Interpretation of MRSASElect Screening Agar at 24 Hours of Incubation

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An incubation time of 24 h at 35 to 38°C is recommended for the optimal performance of MRSASElect (Bio-Rad) chromogenic screening agar. An additional 24 h is required to capture slow-growing methicillin-resistant Staphylococcus aureus (MRSA). However, the normal hours of operation for most laboratories cannot reliably accommodate 24-h interpretation intervals. Daily agar plate interpretations are more likely to occur around 18 h and 42 h of incubation, which may compromise the performance of the chromogenic agar and negatively impact patient infection control efforts. In order to validate the importance of stringent incubation times to plate performance, we evaluated MRSASElect medium at controlled intervals of 24 and 48 h of incubation, using clinical MRSA-screening swabs. A total of 1,071 MRSA-positive and 2,733 MRSA-negative cultures were selected for analysis. Compared to 48-h-incubation results, the sensitivity and specificity of MRSASElect at 24 h were 98.3% and 98.2%, respectively. Only 19 of 1,071 (1.8%) MRSA-positive isolates required 48 h for detection. Holding 24-h-negative plates an additional 24 h resulted in the workup of 253 (6.6%) pink, yet non-MRSA, colonies. The 24-h-positive and negative predictive values of MRSASElect, assuming MRSA prevalences of 1, 5, and 10%, were 35.5 and 99.98%, 74.2 and 99.9%, and 85.9 and 99.8%, respectively. MRSASElect medium held for 24 h incubation is a highly sensitive and specific MRSA-screening tool. Further incubation prolongs the turnaround time for results and creates a significant amount of unnecessary work in the laboratory.

The Society of Healthcare Epidemiology of America (SHEA) currently recommends active surveillance of methicillin-resistant Staphylococcus aureus (MRSA) infections and contact precautions to control its spread (7). In Canada, MRSA infection and colonization rates in hospitals have increased more than 10-fold, from less than 1% of total S. aureus isolates in 1995 to 10% in 2002 (2, 10). Much of this increase can be attributed to surveillance, but the infection rate also increased fivefold in this same period. As a result, many laboratories have experienced substantial increases in their work loads to meet the demands of active MRSA surveillance programs.

While rapid and reliable screening measures are important for the implementation of appropriate and effective infection control measures, they are also important for the efficiency of the work flow in the clinical laboratory. Chromogenic media for the rapid isolation and identification of MRSA from surveillance specimens have recently been developed. MRSASElect (Bio-Rad, Marnes La Coquette, France) is a sensitive and specific chromogenic agar medium used for MRSA screening. Compared to those of other screening-culture media, including other chromogenic media, the sensitivity and specificity of MRSASElect range between 97 to 99% and 90 to 99%, respectively (1, 6, 12). MRSA is presumptively identified as a small pink colony, while white or pink-tinged colonies are disregarded. According to the manufacturer's instructions, MRSASElect plates recover the majority of MRSA isolates following 24-h incubations at 35 to 38°C. However, the length of the incubation presents a challenge for most clinical laboratories, because the majority of specimens are not received until midday or later, which results in incubation periods closer to 18 (day 1) and 42 h (day 2). In this setting, a large number of MRSA-positive cultures may not be identified on day 1. However, previous evaluations of MRSA-screening media have shown that their specificity is reduced after 24 h of incubation due to the breakthrough growth of coagulase-negative staphylococci, which can significantly increase the laboratory work load.

The objective of this study was to evaluate the ability of MRSASElect to detect MRSA from clinical surveillance specimens at 24 h of incubation compared to that at 48 h of incubation in an acute-care diagnostic laboratory setting.

MATERIALS AND METHODS

Over a 10-month period in a 750-bed tertiary care hospital with four intensive care units, MRSA surveillance swabs were collected from nares, perineum, and urine and/or wounds of adult and pediatric hospital patients who were at risk of carrying MRSA. Risk factors included previous hospitalization, residence in a long-term-care facility, and previous MRSA colonization or infection. For the first 5 months, each swab was inoculated directly onto a MRSASElect agar plate. During the latter 5 months, nares and perineum swabs were combined on the same plate, while other specimen types were inoculated individually. The plates were incubated at 37°C in ambient air for 24 h and 48 h, plus or minus 1 h, and pink colonies were selected for further identification as per the manufacturer's instructions. MRSA and non-MRSA S. aureus strains (ATCC 43300 and ATCC 25923, respectively) were used for quality control of the media throughout the study. Confirmatory identification of MRSA by using conventional bacteriology methods for all pink colonies isolated from the 48-h plate served as our reference standard for comparative analyses. The identification of S. aureus was confirmed based on Gram stains; coagulase (slide and tube), catalase, and pyrrolidinyl-arylamidase reactions; and detection of the modified penicillin binding protein 2a by latex agglutination (Denka Seiken, Tokyo, Japan). Antimicrobial susceptibility testing, including testing with cefoxitin, was performed in accordance with the National Committee for Clinical Laboratory Standards susceptibility testing guidelines.

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with Clinical Laboratory Standards Institute recommendations (4). Specimen
collection was designed to capture an approximate 3:1 ratio of true-negative to
true-positive specimens based on estimates of monthly MRSA surveillance swab
volumes and positivity rates. The sensitivity, specificity, and positive and negative
predictive values (PPV and NPV) for 24-h incubations were calculated in com-
parison to the 48-h results.

RESULTS
A total of 1,071 confirmed MRSA-positive specimens (from
285 patients) and 2,733 confirmed MRSA-negative specimens
were selected for analysis. The results are summarized in Table
1. ATCC control strains verified the integrity of the media
throughout the study. The sensitivity, specificity, PPV, and
NPV of MRSASelect at 24 h compared to the results at 48 h
were 98.3, 98.2, 95.6 and 98.3%, respectively. Only 19 (1.8%)
of 1,071 MRSA-positive specimens required 48 h for isolation.
Three specimens from three patients would have been mis-
identified as MRSA negative at 24 h, as no other specimens
were collected from these patients. The remaining 16 speci-
mens from 14 patients had 24-h MRSA-positive results from
previous or concurrent specimens. At 24 h, there were 49
chromogenic (pink) isolates selected that were ultimately iden-
tified as non-MRSA bacteria (false positives). Holding 24-h
negative plates an additional 24 h resulted in the identification of
253 (6.6% of total isolates) false positives and a decrease in
specificity from 98.2% at 24 h to 90.6% at 48 h. In the first 4
months, false positives accounted for 10% of the total positive
isolates. Over subsequent months, the percentage of false pos-
tives decreased to 7%. The percentages of MRSASelect PPV
and NPV (Table 2), assuming an MRSA prevalence of 1, 5,
and 10%, were 35.5 and 100, 74.2 and 99.9, and 85.9 and 99.8,
respectively.

DISCUSSION
For the majority of clinical laboratories, routine “24-h” or
overnight incubation for bacterial culture actually occurs for 16
to 20 h. However, the product monograph for MRSASelect
indicates that optimal performance requires 24 h of incubation
at 35 to 38°C (35°C is standard for most laboratories). To our
knowledge, this is the first study designed to evaluate the per-
fomance of MRSASelect at controlled incubation intervals. At
24 h of incubation, 98.2% of MRSA isolates were identified.
Prior to controlled 24-h incubation periods, our laboratory
incubated MRSASelect overnight (~16 to 18 h) initially, fol-
lowed by another 24 h of incubation. During this period, we
recovered 118 MRSA isolates from 5,242 specimens, with only
83 (70%) recovered after overnight incubation (data not shown),
which indirectly validates the importance of a com-
plete 24-h incubation period. In previous studies, shorter
incubation times of 16 to 20 h resulted in detection of 62 to 81%
of MRSA isolates (3, 8, 13). Conversely, two other studies have
reported ≥95% MRSA detection after 18 to 24 h of incubation
(1, 6). Louie et al. (6) recovered 169 (95%) of 177 MRSA
isolates at 18 to 24 h (from a total of 6,199 specimens), and Ben
Nsira et al. (1) recovered 99 (97%) of 102 MRSA isolates at 18
to 24 h (from a total of 699 specimens). The incubation inter-
vals were not elaborated in either study. Stokas et al. (12)
reported that 98% (108 of 111) of the MRSA isolates were
isolated at 18 h without further incubation.
At 24 h, only 49 specimens yielded chromogenic non-MRSA
isolates (1.8% of the 2,733 negative specimens), underscoring
the specificity of MRSASelect medium. However, reincubating
plates for a further 24 h had a minimal increase in yield; only
19 (1.8%) of 1,071 MRSA isolates required 48 h for detection.
It is not clear why these specimens were not detected at 24 h;
however, low bacterial concentration, inhibitory ingredients in
the media, or small colony variants have been implicated in
other studies with delayed recoveries from MRSASelect and
other chromogenic media (5, 9, 11, 12). The use of antimicro-
bials may also have contributed to the delayed recovery of
these isolates (patient charts were not reviewed).
Reincubation also created further unnecessary work; chro-
mogenic isolates on 253 of 272 (93%) plates at 48 h of incub-
ation proved not to be MRSA, accounting for 83.8% of the
total false positives evaluated. These false positives decreased
the specificity from 98.2 to 90.6% at 48 h, which mirrors the
findings of similar studies. Louie et al. (6) found a decrease in
specificity from 99.5 to 92%, detecting 28 false-positives by
48 h and another 428 at 48 h, and van Loo et al. (13) found a
decrease from 92% to 89%, detecting 4 false positives at 20 h
and 12 more at 48 h. While we did not further identify our
false-positive isolates, false-positive pink colonies have been
found to be coagulase-negative staphylococci, corynebacteria,
enterococci, or one of a small number of Enterobacteriaceae,
Acinetobacter, or Pseudomonas species. (1, 6, 13).

Chromogenic agar assays are subjective; they rely on the
observer to decide if the colony is the “correct” color for
further identification of the organism in question. In the case of
MRSASelect, colonies that are pink tinged or light pink are

<table>
<thead>
<tr>
<th>Incubation period (h)</th>
<th>No. of plates with chromogenica MRSA colonies</th>
<th>No. of plates with chromogenica non-MRSA colonies</th>
<th>No. of plates with nogrowth</th>
<th>No. of plates with nonchromogenicb colonies</th>
<th>% Sensitivity</th>
<th>% Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>1,052</td>
<td>49</td>
<td>925</td>
<td>1,759</td>
<td>98.2</td>
<td>98.2</td>
</tr>
<tr>
<td>48</td>
<td>1,071</td>
<td>302</td>
<td>611</td>
<td>1,620</td>
<td>90.6</td>
<td>90.6</td>
</tr>
</tbody>
</table>

a Pink colonies on MRSASelect.
b White colonies on MRSASelect.

TABLE 2. PPV and NPV of MRSASelect at 24 h of incubation

<table>
<thead>
<tr>
<th>Predictive value</th>
<th>% at indicated % MRSA prevalence:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>35.5</td>
</tr>
<tr>
<td>Negative</td>
<td>100.0</td>
</tr>
</tbody>
</table>
not considered suspicious for MRSA. However, when chromogenic media are initially implemented, it may require some time for observers to become familiar with color variations. Although the difference in the percentage of false positives in the first 4 months and that for the remainder of the study was only 3%, the trend each month was a decrease in the number of false positives selected, particularly at 24 h, for further identification. This indicates a temporal improvement in the accuracy of observer interpretation.

The increasing prevalence and surveillance of MRSA infections subsequently impart significant constraints on laboratory resources and management. Incubation of MRSA Select plates for a full 24 h is a highly sensitive and specific screening approach to MRSA detection. Stringent adherence to this incubation time can minimize the number of false-positive work-ups and decrease the time required to report a MRSA-negative result.

REFERENCES