

What we learned from testing ticks – Multipathogen study in European ticks

*Tanja Mijatovic, PhD
R.E.D. Laboratories CSO &
Lab Responsible*

tmijatovic@redlabs.be

TICK-BORNE INFECTIONS

Vector-borne infections are increasing globally:
Lyme disease is among the most prevalent vector borne infection in the U.S. and Europe and is reaching epidemic levels (Kugeler et al. 2015; Sykes et al. 2014).

The most known, and most investigating concern borreliosis, bartonellosis, babesiosis, rickettsiosis and anaplasmosis. Many other pathogens can worsen the clinical picture and further complicate differential diagnosis.

- Tick-borne diseases, which afflict humans and other animals, are **caused by infectious agents transmitted by tick bites.**
- Tick-borne illnesses are caused by infection with a variety of pathogens.
- ➔ **Whenever possible, get the tick tested for a wide array of pathogens**



Polymicrobial Nature of Tick-borne Infections

➤ mBio. 2019 Sep 10;10(5):e02055-19. doi: 10.1128/mBio.02055-19.

Polymicrobial Nature of Tick-Borne Diseases

Santiago Sanchez-Vicente ¹, Teresa Tagliafierro ², James L Coleman ¹, Jorge L Benach ³, Rafal Tokarz ⁴

- Tick-borne diseases have increased in prevalence in the United States and abroad.
- The reasons for these increases are multifactorial, but **climate change** is likely to be a major factor. One of the main features of the increase is the geographic expansion of tick vectors
- The clinical spectrum of tick-borne diseases can range from asymptomatic to fatal infections, with a disproportionate incidence in children and the elderly.
- **Polymicrobial infection has important consequences for the diagnosis and management of tick-borne diseases.**

Importance of Testing the Ticks

Review > Vet Clin North Am Small Anim Pract. 2022 Nov;52(6):1153-1161.

doi: 10.1016/j.cvsm.2022.06.005.

How Changing Tick-Borne Disease Prevalence in Dogs Affects Diagnostic Testing

Linda Kidd ¹

Affiliations + expand

PMID: 36336415 DOI: [10.1016/j.cvsm.2022.06.005](https://doi.org/10.1016/j.cvsm.2022.06.005)

Abstract

Ixodes scapularis (the deer tick), *Amblyomma americanum* (the lone star tick) and *Rhipicephalus sanguineus* (the brown dog tick) are ticks that commonly parasitize dogs in the United States. In the first part of this article, we will examine their changing epidemiology to illustrate how being aware of their distribution and adapting diagnostic testing to include a broad range of pathogens may improve our ability to identify and help infected patients, especially those with suspected idiopathic immune-mediated disease. We will then discuss how to optimize testing for these pathogens using available panels.

Testing for Tick-borne infections

- Diagnosing Lyme and TBI-related diseases is extremely **challenging**.
- TBI diagnosis **complications** are a result of inadequate testing, mainly focusing on markers for the disease's early stages.
- TBD cases are **commonly misdiagnosed with other illnesses** and even when a proper diagnosis is made, it's often difficult to verify because accurate testing isn't always available.
- Very few tests for Tick-borne diseases (TBD) are approved for clinical diagnosis, thus most of available testing options are “investigational” or “research” tests, aiming to **help the assessment of patients** with Lyme-like complaints.
- **The overall high failure rate of TBI-related testing underscores the necessity for novel approaches**

Phelix Phage Test: Cooperation between Academia, Specialized Testing Lab and Medical specialists

- R&D on this novel approach started in 2015 :
 - Louis Teulières MD, PhD: *Infectious and immune diseases consultation (Paris & Lisbon); PhelixRD Charity (chronic infections and bacteriophages research group)*
 - Jinyu Shan PhD : *Department of Infection, Immunity, and Inflammation, University of Leicester, UK*
 - Martha Clokie PhD, Pr, Head: *Department of Infection, Immunity, and Inflammation, University of Leicester, UK*
- 2018: Technology transfer phase between Leicester University and R.E.D. Laboratories
- Commercialisation started in 2019 with R.E.D. Laboratories, BE
- Ongoing cooperation between Leicester University, Phelix Charity and R.E.D. Laboratories on further Phage-tests development and improvement



UNIVERSITY OF
LEICESTER



Patent WO2018083491A1

Peer-reviewed publication by Shan et al, Frontiers in Microbiology 2021:

<https://www.frontiersin.org/articles/10.3389/fmicb.2021.651217/full>

Novel Testing Approaches : Phage-based Test

The importance of novel testing approaches

The overall high failure rate of tick-borne infection (TBI)-related testing underscores the necessity for novel approaches, i.e. not relying on serology and two-tier testing.

- Phelix Phage Borrelia detection method (Patent WO2018083491A1) consists of targeting the presence of outnumbered prophages part of the **bacteria lysogenic cycle**.
- This very novel concept is that we are no longer looking for the bacteria but for its specific bacteriophage, which must find the bacteria to survive.
- The genetic material of the **bacteriophage** is therefore **specific to the bacteria with which it is associated**.
- The test will search for the presence of specific bacteriophages as witnesses of a specific Borrelia infection.
- Phages come along with the bacteria (**they are transmitted with the bacteria during the tick bite**), and can survive only if they have their specific bacterial host.
- Bacteriophages are present only on active bacterial infections; hence a phage-based test is a direct proof of an active infection.

Phelix Phage Borrelia Test



Manual DNA
Phenol /
Chloroform
extraction

QCs

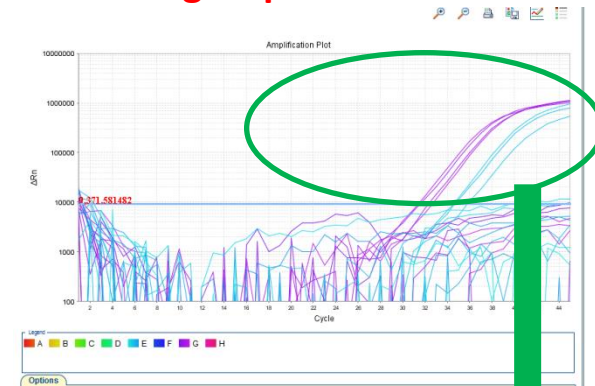
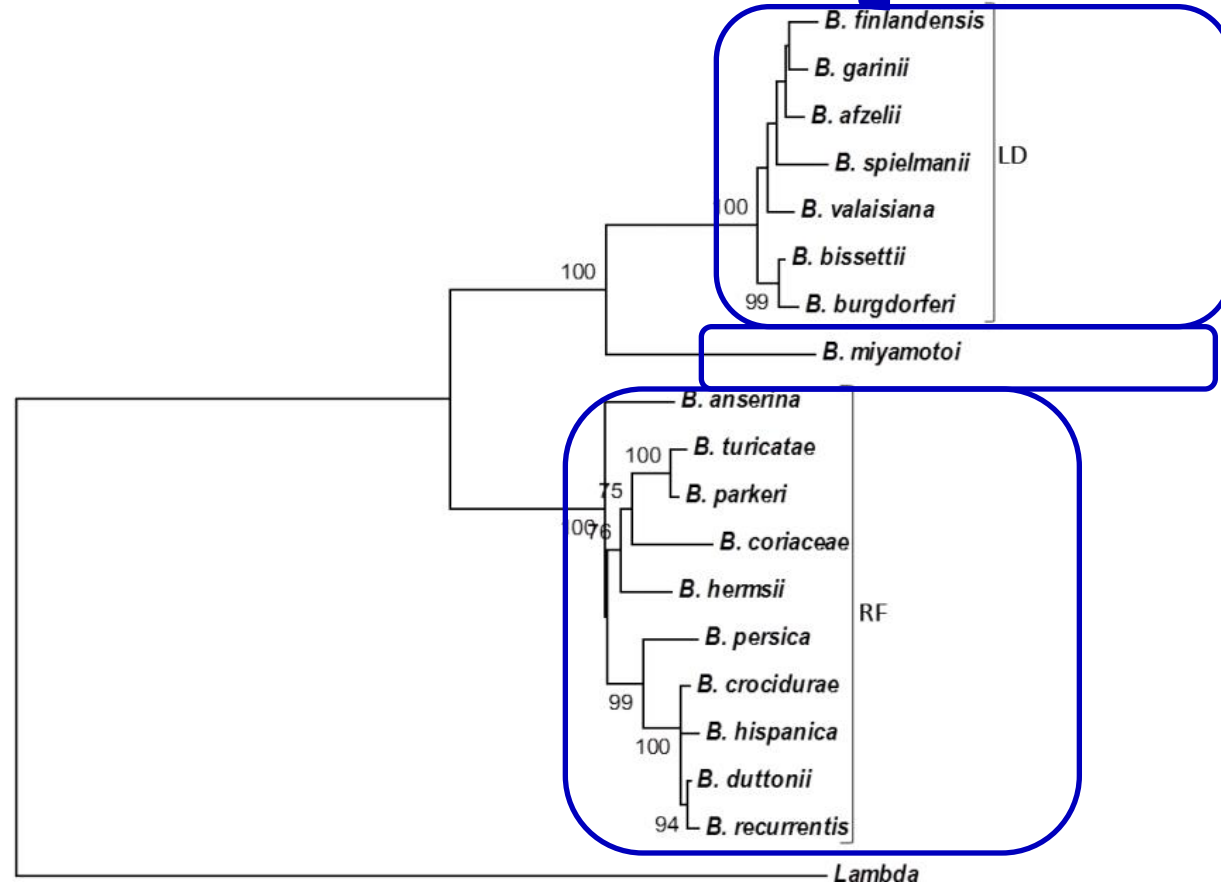
Extraction control PCR
quality of extracted DNA

IAC control PCR
*absence of PCR
inhibitors*

applied
biosystems
by ThermoFisher Scientific



Each sample tested in 3
replicates for 7 different real-
time PCRs, targeting all 3
Borrelia groups



Confirmatory sequencing
for positive-like samples

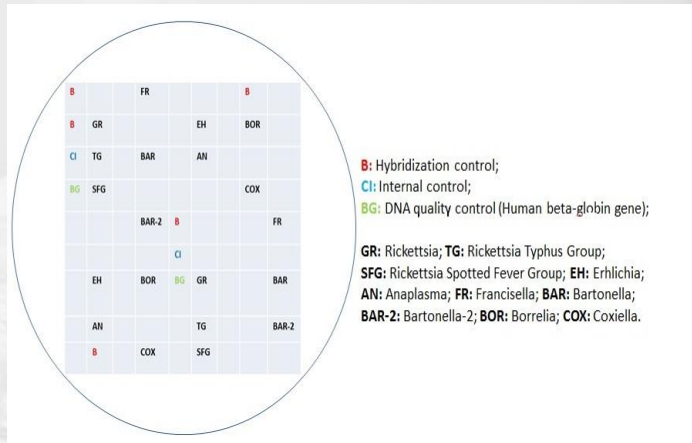
Methods Used For Testing The Ticks

- Whole ticks were mechanically grinded with glass beads and DNA extracted by Phenol-Chloroform method.
- Extracted DNA was analyzed by 2 novel methods, in addition to classic PCRs:

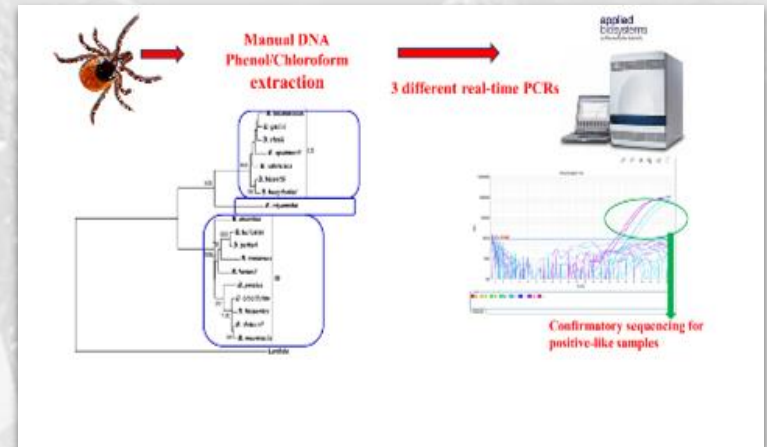
HYBRISPOT TICK-BORNE BACTERIA FLOW CHIP

Simultaneous detection of 7 tick-borne bacteria genera:

Rickettsia
 Ehrlichia
 Anaplasma
 Francisella
 Bartonella
 Borrelia
 Coxiella



PHELIX PHAGE BORRELIA TEST



Results of the Tests Done on Ticks

110 analyzed ticks (BE, FR, NO)

- 75% of analysed ticks were positive for at least 1 pathogen
- 32% of analysed ticks were positive for at least 2 pathogens

	Phage Method		
	B.burgd sl	B. miya	B. RelapsFev
% positive ticks	10	30	42
		14	

	Hybrispot						
	Anaplasma	Bartonella	B. burgd sl	Coxiella	Ehrlichia	Francisella	Rickettsia
% positive ticks	2	9	10	-	3	-	10

	CE IVD qPCR		PCR
	B. burgd sl	Bartonella	Babesia
% positive ticks	2	-	4

Discussion of the Results Obtained on Ticks

110 analyzed ticks (BE, FR, No)

- Only 25% of analysed ticks were negative
- 75% of analysed ticks were positive for at least 1 pathogen
- 32% of analysed ticks were positive for at least 2 pathogens
- none of 3 different methods used evidenced more than 10% of ticks positive for *B. burgdorferi* sI
- 70% of analysed ticks were positive for the strains of the Borrelia Relapsing Fever group (*B. miyamotoi*, *B. hermsii*, etc)
- Seen this high prevalence of Borrelia RF in tested ticks, further supported by similar high percentages found in patients using Phage method, the overall high expansion of undiagnosed TBD cases worldwide might be linked to the screening choice focusing only on *B. burgdorferi* sI and only rarely testing for RF while the later ones seem to be much more prevalent.

Why Taking Phelix Phage Test

- Unlike serology, this **test does not rely on the development of antibodies**, which can take several weeks, and not all patients are able to develop them at all.
- It is the best choice for the **early detection** (antibodies need several weeks to show up....)
- It is more sensitive than conventional *Borrelia* PCR tests that are frequently negative due to low bacterial concentration in the blood.
- Bacteriophages are circulating in the blood, thus the phage method is **suitable for late stage patients**
- It's a **DIRECT test**, in contrast to all existing indirect tests (ELISA, Western BLOT, LTT/ELISPOT tests), evidencing an ongoing bacterial presence
- The overall high expansion of undiagnosed Lyme disease cases worldwide might be linked to the screening choice focusing only on *B. burgdorferi sl* and only rarely testing for *B. relapsing fever* and *B. miyamotoi* while they seem to be much more prevalent, according to our data obtained both on human and tick samples.
- **➔ The utility of the Phage test in a clinical practice : ILADS 2023 : Dr Hope A. McIntyre (Persistent Lyme or Tick-borne Relapsing fever – Could we be studying the wrong infection?)**

Dr Hope A. McIntyre

ILADS 2023 presentation

- I began ordering the RED lab phage borrelia test in my practice in early 202
- A study by the Hopkins Lyme group showed that blood PCR testing for Lyme disease is not that helpful. It does not give any advantage over serologic testing alone in making a diagnosis of Lyme disease
- Since 2021 I tested 74 chronic Lyme/tick borne disease patients with the RED test of which 28 (38%) were positive
 - 16 (22%) were positive for borrelia miyamotoi
 - 13 (18%) were positive for one of the other TBRF group borrelias (B. hermsii, Parkeri or turicatae)
 - 4(5%) had both b miyamotoi and another TBRF borrelia
 - Only 1 patient was positive for Lyme disease, and that patient was from Ukraine, and was positive for European Lyme, both B. Afzelii and B. Garinii.
 - No patients were positive for borreliella burgdorferi

Information obtained from Dr McIntyre

Remember

- Vector-borne infections are increasing globally and there is a lack of accurate testing.
- The overall high expansion of undiagnosed *Borrelia*-related cases worldwide might be linked to the screening choice focusing only on *B. burgdorferi* *sl* and only rarely testing for *B. relapsing fever* and *B. miyamotoi* while they seem to be much more prevalent, according to our data obtained both on human and tick samples.
 - 32% of analysed ticks were positive for at least 2 pathogens
 - none of 3 different methods used evidenced more than 10% of ticks positive for *B. burgdorferi* *sl*
 - 70% of analysed ticks were positive for the strains of the *Borrelia Relapsing Fever* group (*B. miyamotoi*, *B. hermsii*, etc)
- **Whenever possible, get the tick tested!**
- **If a test is not looking for a given pathogen, it is unlikely that it will be detected → enlarge the list of tested targets**

Questions and Contacts

- Check regularly our **website** (www.redlabs.com) for the updates
- News and Blogs : <https://blog.redlaboratories.be/p/news.html>



- **Questions and contact:**
 - General queries, logistics : E-mail to info@redlabs.be
 - Scientific questions : E-mail to tmijatovic@redlabs.be