

Assessing the sensitivity and specificity of three different methods for DNA isolation from *Borrelia burgdorferi*

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Although Lyme disease has recently been acknowledged as a major health threat to both the United States and Canada, controversy exists regarding its diagnosis and treatment. Current testing protocol in the United States consists of ELISA, Western blotting, and protein work to detect *B. burgdorferi* (the causative agent of Lyme disease) proteins in blood or urine specimens. Frequently, these tests result in misdiagnoses, including both false positives and false negatives – this is suggestive of insufficient sensitivity and specificity among these protein-based methods. An alternative testing protocol employs PCR diagnostic kits, however these also face problems with sensitivity and specificity. We hypothesize that the isolation of *B. burgdorferi* from human specimens is the point in the diagnostic procedure that causes the poor results whilst employing the PCR method. The present study aimed to identify the optimal method for isolating *B. burgdorferi* DNA; thus, our study evaluated diagnostic methods that may ameliorate the existing deficiencies in sensitivity and specificity of Lyme disease testing. *B. burgdorferi* has several unique properties, such as its diderm (double membrane) structure that renders it neither Gram negative nor Gram positive, and therefore it is likely that isolating its DNA can be impacted by the different isolation methods and lysis conditions employed. Our study compared the current Norgen Biotek Bacterial Genomic DNA Isolation Kit, another commercially available kit in popular use (Qiagen's QIAamp DNA Blood Mini Kit), and the alkaline hydrolysis method, which is frequently cited in *B. burgdorferi* literature. *B. burgdorferi* DNA was isolated from a bacterial suspension, as well as bacterial suspension spiked in plasma, with a concomitant serial dilution to assess the limit of detection for each method. Results indicate that DNA isolated using Norgen's method consistently yielded greater quantity and quality of *B. burgdorferi* genomic DNA at low bacterial concentration; as such, Norgen's kit presents a highly sensitive method for Lyme disease testing from patient plasma.

Materials & Methods

DNA ISOLATION FROM *B. BURGDORFERI* SUSPENSION

DNA Isolation

- Obtained 5×10^8 , 2.5×10^8 , 1.25×10^8 , or 6.25×10^7 cells of *B. burgdorferi*
- In duplicates, DNA was isolated from each of the four starting concentrations using Norgen's Bacterial Genomic DNA Isolation Kit (Cat. #17900) and Norgen's PCR Purification Kit (Cat. #14400), Qiagen's QIAamp DNA Blood Mini Kit¹, or the alkaline hydrolysis method²

PCR Quantification & Gel Electrophoresis

- 2 μ L of each elution was used in a 20 μ L PCR reaction, using Norgen's *Borrelia burgdorferi* TaqMan PCR Detection Kit (Cat. #TM45200)
- 5 μ L of each elution was loaded onto a 1.2% agarose DNA gel and run for 30 minutes at 150 V
- 4 μ L of Norgen Biotek's High Ranger Ladder was used

DNA ISOLATION FROM *B. BURGDORFERI* SUSPENSION SPIKED IN PLASMA

Plasma Collection

- Whole blood was collected from a healthy donor in EDTA tubes
- Plasma was collected by centrifugation at 600 x g for 5 min, followed by 2000 x g for 10 min³

DNA Isolation

- Obtained 5×10^8 , 2.5×10^8 , 1.25×10^8 , or 6.25×10^7 cells of *B. burgdorferi*. The cells were pelleted by centrifugation at 14,000 RPM for 1 minute. The bacterial pellet was resuspended in 100 μ L of plasma
- In duplicates, DNA was isolated from each of the four starting concentrations using Norgen's Bacterial Genomic DNA Isolation Kit, Qiagen's QIAamp DNA Blood Mini Kit, or the alkaline hydrolysis method (refer to above conditions)

PCR Quantification & Gel Electrophoresis

- Refer to above conditions

Results & Discussion

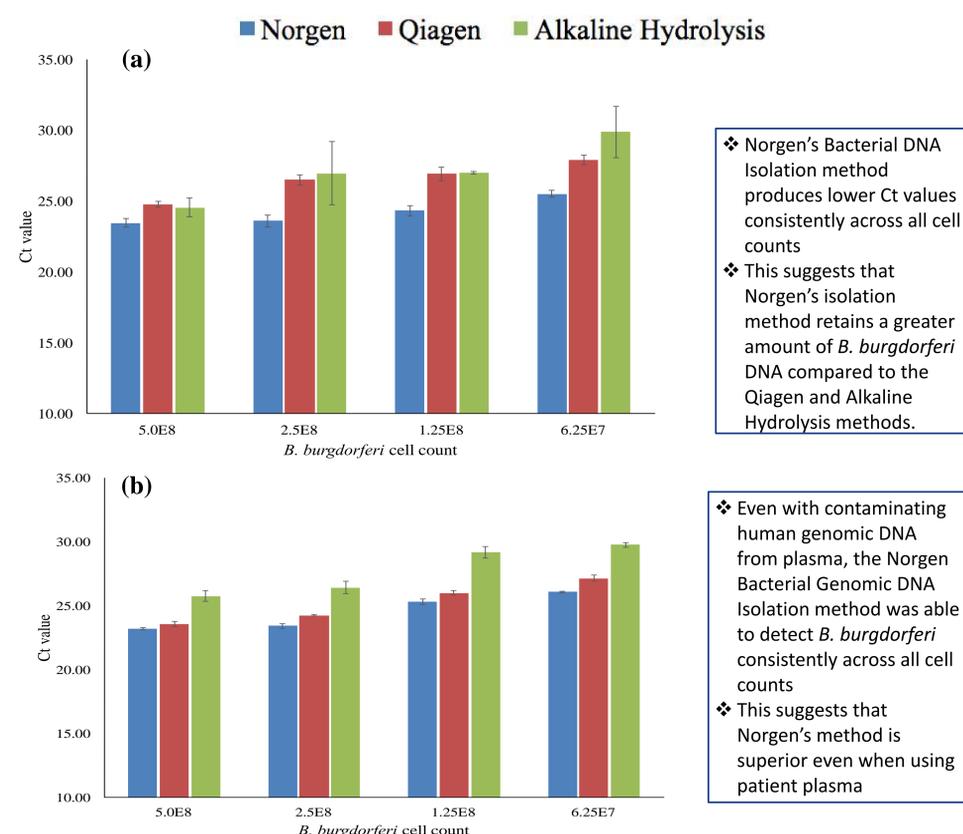


Figure 1. Mean cycle threshold values of *B. burgdorferi* DNA isolated from (a) bacterial suspension alone (pure system), and (b) plasma spiked with bacterial suspension, with standard error.

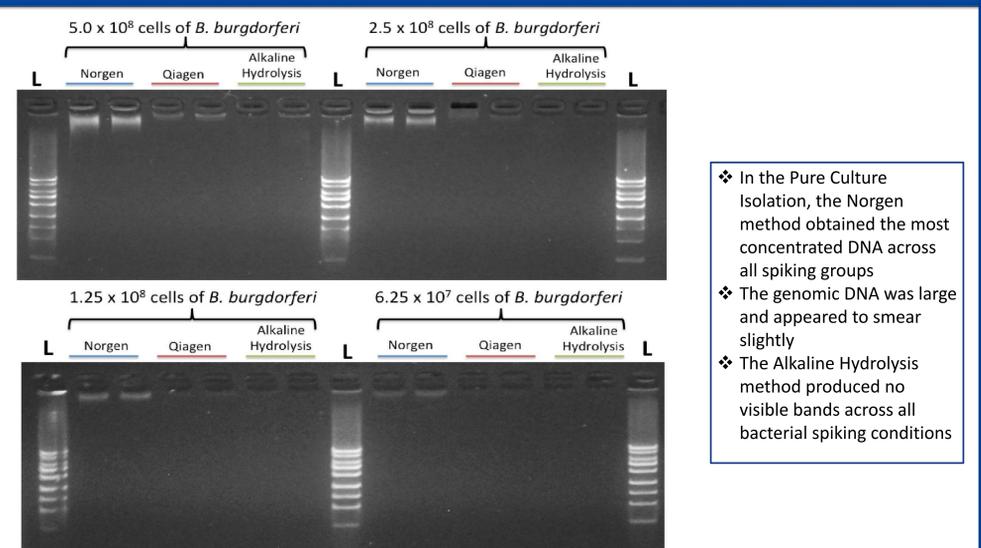


Figure 2. Gel electrophoresis of *B. burgdorferi* DNA isolated from bacterial suspension alone (pure system).

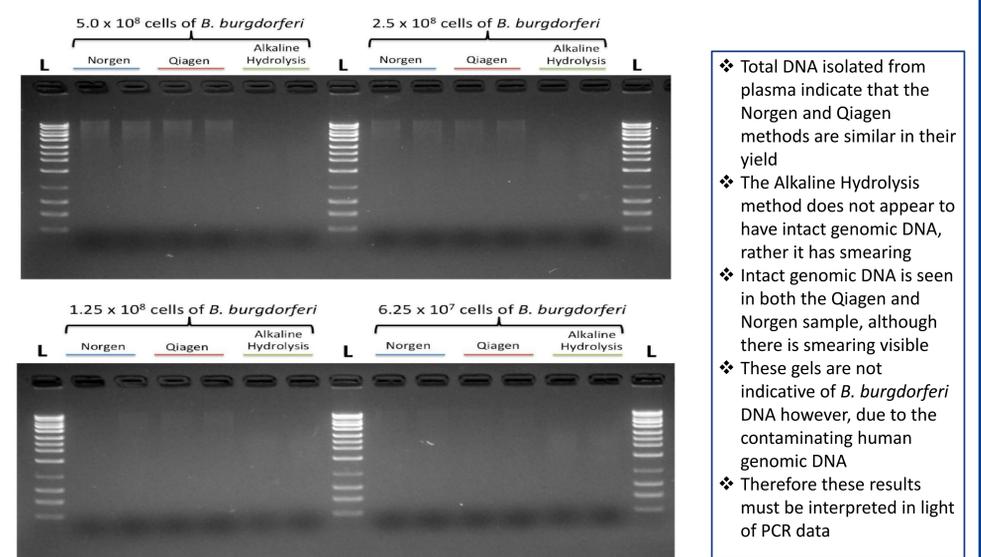


Figure 3. Gel electrophoresis of *B. burgdorferi* DNA isolated from bacterial suspension spiked in plasma.

Conclusions

Isolation from *B. burgdorferi* suspension:

- Norgen's Bacterial Genomic DNA Isolation method resulted in a slightly increased yield of *B. burgdorferi* DNA when compared to the Qiagen and alkaline hydrolysis methods

Isolation from *B. burgdorferi* suspension spiked in plasma:

- Norgen's Bacterial Genomic DNA Isolation method produced a slight greater final yield of *B. burgdorferi* in comparison to the Alkaline Hydrolysis and Qiagen methods

Take-home message:

- Norgen's Genomic Bacterial DNA Isolation kit will allow for the detection of small quantities of *B. burgdorferi* from clinical samples
- Norgen's increased isolation sensitivity at lower concentration of *B. burgdorferi* will allow for an appropriate diagnosis to be made in patients with Lyme Disease

References

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