Comparison of Primers Amplifying Two Different Regions of the 16S Ribosomal RNA Gene for Microbiologic Diagnosis of Cardiovascular Implantable Electronic Device Infection

Zerelda Esquer Garrigos, MD1, M. Rizwan Sohail, MD, FIDSA2, Kerryl Greenwood-Quaintance, MS3, Scott Cunningham, MS MT (ASCP) SM3, Prakhar Vijayvargiya, MD1, Matthew Thoendel, MD, PhD1, Paul a. Friedman, MD4 and Robin Patel, MD, FIDSA, D(ABMM)5.

1)Division of Infectious Diseases (2)Infectious Diseases and Cardiovascular Diseases, Mayo School of Graduate Medical Education (3)Division of Clinical Microbiology (4)Cardiovascular Diseases (5)Divisions of Clinical Microbiology and Infectious Diseases., Mayo Clinic, Rochester MN

Background
- Bacterial cultures are negative in over 13% of cases of Cardiovascular Implantable Electronic Device (CIED) Infections.
- Broad-range PCR and Sanger sequencing could potentially establish a microbiologic diagnosis in these patients.
- We aim to evaluate and compare detection and sequencing performance of primers targeting two different hypervariable regions of the 16S ribosomal RNA (rRNA) gene on sonicate fluid from extracted CIEDs.

Figure 1. Regions of the 16S rRNA gene amplified by the two set of primers utilized

Methods
- Contiguous assemblies were queried using the NCBI BLAST database and results interpreted using CLSI guidelines.
- The organism identified by sequencing was compared to the results of conventional culture.

Results
- Through 2017, devices of patients with suspected CIED infection have been collected in our laboratory from 2012 to 2017.
- We selected 39 of these samples and classified them as culture-positive or culture-negative based on the results of conventional culture. ZymoBIOMICS DNA miniprep Kit was utilized for DNA extraction with modifications.
- Quantitative Real-Time PCR was performed on a Roche LightCycler 1.0 instrument.
- Annealing temperature of 65°C was used for the primers targeting the V3-V4 16S rRNA hypervariable region and 62°C for the V1-V3 primers according to previously published protocols.
- Samples with crossing point (Cp) of <32 or >3 Cps below the negative control were sent for bidirectional Sanger sequencing.
- Sequences were aligned and edited using Sequencher 5.0 software.
- Of those 39 samples, 23 were culture-positive and 16 were culture-negative.
- Of those 23 culture-positive, 19 were PCR-positive using both sets of primers and sequencing of these samples identified the same organism reported on conventional culture.
- Two out of the 23 samples were only PCR-positive using the V1-V3 primers.
- In the culture negative group, 2 specimens were PCR-positive using both sets of primers, identifying Staphylococcus aureus in both.
- The remaining samples were PCR-negative.

Conclusions
- Our results suggest that primer set amplifying the V1-V3 region leads to slightly better detection results compared to the V3-V4 primer set used.
- Molecular testing performed on sonicate fluid from extracted devices may identify pathogens in cases of culture-negative CIED infection.

Table 1 Comparison of the two primer sets amplifying 16S rRNA Gene

<table>
<thead>
<tr>
<th>Study group classification</th>
<th>Detection with V1-V3 primer set</th>
<th>Detection with V3-V4 primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture-positive (n=23)</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Culture-negative (n=16)</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

Acknowledgments
This project was made possible by the Mayo Clinic CTSA grant number UL1TR002377 from the National Center for Advancing Translational Science (NCATS), a component of the National Institutes of Health (NIH); and CTSA Grant Number U11 TR000755 from the National Center for Advancing Translational Science (NCATS). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.
Title Safe Area: Title text should appear within this area

Author/Affiliation Area: Authors, affiliations and subbrand names should snap to the top of this area and flow downward.

Brand Safe Area: The upper title banner section of the poster provides a brand safe area for the logo, title and author/affiliation text. No photos, illustrations, patterns, high-contrast backgrounds, or graphics are allowed within this area. A logo representing another non-Mayo listed contributing affiliation may be placed in upper right corner within green guideline space.

Poster Body Area: Research text, figures, tables and graphs should appear within this area. No photos, illustrations, patterns, high-contrast backgrounds, or graphics are allowed in the margins. Use the text boxes in the template when possible.

Copyright Line: Copyright graphic should appear at bottom right under last text/figure box. Recommend graphic be placed no more than 1.5" from bottom of poster.