Evaluation of S. aureus ID, a New Chromogenic Agar Medium for Detection of Staphylococcus aureus

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S. aureus ID (bioMérieux, La Balme Les Grottes, France) is a new chromogenic agar medium designed to enable the isolation of staphylococci and the specific identification of Staphylococcus aureus. S. aureus produces green colonies on this medium due to production of α-glucosidase. To evaluate this medium, a total of 350 wound swabs were cultured onto S. aureus ID, CHROMagar Staph. aureus, and conventional media routinely used in our laboratory. After 18 to 20 h of incubation, 96.8% of strains formed green colonies on S. aureus ID compared with 91.1% of strains forming mauve colonies on CHROMagar Staph. aureus. A total of 94.3% of strains were recovered within 18 to 20 h with conventional media. The sensitivity was increased after 48 h of incubation to 98.7, 96.2, and 95.6% with S. aureus ID, CHROMagar Staph. aureus, and conventional media, respectively. A total of 97.4% of green colonies on S. aureus ID were confirmed as S. aureus compared with 94.4% of mauve colonies on CHROMagar Staph. aureus. We conclude that S. aureus ID is a highly sensitive and specific medium for the isolation and identification of S. aureus from wound swabs.

Staphylococcus aureus is an important and frequent cause of wound infection and is consequently one of the most common pathogens sought in clinical microbiology laboratories. Diagnosis of S. aureus infection is generally performed by culture of wound swabs onto nonspecific media and confirmation of suspect colonies by biochemical and/or serological tests. Most commonly, this involves testing colonies of staphylococci for agglutination with sensitized latex particles to detect bound coagulase, protein A, and/or specific capsular antigens. A number of culture media have been developed to increase the specificity of S. aureus detection, including mannitol-salt agar and Baird-Parker medium. A more recent approach has been the use of CHROMagar Staph. aureus, which employs chromogenic enzyme substrates in a selective agar medium, allowing the detection of S. aureus with a high degree of sensitivity and specificity. S. aureus ID is another recently described chromogenic medium for the specific detection of S. aureus, but there are no reports of the efficacy of this medium with clinical samples (C. Cotte, N. Fanjat, C. Iletter, D. Monget, S. Orenga, D. Robichon, and C. Roger-Dalbert, Abstr. 102nd Gen. Meet. Am. Soc. Microbiol., abstr. C23, 2002). On S. aureus ID, S. aureus forms distinctive green colonies due to production of α-glucosidase. Other staphylococci generally form white colonies but occasionally produce pink colonies due to the hydrolysis of a second chromogenic substrate for β-glucosidase.

We performed a comparison of S. aureus ID and CHROMagar Staph. aureus for the detection of S. aureus with 350 clinical samples. These media were compared with nonspecific media routinely used in our laboratory for culture of wound swabs; cystine lactose electrolyte-deficient (CLED) agar, aztreonam blood agar (ATM BA), and nalidixic acid blood agar.

MATERIALS AND METHODS

Culture media. S. aureus ID was supplied as preprepared culture plates from bioMérieux, La Balme Les Grottes, France. CHROMagar Staph. aureus was obtained as a dehydrated medium from M-Tech Diagnostics, Warrington, United Kingdom, and was prepared in accordance with the manufacturer’s instructions. CLED agar (CM423; Oxoid, Basingstoke, United Kingdom) was also prepared according to the manufacturer’s instructions.

ATM BA was prepared as follows: Columbia agar (CM331; Oxoid) was prepared according to the manufacturers instructions, cooled to 50°C, and supplemented with 5% horse blood and 4 mg of aztreonam (Bristol-Myers Squibb, Dublin, Ireland)/liter. Nalidixic acid blood agar was prepared by producing an additional batch of Columbia agar, as described above, and supplementing the medium with 5% horse blood and 30 mg of nalidixic acid (Sigma Chemical Company, Poole, United Kingdom)/liter.

Clinical samples. A total of 350 consecutive wound swabs referred from general practitioners was used in this study. All swabs were received in charcoal transport medium. Each swab was emulsified in 1 ml of sterile physiological saline (0.85%), and 50 μl of this suspension was inoculated and spread onto each of the five types of media. This procedure ensured that each culture plate received an equivalent inoculum. All culture plates were incubated at 37°C for 48 h, except for nalidixic acid blood agar, which was incubated anaerobically for 48 h. Other media such as chocolate agar were occasionally used, depending on the patient’s clinical details; however, colonies isolated on these additional media were ignored for the purposes of this study.

All culture plates were interpreted by two laboratory staff members after 18 to 20 h and once again after 48 h of incubation. Media of each particular type were read independently of each other without knowledge of the results obtained on other media. For example, for each batch of culture plates, all of the results obtained on medium A were recorded and concealed before the results from medium B were recorded to minimize bias. The order in which the media were read was changed each day.

Identification of S. aureus. Mauve colonies on CHROMagar Staph. aureus and green colonies on S. aureus ID were regarded as presumptive S. aureus and were confirmed with SlideX Staph Plus (SSP) latex reagent (bioMérieux UK, Basingstoke, United Kingdom) in accordance with the manufacturer’s instructions. Any colonies resembling staphylococci on either CLED, ATM BA, or nalidixic acid blood agar were also tested with SSP latex reagent. SSP reagent has been shown...
to have a specificity comparable to that of the tube coagulase test (14). Therefore, any presumptive *S. aureus* strains which produced the expected colonial appearance on both CHROMagar Staph. aureus and S. aureus ID were regarded as confirmed *S. aureus* if they demonstrated specific agglutination with SSP reagent.

Any staphylococcus strain resembling *S. aureus* on any medium that did not produce agglutination with SSP latex reagent was identified by using API 20 STAPH (bioMérieux UK) and the tube coagulase test as previously described (9). Any strain which agglutinated SSP latex reagent but did not produce green colonies on S. aureus ID and/or mauve colonies on CHROMagar Staph. aureus was also identified by using API 20 STAPH and the tube coagulase test and subcultured onto the relevant chromogenic medium.

All other isolated strains (nonstaphylococci) were presumptively identified based on colonial appearance and the results of standard laboratory tests, e.g., Gram stain, catalase, and oxidase. In addition, any nonstaphylococci that produced a green colony on S. aureus ID or a mauve colony on CHROMagar Staph. aureus were identified fully by using the appropriate API identification kits (bioMérieux UK).

**RESULTS**

One hundred fifty-eight confirmed strains of *S. aureus* were isolated from 350 wound swabs on one or more media within 48 h of incubation. Table 1 shows the number of *S. aureus* strains isolated on each of the respective media. A total of 149 strains were isolated by ATM BA after 18 to 20 h of incubation, and two further strains were recovered on ATM BA after 48 h of incubation. Of the nine strains of *S. aureus* that were not recovered on ATM BA within 18 to 20 h incubation, seven were isolated in small numbers and there were no more than two colonies isolated on any other medium. Two strains were isolated in higher yield on other media (40 to 60 colonies), but both of these cultures yielded a heavy growth of *Pseudomonas aeruginosa* on ATM BA, which probably accounted for the failure to recover *S. aureus* on this medium. All of these un-recovered strains grew well when respread on ATM BA. All strains of *S. aureus* which grew on CLED or blood agar plus nalidixic acid were also recovered on ATM BA within 18 to 20 h, indicating that these media offered no advantage to ATM BA alone for the isolation of *S. aureus*.

Figure 1 shows the typical appearance of *S. aureus* colonies on S. aureus ID medium and CHROMagar Staph. aureus. On S. aureus ID, 153 strains of *S. aureus* were recovered as green colonies after 18 to 20 h of incubation compared with 156 strains after 48 h of incubation. Four of the five strains which were not recovered within 18 to 20 h on *S. aureus* ID were present in small numbers, and there were no more than two colonies isolated on any other medium. After 48 h of incubation, two of these strains grew on *S. aureus* ID, each as a single colony. All four strains produced characteristic green colonies when respread on *S. aureus* ID. One strain was isolated at a higher yield (>50 colonies on all test media) but only generated colored colonies on either *S. aureus* ID or CHROMagar Staph. aureus after 48 h of incubation. This strain of methicillin-resistant *S. aureus* (MRSA) retained this characteristic and, when respread on both chromogenic media, required 48 h of incubation to generate typical coloration on either medium.

On CHROMagar Staph. aureus, 144 strains were recovered as mauve colonies after 18 to 20 h of incubation compared with 152 strains after incubation for 48 h. Six of the 14 strains which were not recovered within 18 to 20 h were present in small numbers, as there were no more than two colonies isolated on any other medium. Two of these strains were isolated as mauve colonies after 48 h of incubation. Seven of the 14 strains not recovered on CHROMagar Staph. aureus within 18 to 20 h were isolated at higher yields on other media (10 to 100 colonies on both *S. aureus* ID and ATM BA). It was perhaps notable that five of these cultures yielded a heavy growth of enterococci (>100 colonies), which generated deep blue colo-

TABLE 1. Numbers of *S. aureus* strains isolated on different media

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. of strains (including MRSA)</th>
<th>MRSA</th>
<th>Sensitivity (at 18–20 h)</th>
<th>Sensitivity (at 48 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>158 (23)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aureus ID</td>
<td>153 (156)</td>
<td>22 (23)</td>
<td>96.8</td>
<td>98.7</td>
</tr>
<tr>
<td>CHROMagar Staph. aureus</td>
<td>144 (152)</td>
<td>21 (22)</td>
<td>91.1</td>
<td>96.2</td>
</tr>
<tr>
<td>ATM BA</td>
<td>149 (151)</td>
<td>23</td>
<td>94.3</td>
<td>95.6</td>
</tr>
<tr>
<td>CLED BA</td>
<td>130</td>
<td>21</td>
<td>82.3</td>
<td>82.3</td>
</tr>
<tr>
<td>NALI BA</td>
<td>17</td>
<td>6</td>
<td>10.8</td>
<td>10.8</td>
</tr>
</tbody>
</table>

* Numbers in parentheses indicate total strains isolated after 48 h of incubation.
* Total number of strains.
* NALI BA, nalidixic acid blood agar.

**FIG. 1.** Culture of an abscess swab showing growth of *S. aureus* as green colonies on S. aureus ID medium (A) and growth of mauve *S. aureus* colonies and blue *Enterococcus faecalis* colonies on CHROMagar Staph. aureus (B).
on CHROMagar Staph. aureus. All strains of S. aureus that were not recovered as mauve colonies on CHROMagar Staph. aureus. aureus produced characteristic mauve colonies when resuspended on this medium after overnight incubation, except for one strain of MRSA which required 48 h of incubation to generate color (as mentioned above).

Table 2 shows the number of other strains which grew on the test media, and Table 3 shows the relative specificities of the chromogenic reactions for detection of S. aureus. Coagulase-negative staphylococci grew well on S. aureus ID and usually grew as white colonies, although occasional strains generated pink colonies. Two strains of Staphylococcus sciuri grew as green colonies and were indistinguishable from S. aureus on S. aureus ID, whereas strains of Staphylococcus schleiferi and Staphylococcus simulans resembled S. aureus on CHROMagar Staph. aureus. Micrococcus luteus also produced false-positive reactions on both chromogenic media. Some strains of corynebacteria and hemolytic streptococci occasionally produced small mauve colonies on CHROMagar Staph. aureus. None of these false-positive strains demonstrated specific agglutination when tested with SSP latex reagent. No strains of S. aureus were found in this study which failed to react with SSP reagent.

### DISCUSSION

Isolation of S. aureus from wound samples is usually accomplished with the use of conventional media such as blood agar. Such media have the advantage that they may also be useful for the simultaneous isolation of other pathogens such as streptococci. The disadvantage of such media is that confirmatory tests are necessary to differentiate S. aureus from other staphylococci. Performing such tests on all colonies resembling staphylococci can be time-consuming and labor intensive. For example, in one study (8), it was calculated that an average of 4.55 latex agglutination tests were performed on suspect colonies for each strain of S. aureus actually identified. The use of chromogenic media, if sufficiently sensitive and specific, can potentially reduce the number of confirmatory tests that are necessary for the detection of S. aureus (4, 8).

The ideal characteristics of any candidate chromogenic medium are the detection of S. aureus with high specificity and an isolation rate at least comparable to conventional media after 18 to 20 h of incubation. In this study, the chromogenic reactions of both S. aureus ID and CHROMagar Staph. aureus were highly sensitive for S. aureus, and only one strain was detected which failed to generate color after 18 to 20 h of incubation. This strain was confirmed as MRSA, and larger numbers of distinct MRSA strains should be examined to further evaluate the usefulness of both chromogenic media for isolation of MRSA.

Both media were also highly specific (97.4% for S. aureus ID; 94.4% for CHROMagar Staph. aureus), with few other species resembling S. aureus. This specificity increased to 100% when either medium was used in conjunction with SSP latex reagent.

The main difference between the media was in their selectivities, and this was the biggest factor influencing their relative sensitivities. Both chromogenic media were substantially better...
than ATM BA for the inhibition of coliforms and *P. aeruginosa*, and *S. aureus* ID was substantially better than any of the other media for the inhibition of enterococci (Table 2). This latter feature probably accounted for the superior sensitivity of *S. aureus* ID compared with CHROMagar Staph. aureus after 18 to 20 h of incubation. There were six instances where at least 50 colonies of *S. aureus* were recovered on both *S. aureus* ID and ATM BA and no mauve colonies were recovered on CHROMagar Staph. aureus. In five of these instances, the cultures on CHROMagar Staph. aureus yielded greater than 100 colonies of enterococci, which produced a deep blue coloration. It is possible that the presence of the enterococci, and their associated intense blue chromogenic reaction, masked the presence of *S. aureus* colonies.

In this study, the sensitivity of CHROMagar Staph. aureus was 91.1%, which compares favorably with the study of Carricajo et al. (4), who reported a sensitivity of 92% for CHROMagar Staph. aureus after 24 h of incubation. Gaillot et al. (8) examined the performance of CHROMagar Staph. aureus with 2,000 consecutive clinical samples and reported a sensitivity of 95.5% after 24 h of incubation. The lower sensitivity obtained in the present study may be due to the fact that CHROMagar Staph. aureus was compared with ATM BA, which is more effective for the isolation of *S. aureus* than blood agar (14) and/or chocolate, as used by Carricajo et al. (4) and Gaillot et al. (8), and also to the fact that some strains of *S. aureus* may have been missed without the use of *S. aureus* ID.

We conclude that *S. aureus* ID is highly effective for the isolation and presumptive identification of *S. aureus* from wound samples and that it compares favorably with both conventional media and CHROMagar Staph. aureus. Further studies are required to examine the utility of *S. aureus* ID for the isolation of *S. aureus* from other types of sample, such as sputum, where *S. aureus* may present as atypical colonies on culture media (8). The use of *S. aureus* ID is much less labor intensive than conventional methods and requires fewer reagents for confirmation of suspet colonies of *S. aureus*. Chromogenic media for *S. aureus* may be supplemented with appropriate antimicrobials (e.g., oxacillin) for the detection of MRSA (1, 10, 11). *S. aureus* ID could potentially be supplemented with antimicrobials to facilitate the specific detection of MRSA or vancomycin-resistant *S. aureus* (5). The fact that *S. aureus* ID has already been shown to have excellent selectivity, particularly against enterococci, may prove to be advantageous in this context.

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**REFERENCES**


