Performance evaluation and further development of PCR and microarray-based Prove-it™ Sepsis assay

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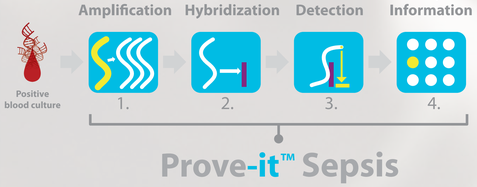
Päivi Tissari (1), Eveliina Tarkka (1), Sointu Mero (1), Laura Savolainen (1), Martti Vaara (1), Alimuddin Zumla (2), Jim Huggett (2), Caroline Carder (2), Vanya Gant (2), Anne Aittakorpi (3), Sanna Laakso (3), Merja Lindfors (3), Niklas Kumlin (3), Heli Piiparinen (3), Minna Mäki (3)

(1) Division of Clinical Microbiology, Helsinki University Hospital Laboratory HUSLAB, Finland
(2) Department of Clinical Microbiology, University College London Hospitals NHS Foundation Trust, and University College London Medical School, Centre for Infectious Diseases and International Health, UK
(3) Mobidiag Ltd, Finland

INTRODUCTION

Superior clinical management of septic patients may result from the more rapid speciation of organisms in positive blood cultures. The Prove-it™ Sepsis assay is a rapid, broad-range PCR and microarray-based assay designed to identify the majority of bacterial pathogens responsible for sepsis from positive blood cultures *(figure 1)*. The pathogen panel covers 50 Gram-negative and Gram-positive bacterial species; 24 at the species level and 26 at the taxon level *(figure 2)*. It also reports methicillin resistance by detecting the mecA gene in addition to differentiating between Staphylococcus aureus, Staphylococcus epidermidis and coagulase negative staphylococci (CNS). DNA is extracted from a positive blood culture and the topoisomerase and mecA gene regions are amplified by PCR. These PCR products are subsequently overlaid on the Prove-it™ TubeArray, where hybridization is detected in a single reaction. Detection is by solidstate hardware, and interpretation and reporting is via Prove-it™ Advisor software. The assay time is 3 hours including the DNA extraction.

Figure 1. Prove-it™ Sepsis Workflow.



METHODS

3318 blood samples collected from patients with suspected sepsis were analysed. Blood culture bottles of BacT/ALERT 3D (bioMérieux) in HUSLAB and BACTEC 924 (Becton Dickinson) in UCLH were incubated for a total of 6 days or until flagged as positive. In both laboratories, DNA was extracted from 100 µl of blood culture using the automated DNA extraction instrument easyMAG (bioMérieux) prior to the Prove-it™ Sepsis assay. Conventional blood cultures were conducted in parallel and results were only revealed for the comparison at statistical analysis stage. Discordant results between the conventional methods and Prove-it™ Sepsis were further studied by DNA sequencing and case-by-case review of original microbiology laboratory data.

Figure 2. Prove-it™ Sepsis pathogen panel.

Gram-negative	Gram-positive	Antibacterial resistance
Neisseria meningitidis	*Staphylococcus aureus*	*methicillin resistance marker mecA*
Enterobacter aerogenes	*Staphylococcus epidermidis*	
Enterobacter cloacae	*Coagulase negative Staphylococcus†*	
Escherichia coli	*Streptococcus pyogenes*	
Klebsiella oxytoca	*Streptococcus agalactiae*	
Klebsiella pneumoniae	*Streptococcus dysgalactiae subsp. equisimilis*	
Proteus mirabilis	*Streptococcus pneumoniae*	
Proteus vulgaris	*Enterococcus faecalis*	
Salmonella enterica subsp. enterica§	*Enterococcus faecium*	
Serratia marcescens	*Listeria monocytogenes*	
Enterobacteriaceae family‡	*Clostridium perfringens*	
Acinetobacter baumannii		
Pseudomonas aeruginosa		
Stenotrophomonas maltophilia		
Haemophilus influenzae		
Campylobacter jejuni/coli		
*Bacteroides fragilis group**		

§ Salmonella enterica subsp. enterica covers at least the following serovars: Enteritidis, Oranienburg,Othmarschen, Paratyphi, Stanley, Typhi, Typhimurium, Virchow, Group A,B,C,D

‡ Enterobacteriaceae covers at least the following species: Citrobacter amalonaticus, Citrobacter freundii, Citrobacter koseri, Citrobacter braakii, Enterobacter hormaechei, Enterobacter sakazakii, Kluyvera intermedia, Morganella morganii, Pantoea agglomerans, Providencia rettgeri, Providencia stuartii, Yersinia enterocolitica, Yersinia pseudotuberculosis

** Bacteroides fragilis covers at least the following species: B. fragilis, B. vulgatus*

†Coagulase negative Staphylococcus covers at least the following species: S. capitis, S. lugdunensis, S. haemolyticus, S. hominis, S. saprophyticus, S. warneri, S. xylosus

RESULTS

Of the analyzed 3284 blood cultures, 2107 yielded a pathogen by conventional techniques. Of these, 300 samples contained microbes not covered by Prove-it™ Sepsis, and an additional 137 cultures contained more than one organism. Sensitivity and specificity for Prove-it™ Sepsis were 94,7 % and 98,8%, respectively *(figure 3)*. Of particular significance was the assay´s faultless ability to differentiate MRSA from MSSA and from coagulase negative staphylococci. Furthermore, it provided a result on average one day earlier than the reference methods *(figure 4)*.

Figure 3. The performance evaluation analysis of Prove-it™ Sepsis

	Prove-it™ Sepsis **Positive**	Prove-it™ Sepsis **Negative**	
Reference method Positive	1696 True positive	94 False negative	Sensitivity 94,7%
Reference method Negative/ positive*	18 False positive†	1476 True negative	Specificity 98,8%

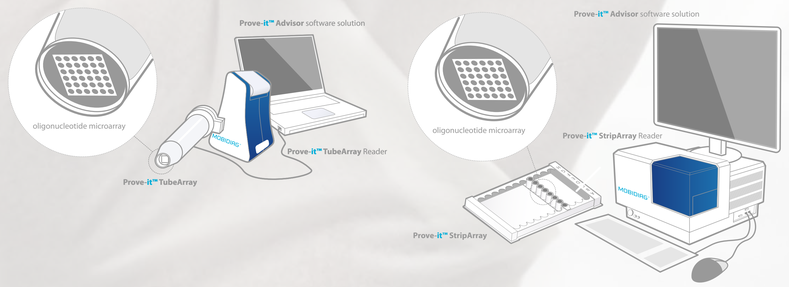
* Blood culture positive samples including a pathogen not covered by Prove-it™ Sepsis

Figure 4. Examples of the measured differences in the analysis time between the Prove-it™ Sepsis assay and the reference method.

Sample	Date of positive bwlood culture	Date of bacterial speciation		Time difference
		Prove-it™ Sepsis	Reference	
Staphylococcus epidermidis	5 Aug	5 Aug	6 Aug	18 h 44 min
Staphylococcus epidermidis	6 Aug	6 Aug	7 Aug	18 h 8 min
Enterococcus faecalis	4 Aug	4 Aug	6 Aug	1 d 19 h 8 min
Escherichia coli	6 Aug	6 Aug	7 Aug	17 h 59 min
Escherichia coli	6 Aug	6 Aug	7 Aug	18 h 24 min
Streptococcus pyogenes	23 July	23 July	24 July	18 h 41 min
Streptococcus pyogenes	23 July	23 July	24 July	18 h 40 min
Staphylococcus aureus	6 Aug	6 Aug	7 Aug	18 h 19 min
Staphylococcus aureus	7 Aug	7 Aug	8 Aug	18 h 6 min

d= day, h= hour, min= minutes.

Figure 5. Mobidiag platforms.



CONCLUSIONS

Prove-it™ Sepsis was considered to be a fast, robust, and high performance diagnostic platform which is easily implemented into everyday laboratory workflow. Both study sites identified patient cases where timely information provided by Prove-it™ Sepsis would have significantly improved patient management. Examples here include more rational management and antibiotic choice subsequent to earlier differentiation of "Gram-positive cocci in clumps" into MRSA, MSSA, or coagulase negative staphylococci, and earlier speciation of Gram-negative organisms.

After the performance evaluation study, the pathogen panel of Prove-itTM Sepsis is further configured for detection of Candida spp. and new bacterial targets, i.e., Neisseria non-meningitidis, Kingella kingae, and Propionibacterium acnes. The assay identifies now 60 out of the 300 samples that were not covered during the performance evaluation, increasing the pathogen coverage from 86% to 89%. Prove-it™ Sepsis has also been evaluated with highly promising results for the diagnostics of bone and joint infections using bone biopsy and articular fluid as sample types.

These evaluation studies have demonstrated excellent performance for this DNA-based diagnostic platform for various diagnostic applications *(figure 5)*. The earlier speciation provided by Prove-it™ Sepsis could contribute to faster, more evidence-based patient management and positive outcomes.

Ordering information
sales@mobidiag.com
+358 41 545 7902

Mobidiag Ltd.
Biomedicum 2U Helsinki
Tukholmankatu 8
FIN-00290 Helsinki
FINLAND

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www.mobidiag.com