The Mobidiag Prove-it™ Sepsis PCR and microarray platform: a 2 centre study evaluating performance for speciation of blood culture bacterial isolates within 3 hours.

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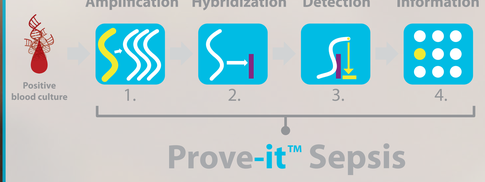
INTRODUCTION AND PURPOSE

Superior clinical management 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. It also claims to detect the mecA gene sequence in addition to differentiating between Staphylococcus aureus and coagulase negative staphylococci (CNS). DNA is extracted from a positive blood culture and proprietary regions 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 solid-state hardware, and interpretation and reporting is via **Prove-it™ Advisor** software.

Figure 1. **Prove-it™ Sepsis** Workflow.



MATERIALS AND METHODS

We compared this system with conventional microbiological identification in two major teaching hospitals in Helsinki and London in 3298 distinct blood culture samples from patients with suspected sepsis. Workflow for this system is shown in Figure 1. Traditional blood culture results were only revealed at statistical analysis stage.

Discordant results between the two methods were further analyzed by DNA sequencing and case-by-case review of original microbiology laboratory data. 1674 blood cultures included a pathogen covered by the **Prove-it™ Sepsis** assay (Figure 2.), leaving 302 currently not included in the system.

Figure 2. **Prove-it™ Sepsis** pathogen panel.

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

We analysed 3298 blood cultures , of which 2113 yielded a pathogen by conventional techniques. Of these, 302 organisms were not covered by appropriate probes, and an additional 137 cultures contained more than one organism. Sensitivity and specificity for the **Prove-it™ Sepsis** system were 94,7 % and 98,7%, respectively (Figure3.).

The system provided a result on average 18 hours earlier than conventional systems (Figure 4.). Of particular significance was its faultless ability to differentiate MRSA from MSSA and from coagulase negative staphylococci. Discrepant result analysis revealed 94 cases where the system's sensitivity limits or multiplexing capacity were likely exceeded; a few other cases were likely due to cross-hybridization, failure to isolate by traditional growth-dependent systems in bacterial mixtures, or unknown reasons.

The system was fast, reliable, robust, and rapid, with analysis taking less than 10 seconds per sample.

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 Positive / Negative	20 False positives	1479 True negative	Specificity 98,7 %

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 blood 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.

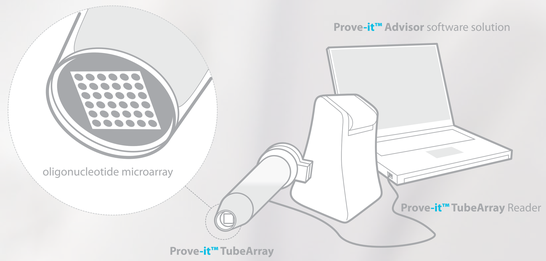
CONCLUSIONS

This study demonstrates excellent performance for this DNA-based identifications platform. Both centres identified cases where timely information which only this system would have significantly improved patient management.

Examples here include more rational management and antibiotic choice subsequent to earlier differentiation of "Gram+ve cocci in clumps" into MRSA, MSSA, or coagulase negative staphylococci, and earlier speciation of Gram negative organisms.

Once primers and probes for additional targets (specifically *Candida* spp.) are validated, we aim to perform a cost/benefit trial where decisions made with results provided by this simple, rapid, and robust diagnostic platform will be analysed for their impact on better patient outcome.

Figure 5. Mobidiag package.



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