Automated Scoring of Chromogenic Media for the Detection of MRSA using the WASPLab Image Analysis Software

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Running Title: Automated Image Analysis of Chromogenic Agar for MRSA.

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Abstract

Recently systems have been developed to create total laboratory automation for clinical microbiology. These systems allow for automation of specimen processing, specimen incubation and imaging of bacterial growth. In this study we used the WASPLab to validate software that discriminates and segregates positive and negative chromogenic MRSA plates by recognition of pigmented colonies. A total of 57,690 swabs submitted for MRSA screening were enrolled in the study. Four sites enrolled specimens following their standard of care.

Chromogenic agar used at these sites included: MRSASelect (Bio-Rad Laboratories, Redmond, WA), ChromID MRSA (BioMerieux, Marcy-l’Etoile, France) and CHROMagar MRSA (BD Diagnostics, Sparks, MD). Specimens were plated and incubated using the WASPLab. The digital camera took images at 0 and 16-24h and the WASPLab software determined the presence of positive colonies based on a HSV (Hue, Saturation, Value) score. If the HSV score fell within a defined threshold, the plate was called positive. Performance of the digital analysis was compared to manual reading. Overall the digital software had a sensitivity of 100% and a specificity of 90.7% with the specificity ranging between 90.0 and 96.0 across all sites. Results were similar when using the three different agars with sensitivity observed to be 100% and specificity ranging from 90.7 and 92.4%. These data demonstrate that automated digital analysis can be used to accurately sort positive from negative chromogenic agar cultures regardless of pigmentation produced.
Introduction:

Automation in clinical chemistry and hematology laboratories has been widely available for years, but it has only been recently that clinical microbiology has adapted to these changes. Initial advances in automation of the microbiology lab include continuously-monitored blood culture, mycobacterial growth and automated antimicrobial susceptibility testing systems. Numerous studies have demonstrated the benefit of these systems in reducing turnaround (TAT), reducing labor costs and improving patient care (1-4). The success and impact of these systems have opened the door to further automation including the processing of microbial specimens. Similar to incorporation of automation in other parts of the laboratory, studies have demonstrated that incorporation of automated specimen processors can improve patient care by producing more isolated colonies than manual plating, reduced laboratory costs and reduced plate contamination (5-7).

Manufacturers have improved on previous specimen processors by adding conveyor/track systems to move plates into incubators, programmable software to adapt to various laboratory protocols and digital cameras to image plates at various time points that can be accessed at work stations using a computer and high definition monitor. The goal of these systems is to create full laboratory automation that process specimens, incubate plates, images plates for interpretation and picks colonies for further culture workup. To date, the Kiestra Total Laboratory Automation (BD Kiestra B.V., Drachten, NL), and the WASPLab (Copan, Brescia, IT) have been marketed to clinical laboratories and include several of the above features. Although the technology may not yet be able to identify organisms based on colony
morphology, digital imaging can currently identify the presence of colonies on a plate and
distinguish between different colors, such as those found on chromogenic agars.

Chromogenic agars are specific media that take advantage of differences in pathogen
metabolism by creating enzymatic reactions specific for target organism such as vancomycin
resistant \textit{enterococci} (VRE), group \textit{B streptococcus} (GBS) and methicillin resistant \textit{S. aureus}
(MRSA) (8-10). When the target is present, substrates produced during growth interact with
the chromogen to produce pigmentation (red, pink or green). With digital imaging software
capable of distinguishing differences in pixel color, chromogenic agar is ideal for digital
automation as color thresholds can be created to detect target growth.

WASPLab Chromogenic Detection Module (CDM) is a software that analyzes digital
images for a customizable target color, by converting RGB images into a 3 dimensional space
composed of Hue, Saturation and Value (HSV) creating a “bubble shaped” tolerance level for
defining “non negative” media plates. Figure 1 demonstrates with a “bubble” the target
definition space. To detect “non negative”/negative plates, the software analyses every pixel
(each media plate image is composed by 27 million pixels) in the image looking for the selected
color pattern within the specified tolerance. Plates containing pixels with HSV values within the
set parameters are marked as “non negative” whereas plates are marked negative if no pixel
contains a HSV score outside of the parameters. We hypothesize implementation of the CDM
software into the WASPLab can accurately sort chromogenic MRSA plates as “non negative” or
negative.
To evaluate performance of the CDM software, we performed a multisite evaluation of the WASPLab to identify MRSA from swabs cultures plated to various chromogenic agars. Four sites enrolled a total of 57,690 swabs that were collected for MRSA screening. Swabs were automatically plated by the WASPLab to chromogenic agar and images were read by the CDM software and compared to a manual read for detection of positive MRSA plates. To demonstrate the robustness and accuracy of the CDM software, 3 different chromogenic media were tested: MRSASelect (Bio-Rad Laboratories, Redmond, WA), ChromID MRSA (BioMerieux, Marcy-l’Etoile, France) and BD CHROMagar MRSA (BD Diagnostic, Sparks, MD). The CDM software threshold is set for each manufacturer’s agar, as pigmentation varies between plates (MRSASelect pink, ChromID MRSA green, CHROMagar MRSA mauve).

Materials and Method:

Specimen processing

Four laboratories from various geographical locations were involved in this study. These sites included: A.O. Ospendale Niguarada (Milano, Italy), PAMM laboratories (Veldhoven, Netherlands), CHU de Quebec (Quebec City, Canada) and Hamilton General Hospital (Ontario, Canada). All 4 laboratories involved in this study routinely perform MRSA screens using ESwab (Copan, Brescia, IT and Murrieta, CA, USA) collected from anterior nares, throat, perineum, or open wounds and then plated onto chromogenic agar. For this study, ESwabs received by the laboratory were enrolled into the study and tested according to laboratory standard of care. Briefly, swabs were loaded into the WASPLab for plating on chromogenic agar. One site performed an enrichment step using nutrient broth (10g/L lab-lemco powder, 10g/L Peptone,
and 5.0g/L NaCl) (Oxoid, Basingstoke, UK) which was incubated 18-24h at 37°C prior to plating. Once plated, the WASPLab transferred the plate to the WASPLab incubator where the on board camera collected a time point 0 image. The plates were then incubated at 35-37°C for 16-24 hours, depending on the laboratory standard operating procedures and manufacturer’s instructions for use. After the established incubation period, a second image was collected, saved and used for both automated and manual reading. Approval by each site’s institutional review board or oversight committee was obtained prior to any specimen enrollment.

**Automated Digital Analysis of Chromogenic Media**

The Chromogenic Detection Module (CDM), image analysis software scans the image looking for colored pixels on the surface of the plate, which is compared to the time point 0 plate to identify growth. Depending on the chromogenic plate used (green, pink, or mauve colonies) a HSV threshold was set that reported plates as “non negative” or “negative” for MRSA. In this study, colonies containing HSV values that fall within the tolerance threshold were reported as automation positive (AP). In the absence of typically colored colonies, the specimen was reported as automation negative (AN).

**Manual reading of chromogenic plates**

Technologists reading plates manually were blinded to the results of the software. After 16 to 24 hours of inoculation, a technologist individually reviewed each plate’s digital image (same image used for automation analysis). Depending on the chromogenic media used by the laboratory, the technologist looked for colonies containing the color indicated in the package insert (MRSASelect - pink, ChromID MRSA - green, CHROMagar MRSA - mauve). Each plate was
scored as manual positive (MP) or manual negative (MN) by the technologist based on the
presence of indicated colonies. Colonies that technologists identified as questionable (hue
differences) were removed from the incubator and a Gram stain, catalase and latex
agglutination test was performed to further determine the presence of \textit{S. aureus}.

\textit{Discrepant analysis}

Data analysis was performed retrospectively so discrepant specimens were not available
for further work-up. To reconcile these discrepant specimens, the digital images were sent
back to the corresponding laboratories to be reviewed by a supervisor or the laboratory
director. Each image was reviewed and all discordant results were reported as having either:
excess matrix background creating pigmentation of the agar (residual Matrix), a borderline
colony color that would not be worked up by the laboratory (borderline colors) or plates where
the technologist missed a positive colony (automation positive 2\textsuperscript{nd} manual positive).

\textit{Statistical analysis}

Results from the software's digital analysis were compared to the technologist’s manual
read as the true value. Performance characteristics, including sensitivity and specificity, were
calculated using standard methods. Ninety five-percent confidence intervals were calculated
by using a binomial expansion.

\textit{Results:}

\textit{Comparison of the automatic imaging to manual detection of MRSA positive chromogenic agar}
Images taken by the onboard camera are a composite image that uses several light sources and several lighting intensities that simulate manual reading of a plate. Representative images of plates with no growth, plates positive for MRSA and plates with growth lacking pigmentation are shown in Figure 2. Technologists performing manual interpretation used similar images to determine if the plates were positive for MRSA.

In total, 57,690 swabs were enrolled and tested at 4 different locations. Overall prevalence of MRSA was observed to be 2.4% and ranged from 2.1-7.3% at the testing sites. Of the 57,690 plates analyzed, 1,367 plates were called positive for MRSA by both automation and manual reading (Table 1). The CDM software reported an additional 5,210 (9.0%) plates as “non negative” that were manually read as negative. Importantly, automatic imaging did not read any manual positive plates as negative. Together these data demonstrated an overall sensitivity of 100% (99-100%, 95% CI) and a specificity of 90.7% (90-91%, 95% CI). Data was similar across all four sites with specificity ranging from 90.0-96.0%.

**Analysis of Manual Negative, Automation Positive plates**

In an effort to reduce false negative results, the threshold “bubble” was large for all testing. Use of a conservative threshold resulted in a manual negative/automate positive (MN/AP) rate of 9.0% (5210/57,690). Re-examination of these MN/AP plates by a supervisor or laboratory director identified three different types of discrepancies, which we have categorized as (1) Automation Positive 2nd Manual Positive, (2) Residual Matrix, or (3) Borderline Colors. An example of each of these categories is shown in Figure 3. Automation Positive 2nd Manual Positive were the least common representing 2.9% (153/5,210) of discrepant results found in
this study (Table 2). These results are defined as small colonies that were not visually detected by initial manual examination but upon review should have been called positive by the laboratory, suggesting that the CDM was correct. Residual Matrix represents 22.8% (1,189/5,210) of the discrepant specimens and are comprises of plates containing colorimetric agar not associated with microbial growth. The most common discrepancy was Borderline Colors, where the CDM software calculated scores within the threshold, but manual examination did not detect any positive color. This class represented 74.2% (3,868/5,210) of discrepant results and is due to the conservative setting of the threshold designed to prevent false negative results.

Comparison of 3 chromogenic media for the detection of MRSA from swabs

 Sites participating in the study only used chromogenic agar outlined in their standard of care and not all sites used the same chromogenic agar. Agars used in this study were: MRSASelect (Bio-Rad Laboratories, Redmond, WA), ChromID MRSA (BioMerieux, Marcy-l’Etoile, France) and BD CHROMagar MRSA (BD Diagnostic, Sparks, MD). Sensitivity for each chromogenic agar used was equivalent when using the CDM software (Table 3). The specificity for the three chromogenic agars was observed to be 90.7% (MRSASelect), 92.4% (ChromID) and 90.7% (BD CHROMagar).

Discussion:

To date there have been limited studies demonstrating the benefits to implementation of full laboratory automation in clinical microbiology laboratories. A recent report observed an increase of approximately 2 fold in the laboratory production index (number of samples/staff
members/day) when using full lab automation (5). Although data demonstrating improvements in efficiency associated with full laboratory automation are limited, further studies documenting efficiency are needed.

Currently, laboratories that are performing MRSA screening receive specimens throughout the day and manually plate each specimen to chromogenic agar. These plates are then incubated for 18-24 hours; however, in practice this time can vary based on available staff and operation hours. After incubation each plate is observed by a technologist and reported as positive or negative with staff reading hundreds of plates a day. Laboratories incorporating the WASPLab into the workflow would load specimens into the instrument where the instrument would process, track, incubate, image the specimen and separate plates as “non negative” and negative. Twenty-four hours later a technologist would interface with a WASPLab workstation to perform analysis of specimens. When imaging negative plates, up to 40 plates can be observed on the screen at one time, confirmed negative and discarded with a single click. Although for this study each plate was viewed individually, this workflow would have reduced the amount screen images viewed by technologist from the 51,113 to 1,278 (negative plates/40 images per screen). “Non negative” plates are called up individually and the technologist can score these similar to previous workflow, but without the need to physically obtain the plate. Quick removal of negative plates will ease the burden of large volume screens. Automation of digital imaging could also help laboratory workflow as plates are always imaged within the appropriate time frame potentially aid in reducing turnaround time. Analyzing chromogenic media at 16-24 hours is important as specificity is lost as the plate
incubates beyond the recommended duration (break through growth, degradation of
products). In a study by Joubrel et al. observed that specificity for detection of *Salmonella* on
chromogenic agar decreased as incubation periods increased from 24 to 48 hours. The
specificity was reported as 91% at 24 hours and was reduced to 84% at 48 hours post
inoculation (11), which is consistent with other studies evaluating various chromogenic media
(12-14). Laboratories that become delayed in plate reading may overcall chromogenic media
resulting in over treatment of patients. Implementation of CDM would allow the technologist
to review the plate as if it was read at 24 hours, ensuring optimal specificity on the
chromogenic agar. In addition, laboratories that cannot support testing over the weekend
could allow screens to be ordered on Friday that were reported on Monday without loss of
specificity.

This is the first high volume, multisite study demonstrating the ability of full lab
automation to perform image analysis on different chromogenic media. For this study,
thresholds in the WASP Lab CDM software were set to ensure that any true positive was
detected by the imaging software. Discrepant resolution demonstrated that the software over
reported positive results due to minor pigmentation that are not associated with positive
specimens or pigmentation of the agar due to residual matrix. These specimens are easily
identifiable on a monitor and can be reported as negative by the technologist. No false
negative plates were identified during this study, demonstrating that the conservative
thresholds set allowed the CDM to be highly sensitive. Interestingly, the CDM identified 153
specimens that were positive after a second review of the digital image. These data suggest
that the CDM software may be more sensitive than manual observation.
The comparison of chromogenic agar was not a direct comparison because we did not evaluate all media types at all sites, which is a limitation to this study. All media types in this study had similar sensitivity and specificity; however, as each specimen was only tested on one media, testing was not a direct specimen to specimen comparison. Specimen enrollment was high at all sites and no differences were observed suggesting that specimen variability did not affect outcomes. In addition, this study was designed to blind the technologist from the software’s results to remove bias, which is essential as chromogenic plates are reliant upon the technologist judgment of growth and color. Data analysis was performed after all testing was completed removing the ability of the laboratory to perform confirmatory testing. Due to this limitation discordant results that contained either borderline colors or automation positive 2nd manual positive colonies could not be classified as MRSA or pigmented breakthrough growth.

Findings from this study demonstrated that automation can accurately remove negative plates and identify plates that can be misread by a manual observation. Currently, the software cannot be used without technologists support as 5,057 false positive results were reported. However, segregating 88.6% of chromogenic plates will reduce the time and cost on clinical laboratories performing high volume screens. Studies measuring TAT, patient outcomes and cost analysis will help aid clinical directors in determining the utility of automated digital analysis.

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Figure 1: HSV Color Space, where H (Hue) represents the Type of color, S (Saturation) represents the Intensity of the color and V (Value) represents the Brightness of the color. The “bubble” is the visual representation of the threshold volume in this three-dimensional space.
Figure 2: Representative examples of chromogenic media generated by WASPLab imaging:

- Negative chromogenic plate containing no growth (A.)
- Positive chromogenic plate containing MRSA (B.)
- Chromogenic plate with non-MRSA growth, small white colonies (C.).
Figure 3 Representative examples of Manual Negative, Automation Positive plates generated by WASPLab CDM software. Automation Positive 2nd Manual Positive showing a small colony not visually detected by manual examination but accurately identified as positive by the CDM (A1 and A2.) Residual Matrix on the plate showing lack of growth, but containing color due to the
presence of specimen matrix (B.) and a Borderline Color plate demonstrating similar color
colonies (C1 and C2).
<table>
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<tr>
<th>Clinical test site</th>
<th>No. of specimens tested</th>
<th>Results (no.)</th>
<th>Performance (% [95% CI])</th>
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</thead>
<tbody>
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<td>MP/AP</td>
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<tr>
<td>1</td>
<td>5604</td>
<td>119</td>
<td>5266</td>
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<td>2</td>
<td>41064</td>
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<td>36333</td>
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<td>3</td>
<td>2217</td>
<td>162</td>
<td>1898</td>
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<td>4</td>
<td>8805</td>
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<td>7616</td>
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<tr>
<td>Total</td>
<td>57690</td>
<td>1367</td>
<td>51113</td>
</tr>
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</table>

*MP/AP, manual Pos automation Pos; MN/AN, manual Neg/automation Neg; MN/AP, manual Neg/automation pos; MP/AN, manual pos/automation Neg.

b CI, confidence interval.
<table>
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<tr>
<th>Discrepant Category</th>
<th>MN/AP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Automation Positive 2&lt;sup&gt;nd&lt;/sup&gt; Manual Positive</th>
<th>Residual Matrix</th>
<th>Borderline Colors</th>
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</thead>
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<tr>
<td>Number of plates</td>
<td>5210</td>
<td>153</td>
<td>1189</td>
<td>3868</td>
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<sup>a</sup> Manual Negative/Automation Positive
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<tr>
<th>Chromogenic media</th>
<th>No. of specimens tested</th>
<th>Results (no.)</th>
<th>Performance (% [95% CI])</th>
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<tr>
<td></td>
<td></td>
<td>MP/AP MN/AN</td>
<td>Sensitivity Specificity</td>
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<td>MRSASelect</td>
<td>46668</td>
<td>799 41599 4270 0</td>
<td>100 (99-100) 90.7 (90-91)</td>
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<tr>
<td>chromID MRSA</td>
<td>2217</td>
<td>162 1898 157 0</td>
<td>100 (97-100) 92.4 (91-93)</td>
</tr>
<tr>
<td>BD Chromagar MRSA</td>
<td>8805</td>
<td>406 7616 783 0</td>
<td>100 (99-100) 90.7 (90-91)</td>
</tr>
</tbody>
</table>

*MP/AP, manual Pos/automation Pos; MN/AN, manual Neg/automation Neg; MN/AP, manual Neg/automation Pos; MP/AN, manual pos/automation Neg.

CI, confidence interval.
References:


