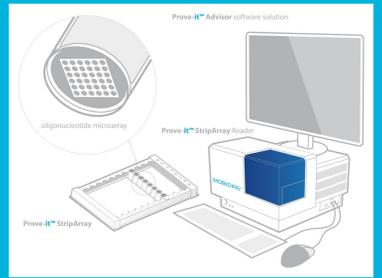


Evaluation of automated DNA extraction devices for sepsis diagnostics

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INTRODUCTION AND PURPOSE

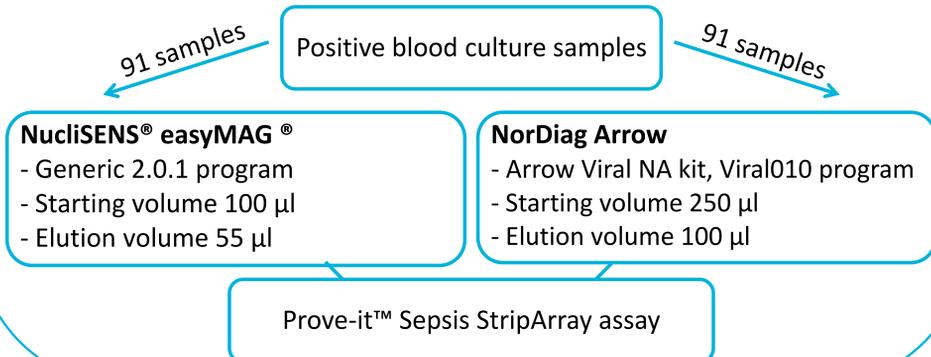
High yearly rates of septic patients increase the pressure of the faster and more reliable bacterial identification methods to decrease use of broad-spectrum antibiotics in patient's therapy. Novel molecular methods for bacterial identification usually require efficient sample processing and DNA extraction, continuing to the amplification steps.

The aim of this study was to bring together two aspects of novel technologies for sepsis diagnostics: automated DNA extraction together with a PCR and microarray based bacterial identification method. We compared two automated, *in vitro* diagnostic labeled DNA extraction devices, NorDiag Arrow (Norddiag) and NucliSENS® easyMAG® (bioMérieux), using positive blood culture samples. We analyzed the DNA extracts with the PCR and microarray-based Prove-it™ Sepsis assay (Mobidiag), which is designed to identify over 60 Gram-negative and Gram-positive bacterial species from positive blood cultures.

MATERIALS AND METHODS

A set of 91 positive blood culture samples from patients with suspected sepsis were collected and analyzed with the conventional culture based bacteriological method (HUSLAB, Finland). Simultaneously samples were extracted with the two devices and analyzed with the Prove-it™ Sepsis colorimetric assay according to the manufacturer's instructions. The obtained results were compared to those of the conventional blood culture method and any discrepancies were further analyzed by sequencing.

DNA extraction procedures for Prove-it™ Sepsis StripArray assay



RESULTS

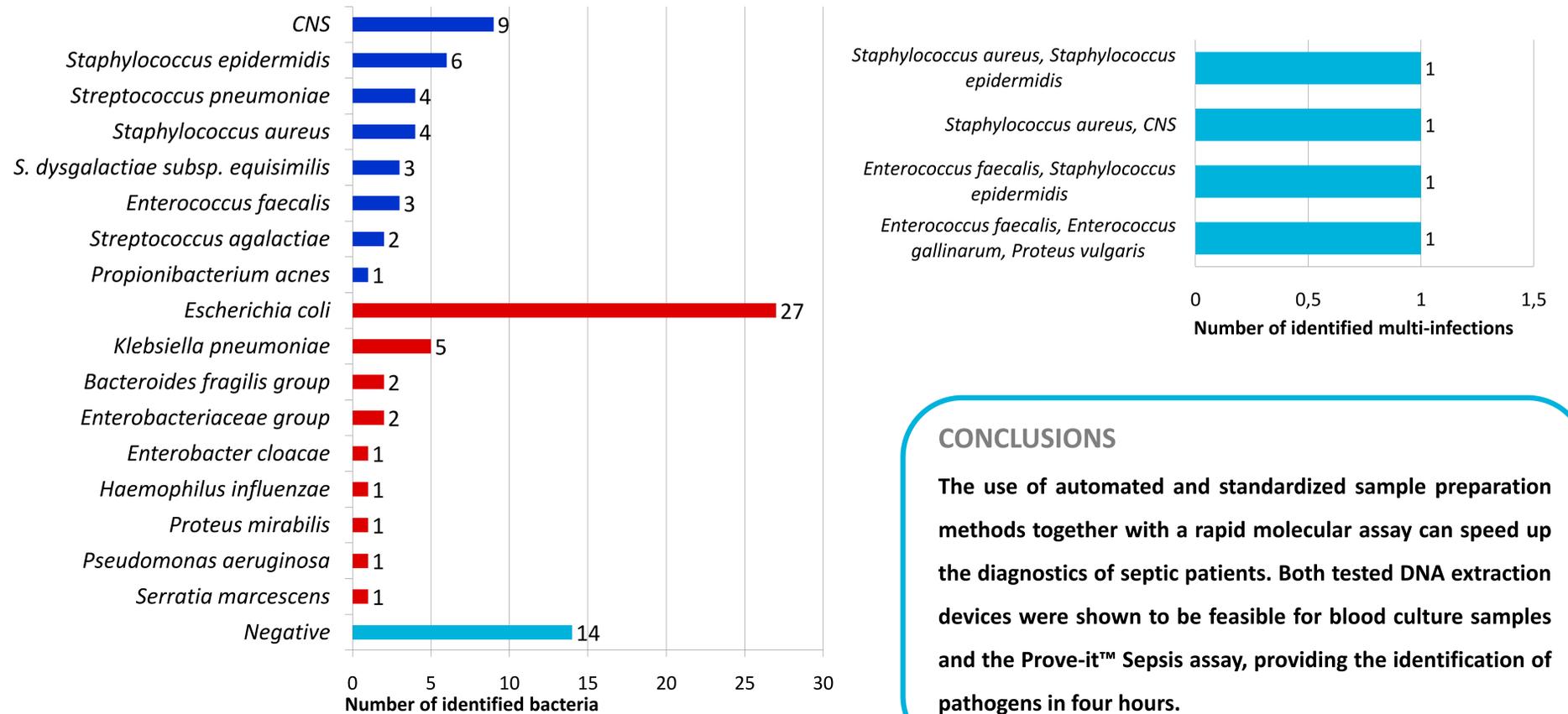
Comparison of extraction devices using Prove-it™ Sepsis assay

The functionality of the two extraction devices was evaluated using a set of 91 positive blood culture samples. The concordance of the results was 100 % meaning that no difference was observed between the performance of Arrow and easyMAG® with regard to the result reporting of Prove-it™ Sepsis. 77 bacterial findings were reported from the blood culture samples. 14 samples were reported as negative, containing bacteria not belonging to the pathogen panel of the assay (Figure 1).

Analysis of discrepancies between Prove-it™ Sepsis assay and conventional blood culture method

Eight different types of discrepancies were found (12 out of 91 samples). Of note was that *Streptococcus dysgalactiae* subsp. *equisimilis* was identified from two samples by Prove-it™ Sepsis, but the conventional method reported *Streptococcus pyogenes* findings. The DNA sequencing confirmed the *S. dysgalactiae* subsp. *equisimilis* findings. Also *Streptococcus pneumoniae* was detected and confirmed from two samples for which conventional method was reported negative.

Figure 1. The bacterial identification by Prove-it™ Sepsis assay when DNA was extracted with Nuclisens® easyMAG® and Norddiag Arrow. A total 41 Gram-negative bacteria, 32 Gram-positive bacteria, 4 multi-bacterial and 14 negative findings were reported.



CONCLUSIONS

The use of automated and standardized sample preparation methods together with a rapid molecular assay can speed up the diagnostics of septic patients. Both tested DNA extraction devices were shown to be feasible for blood culture samples and the Prove-it™ Sepsis assay, providing the identification of pathogens in four hours.