Screening tests have recently been introduced that detect single molecular targets specific to methicillin-resistant *Staphylococcus aureus* (MRSA) (21, 23). These offer significant advantages over culture, which takes 2 to 5 days to produce a positive result, and their use has been associated with reduced transmission of MRSA in both intensive care unit (ICU) and surgical settings (4, 11, 12). One of the most widely used commercial screens is the BD GeneOhm MRSA assay (BD Diagnostics, Becton Dickinson, NJ), simultaneously detects targets in the staphylococcal cassette chromosome mec (SCCmec) and *S. aureus*-specific orfX genes. It has been evaluated for use on individual swabs taken from nasal and extranasal sites and from pooled samples, using the Smart-Cycler II (Cepheid, Sunnyvale, CA) PCR format (2, 6–8, 14). With the adoption of universal MRSA screening for hospital admissions (5), however, the volume of samples is set to increase significantly, necessitating an evaluation of alternative, higher-throughput platforms.

The aim of the present study was to evaluate the performance of the BD GeneOhm MRSA assay on the Rotor-Gene 6000 thermal cycler, using samples taken directly from pooled MRSA screens. Results were compared with the same assay performed on the Smart-Cycler II platform and overnight broth culture. Samples yielding discrepant results were subjected to detailed analysis with an in-house PCR and patient note review. A total of 1,428 pooled MRSA screens were tested. Sensitivities and specificities of 85.3% and 95.8% for the Rotor-Gene and 81% and 95.7% for the Smart-Cycler were obtained, compared with broth enrichment. The sensitivity of the BD GeneOhm assay was increased to 100% when the results of in-house PCR and patient note review were taken into account. This study demonstrates that the Rotor-Gene 6000 thermal cycler is a reliable platform for use with the BD GeneOhm assay. It also proves that commercial PCR can be performed direct on pooled samples in selective broth, without the need for overnight incubation.

**Evaluation of the BD GeneOhm Assay Using the Rotor-Gene 6000 Platform for Rapid Detection of Methicillin-Resistant *Staphylococcus aureus* from Pooled Screening Swabs**

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As health services move toward universal methicillin-resistant *Staphylococcus aureus* (MRSA) screening for hospital admissions, the most cost-effective approach is yet to be defined. In this study, one of the largest to date, we evaluated the performance of the BD GeneOhm MRSA assay on the Rotor-Gene 6000 thermal cycler, using samples taken directly from pooled MRSA screens. Results were compared with the same assay performed on the Smart-Cycler II platform and overnight broth culture. Samples yielding discrepant results were subjected to detailed analysis with an in-house PCR and patient note review. A total of 1,428 pooled MRSA screens were tested. Sensitivities and specificities of 85.3% and 95.8% for the Rotor-Gene and 81% and 95.7% for the Smart-Cycler were obtained, compared with broth enrichment. The sensitivity of the BD GeneOhm assay was increased to 100% when the results of in-house PCR and patient note review were taken into account. This study demonstrates that the Rotor-Gene 6000 thermal cycler is a reliable platform for use with the BD GeneOhm assay. It also proves that commercial PCR can be performed direct on pooled samples in selective broth, without the need for overnight incubation.

**MATERIALS AND METHODS**

The study was conducted at Kings College Hospital, a 940-bed London teaching hospital in South London, England.

**Specimen collection and processing.** Routine MRSA screening swabs were taken from patients being admitted to or resident in our hospital. Unlike many previous studies, there was no preselection of samples according to previous MRSA status or patient type (2, 6–8, 14). The sites sampled included anterior nares, groin/perineum, and throat in addition to skin breaks/wounds and indwelling device insertion sites, where appropriate. Either Stuart’s or Amies medium (Copan Diagnostics, Italy), with or without charcoal, was used for transport of swabs (14). To minimize sampling error at sites with low bacterial counts, we avoided the use of double-headed or duplicate swabs.

**Culture.** Pooled swabs from individual patients were inoculated into 5 ml selective mannitol broth (product code EB1016C; Oxoid Ltd.), with or without charcoal, was used for transport of swabs (14). To minimize sampling error at sites with low bacterial counts, we avoided the use of double-headed or duplicate swabs.

**Culture.** Pooled swabs from individual patients were inoculated into 5 ml selective mannitol broth (product code EB1016C; Oxoid Ltd.). After overnight incubation at 37°C, broths with a positive chromogenic indicator change on visual inspection (approximately 40% of the total) were subcultured onto Brilliant MRSA agar (product code P011621A; Oxoid Ltd.). After at least 18 h of incubation at 37°C, “denim blue” colonies were identified by standard methodology (13).

**LoD testing.** For limit of detection (LoD) testing, a laboratory strain of MRSA was cultured overnight at 37°C to late log phase (10^8 to 10^9 CFU/ml). Log dilutions were made in nutrient broth (10^{-1} to 10^{-5}), and 50 μl from each dilution was plated onto blood agar and incubated overnight in air at 37°C for CFU counts. One hundred microliters from each of these dilutions was transferred to sterile cotton-tipped swabs (in triplicate) and placed in 5 ml Oxoid indicator broth, incubated for 2 h. Fifty microliters was taken from each culture, and CFU counts were performed. A further 1 ml was removed and processed as described below for PCR analysis.

**PCR.** The commercial PCR used was the BD GeneOhm MRSA assay (Becton Dickinson, Franklin Lakes, NJ). After 2 h of incubation at 37°C, each enrichment broth was vortexed for 30 s. A 1-ml aliquot was centrifuged at 13,000 × g for 5 min at room temperature, supernatants were discarded, and the pellet was resuspended in 1 ml of BD GeneOhm sample buffer. Each suspension was transferred to a BD GeneOhm lysis tube for DNA extraction according to the manufacturer’s instructions. The pellet was resuspended in 50 μl of BD sample buffer, and 2.8 μl was used for the PCR, which was performed according to the manufacturer’s instructions with the Smart-Cycler II platform (Cepheid, Sunnyvale, CA).
TABLE 1. Limit of detection analysis for MRSA PCR from selective broth

<table>
<thead>
<tr>
<th>CFU count from 50-μl Oxiid culturea</th>
<th>Result by:b</th>
<th>Smart-Cycler</th>
<th>Rotor-Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>477</td>
<td>+ + +</td>
<td>+ + +</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>10</td>
<td>+ + +</td>
<td>+ + +</td>
<td>+ + +</td>
</tr>
<tr>
<td>0</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
<tr>
<td>0</td>
<td>- - -</td>
<td>- - -</td>
<td>- - -</td>
</tr>
</tbody>
</table>

a The average of three readings is shown.
b The Smart-Cycler and Rotor-Gene assays were carried out in triplicate, and the numbers of positive (+) or negative (−) results are indicated.

GeneOhm MRSA assay reagents run on the Rotor-Gene 6000. Samples for the Rotor-Gene assay were processed as described above, using 28 μl of BD master mix and 3 μl of the same lysate used for the Smart-Cycler II. The program consisted of a 10-min hold at 95°C, followed by 45 cycles of 95°C for 5 s, 59°C for 15 s with acquisition on green (MRSA) and yellow (internal control), and 72°C for 20 s.

In-house real-time PCR assays run on the Rotor-Gene 6000. Discrepant samples were analyzed by an in-house PCR assay targeting the sa442 and mecA genes. The assays were based on the method described by Reischl et al. (18), using newly designed primers for the mecA gene and TaqMan probes for both mecA and the 5.5-mecA-specific fragment, Sa442. The two targets were amplified and detected in separate reactions: mecA amplification mixtures contained 15 μl Qiagen QuantiTect probe mix, 10 pmol each of forward and reverse primer (MecF1, 5′-TAG CAC TCG AAT TAG GCA GT-3′; and MecR1, 5′-GCA GTA CCG GAT TTG CCA AT-3′), and 5 pmol of probe (MecP1 6-carboxy-fluorescein [FAM]-ATC ACT TGG TAT ATC TTC ACC AAC ACC TAG GTA CCG GAT TTG CCA AT-3′; and MecR1, 5′-GCA GTA CCG GAT TTG CCA AT-3′); and Sa442 amplification mixtures contained 15 μl Qiagen QuantiTect probe mix, 10 pmol each of Sa442-F and Sa442-R (18), and 5 pmol of probe (Sa442P1 CAL fluor red 610-TGT GTG CTG TAT GTA AAA GCC GTC TGT GTA ATA ATC T-BHQ2) in a total volume of 30 μl. For both targets, 3 μl of the same lysate as used for the Smart-Cycler II and Rotor-Gene 6000 PCRs was used as a source of template. The program used was a 10-min hold at 95°C, followed by 45 cycles of 95°C for 5 s, 59°C for 15 s with acquisition on green (mecA) and orange (sa442), and 72°C for 20 s.

Patient NR. Electronic patient records were reviewed for all patients with discrepant results. Patients were classified as note review (NR) positive if at least one of three screens and the screen immediately following the test sample, where available, were recorded. Results from clinical samples processed within this period were also recorded.

Discrepant analysis. PCR extracts for samples yielding PCR-positive, culture-negative (P+ C−) or PCR-negative, culture-positive (P− C+) results were tested by in-house PCR. Patient note review (NR) was conducted, and results from the three previous MRSA screens and the screen immediately following the test sample, where available, were recorded. Results from clinical samples processed within this period were also recorded.

Forty-two samples positive by the BD GeneOhm assay on the Smart-Cycler and Rotor-Gene were negative by culture. Thirty-three (79%) of these tested positive by in-house PCR (mecA and sa442). Twenty-two of these (53% of total) came from patients who were MRSA positive by NR and therefore likely represent culture false negatives. The six P+C− samples testing negative for mecA and/or sa442 were from patients who were NR MRSA negative. These are likely to represent false positives by the BD GeneOhm assay. Results for analysis of P+ C− discrepant samples are shown in Table 3.

TABLE 2. Comparison of BD GeneOhm MRSA assay with culture

<table>
<thead>
<tr>
<th>Result</th>
<th>No. of samples with result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corbett Rotor-Gene 6000</td>
<td>Smart-Cycler</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive</td>
<td>116</td>
</tr>
<tr>
<td>Negative</td>
<td>1,312</td>
</tr>
<tr>
<td>Total</td>
<td>1,428</td>
</tr>
</tbody>
</table>

TABLE 3. Analysis of 42 P+ C− discrepant samples

<table>
<thead>
<tr>
<th>Result</th>
<th>No. of samples with NR resulta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Sa442 and MecA positive</td>
<td>22</td>
</tr>
<tr>
<td>Sa442 and/or MecA negative</td>
<td>1</td>
</tr>
</tbody>
</table>

a Samples GeneOhm assay positive on the Smart-Cycler and Rotor-Gene.
b Three MRSA screens were not taken before the test sample.

RESULTS

LoD testing. The limits of detection (LoD) for the BD GeneOhm MRSA assay performed on selective broth cultures were ca. 10 CFU for the Smart-Cycler II and 0 to 10 CFU for the Rotor-Gene 6000. The results are shown in Table 1.

Analysis of clinical samples. A total of 4,128 pooled MRSA screens were tested by PCR and culture. The overall results are shown in Table 2. One hundred sixteen screens (8.1%) were positive by culture, 150 (10.5%) by PCR on the Smart-Cycler, and 154 (10.8%) by PCR on the Rotor-Gene 6000. Ninety-four of the PCR-positive screens by Smart-Cycler and 99 by Rotor-Gene were culture positive, yielding sensitivities and specificities of 81% and 95.7% for the Smart-Cycler and 85.3% and 95.8% for the Rotor-Gene, respectively. Based on an MRSA prevalence rate of 8.1% by broth culture, the positive predictive values (PPV) and negative predictive values (NPV) were 63% and 98% for the Smart-Cycler and 64% and 99% for the Rotor-Gene, respectively.

DISCUSSION

Rapid screening for MRSA has been shown to reduce transmission and cross-infections in surgical and ICU patients (4, 11, 12). This is important given that each surgical site infection is estimated to cost an average of £4,200 (22) and is associated with significant increases in length of stay and mortality (3, 10).
In this study, we demonstrated that the BD GeneOhm PCR assay, which is validated for use on the Smart-Cycler II, works equally well on the Rotor-Gene 6000. This unit has more than four times the capacity of a Smart-Cycler II block and is easily adapted for use with robot technology (1), which should be of interest for laboratories wanting to adapt this assay to an existing platform or where a greater degree of automation is required: e.g., with universal screening (5).

The sensitivities obtained for the BD GeneOhm assay (81% on the Smart-Cycler and 85% on the Rotor-Gene) were similar to published rates for pooled samples using broth culture as a comparator (6, 14, 15). In the present study, most C+P− samples were positive by in-house PCR and/or note review, suggesting a degree of insensitivity of the commercial PCR. This may reflect our unlicensed application of the technology (performing PCR direct on selective broth, the use of Amies transport medium, and/or the inclusion of swabs from extra-nasal sites) rather than an inherent flaw in the assay.

A slight increase in sensitivity was seen when the IDI-MRSA assay was performed on the Rotor-Gene 6000 compared with the Smart-Cycler. Such differences have been noted previously, with more consistent threshold cycle (CT) values obtained by using the Rotor-Gene (1). This may be due to the greater temperature uniformity in the reaction chamber of the Rotor-Gene, reducing tube-to-tube variation (16). The Rotor-Gene also showed a slight increase in sensitivity in the LoD assay, with 2/3 runs detecting down to 2.0 × 10^1 CFU/ml compared with 2.0 × 10^2 CFU/ml for the Smart-Cycler. A similar study by Rossney et al. demonstrated higher LoDs for Smart-Cycler (2.0 × 10^3) compared to culture from Stuart’s medium (1.4 × 10^3), although differences in methodology may have accounted for this (19).

High rates of C−P+ samples have been reported in several studies (12, 19), leading some investigators to recommend culture of all PCR-positive samples (7, 20). This discordance was particularly marked when PCR was compared with culture on chromogenic agar (12) and is due in large part to the insensitivity of the latter (21). In the present study, 22 of the 43 culture-negative samples were positive by SmartCycler, Rotor-Gene, mecA, sa442, and note review. These are therefore likely to represent culture false negatives, and their inclusion as true PCR positives in the analysis would have increased the sensitivity of the BD GeneOhm MRSA assay to 100% compared with broth culture. The mechanism for this C−P+ discrepancy is unclear. Sampling error may occur when sites with low numbers of organisms are swabbed (23). This was excluded in the present study by performing PCR directly on the broth cultures rather than dual/repeat swabbing of sites. Community-acquired MRSA (CA-MRSA) strains are commonly susceptible to ciprofloxacin (9) and may therefore be missed when this antibiotic is used as a selective agent in enrichment broths. A recent study in the United Kingdom suggested that such strains account for less than 2% of MRSA isolates (17); however, this is unlikely to account for the high levels of C−P+ seen in this and other United Kingdom-based studies using broth culture (14, 19). Of the C−P+ samples seen here, more than 80% had detectable mecA by in-house PCR. It is possible that the remainder represented MRSA strains with genetic excisions within the SCCmec region of the chromosome (23). These so-called “empty cassette” variants are phenotypically susceptible to oxacillin and cefoxitin but give a positive result by commercial PCRs, such as the BD GeneOhm, which target sequences upstream from the mecA gene. Unfortunately, sequencing of the SCCmec region, which would be required to confirm this, was not available to us at the time of this study. It is also possible that recent mupirocin or chlorhexidine use in patients with positive MRSA screens (at least 50% of the C−P+ screens in this study) may have affected culture results, although there was no evidence for this from the electronic notes. The potential for the BD GeneOhm MRSA assay and other molecular tests to detect residual DNA rather than viable organisms exists, and this is an important factor if PCR is to be adopted as a “test of cure” as well as a screen for MRSA.

The methodological approach used in this study (i.e., performing the BD GeneOhm assay directly on liquid culture) has been investigated previously (7). The assay was performed on overnight cultures of pooled nasal and rectal swabs from patients deemed to be high risk for MRSA. The sensitivity of PCR was slightly higher than that in the present study, but results were not available until the day after sampling. It is possible that the sensitivity of our assay might be improved by increasing the incubation time from 2 to 4 h. This would require receipt and processing of samples early in the day, however, if a same-day result is to be achieved as sample “setup” takes ca. 2 h in addition to the broth incubation and PCR steps. One obvious advantage of sampling directly from broth is the dilution of PCR inhibitors. In the present study, only 0.8% samples required reamplification. This rate, similar to that seen in this study above (7), compares well with observed inhibition rates seen when PCR was performed directly on perineal swabs (12%), rectal swabs (12%), and pooled swabs from multiple sites (5%) (8).

The dual-test approach used in the present study could be tailored to different health care settings. If the primary goal in this study had been the rapid confirmation of PCR positives, only ca. 10% of broths would have required subculture to chromogenic agar, compared with the 40 to 50% that are routinely processed in our laboratory. This would have reduced costs considerably. If sensitivity is the overriding priority, then PCR of all samples and subculture of “indicator-positive” broths would be required. On the basis of this study, dual testing could be expected to increase the sensitivity of either culture or PCR alone by up to 15%. This approach might be useful for patients in high-risk specialties such as cardiothoracic, vascular, or orthopedic surgery.

As health services move toward universal MRSA screening for hospital admissions, the most cost-effective approach is yet to be defined. While molecular methods offer advantages in

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**TABLE 4. Analysis of 15 P−C+ discrepant samples**

<table>
<thead>
<tr>
<th>Result</th>
<th>No. of samples with NR result:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Sa442 and MecA positive</td>
<td>9</td>
</tr>
<tr>
<td>Sa442 and/or MecA negative</td>
<td>5</td>
</tr>
</tbody>
</table>

a Samples PCR negative on repeat testing (Rotor-Gene 6000) with the BD GeneOhm assay.

b Two samples were from the same patient.
terms of rapidity, unease exists over cost and capacity as well as the high rates of discrepancy reported by some investigators. The present study, one of the largest to compare commercial PCR with broth culture, adds valuable information to this debate while offering an alternative platform for commercial MRSA testing in hospital laboratories.

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