Portrait Staph ID/R: A Novel Molecular Diagnostic Test for Simultaneous Identification of Staphylococcus species and Detection of the *mec*A gene Directly from Positive Blood Cultures.

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Abstract

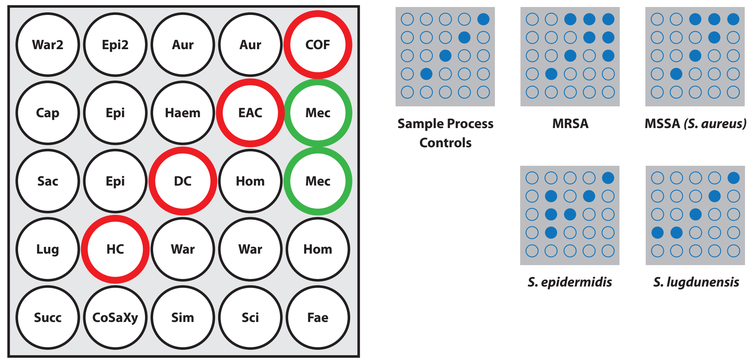
Background: Staphylococcal bacteremia is associated with high morbidity and mortality. The Portrait Staph ID/R Blood Culture Panel from Great Basin Diagnostics (Salt Lake City, UT) is a rapid, automated, DNA multiplex assay performed on the Portrait Dx Analyzer for simultaneous identification (ID) of *Staphylococcus aureus* and *Staphylococcus* species and the detection of *mec*A gene directly from positive blood cultures. For the Portrait Staph ID/R test all that is required of the operator is to add an aliquot directly from a positive blood culture bottle. The assay utilizes thermophilic helicase-dependent amplification (tHDA) technology to amplify specific sequences from Staphylococcal genomic DNA. tHDA is coupled with a hot start approach, RN2, which utilizes primers that are inactive until hybridized to target DNA at elevated temperatures, wherein RNase H2 removes a 3'-terminal blocking group, permitting DNA amplification. Multiple species specific staphylococcal DNA probes are immobilized on a modified silicon chip surface to enable eye visible detection of amplified DNA. The combination of isothermal amplification and chip-based eye visible signal creates a low cost, scalable platform. The objective of this preliminary study was to investigate the performance of the Portrait Staph ID/R compared to standard microbiological methods in our laboratory.

Methods: Thirty two positive blood culture bottles (BD BACTEC™ PLUS) yielding Gram positive cocci in clusters were analyzed using the Portrait Dx System. Results were simultaneously compared to the coagulase test and VITEK 2 ID/Antibiotic Susceptibility Test (AST) system (bioMerieux) with confirmation of oxacillin (OX) resistance by the CLSI cefoxitin (FOX) disk diffusion test. The time of ID and mecA gene detection was evaluated.

Results: All blood cultures were positive for *S. aureus* (n=9) or coagulase-negative staphylococci [CNS] (n=23) by conventional culture methods. The Portrait Staph ID/R correctly ID 32/32 to the genius-level and 30/32 to the species-level including 2 mixed cultures. Overall, 17/32 samples were OX/FOX resistant. The Portrait Staph ID/R detected the mecA gene in 5/6 OX resistant *S. aureus* and 9/11 FOX resistant CNS. On repeat testing, false negative *mec*A (3 samples) and 3 initial invalid test runs results were all resolved and in agreement by Portrait Staph ID/R and reference methods. The mean time to ID and mecA detection by Portrait Staph ID/R was 90 min. with minimal hands-on time.

Conclusion: The performance characteristics of the Portrait Staph ID/R in our laboratory compared favorably with conventional culture and AST methods. The described multiplex technology provides valuable information beyond the initial Gram stain in less than 90 min. Having more specific information about the organism could have a positive impact on initial therapy and help discriminate contaminated blood cultures.

Figure 2. Bacterial Identification Array



Feature	Staph species	Feature	Staph species
Succ	succinus	Hom	hominis (replicate)
CoSaXy	cohnii, saprophyticus, xylosus	Mec	***mecA*, methicillin resistance gene**
Sim	simulans	Cap	capitis
Sci	sciuri	Epi	epidermidis (replicate)
Fae	Entercoccus faecalis	Haem	haemolyticus
Lug	lugdunensis	EAC	Extraction-Amplification Control
HC	Hyb. Control	Mec	***mecA*, methicillin resistance gene**
War	warneri	War2	warneri variant
War	warneri (replicate)	Epi2	epidermidis variant
Hom	hominis	Aur	aureus
Sac	saccharolyticus	Aur	aureus (duplicate)
Epi	epidermidis	COF	Chip Orientation Feature
DC	Detect Control		

Capture probes are immobilized to the silicon surface. A diagonal set of control features verifies that chip orientation (COF), DNA **extraction** and **amplification** (EAC), and **detection** (HC, DC) functioned properly, validating test results.

Limit of Detection

***mecA* LOD** = 10^4 CFU/mL (3 CFU input)

Staph marker LOD = 10^5 CFU/mL (30 CFU input)

Blood culture samples were quantitated to determine CFU/mL, then serially diluted using a blood culture control. Staph ID/R was performed manually in 96-well format. *mecA* detection is more sensitive than species detection due to strength of capture probe for this sequence. LODs are on par with real-time PCR methods.

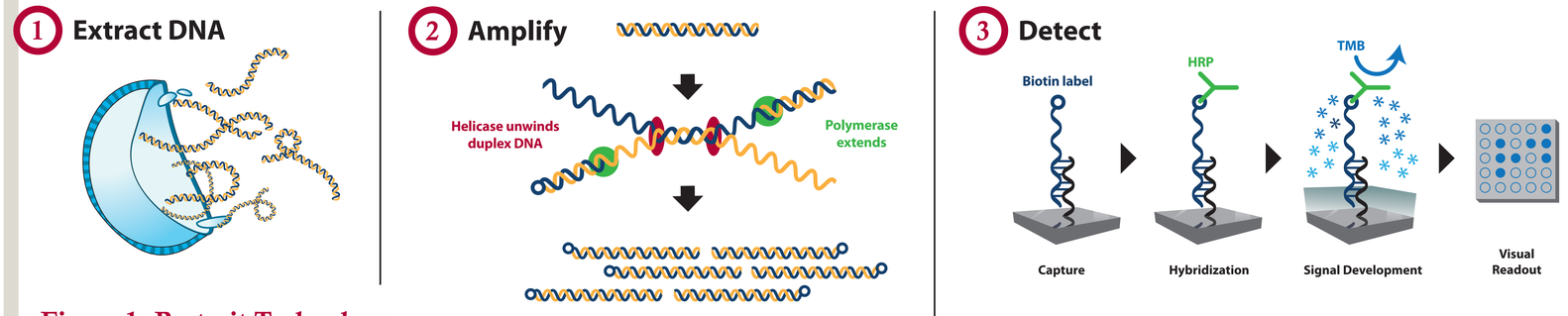


Figure 1. Portrait Technology

Introduction

Staphylococci are a major cause of hospital and community-acquired infections, leading to serious infections associated with significant rates of morbidity and mortality. The Portrait Staph ID/R Blood Culture Panel is a rapid DNA multiplex assay (**Figure 1, Portrait Technology**) that simultaneously identifies *Staphylococcus aureus* and most clinically relevant *Staphylococcus* species and detects the *mec*A gene for methicillin resistance directly from positive blood cultures (**Figure 2**). The purpose of this preclinical study was to assess the workflow and performance of the prototype Portrait Staph ID/R System compared to standard microbiological methods used in our laboratory.

Bacterial DNA is **Extracted** by enyzmatic lysis, then **Amplified** via Helicase Dependent Amplification (HDA). HDA represents an isothermal alternative to PCR in which strand separation is accomplished by a helicase rather than by heat denaturation. After amplification for 30 min at 65°, **Detection** occurs: amplicon binds to species-specific capture probes. After washing, an anti-biotin antibody conjugated to Horse Radish Peroxidase (HRP) produces a visual signal via precipitation of a tetramethylbenzidine cleavage product onto the silicon surface

Methods

Study Design. Positive blood culture bottles (BD BACTECTM PLUS) detected on the BACTEC 9240 blood culture instrument were subject to a Gram stain and routine culture. Those samples yielding Gram positive cocci in clusters were identified by standard microbiological methods for bacterial identification and resistance profile and an aliquot of the blood culture sample was analyzed using the Portrait Staph ID/R.

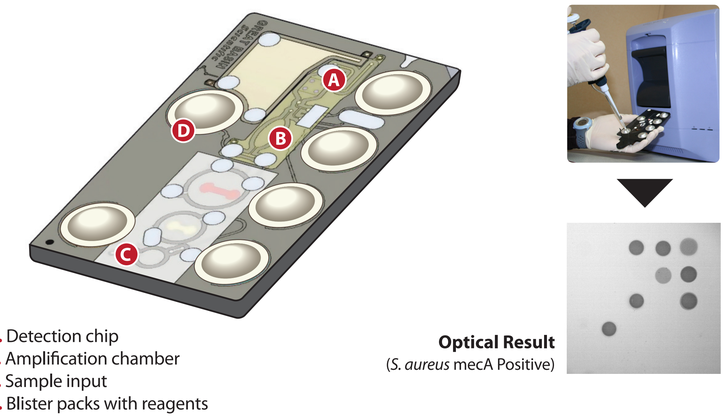
Organism Identification. Identification of the Staphylococcus genus was determined by colony morphology and a negative catalase test. Identification of *S. aureus* was determined by the tube coagulase test. Coagulase negative Staphylococci were identified by using the Vitek 2 ID card.

Antibiotic Susceptibility. Methicillin susceptibility was determined using the Vitek 2 AST or cefoxitin disk diffusion methods. The cefoxitin disk method was used to confirm methicillin resistance according to CLSI recommended procedure.

Portrait Staph ID/R Automated System. The Portrait Staph ID/R Blood Culture Panel procedure was performed according to the manufacturer's instructions. **See Figure 3.**

Results were compared for discordant organism identification/ and methicillin susceptibility. Samples were frozen at -85° C for resolution testing of discordant results. The time to final result was also determined.

Figure 3. Portrait Staph ID/R Automated System



A. Detection chip
B. Amplification chamber
C. Sample input
D. Blister packs with reagents

Optical Result
(*S. aureus* mecA Positive)

The Staph ID/R assay is built into a injection-molded card. Reagents are lyophilized or placed in blister packs. In the instrument, optical sensors control motors that propel 10s to 100s of uL through channels and chambers. This mesofluidic-scale design and injection-molded plastic card, in combination with isothermal amplification and human eye-visible signal, enables a low-cost card and instrument.

The operator inserts ~50 uL blood culture into the sample port as shown, inserts the card into the desktop instrument, and initiates the test. Software automatically returns a result within 90 min. The report details the presence of staphylococci, specifically identifies the 12 Staph. species deemed most relevant, and indicates status of the drug resistance gene *mec*A.

Results

In a blinded study, 32 blood culture samples representing 28 patients were positive for Gram positive cocci in clusters. Three additional samples gave invalid results by Portrait Staph ID/R. The distribution of Staphylococcal species and identification results by Vitek versus Portrait Staph ID/R is shown in **Table 1**. There was complete agreement to the genus level (32/32) and only 2 discordant results by Portrait Staph ID/R at the species level. Discordant results were resolved on repeat testing for 1 sample, while the other sample was positive for *S. auricularis*, which is not included in the Portrait Staph ID/R species specifc DNA capture probes.

The results for the detection of methicillin resistant Staphyloccocci by conventional AST and Portrait Staph ID/R are shown in **Table 2.** A total of 17 samples were OX/FOX resistant. The Portrait Staph ID/R detected 5/6 resistant *S. aureus* and 9/11 resistant coagulase-negative Staphylococci. One sample was Portrait Staph ID/R positive for mecA and cefoxitin susceptible by disk diffusion. All discordant results were resolved on repeat testing and in agreement by both methods.

The mean time from when the positive Gram stain was called to the floor and culture set-up to final results was 90 minutes and 32.5 hour for Portrait Staph ID/R and standard microbiological methods, respectively.

Table 1. Identification of Staphylococcus species by Vitek ID versus Portrait Staph ID/R.

Species	Vitek ID	Portrait Staph ID/R	Discordance
S. aureus	9	9	
S. auricularis	1	0	Portrait: *Staph Other*
S. epidermidis	15	15	
S. hominis	2	2	
S. lugdunensis	3	2	Portrait: *S. warneri / S. aureus*
S. epi + S. haemolyticus*	1	1	*not re-isolated by culture
S. epi + S. hominis	1	1	
Overall	**32**	**30**	

Table 2. Detection of methicillin resistant Staphylococci by Vitek AST (OX) and/or cefoxitin (FOX) disk diffusion versus Portrait Staph ID/R

Species	OX/FOX-Resistant	Portrait Staph ID/R *mecA* Pos	Discordance
S. aureus	6	5	OX-R, Portrait *mec*A Neg
S. auricularis	0	0	
S. epidermidis	8	8	FOX-R, Portrait *mec*A Neg FOX-S, Portrait *mec*A Pos
S. hominis	2	1	FOX-R, Portrait: *mec*A Neg
S. lugdunensis	0	0	
S. epi + S. haemolyticus*	0	0	
S. epi + S. hominis	1	1	
Overall	**17**	**15**	

Conclusions

- The performance of the Portrait Staph ID/R in this preliminary study was found to be highly favorable compared to standard identification and AST method.
- The Portrait Staph ID/R instrument is a small, automated bench-top analyzer with low cost, disposable cartridges for performing on demand testing during any shift. The combination of isothermal amplification and chip-based eye visible signal also creates a low cost, scalable platform.
- The immediate benefit of the Portrait Staph ID/R is the minimal sample handling by a laboratory technologist (sample in/result out).
- The Protrait Staph ID/R can identify a number of Staphylococcal species which are increasingly being identified in true infections that are not effectively detected by current molecular methods.
- The decreased time to results has benefits of improved treatment decisions and patient outcomes, and potential savings in hospital costs.
- Recent improvements in the Portrait Staph ID/R performance are currently under investigation.