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Version 1.1 - April 2025

Evaluation of the EasySeq Full-Length 16S sequencing kit for bacterial identification in clinical samples: a multicentre study

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Introduction

Bacterial culture remains an essential method for **pathogen identification** but is often inadequate for fastidious bacteria or complex infections. Its **sensitivity** is further diminished by prior **antibiotic exposure**.

The **molecular** diagnostic revolution provided numerous options for **pathogen detection** or **identification** like syndromic testing by qPCR, Sanger Sequencing or Next-Generation sequencing. Each of these methods offer a different detection spectrum.

In this **consortium**, we compare different molecular methods of 9 different institutes with the **NimaGen**'s EasySeq Full-Length 16S sequencing kit in combination with the **Emweb**'s Genome Detective 16S pipeline, aiming to assess its suitability for **clinical diagnostics**.

Methods

Nine major medical centres in the Netherlands and Belgium processed **20 clinical samples** each from sterile sites (synovial fluid, cerebrospinal fluid, and tissue), ranging from negative (n=5) to polymicrobial infections.

Each centre applied **16S-based routine molecular diagnostics (MDx)** and the **EasySeq™ Full-Length 16S Library Prep Kit (NimaGen)** for Oxford Nanopore Technologies (ONT) sequencing. All centres worked according to manufacturers protocol and used the same lotnumbers of reagents and MinION flowcells.

Sequence data were analyzed using the Default Bacterial Analysis (16S) pipeline available at **GenomeDetective.com** (Emweb).

To assess **interlaboratory performance** and **reproducibility**, all centres processed Microbial Community DNA Standard II (Zymo, cat# D6310) in triplicate.

Table 1: Contributing centres and their routine 16S-based MDx

Centres	**Routine 16S-based MDx**
Isala	16S Sanger sequencing
UMCG	16S Sanger sequencing
Labmicta	qPCR
AUMC	IS-pro
LUMC	16S micPCR/Illumina sequencing
RUMC	16S RC-PCR Nimagen, Illumina sequencing
Maasstad ziekenhuis	16S ONT sequencing
Jessa ziekenhuis	16S modified ONT sequencing
MUMC	16S Sanger sequencing

Results

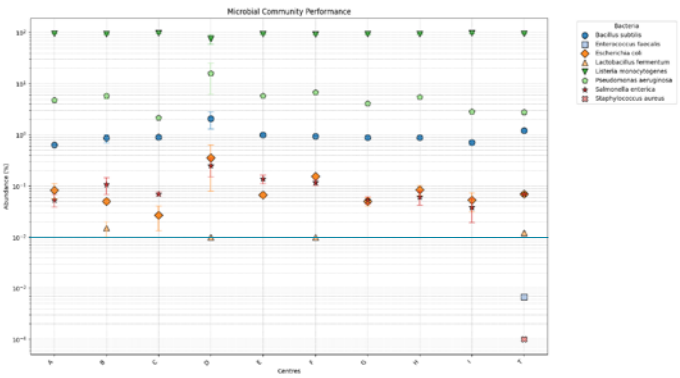

Figure 1: Microbial community DNA standard II performance for all centres tested in triplicate. Centres are indicated by A to I. Centre D only tested in duplo for which was normalized (SEM). 10K reads were subsetted prior analysis to normalize comparison. T indicates the theoretical input of the standard. The blue line in the figure represents 0.01% abundance. With our methods, detecting bacteria with an abundancy below this threshold is not feasible.

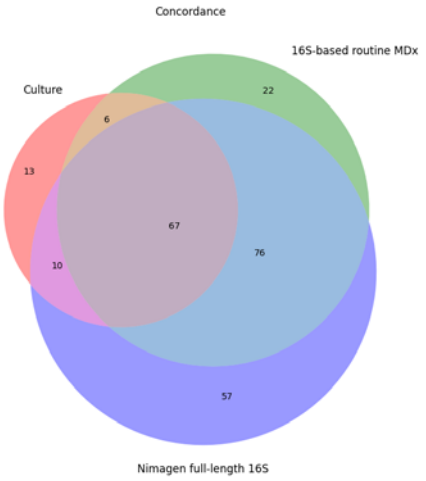

Figure 2: VENN diagram of bacterial taxa found. 16S-based routine MDx methods are shown in Table 1. Negative Processing Controls revealed bacterial reads (kitome), requiring correction before data visualization. Without this adjustment, taxa uniquely detected by the NimaGen Full-Length 16S kit in combination with the Genome Detective 16S pipeline, would exceed several hundred. Results are shown for 161/180 samples (89.44%) after excluding 19 samples due to failures in library preparation, sequencing and/or data analysis.

Discussion/Conclusion

Based on qualitative results, the EasySeq Full-Length 16S kit shows **high concordance** with routine diagnostic methods. It frequently detects **more bacterial** species than other methods and demonstrates the capability to identify a **high diversity** of bacteria within a single primary sample. The performance of the **Microbial Community DNA Standard** varies slightly between centres but remains consistent within triplicates. Detected bacteria follow the expected order of decreasing absolute load (Figure 1). The **Nimagen Full-Length 16S kit for ONT** in combination with the **Emweb Genome Detective 16S pipeline** may be of great diagnostic value, due to the fast work-up and detection of (multiple) fastidious, clinically relevant bacteria; however, users should be **aware** of the potential influence of **kitome** and **intersample signals**.

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This consortium study is supported by Nimagen and Emweb bv.