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

Introduction
We describe a sample input-out molecular diagnostic platform that combines the sensitivity of nucleic acid amplification with the multiplex capability of chip-based detection.

Bacterial Identification Array

<table>
<thead>
<tr>
<th>Feature</th>
<th>Staph species</th>
<th>Non-Staph species</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU/mL</td>
<td>10^7</td>
<td>10^6</td>
</tr>
<tr>
<td>S. aureus</td>
<td></td>
<td>S. lugdunensis</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td></td>
<td>S. warneri</td>
</tr>
<tr>
<td>S. aureus (duplicate)</td>
<td></td>
<td>S. capitis</td>
</tr>
<tr>
<td>S. aureus (variant)</td>
<td></td>
<td>S. epidermidis</td>
</tr>
<tr>
<td>S. warneri</td>
<td></td>
<td>S. sciuri</td>
</tr>
<tr>
<td>S. simulans</td>
<td></td>
<td>S.War2</td>
</tr>
</tbody>
</table>

Capture probes are immobilized on the silica surface. A diagnostic set of control features verifies that chip orientation (CFU/mL) extraction (EAC) and amplification (EAC) and detection (PCR) function properly, validating test results.

Test Automation: Sample In / Result Out

- Extract DNA
- Amplify
- DETECT

Optical Result

- Sample Input
- Result Output
- Optical Result Readout
- CFU/mL 10^7 10^6 10^5 10^4 0

Limit of Detection

- CFU/mL = 10^7 (EAC input)
- Staph rod: CFU/mL = 10^7 (EAC input)

<table>
<thead>
<tr>
<th>Method</th>
<th>Signal</th>
<th>LOD (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR</td>
<td>HCR</td>
<td>10^3</td>
</tr>
<tr>
<td>Staph ID/R</td>
<td>EAC</td>
<td>10^4</td>
</tr>
</tbody>
</table>

Clinical Testing, Staph ID/R

- Optimal Result
- Sample Input
- Result Output
- Optical Result
- S. aureus: 10^7
- S. warneri: 10^6
- S. capitis: 10^5
- S. sciuri: 10^4
- S. War2: 10^3

Methicillin resistance marker

- S. aureus: meca
- Non-Staph: meca

Molecular diagnostic approaches utilizing real-time PCR have shortened the time to MRSA identification from 48-72 hours to < 2 hours after isolation of a positive blood culture. However, sets of Staph 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 format using frozen retrospective blood culture samples. Representative Staph ID/R visual images are shown.

Clonality

- S. aureus
- S. warneri
- S. capitis
- S. sciuri
- S. War2

Specificity: SNP Discrimination

- S. aureus: meca
- Methicillin resistance
- Staph identification

Staph ID/R Chip

- S. aureus
- S. warneri
- S. capitis
- S. sciuri

Conclusions: Staph ID/R

- Rapid, cost-effective, and sensitive detection of Staph species and methicillin resistance
- Simple and rapid detection in 75 minutes
- Suitable for point-of-care testing
- Cost-effective and easy to use

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- Great Basin Scientific, Salt Lake City, UT; 2. Clarian Health Partners Methodist Hospital, Indianapolis, IN; 3. Children’s Memorial Hospital, Chicago, IL. Contact: bhicke@gbscience.com or rjenison@gbscience.com

Results for 135 blinded samples, mainly from pediatric ICU patients, are tabulated and compared to identification systems and coagulate testing. Discrepant results were resolved by DNA sequencing at the 16S rDNA sequence.

Staph ID/R distributes 12 Staph. species deemed most relevant, and within 75 min (test speed is not yet optimized). The report details the presence of MRSA, Staph ID/R and Clinical Site. The platform now in production, a variety of tests are in development.