Low Cost Molecular Diagnosis of Hospital Acquired Infection: Staph ID/R

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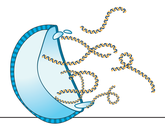
Introduction

We describe a sample in/result out molecular diagnostic platform that combines the sensitivity of nucleic acid amplification with the multiplex capability of chip-based detection. A desktop instrument runs sample in/result out tests in a disposable card format. With this platform now in production, a variety of rapid, simple, and inexpensive tests are in development.

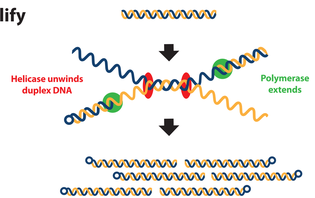
Initial focus is on hospital-acquired infections. *Staphylococcal* species, including the "superbug", Methicillin-Resistant *S. aureus* (MRSA), are the main causative agents of blood stream infections. Here we demonstrate the Staph ID/R assay for MRSA detection at Point of Care. In addition to MRSA, Staph ID/R detects an array of species that are increasingly recognized as blood stream pathogens. Test results for 135 clinical samples are reported.

Portrait Technology

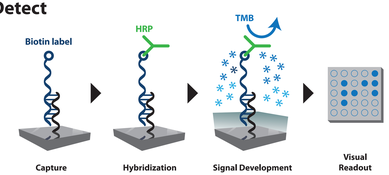
1 Extract DNA



2 Amplify



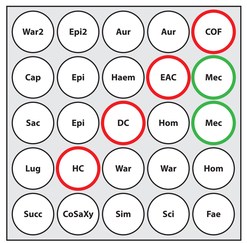
3 Detect



Bacterial DNA is **Extracted** by enzymatic 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

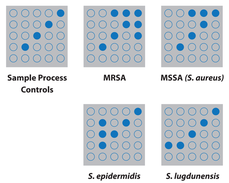
Bacterial Identification Array

Control Features **MecA Gene**



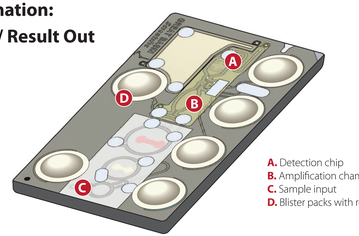
Feature	Staph species
Succ	succinus
CoSaXy	cohnii, saprophyticus, xylosus
Sim	simulans
Sci	sciuri
Fae	Entercoccus faecalis
Lug	lugdunensis
HC	**Hyb. Control**
War	warneri
War	warneri (replicate)
Hom	hominis
Sac	saccharolyticus
Epi	epidermidis
DC	**Detect Control**

Feature	Staph species
Hom	hominis (replicate)
Mec	***mecA*, methicillin resistance gene**
Cap	capitis
Epi	epidermidis (replicate)
Haem	haemolyticus
EAC	**Extraction-Amplification Control**
Mec	***mecA*, methicillin resistance gene**
War2	warneri variant
Epi2	epidermidis variant
Aur	aureus
Aur	aureus (duplicate)
COF	**Chip Orientation Feature**



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.

Test Automation: Sample In / Result Out



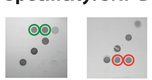
A. Detection chip
B. Amplification chamber
C. Sample input
D. Blister packs with reagents

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.

Optical Result

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 75 min (test speed is not yet optimized). The report details the presence of staphylococci, specifically identifies the 12 Staph. species deemed most relevant, and indicates status of the drug resistance gene *mecA*.

Specificity: SNP Discrimination



S. aureus ***S. warneri***

Portrait technology has the capacity to discriminate single nucleotide polymorphisms (SNPs), as indicated by the ability to separate two species that differ by a single nucleotide in the capture probe region. Samples were processed by manual 96-well format, with 4 min hybridization, 1 min wash, 4 min conjugated Ab, and 2 min color development, all at 53°.

Limit of Detection

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

CFU/mL	10^7	10^6	10^5	10^4	0

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

CFU/mL	10^7	10^6	10^5	10^4	0

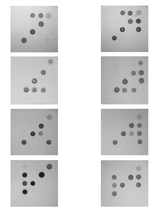
Blood culture samples were quantitated to determine CFU/mL, then serially diluted using a blood culture control. Staph ID/R was performed manually in 96-well format. *mecA* detection is more sensitive than species detection due to strength of capture probe for this sequence. LODs are on par with real-time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

Clinical Testing, Staph ID/R

Optical Result Readout

Control Features Indicate Valid Test:
Result = non-Staph

Methicillin resistance marker



Other species

Molecular diagnostic approaches utilizing real-time PCR have shortened the time to MRSA/MSSA identification from 48-72 hr to < 2 hr after indication of a positive blood culture. However, an array of Staph spp. that are not effectively detected by these approaches are increasingly being identified in true infections. We therefore tested the Staph ID/R assay in 96-well manual format using frozen retrospective blood culture samples. Representative Staph ID/R visual images are shown.

Species Identification: Staph ID/R and Clinical Site

Species	Staph ID/R	Clinical Sites (2)
S. aureus	37/37	37/37
S. epidermidis	51/51	49/51
S. warneri	10/10	8/9
S. hominis	10/10	6/6
S. haemolyticus	3/3	0/3
S. capitis	3/3	2/3
S. lugdunensis	2/2	0/2
S. cohnii	1/2	1/2
Other *Staph.*	3/3	2/3
Non *Staph.*	15/17	16/17
Overall	98% Correct	91% Correct

Methicillin resistance: Staph ID/R and Clinical Site

	Staph ID/R	Clinical Sites (2)
Methicillin resistance	64/64 = 100%	59/63 = 94%

Results for 135 blinded samples, mainly from pediatric ICU patients, are tabulated and compared to identification performed at the clinical sites, Children's Memorial in Chicago, and Clarian Health Partners Methodist Hospital in Indianapolis. Clinical sites used automated phenotypic identification systems and coagulase testing. Discrepant results were resolved by DNA sequencing at the 16S rDNA and *rpoB* loci, and by streaking cells onto chromogenic agar plates.

Details on Misidentified Species. *S epidermidis:* 2 not detected in polymicrobial cultures; *S warneri:* 1 misidentified as *S. cohnii*; *S. haemolyticus:* 1 misidentified as *S. auricularis* and 2 not detected in polymicrobial cultures; *S. capitis:* 1 misidentified as *S. hominis*; *S. lugdunensis:* 1 identified as CoNS without follow-up, 1 not detected in a polymicrobial culture; *S. cohnii:* 1 not detected in polymicrobial culture (Clinical Sites and Staph ID/R); Other Staph: 1 misidentified as *S. lugdunensis*; Non-Staph: 1 misidentified as *S. warneri* (Clinical Site) and 2 not detected in polymicrobial cultures where other Staph where found (Staph ID/R).

Conclusions: Staph ID/R

Portrait™ Technology is now integrated into a device that provides simple, rapid, and inexpensive detection of hospital-acquired infections. With this platform now in production, a variety of tests are in development.

GREAT BASIN Scientific

Simple:	Sample in / result out
Rapid:	Results in less than 75 minutes
Accurate:	>98% correct on 135 clinical samples
Sensitive:	LOD is on par with real-time PCR
Specific:	Distinguishes SNPs to resolve species
Inexpensive:	Mesofluidics, not microfluidics Visual signal = Digital camera optics Injection molded card No thermal cycling required