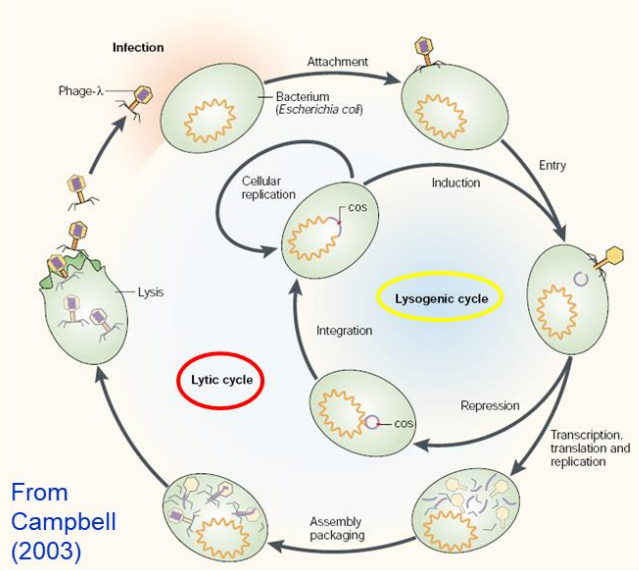
BORRELIA PHELIX PHAGE TEST:

A Breakthrough way to detect Borrelia-related infection,

and an answer to non-diagnosed ill people

What are bacteriophages?

Phages belong to the simplest and most primitive form of life (viruses). They are extraordinarily specific for the bacterial host that they infect to propagate. They can rapidly infect their host and insert its genetic material into their host. As a result, they produce large numbers of copies that can further infect (and in some specific cases decimate) the bacteria causing the infection. They can also make the bacteria vulnerable to classic treatment by altering their genetic material.



•The **lysogenic cycle**: The phage *infects* a bacterium and inserts its DNA into the bacterial chromosome, allowing the phage DNA (now called a **prophage**) to be copied and passed on along with the cell's own DNA.

•The **lytic cycle**: The phage infects a bacterium, hijacks the bacterium to make lots of phages, and then kills the cell by making it explode (*lyse*).

Bacteriophages are ubiquitous and are part of the natural 'ecosystem' of the bacteria living and replication cycles. We have decided to focus on bacteriophages as a target for direct detection of an infection, as they are specific and outnumber the pathogen bacterial population.

Why to use bacteriophages as a diagnostic tool?

Tick-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). While most ticks have the capacity to transmit a number of pathogens that cause human disease, Lyme disease is the most widely known tick-borne disease and is caused by bacteria of the genus *Borrelia*; typically *Borrelia burgdorferi* which are gram-negative spirochete bacteria. *Borrelia* are divided into "genospecies"; the most common include *B. burgdorferi sensu stricto, B. garinii, B. afzelii, B. spielmanii, B. bavariensis* and the newly identified *B. miyamotoi*. 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.

PCR-based assays are being used more often in clinical settings, and they have several distinct advantages over serology. They detect the presence of infectious agents directly. Unlike serology, they don't rely on the development of antibodies, which can take several weeks, and not all patients are able to develop them at all. Nevertheless, PCR testing has limitations as well. The current bottleneck in PCRs detecting of *Borrelia* is their low sensitivity.

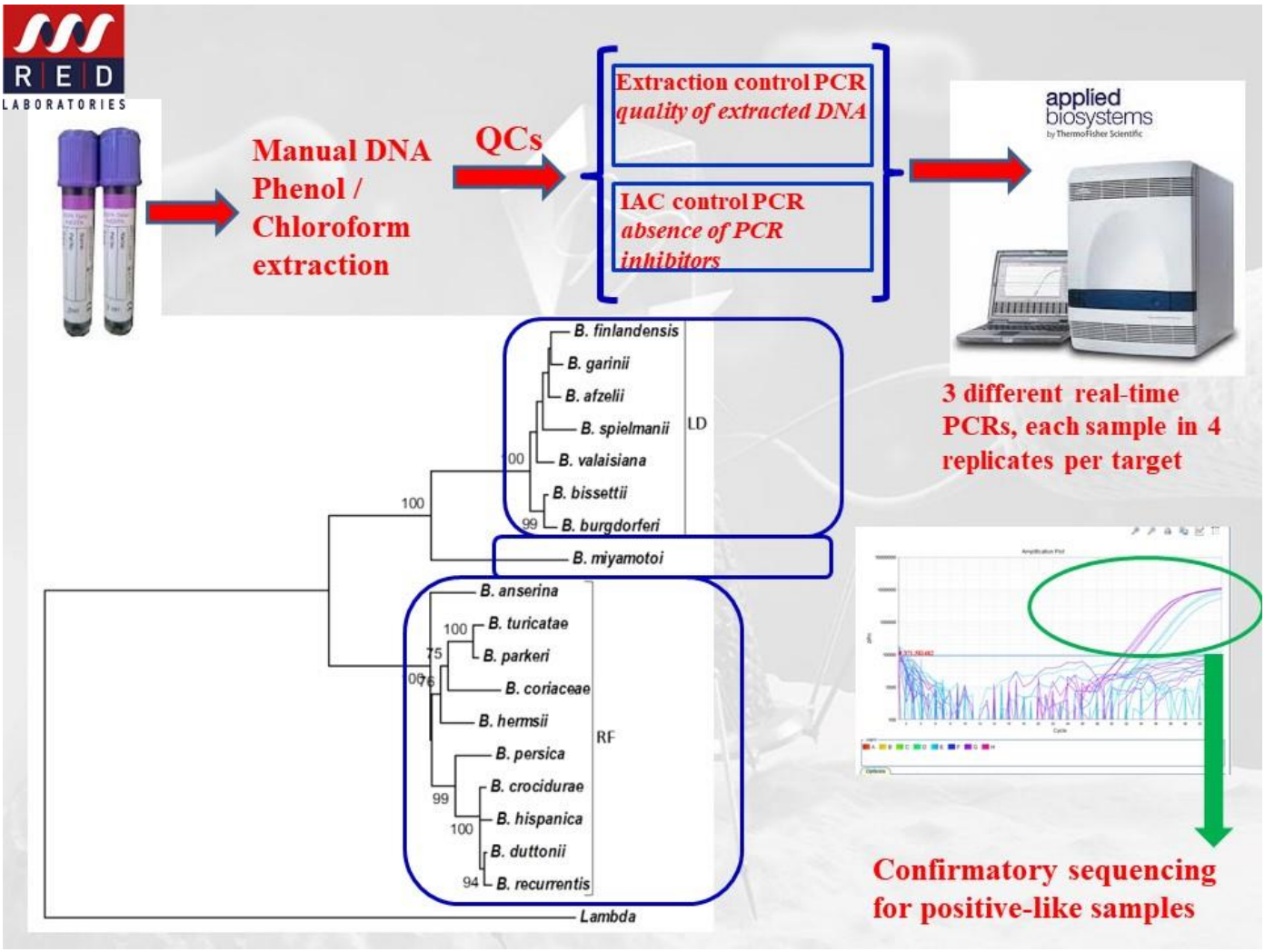
We have developed a direct and specific method which is more sensitive. Our detection method (Patented by Phelix R&D and Leicester University, WO2018083491A1) consists of targeting the presence of outnumbered prophages part of the bacteria lysogenic cycle. More precisely, one way to increase the sensitivity of a PCR assay is to increase the number of PCR targets. In contrast with the current PCR methods in Lyme diagnosis amplifying bacterial genomic DNA regions that have only one copy in each bacterium (such as bacterial 16S rRNA gene, RecA gene, and 5S-23S intergenic region), we propose a more sensitive, phage-based PCR assay for detecting Lyme Disease and Relapsing Fever caused by *Borrelia species*. To do this, we target phage DNA sequences specific to all *Borrelia* species: Lyme Disease and Relapsing Fever. Bacteriophages could become a diagnostic tool based on the principle that if there are phages it is because there are bacteria. Thus the 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.

This approach offer an increased sensitivity due to the fact that there is much more circulating phages compared to bacteria (average 1à to 100 phages per bacteria), therefore it's accurate and more sensitive than conventional PCR tests that are frequently negative due to low bacterial concentration in the blood. It's also a DIRECT test, evidencing bacterial own genetic material in the body, in contrast to all existing indirect tests (ELISA, Western BLOT, LTT/ELISPOT test). Secondly, it makes it possible to differentiate between the different subtypes of bacteria (*B. burgdorferi s.l., B. miyamotoi*, ...), as well as the relapsing fevers. Finally, this test is also the very best choice for the early detection (remember that antibodies need several weeks to show up….).

What is Phelix Phage Test?

Phelix Phage test (patent n° WO2018083491A1) is performed on whole blood, but can be also used for any other material suspected to contain given pathogen (like biopsy, punction liquid from a swollen joint, cerebrospinal fluid or ticks). The very first step is to extract the DNA from the provided sample using a specific manual method to ensure the best possible recovery of the pathogenic DNA. The extracted DNA is first subjected to two quality verification steps: (i) to ensure the quality of extracted DNA and (ii) to ensure the absence of inhibitors to the PCR reaction. If the sample passes these two quality control steps, it is then subjected to three real-time PCRs using proprietary primers and probes for specific phage detection. The 3 PCRs aim to detect the following targets (i) Borrelia miyamotoi, (ii) Borrelia burgdorferi sensu lato (B. burgdorferi s.s., B. bissetti, B. bavariensis, B. valaisiana, B. afzelii,B.

garinii), (iii) Relapsing fever (B. hermsii, B. recurrentis, B. crocidurae, B. duttonii). Each target is tested in 4 replicates. If the sample shows amplification it is likely to be positive. These amplified fragments are then analyzed by sequencing to confirm the positivity of the sample (i.e. to exclude false positives).



Significance

It is important to analyze the test result within the clinical context of the tested person. PCR Phage test result is reported as positive or negative. A positive result means that at the time of the test, the patient had Borrelia present in its body. Positive result in absence of symptoms suggests that, despite the presence of bacteria, the immune system seems to control the situation. In the case of a positive test after a recent or previous serological diagnosis, it is important to discuss about the possibility of a late stage/ post-lyme syndrome or chronic form of borreliosis. A negative result means that at the time of the test and in a given sample, the presence of Borrelia was not evidenced. It is important to remember that in late stages the number of bacteria is very low and mostly hidden in tissues and biofilms; accordingly the number of circulating phages will be low also. It is advisable to get retested at the period when the worsening of symptoms is noticed and/or after a use of an appropriate biofilm breaker. Remember that ticks are carrying and transmitting many other pathogens (*Bartonella, Rickettsia, Anaplasma*, etc) that all give very similar symptoms. Thus a negative *Borrelia* test with persistent symptoms might suggest the presence of other pathogens. Finally, it is also important to remember that in late stages the symptoms are

reflecting profound immune (*see https://www.frontiersin.org/articles/10.3389/fimmu.2020.01560/full.*) and gastrointestinal dysregulations as a consequence of previously untreated vector-borne disease(s). Chronic inflammation and low grade infections are the resulting complications of uncovered vector-borne diseases and thus need to be addressed in addition to testing for a presence of a specific pathogen.

Overall Findings

This method, publicly available since July 2019, is efficiently used to assess both human samples and ticks. The aggregated data are showing average 30 % negative results and 70% positive among which over 60 % indicated the presence of specific phages for Borrelia Relapsing Fever Group in general, and Borrelia miyamotoi in particular (unpublished data). Furthermore, ticks from 2019 and 2020 have been analysed by the same method. The obtained results on ticks showed that over 60% were found positive for Borrelia miyamotoi and only 15% for B. burgdorferi sl (unpublished data). Additionally, B. burgdorferi sl test design was further assessed using Lyme Disease biobank panel including early stage patient samples and control samples.

Thus 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. miyamotoi and other Relapsing fever Borrelia species while the later ones seem to be growingly prevalent.

Practical information

Phelix Phage Borrelia Test is mainly performed on blood (2 EDTA lavender-top tubes) but it can be also done on other types of biological sample (urine, serum, lumbal puncture, biopsy, the tick etc, as per need). Kits are sent on request (Email to **info@redlabs.be**). Requisition forms can be downloaded here : https://redlabs.be/ordering-tests/request-forms/.

References

First peer-reviewed publication by Shan et al, Frontiers in Microbiology 2021: https://www.frontiersin.org/articles/10.3389/fmicb.2021.651217/full

Patent: WO2018083491A1

This new approach, developed by Phelix R&D and the Laboratory directed by Pr Matha Clockie at University of Leicester, was presented on over 15 international Conferences by Dr Jinyu Shan (PhD, Phelix Phage test PI at the University of Leicester), Dr Louis Teulières (MD, PhD, test co-inventor) and Dr Tanja Mijatovic (PhD, CSO & Lab director of R.E.D. Laboratories that perform this test).

To know more about the test, you can visit **https://redlabs.be/phelix-phage-borrelia/**