testing has historically been the mainstay of laboratory diagnosis, this approach is fraught with pitfalls. Detection of *Ehrlichia* DNA via PCR has become more widely available, but often only through reference laboratories. Therefore, we sought to (i) assess diagnostic testing practices, (ii) quantify the proportion of samples eligible for PCR testing, and (iii) estimate the potential impact of PCR at an academic center in a high-incidence area. Overall, we found that the vast majority of patients did not undergo PCR testing, even as rates of serodiagnostic algorithm completion (i.e., testing of acute and convalescent samples) were low (18.4%). These findings show that there is a need to educate providers on the availability and the advantage of PCR testing. Furthermore, the relatively low proportion of individuals with fever supports recent changes to the clinical criteria used for surveillance.

## Background

- » 10-fold increase in ehrlichiosis cases since 2000
- » Frequent misdiagnosis, can have severe consequences (fatal if untreated)
- » Non-specific clinical presentation
- » Standard diagnostic: paired acute and convalescent serum samples using IFA to detect immunoglobulin G antibodies.
- » Serodiagnosis has many shortcomings:
  - » No detectable levels of IgG → inconclusive negative acute titer
  - » IgG levels from prior infection → unreliable positive acute titer
  - » Time consuming: two titers taken weeks apart, ≥ 4-fold increase to confirm diagnosis
  - » Incomplete diagnostic algorithm: few patients return for convalescent testing
  - » Diagnostic shortcomings cause clinicians to make treatment decisions without objective evidence
- » PCR detects *Ehrlichia* DNA with high sensitivity and specificity
  - » Eliminates need for convalescent specimens to confirm diagnosis

## Objectives

In preparation for implementation of a laboratory developed Ehrlichia PCR, we sought to...

1. Assess current diagnostic testing practices
2. Quantify the proportion of serologically tested patients that would be eligible for PCR testing
3. Estimate the potential impact of PCR on the diagnosis and management of ehrlichiosis at UNC

## Methods

- » Retrospective chart review of those tested for *Ehrlichia* over a 12-month period via IFA, at UNC McLendon Clinical Laboratories, or via PCR, as a “send out” test to Mayo Clinic
- Stratified patients by 3 criteria:
  1. Test appropriateness
    - » “Appropriately tested” = met 2008 CSTE clinical criteria of fever + one other symptom
  2. PCR eligibility
    - » Antibiotics rapidly clear *Ehrlichia* DNA from blood
    - » Eligible = patients prescribed antibiotics day of or after acute titer taken
  3. Epidemiological Case Classification
    - » Threshold for positive serological test: ≥1:64 IgG titer
    - » Confirmed: ≥4-fold titer increase or positive PCR and clinical criteria met
    - » Probable: 1+ positive serological test, clinical criteria met
    - » Suspect: 1+ positive serological test, clinical criteria not met

## Results

Figure 1. Study classification schema

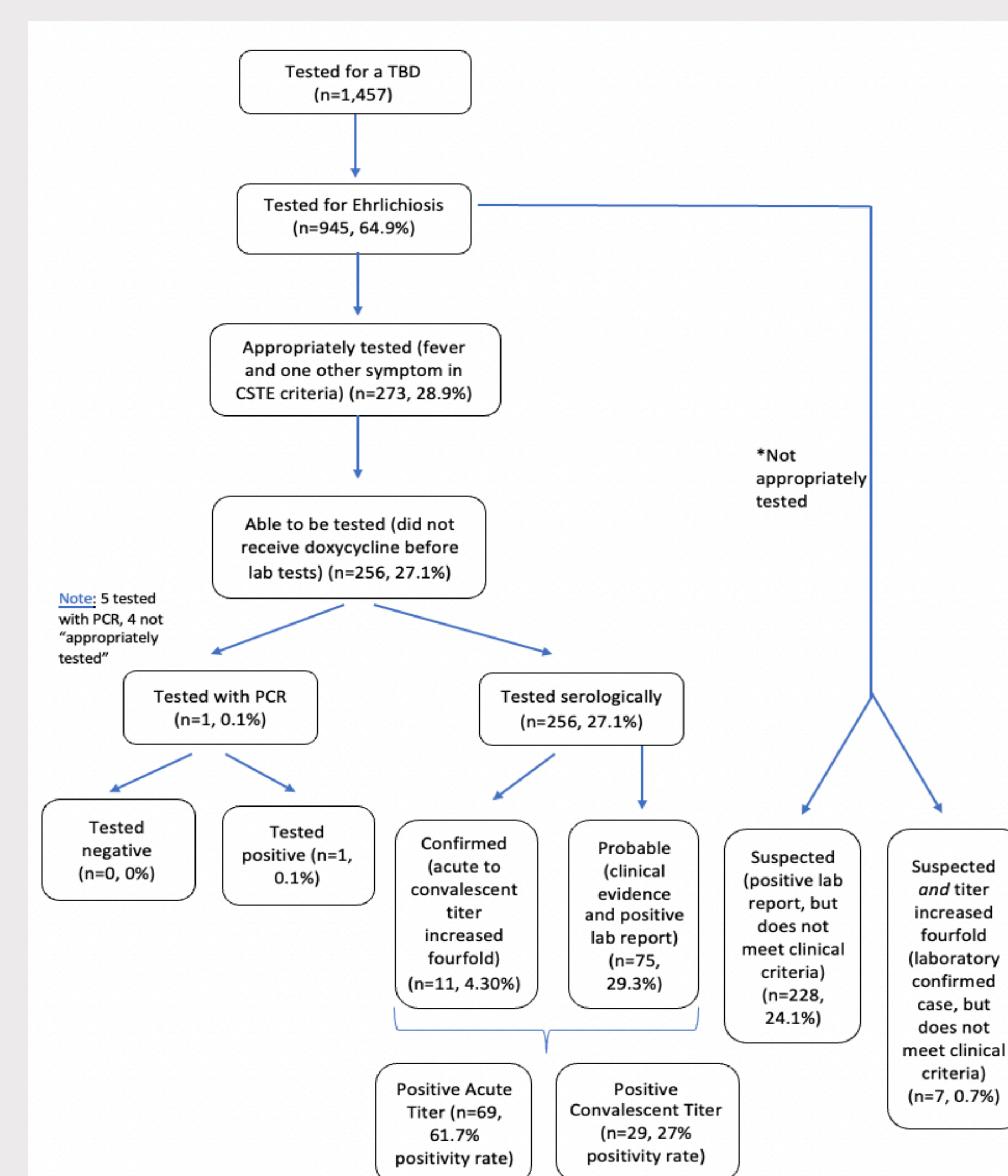


Table 1. Clinical Symptoms Among Individuals Tested For Tick-Borne Disease at Acute Visit Within the UNC Health System, March 24, 2022 – April 14, 2023

Clinical Symptoms of a TBD	Patient No. (%)
Meet Clinical Criteria	273 (28.9)
Fever + Headache	151 (55.3)
Fever + Myalgia	152 (55.7)
Fever + Anemia	92 (33.7)
Fever + Thrombocytopenia <sup>1</sup>	65 (23.8)
Fever + Hepatic Transaminase Elevation <sup>2</sup>	78 (28.6)
Fever + Leukopenia <sup>3</sup>	42 (15.4)
Bite Noticed in Two Weeks Prior	77 (28.3)
Does Not Meet Clinical Criteria	672 (71.1)
Fever	14 (2.1)
Headache	145 (21.6)
Myalgia	168 (25)
Anemia	50 (7.3)
Thrombocytopenia	28 (4.2)
Hepatic Transaminase Elevation	47 (7)
Leukopenia	26 (3.9)
Bite Noticed in Two Weeks Prior	205 (30.5)
No Symptoms or Bite Noticed	69 (10.3)

<sup>1</sup> Platelets < 150  
<sup>2</sup> AST or ALT > 145  
<sup>3</sup> WBC < 4.0

## Discussion

- » Reliance on serological testing, despite low rates of paired testing (18.4%, 47/256)
- » Convalescent testing needed to interpret serological results accurately
  - » 61.7% vs. 27.0% : acute vs. convalescent positivity rate
  - » Differing values in over half paired samples
  - » Patients with ehrlichiosis go undetected, untreated
- » The number of patients for whom testing was ordered, but were not empirically treated with doxycycline is concerning. With sufficient suspicion to order testing, patients should have received empirical treatment, as without a convalescent draw an acute titer can not be used to diagnose or rule out ehrlichiosis.
- » With improvements in the turn-around time of PCR, positive results can offer a more timely confirmation of diagnoses and ensure appropriate treatment plan.
- » Why is *Ehrlichia* PCR underutilized?
  - » Lack of capability or resources to perform test
  - » Time for send-out PCR tests (~ 6.2 days)
  - » Lack of awareness of availability or that PCR is preferred for acute diagnosis
- » For optimal implementation of *Ehrlichia* PCR, providers must be educated on the use of PCR to confirm ehrlichiosis cases and testing needs to be performed in-house with rapid turnaround time.
- » Low proportion of “appropriately tested” individuals supports recent changes to CSTE criteria, which eliminated fever as necessary to meet clinical criteria for cases with confirmatory laboratory evidence
  - » Similar positivity rates for not “appropriately tested” individuals suggests fever is not always a symptom of ehrlichiosis

Table 2. Clinical Symptoms and Titer of Individuals Confirmed for Ehrlichiosis Within UNC Health System, March 24 2022 - April 14, 2023

Study ID	Clinical Symptoms					Serological Testing				
	Fever	Headache	Myalgia	Anemia	Thrombocytopenia	Hepatic Transaminase Elevation	Leukopenia	Acute Ehrlichia IgG titer	Convalescent Ehrlichia IgG titer	Days Between Testing
548	X	X	N/A	X				≥1:64	1:256	19
549	X			X	X	X		1:128	1:1024	30
540	X	X		X		X		1:128	1:1024	18
765	X	X		X				<1:64	1:128	17
995	X	X	X	X	X			<1:64	1:1024	19
951	X	X		X	X	X	X	≥1:64	1:1024	22
1278	X		X		X			1:128	1:512	16
1397	X		X			X		<1:64	1:128	29
1339	X		X		X	X		<1:64	1:128	29
1370	X			X	X		X	1:128	1:512	24
1466	X			X	X			<1:64	1:512	21
1655	X	X	X		X	X	X	<1:64	1:128	8

- » 18.4% (47/256) underwent convalescent testing
- » 55.3% (26/47) patients with paired titers had differing values
  - » 10.6% (5/47) reverted from a positive to a negative titer
  - » 12.8% (6/47) seroconverted seroconverted (i.e., non-reactive to ≥1:64), representing half of confirmed cases (Table 2)
  - » 19.1% (9/47) had not change in titer
- » 33.9% (228/673) not “appropriately tested” patients had 1+ positive *Ehrlichia* test
  - » 28.7% (193/673) with positive acute titer
  - » 13.4% (90/673) with positive convalescent titer
  - » 7 with ≥ 4-fold increase between titers
- » Median of 22 days between tests (IQR 17-32)
- » 38.8% of eligible patients had a negative acute *Ehrlichia* IgG titer and did not return for a convalescent draw, but were put on antibiotics.
- » 22.7% of eligible patients had a negative acute *Ehrlichia* IgG titer and did not return for a convalescent test, but did not receive antibiotics.