# Fast and Reliable 16S Diagnostics for Prosthetic joint Infections

## Validation of an Oxford Nanopore-Based 16S rRNA Gene Sequencing Method for Rapid Bacterial Identification in Foreign Body and Sterile Site Infections

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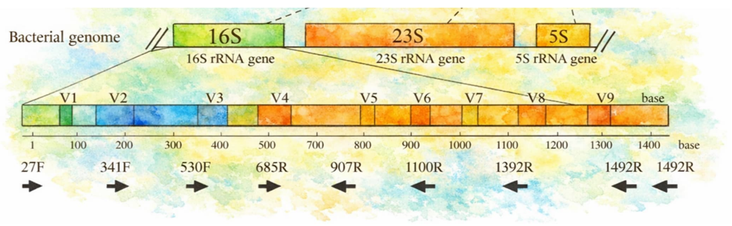
## Summary

An in-house Oxford Nanopore Technology (ONT) based full length 16S gene sequencing workflow was validated against culture and Sanger sequencing, across five different criteria for sigificant findings. A commercial spike-in control improved interpretation and contamination detection. Ligation-based barcoding allowed co-sequencing of multiple amplicons from different assays to be sequenced on the same flow cell, increasing time- and cost-efficiency.

## Background

ONT sequencing enables analysis of the full-length 16S gene which improves species identification.




For adequate diagnostics, criteria are needed to distinguish true pathogens from background signals and contaminating flora. We aimed to validate an ONT-based 16S gene sequencing workflow by systematically comparing criteria for defining significant pathogen levels and addressing challenges in distinguishing clinically relevant organisms.

A secondary aim was to develop a rapid, scalable workflow enabling co-sequencing of 16S with other PCR amplicons, making it suitable for laboratories that do not fill an entire flow cell with each run.

### Evaluated criteria for a True Positive Finding

**Baseline requirements**
A species must have ≥50 reads to be considered a positive finding.

**Five sets of criteria were evaluated**

1) RA* ≥5% AND total no. of reads >1000
2) RA ≥2,5% AND total no. of reads >1000
3) RA ≥1% AND total no. of reads >1000
4) RA ≥1% AND result invalid if ≥90% of reads unassigned
5) RA ≥2,5% AND result invalid if ≥90 % reads unassigned

(*Relative Abundance)

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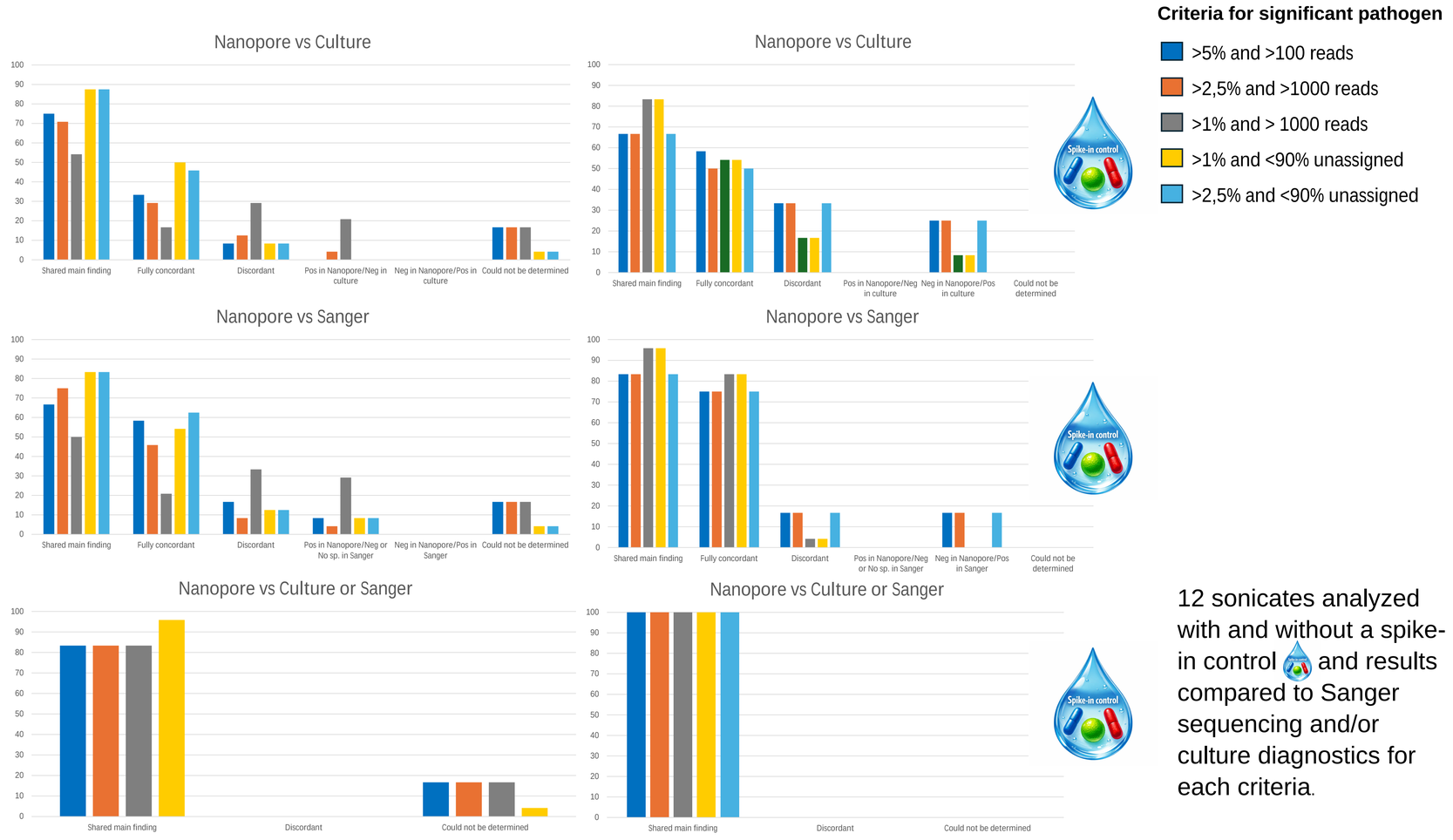


12 sonicates analyzed with and without a spike-in control and results compared to Sanger sequencing and/or culture diagnostics for each criteria.

## Results

**Concordance** between our Nanopore 16S method and the two comparison methods varied across evaluated criteria from 87% to 95% for sonicated implants and from 55% to 83% for tissues.

Incorporating a **spike in control** improved concordance to 100% for sonicates and 83% to 89% for tissues, and greatly aided results interpretation.

**Limit of detection** in tissue samples spiked with bacterial mixture was 2-8x$10^2$ CFU per species, except for *S. aureus* which required 6–7 × $10^3$ CFU.

**Final criteria for true positives**
A species is accepted only if

- ≥50 reads
- Relative Abundance ≥ 1 %
- > 1000 total reads in the sample

**Conclusions:**

Applying thresholds for defining clinically significant pathogen levels and the use of a multi-species process control substantially improved results interpretation and inter assay stability control in clinical 16S analyses of prosthetic joint infections.

## Materials and methods

A total of 87 sonicated prosthetic implants and 18 tissue samples from suspected foreign-body infections were included. 12 sonicates and all tissue samples were also processed with and without a commercial spike-in control to assess PCR inhibition.

Samples were homogenised, pretreated with mutanolysin and DNA extracted using MagDEA Dx SV. Duplicate PCR was performed with primers 27F/1492R and amplicons were barcoded with the ONT Native Barcoding Kit (v14) and sequenced on a GridION platform.

Bioinformatics analysis was performed in Geneious Prime using Emu for species identification (workflow avaliable at: https://github.com/clinical-genomics-uppsala/Geneious_metabarcoding).

The Limit of Detection (LOD) was evaluated using tissue samples spiked with a defined bacterial mock community.

All criteria were compared with Sanger sequencing and culture based diagnostics.

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