**Borrelia** bacteriophages for diagnosis of Lyme Disease (LD) and Relapsing Fever (RF)

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**Facts about Lyme disease (LD) and Relapsing Fever (RF)**

- Bacterial infection, caused by *Borrelia* spp.
- Vector-borne disease
- Global distribution
- Problematic in laboratory diagnostics

**Global distribution of hard-bodied ticks (LD&RF)**

*(Gerold Stanek, et al., 2012)*

**Global distribution of soft-bodied ticks (RF)**

**Lyme disease is one of tick-borne diseases**

One single tick bite can potentially give you:

**Bacterial infection:**
- Lyme disease or borreliosis (*Borrelia*)
- Relapsing fever (*Borrelia*)
- Rocky Mountain spotted fever (*Rickettsia*)
- Ehrlichiosis and anaplasmosis (*Rickettsia*-like bacteria)
- Bartonellosis (*Bartonella*)

**Viral infection:**
- Tick-borne encephalitis
- Powassan virus/deer tick virus

**Protozoan** (Babesiosis)

**Life cycle of typical Lyme Borrelia species**

29/07/2017
A brief summary of current methods used in detecting *Borrelia*

<table>
<thead>
<tr>
<th>Diagnostics</th>
<th>Remit</th>
</tr>
</thead>
</table>
| Antibody-based | • Give indirect evidence  
• Low sensitivity  
• Can’t distinguish active and non-active *Borrelia* presence  
• Can’t distinguish different *Borrelia* sub-types |
| Bacterial DNA-based | • Direct evidence of *Borrelia* presence  
• Low sensitivity  
• Can’t distinguish live and dead *Borrelia*  
• Might be able to tell different *Borrelia* sub-types |
| Lymphocyte transformation test | • Provide indirect evidence  
• Variable sensitivity  
• Can only detect lymphocytes that have been in contact with *Borrelia* within 45±15 days, thus limited in application  
• Distinguish active *Borrelia*?  
• Distinguish different *Borrelia* sub-types? |

A comparison of the current PCR diagnostic methods

<table>
<thead>
<tr>
<th>Lyme PCR kit</th>
<th>Manufacturer/Institution</th>
<th>Target organ</th>
<th>Temperature (°C)</th>
<th>Range of melting</th>
<th>Stability of template</th>
<th>Utility to give strain profile</th>
<th>Impact on diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDC</td>
<td>Centers for Disease Control</td>
<td>Whole blood</td>
<td>95</td>
<td>65-85</td>
<td>Yes</td>
<td>Can distinguish among Lyme genotypes</td>
<td>Very sensitive</td>
</tr>
<tr>
<td>Lyme disease PCR</td>
<td>Lambda Laboratories Inc.</td>
<td>Whole blood</td>
<td>95</td>
<td>65-85</td>
<td>Yes</td>
<td>Can distinguish among Borrelia genotypes</td>
<td>Very sensitive</td>
</tr>
<tr>
<td>Yonmi</td>
<td>Yonmi Biotechnology Inc.</td>
<td>Whole blood</td>
<td>95</td>
<td>65-85</td>
<td>Yes</td>
<td>Can distinguish among Borrelia genotypes</td>
<td>Very sensitive</td>
</tr>
<tr>
<td>B. Tachibana 4 PCR</td>
<td>B. Tachibana Inc.</td>
<td>Whole blood</td>
<td>95</td>
<td>65-85</td>
<td>Yes</td>
<td>Can distinguish among Borrelia subtypes</td>
<td>Very sensitive</td>
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<tr>
<td>ThermoFisher</td>
<td>ThermoFisher Scientific Inc.</td>
<td>Whole blood</td>
<td>95</td>
<td>65-85</td>
<td>Yes</td>
<td>Can distinguish among Borrelia subtypes</td>
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<tr>
<td>Pfizer</td>
<td>Pfizer Inc.</td>
<td>Whole blood</td>
<td>95</td>
<td>65-85</td>
<td>Yes</td>
<td>Can distinguish among Borrelia subtypes</td>
<td>Very sensitive</td>
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<tr>
<td>PCR Micro-gPCR</td>
<td>PCR Micro Ltd.</td>
<td>RBC, RBC</td>
<td>95</td>
<td>65-85</td>
<td>Yes</td>
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<tr>
<td>Biolog S.</td>
<td>Biolog S. Ltd</td>
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New Lyme diagnostics needed!

Bacteriophages (phages) are viruses that infect bacteria

Frederick Twort  
Felix d’Herelle

Phage therapy: therapeutic use of phages to treat bacterial infection

In 1915, the well-known British journal *The Lancet* published an article written by Frederick Twort about "the transmissible bacterial lyses" (Twort, 1915), in which Twort described his observation of the "external edges of the colonies of *Staphylococcus*". He managed to filter the appropriate cultures of *Staphylococcus* and spotted the filtrate on the lawn of different *Staphylococcus* strains. Thus, he received a clear zone of lysis again and again. However, Twort could not explain the observed event and provided only its description. This was the very first publication on bacteriophages. d’Herelle read this article, which reminded him of his own observations in Mexico and...
Phage images taken by transmission electron microscope (TEM) under a magnification of around 100,000

Life cycle: lytic and lysogenic

Use of phages as a diagnostic tool
- Phages residing inside *Clostridium difficile* can be used as marker to indicate the presence of *C. difficile*

Phages residing within *Borrelia* strains were tightly correlated to the identity of their bacterial hosts

Method development
Phage-based PCR: Journey so far

- PCR was validated against all known bacteria using in silico PCR (http://insilico.ehu.eus/PCR/).
- ‘Wet PCR’ were performed against LD&RF Borrelia strains and the following bacteria in the lab, such as *Clostridium difficile*, *Burkholderia thailandensis*, *E. coli*, *Salmonella*, *Legionellae*, and *Haemophilia* strains. None of these bacteria generated any PCR products.

![Fig. 1](image1.png)

Method development: against five sera

- We applied both phage and bacterial PCRs against five serum samples obtained from Lyme positive patients. Four out of five serum samples generated PCR products with phage PCR (top panel), while only one serum samples produced positive with bacterial PCR (bottom panel).

![Phage PCR vs. Bacterial PCR](image2.png)

Phage-based PCR amplification curves: positives, negatives, and internal controls

- Different primers/probes against the same *Borrelia*
- The same primer/probe against serial diluted *Borrelia*
- The same primer/probe against different *Borrelia*
- The internal control primer/probe against serial diluted internal control DNA

Comparison of the current and phage tests to determine *Borrelia* presence: Results from 22 clinically confirmed patients

22 patients showed typical ‘Lyme clinical symptoms’ were examined using the current test and phage test, respectively. Two patients showed both serologic and phage positive. 18 patients showed phage positive but serologic negative. Two patients were negative for both tests.

![Current test vs. Phage test against 22 patients with Lyme clinical symptoms](image3.png)

Comparison of the current and phage tests to determine *Borrelia* presence: Results from a cohort of 96 patients

96 patients were examined using the current (ELISA and WB) and phage tests, respectively. 15 patients were positive for both the current and phage tests. 73 patients were positive for phage test, but negative for the current test. Eight patients were negative for both tests.

![Current test vs. Phage test against 96 patients](image4.png)
Comparison of the current and phage tests to determine *Borrelia* presence: Results from 11 patients who have been bitten by ticks

11 patients can recall tick bite. Two patients showed both serologic and phage positive. Eight patients were phage positive but serologic negative. One patient was negative for both tests.

Comparison of GeneProof (commercial PCR) and phage tests to determine *Borrelia* presence: Results from 66 patients within the 96-patient cohort

66 patients were random selected from the 96-patient cohort. Nine patients showed both GeneProof and phage positive, while 54 patients showed phage positive and GeneProof negative. 3 patients showed negative for both tests.

How to avoid false positive?

- PCR primers/probes were designed only specific for Lyme *Borrelia*. This was bioinformatically (PCR primer BLAST and *in silico* PCR) and experimentally (targeting various LD&RF *Borrelia* strains) confirmed.
- For every PCR, there were positive and negative controls.
- PCR products generated against serum samples were sequenced and blasted against NCBI database. The top 100 hits were all *Borrelia*.

How to avoid false negative?

False negative:
- For every PCR, there were positive and negative controls.
- An internal control DNA is to be added into each PCR reaction (developing).
- This internal control DNA has been identified, synthesised, primer/probe designed, amplification efficiency determined.

PCR amplification curves: internal controls

The internal control will service as a normaliser to enable a quantitative estimation of ‘copy number’ of *Borrelia* load.

This will enable phage PCR to measure the relative *Borrelia* load for each case.
Phage test
- Direct evidence of Borrelia presence
- Can’t distinguish active and non-active Borrelia presence
- Can’t distinguish different Borrelia sub-types
- Could distinguish active and non-active Borrelia presence (Pilot study)
- Could be developed to have the ability to distinguish different Borrelia sub-types (ongoing)

Diagnostics

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<td>• High sensitivity and specificity (Pilot study)</td>
</tr>
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Further development

- More controls (healthy and sick) will be needed to figure out the false positive (specificity).
- Random confirmed Lyme patients will be needed to figure out the false negative (sensitivity).
- Primers/probes targeting relapsing fever strain B. miyamotoi has been designed, validated in the lab. We are currently testing them against sera.
- A multiplex PCR will be designed to identify LD&RF Borrelia strains by a single PCR run.

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Mr Faizal Patel

Thank you for listening!