**ASM Microbe 2019 Abstract Summary**

**Karius® Test for Pathogen Detection in Prosthetic Joint Infections**

**Poster Session CIV01—Clinical Studies of Adult Infectious Diseases**
**Sunday | June 23 | 11:00 AM–1:00 PM**

**Poster CIV-170**

**Sequencing of Plasma Cell-Free DNA for Pathogen Detection in Prosthetic Joint Infections**

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**Study Design**

Plasma samples from peripheral blood of 25 patients undergoing surgical interventions for suspected prosthetic joint infections (PJI) were tested using the Karius Test. These included 22 culture-positive PJI cases. Joint fluid samples from all patients were also sequenced for intracellular DNA using the MolYsis Basic5 kit (Molyzym) and BIOstic Bacteremia DNA isolation kit (Qiagen), and standard Illumina sequencing.

**Results**

1. **Comparison with culture**—The Karius Test detected the same organism in 13 out of the 22 culture-positive cases with detection of at least one additional organism in 6 cases. Optimizing the analytical pipeline for increased sensitivity identified the PJI microbe in three additional cases.

2. **Utility for monitoring PJI**—In 5 cases Karius Test results were compared between samples collected before and after surgical and antibiotic treatment. The microbes detected pre-treatment were no longer detected post-treatment.

3. **Comparison with joint fluid sequencing**—Joint fluid sequencing detected the same organism in 19 out of the 22 culture-positive cases. The Karius Test results aligned with 13 of these cases.
Sequencing of plasma cell-free DNA for pathogen detection in joints infections

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INTRODUCTION

• 1 million joint replacement surgeries a year in the U.S.
• 2% result in a prosthetic joint infection (PJI)
• Culture based diagnosis requires invasive joint aspirations
• 20% of PJI cases are culture-negative

OBJECTIVES

• Test if plasma microbial cell-free DNA (mcfDNA) sequencing detects PJI infections
• Compare plasma mcfDNA sequencing, joint fluid DNA sequencing & culture
• If all 3 results align, test if plasma mcfDNA sequencing quantitatively tracks clearance

Sample Preparation

Karius mcfDNA sequencing from plasma
• The Karius test is a CLIA certified/CAP accredited NGS test that detects mcfDNA
• 5 ml of blood is collected (KNGS test that detects mcfDNA)
• mcfDNA is extracted from plasma
• NGS is performed (Illumina)
• Human reads are removed and remaining sequences are aligned to a curated database of >1400 organisms
• Organisms present above a statistical threshold are reported and their mcfDNA is quantified

RESULTS

Plasma mcfDNA sequencing detects the pathogen in 62.2% of cases (23 of 37)
* Indicates pretreatment with antibiotics

Table: Percentage of cultured organisms

<table>
<thead>
<tr>
<th>Patient</th>
<th>Blood mcfDNA sequencing</th>
<th>Joint fluid sequencing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>80%</td>
<td>85%</td>
</tr>
<tr>
<td>Patient 2</td>
<td>70%</td>
<td>78%</td>
</tr>
<tr>
<td>Patient 3</td>
<td>90%</td>
<td>92%</td>
</tr>
<tr>
<td>Patient 4</td>
<td>75%</td>
<td>77%</td>
</tr>
<tr>
<td>Patient 5</td>
<td>85%</td>
<td>88%</td>
</tr>
</tbody>
</table>

CONCLUSIONS

Plasma mcfDNA sequencing can non-invasively detect pathogens in infected prosthetics joints

Immediate use for plasma mcfDNA sequencing:
• Culture-negative cases
  • *note: plasma mcfDNA can detect pathogens from patients pretreated with antibiotics
  • Rapid diagnostic decisions
  • Infection clearance: before reimplantation ensure microbe is undetectable

Developing sequencing applications for infections:
• Hospital-specific strain database
• AMR gene identification

Synovial Fluid DNA Sequencing Bioinformatics

Synovial fluid DNA analysis:
• Human DNA filtering & QC
• Remove microbes common across a samples in a batch as putative contaminants
• MAD decision boundary
• Qualitative thresholds
• Areas for improvement
• Technical controls for contamination