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16S rDNA Sequencing Diagnosis of Spirochetemia in Lyme and related Borrelioses

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Conflict of interest: *Sin Hang Lee is an employee of Milford Hospital. Milford Medical Laboratory-affiliated with Milford Hospital offers commercial diagnostic tests, including DNA sequencing tests for Lyme and related borrelioses, to the patients and health care providers.

Presentation at Lyme Borreliosis and Tick Borne Illnesses: Diagnostics, Emerging Pathogens and Avenues for New Research on November 8, 2014 4:30-5:30 pm Roundtable Discussion on future research and collaborations

Massachusetts General Hospital, O'Keeffe Auditorium

55 Fruit Street, Boston, MA

Introduction

Reliable diagnosis of early Lyme disease plays a pivotal role in “curing” the infection with timely appropriate antibiotic treatment in most cases, thus preventing the infection from going into chronic phase which may cause debilitating tissue damage. However, Lyme and related borrelioses are systemic bacterial infections caused by *Borrelia burgdorferi sensu lato*, *Borrelia miyamotoi* and probably other unnamed borrelial species in the Northeast of the US [1-4]. As for any emerging infectious diseases whose causative agents are difficult to culture, e.g. the Ebola virus, polymerase chain reaction (PCR) amplification of a signature segment of the genomic nucleic acid of the causative agent followed by DNA sequencing of the amplicon is the standard laboratory diagnostic approach [5]. Analysis of a signature sequence of the borrelial 16S rRNA gene (16S rDNA) can detect the pathogens of *B. burgdorferi sensu lato* [6-8], *B. miyamotoi* [1,3,4,9] and even unnamed novel pathogenic borrelial species [3] in patient samples.

Methods

Shifting the 21-base LD-specific PCR primer pair originally developed by Marconi [6] upstream for another 3 nucleotides created a pair of “genus-specific” M1/M2 primers which can amplify a highly conserved 357-bp segment of the 16S ribosomal RNA gene (16S rDNA) of all *B. burgdorferi sensu lato* species and the correspondent 358-bp segment of the relapsing fever borreliae, including *B. miyamotoi*. The same-nested PCR amplicons were used as the templates for direct Sanger DNA sequencing.

The relative positions of the LD1/LD2 v the M1/M2 primers are illustrated as follows.

LD1 ATGCACACTGGTGTTAACTA (819-842) LD2 GACTTATCACCGGAGTCCTTA (1153-1173) [6]
M1 5'-ACGATGCACACTGGTGTTAA-3' (816-839) M2 5'-TCCGACTTATCACCGGAGTC-3' (1156-1176) [3,4]



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The highly conserved sequence segment with hypervariable regions of the 16S rDNA of *B. miyamotoi* and *B. burgdorferi* in this segment are listed as follows.

B. miyamotoi (358 bases)

ACGATGCACACTGGGTGTTAA TCAGAAAGGTTAGTACCGAAGCTAACGTGTTAAGTGTGCCGCTGGGAGTAGTTCGCAAGAACTCAAAGGAATTGACGGGGCCGCACAAGCGGTGGAGCATGTGGTTAATTCGATGATACGCGAGGAA
CCTTACCAGGGCTTGACATATACAGGATATAGTTAGAGATAACTATTTCCCGTTTGGGGTCTATACAGGTGCTGCATGTTGTCTGCAGCTGTGCCGTGAGGTGTGGGTTAAGTCCCGCAACGAGCGCAACCTTGTGTCTGTTACCGATGTAA
AGATGGGACTCAGACGAGACTGCCGGTATAAGCCGGA

B. burgdorferi (357 bases)

ACGATGCACACTGGGTGTTAA CTAAGTGTAGTACCGAAGCTAACGTGTTAAGTGTGCCGCTGGGAGTAGTTCGCAAGAGTCAAAGGAATTGACGGGGCCGCACAAGCGGTGGAGCATGTGGTTAATTCGATGATACGCGAGGAA
CTTACCAGGGCTTGACATATAGGATATAGTTAGAGATAACTATTTCCCGTTTGGGGTCTATACAGGTGCTGCATGTTGTCTGCAGCTGTGCCGTGAGGTGTGGGTTAAGTCCCGCAACGAGCGCAACCTTGTGTCTGTTACCGATGTAA
GTGGGACTCAGATAAGACTGCCGGTATAAGTCGGA

The inter-primer hypervariable regions of the 357/358 highly conserved segment of the 16S rDNA of the borrelial species are summarized in Table 1.

Table 1 Alignment of the hypervariable bases in a highly conserved borrelial 16S rDNA sequence (Modified)

Borrelia Locus ID#	Variable nucleotide bases using <i>Borrelia miyamotoi</i> JF951379.1 as position reference																						
RF Group:	764-766	768	770	817	826	856	898	900	908	923	929	943	946	949	960	963	999	1040	1043	1062	1064	1077	1078
<i>miyamotoi</i> JF951379.1	TCG	A	G	<u>I</u>	<u>A</u>	C	G	A	A	C	A	C	T	C	T	G	<u>C</u>	G	G	A	A	C	G
<i>hermsii</i> DQ855530.1	TCG	A	G	C	G	C	G	A	A	C	G	C	T	C	T	G	T	A	G	A	A	C	G
<i>coriaceae</i> AF210136.1	TCG	A	G	C	G	C	A	A	A	C	G	C	T	C	T	G	T	A	G	A	A	T	G
<i>thelleri</i> DQ872186.1	TCG	A	G	C	G	C	G	A	A	C	A	C	C	C	T	G	T	G	G	A	A	C	G
<i>duttonii</i> AB113315.1	TCG	A	G	C	G	C	G	A	A	C	G	C	T	C	T	G	T	G	G	A	A	C	G
<i>parkeri</i> AY604975.1 ^a	TCG	A	G	C	G	C	G	A	A	C	G	C	T	C	T	G	T	A	G	A	A	C	G
<i>turicatae</i> U42299.1 ^a	TCG	A	G	C	G	C	G	A	A	C	G	C	T	C	T	G	T	A	G	A	A	C	G
<i>crocidurae</i> JX292910.1	TCG	G	G	C	G	C	G	A	A	C	G	C	T	C	T	G	T	A	G	A	A	C	G
<i>lonestari</i> AY442141.1	TCG	A	G	C	G	C	G	A	A	C	A	C	C	T	T	G	T	G	G	A	A	C	G
<i>recurrentis</i> CP000993.1	TCG	G	G	C	G	T	G	A	A	C	G	C	T	C	T	G	T	A	G	A	A	C	G
CDC unnamed KM052618	TCG	A	G	C	G	C	A	G	G	C	G	C	T	C	T	G	T	A	G	A	A	T	G
BB sensu lato Complex																							
<i>burgdorferi</i> NR_03929.1 ^c	CTA	A	-	C	G	C	G	A	A	T	A	T	T	C	T	A	T	G	A	T	G	T	A
<i>valaisiana</i> AB091815.1	CTG	A	-	C	G	C	G	A	A	T	A	T	T	C	T	A	T	G	A	T	A	T	A
<i>spielmanii</i> HE582779.1	CTA	A	-	C	G	C	G	A	A	T	A	T	T	C	C	A	T	G	A	T	A	T	A
<i>afzelii</i> NR_074840.1	CTA	A	-	C	G	C	G	A	A	T	A	T	T	C	T	A	T	G	A	T	A	T	A
<i>lusitanae</i> AB091822.1	CTA	C	-	C	G	C	G	A	A	T	A	T	T	C	T	A	T	G	A	T	G	T	A

Footnote: RF = Relapsing Fever; BB = *Borrelia burgdorferi*. "-" = gap. The nucleotide sequences used for base alignment in this table were retrieved from the National Center for Biotechnology Information database. The nucleotide bases T, A and C (underlined) at positions 817, 816 and 999, respectively, are unique for *B. miyamotoi*.

^aDiscordant 16S rDNA is the accepted basis for borrelial species differentiation. However, isolates of different relapsing fever species with an identical DNA sequence in this highly conserved 16S rDNA segment may be found in the GenBank database.

^bThe novel 16S rDNA sequence of a *Borrelia* isolate in a serum sample of a patient diagnosed with neurologic Lyme disease (CDC54) is closely related to those of relapsing fever borreliae, but has two "G" bases (underlined) at positions 900 and 908 which are occupied by an invariant "A" in all known borrelial species.

^cThe species of *B. burgdorferi* sensu stricto, *B. bissettii* (NR_102956.1), *B. garinii* (NR_074854.1), and *B. americana* (HM802226.1) have an identical DNA sequence in this highly conserved 16S rDNA segment.

Results

DNA sequencing-based detection of *B. burgdorferi*, *B. miyamotoi*, and mixed *B. burgdorferi* and *B. miyamotoi* in patient blood samples are illustrated in the following electropherograms.

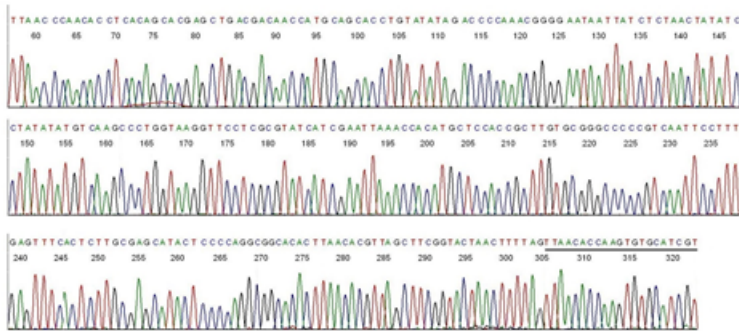


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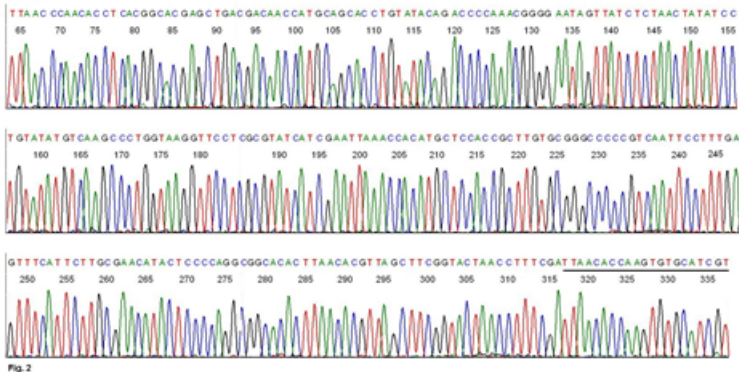
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Partial sequence including the V-regions 5 and 6 of the 16S rDNA of *Borrelia burgdorferi* sensu lato (M1 primer site underlined)



Partial sequence including the V-regions 5 and 6 of the 16S rDNA of *Borrelia miyamotoi* (M1 primer site underlined)



Co-infection by *B. miyamotoi* and *B. burgdorferi* in a Patient with Spirochetemia (case #8)

A 107-base segment of the base-calling DNA sequencing electropherogram showing two superimposed DNA sequences, one of *B. burgdorferi* and one of *B. miyamotoi*. These are three characteristic double base peaks at positions 770, 817, and 826 (Table 1) in this signature sequence, representing concomitant borrelial infections by these two species in one patient blood sample. The M2 primer was used as the sequencing primer.

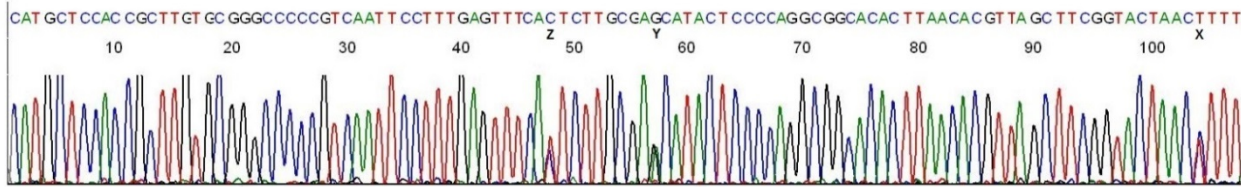
Only pairing of #1 and #2 of eight possible individual DNA sequences listed as follows resulted in 100% ID matches with two superimposed 16S rDNA sequences of borrelia species in the GenBank database.



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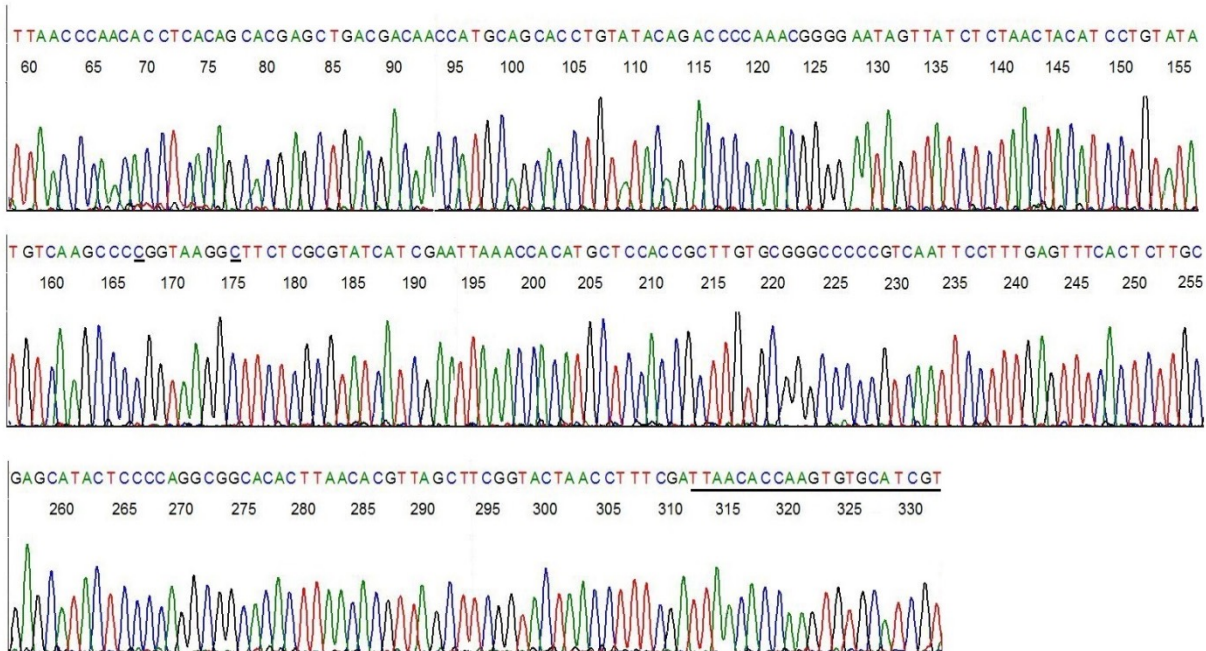
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- #1 *B. burgdorferi*
CATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCACTCTTGGCGAGCATACTCCCCAGGCGGCACACTTAACACGTTAGCTTCGGTACTAACTTTT
- #2 *B. miyamotoi*
CATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCACTCTTGGCGAGCATACTCCCCAGGCGGCACACTTAACACGTTAGCTTCGGTACTAACTTTT
- #3
CATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCACTCTTGGCGAGCATACTCCCCAGGCGGCACACTTAACACGTTAGCTTCGGTACTAACTTTT
- #4
CATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCACTCTTGGCGAGCATACTCCCCAGGCGGCACACTTAACACGTTAGCTTCGGTACTAACTTTT
- #5
CATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCACTCTTGGCGAGCATACTCCCCAGGCGGCACACTTAACACGTTAGCTTCGGTACTAACTTTT
- #6
CATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCACTCTTGGCGAGCATACTCCCCAGGCGGCACACTTAACACGTTAGCTTCGGTACTAACTTTT
- #7
CATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCACTCTTGGCGAGCATACTCCCCAGGCGGCACACTTAACACGTTAGCTTCGGTACTAACTTTT
- #8
CATGCTCCACCGCTTGTGCGGGCCCCGTCAATTCCTTTGAGTTTCACTCTTGGCGAGCATACTCCCCAGGCGGCACACTTAACACGTTAGCTTCGGTACTAACTTTT

Bacterial residues in archived serum of a treated patient with “neurologic Lyme disease” from the CDC

Lee SH et al. *Int J Mol Sci*, 2014; 15:4284-4298. (sequence deposited in GenBank under accession number KM052618)



Discussion on future research and collaborations

There is a need to develop reliable diagnostic tests for causative agents of Lyme and related borrelioses at the early stage of the infection for timely appropriate treatment. To encourage technology innovation, it is recommended that blind-coded simulated blood samples spiked with various species of known borreliae or blank be distributed to all clinical



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laboratories performing Lyme disease testing for a bacteriology proficiency survey, as routinely conducted by the College of American Pathologists for *Neisseria gonorrhoeae*. The laboratories which return the correct answers will be invited to further develop a generally accepted diagnostic protocol to be used by hospitals located in Lyme disease-endemic areas. To be of use for timely patient care, the results must be generated within 7 days.

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