

Chromogenic Media vs Real-Time PCR for Nasal Surveillance of Methicillin-Resistant *Staphylococcus aureus*

Impact on Detection of MRSA-Positive Persons

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Abstract

Surveillance for methicillin-resistant Staphylococcus aureus (MRSA) colonization can be an important element for infection control programs when managing a multidrug-resistant pathogen such as MRSA. The sensitivity and speed of laboratory testing affects the proportion of appropriate isolation days captured, which determines the success or failure of a MRSA control program. Chromogenic culture, CHROMagar MRSA (BBL, Becton Dickinson, Sparks, MD) and MRSASelect (Bio-Rad, Hercules, CA), with and without broth enrichment and real-time polymerase chain reaction (PCR; BD GeneOhm MRSA, BD Diagnostics, San Diego, CA), were compared and found to have a wide range of sensitivities (78.5%-98.2%), specificities (91.6%-100.0%), and turnaround times (2-72 hours). Real-time PCR provided the most rapid results and demonstrated the highest sensitivity followed by broth-enriched culture and then direct plating for MRSA detection in nasal swabs. There was no substantial difference in the labor required for any of the 3 approaches.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen causing bacteremia, pneumonia, and skin and soft-tissue infections that result in significant morbidity, mortality, and longer hospital stays.¹ MRSA has now reached epidemic levels worldwide, including in US health care.² Nasal colonization with MRSA serves as the most common reservoir for nosocomial transmission and is the major risk factor for subsequent infection.³ Guidelines for actions to limit the transmission of MRSA by the Society for Healthcare Epidemiology of America and the Centers for Disease Control and Prevention advocate increased MRSA colonization surveillance as a tool for enhanced activity whenever routine infection control practice fails to lower infection rates.^{4,5} Optimal surveillance methods need diagnostic testing that is sensitive, specific, and rapid with a high negative predictive value so that MRSA-colonized patients can be identified quickly and placed into contact isolation. Published reports on methods suggest a variety of approaches and confound the determination of which technique is most effective.

The purpose of this study was to evaluate 2 chromogenic media, CHROMagar MRSA (BBL, Becton Dickinson, Sparks, MD) and MRSASelect (Bio-Rad, Hercules, CA), with and without broth enrichment, and compare culture-based performance with the BD GeneOhm MRSA real-time polymerase chain reaction (PCR) assay (BD Diagnostics, San Diego, CA) regarding sensitivity, specificity, labor, and turnaround time for MRSA detection.

Materials and Methods

Patient Population and Specimen Collection

NorthShore University HealthSystem, formerly Evanston Northwestern Healthcare, Evanston, IL, is an academic organization in the northern suburbs of Chicago, IL, that at the time of this study covered 3 hospitals, 850 beds, and approximately 45,000 annual admissions. Nasal samples were collected on admission using premoistened, double-headed, rayon-tipped swabs (CultureSwab, BBL, Becton Dickinson) with both swabs rubbed inside one nostril followed by the other, yielding a paired swab sample.

Real-Time PCR for MRSA

One swab from the nasal specimen was broken off into a screw-top microcentrifuge tube containing 200 μ L of 1 U/ μ L achromopeptidase (Sigma-Aldrich, St Louis, MO) in 1 \times TE (10 mmol/L of Tris, pH 8.0, and 1 mmol/L of EDTA; Sigma-Aldrich). Swabs in the achromopeptidase solution were vortexed for 5 to 10 seconds, and the samples were incubated at 37°C for 15 minutes. Then samples were incubated at 99°C for 5 minutes and placed at 4°C until used directly in real-time PCR with the BD GeneOhm MRSA assay.⁶

To evaluate the use of a broth-enrichment step before PCR (further described subsequently), a 50- μ L aliquot of enrichment broth was centrifuged at more than 10,000g for 1 minute. The supernatant was removed and the cell pellet resuspended in 200 μ L of the achromopeptidase solution. The sample was processed as above.

The real-time PCR reaction was performed following the manufacturer's protocol. BD GeneOhm MRSA master mix was hydrated, divided into aliquots, and placed in SmartCycler reaction tubes to which 2.8 μ L of sample was added. Real-time PCR was executed by using the SmartCycler instrument (Cepheid, Sunnyvale, CA) with the BD GeneOhm MRSA assay PCR protocol. A positive control sample (supplied with the kit) and a negative control sample (processed blank achromopeptidase sample) were included in each run.⁶

Chromogenic Culture for MRSA

Based on the clinical microbiology laboratory real-time PCR results, a convenience sample of 250 negative and 250 positive specimens were selected and assigned to a sequential study number. The second swab from these nasal specimens was used for culture. To remove any plate selection bias, the specimens were evenly distributed between both media for first plating. Odd-numbered study specimens were plated first to CHROMagar MRSA and then plated to MRSASelect, and even-numbered study specimens were plated to MRSASelect first and then CHROMagar MRSA. After direct plating, all swabs were inoculated into 5 mL of tryptic soy broth without NaCl (TSB; Remel, Lenexa, KS) for broth enrichment. After

24-hour incubation at 35°C, a 50- μ L aliquot of broth was removed to use for PCR and another sample of broth was plated to Columbia colistin-nalidixic agar (CNA) with 5% sheep blood (Remel), MRSASelect, and CHROMagar MRSA.

All plated media were incubated at 35°C for a total of 48 hours, with examinations at 24 and 48 hours. Operators blinded to the PCR results performed the culture analysis. All *S aureus* isolates were confirmed by using the Staphaurex latex agglutination test (Murex Biotech, Dartford, England), and MRSA was confirmed by detection of the *mecA* gene using a validated in-house real-time PCR assay.⁷ The manufacturers' procedures for interpretation of chromogenic culture results are listed in **Table 1**.

Data Analysis

The sensitivity and specificity for culture were calculated using 2 "gold standards." First, a true culture positive was defined as a sample with confirmed MRSA recovered from any culture. Second, a true-positive was defined as a sample with confirmed MRSA isolated from any culture and a PCR-positive result from a patient with a history of MRSA recovered from any source within the prior year. Differences in performance between the test results were assessed by using the χ^2 statistic. The institutional review board of NorthShore University HealthSystem approved this research.

Results

Between April 2007 and October 2007, 500 specimens were selected for this study based on the clinical laboratory PCR results, and 5 samples were subsequently excluded owing to possible contamination of the broth enrichment, leaving a total of 495 for analysis. During the evaluation,

Table 1
Chromogenic Culture Interpretation From Manufacturers' Current Product Inserts

CHROMagar MRSA (BBL, Becton Dickinson, Sparks, MD)
24 (\pm 4)-h incubation
Mauve colonies: MRSA
No mauve colonies: reincubate
48 (\pm 4)-h incubation
Mauve colonies: perform coagulase or latex agglutination
Positive: MRSA
Negative: no MRSA
No mauve colonies: no MRSA
MRSASelect (Bio-Rad, Hercules, CA)
18- to 28-h incubation
Small pink colonies: MRSA
No pink colonies: no MRSA
Incubation beyond 28 hours not recommended

MRSA, methicillin-resistant *Staphylococcus aureus*.

all suspect colonies underwent a full identification including latex agglutination and *mecA* testing to confirm MRSA. The full identification of colonies from cultures allowed for a supplementary analysis component to the accuracy determination. This revealed that not all of the mauve or pink colonies observed growing on the chromogenic media were actually confirmed to be MRSA, some of which were samples that had true MRSA isolated from a different method. Therefore, the true sensitivity was lower when confirmatory testing for MRSA was performed **Table 2** and **Table 3**.

There were a total of 171 samples that grew confirmed MRSA on any media: 163 samples on both chromogenic media, 4 samples on CHROMagar MRSA only, and 4 samples on MRSASelect only. For CHROMagar MRSA, a total of 167 samples had MRSA isolated: 146 on direct plating and broth enrichment, 5 on direct plating only, and 16 in broth enrichment only. For MRSASelect, including a 48-hour reading, a total of 167 samples produced MRSA: 142 on direct plating and broth enrichment, 1 on direct plating only, and 24 in broth enrichment only.

Table 2
Performance Characteristics of CHROMagar MRSA and MRSASelect Using Direct Plating and With Broth Enrichment Compared to 171 True-Positives Defined as Samples With MRSA Isolated From Any Culture*

	CHROMagar MRSA				MRSASelect	
	Direct Plating		Broth Enrichment		Direct Plating	Broth Enrichment
	Mauve Colonies [†]	Mauve <i>S aureus</i> [‡]	Mauve Colonies [†]	Mauve <i>S aureus</i> [‡]	Pink Colonies [§]	Pink Colonies [§]
Incubation time (h)	24	48	24	48	24	24
True-positive	146 (145)	150 (150)	160 (156)	161 (161)	146 (134)	167 (163)
False-negative	25 (26)	21 (21)	11 (15)	10 (10)	25 (37)	4 (8)
False-positive	2 (0)	0 (0)	1 (0)	1 (0)	8 (0)	29 (0)
True-negative	322 (324)	324 (324)	323 (324)	323 (324)	316 (324)	295 (324)
Sensitivity (%)	85.4 (84.8)	87.7 (87.7)	93.6 (91.2)	94.2 (94.2)	85.4 (78.4)	97.7 (95.3)
Specificity (%)	99.4 (100.0)	100.0 (100.0)	99.7 (100.0)	99.7 (100.0)	97.5 (100.0)	91.0 (100.0)

MRSA, methicillin-resistant *Staphylococcus aureus*.

* Values in parentheses are for mauve or pink colonies that were confirmed to be MRSA. The differences in performance for the combined CHROMagar MRSA and MRSASelect results when comparing direct plating and broth enrichment were significant at $P < .0001$.

[†] Mauve colonies visible on the CHROMagar MRSA sample plate that may or may not have been MRSA but, nevertheless, corresponded to a sample with confirmed MRSA isolated from any culture method.

[‡] Mauve colonies visible on a CHROMagar MRSA sample plate that were confirmed to be *S aureus* and may or may not have been MRSA but, nevertheless, corresponded to a sample with confirmed MRSA isolated from any culture method.

[§] Pink colonies visible on an MRSASelect sample plate that may or may not have been MRSA but, nevertheless, corresponded to a sample with confirmed MRSA isolated from any culture method.

Table 3
Performance Characteristics of CHROMagar MRSA and MRSASelect for Direct Plating and With Prior Broth Enrichment Compared to 186 True-Positives Defined as Samples With MRSA Isolated From Any Culture and PCR-Positive Results From a Patient History of MRSA Within the Prior Year*

	CHROMagar MRSA				MRSASelect	
	Direct Plating		Broth Enrichment		Direct Plating	Broth Enrichment
	Mauve Colonies [†]	Mauve <i>S aureus</i> [‡]	Mauve Colonies [†]	Mauve <i>S aureus</i> [‡]	Pink Colonies [§]	Pink Colonies [§]
Incubation time (h)	24	48	24	48	24	24
True-positive	146 (145)	150 (150)	160 (156)	161 (161)	146 (134)	168 (163)
False-negative	40 (41)	36 (36)	26 (30)	25 (25)	40 (52)	18 (23)
False-positive	2 (0)	0 (0)	1 (0)	1 (0)	8 (0)	26 (0)
True-negative	307 (309)	309 (309)	308 (309)	308 (309)	301 (309)	283 (309)
Sensitivity (%)	78.5 (78.0)	80.6 (80.6)	86.0 (83.9)	86.6 (86.6)	78.5 (72.0)	90.3 (87.6)
Specificity (%)	99.4 (100.0)	100.0 (100.0)	99.7 (100.0)	99.7 (100.0)	97.4 (100.0)	91.6 (100.0)

MRSA, methicillin-resistant *Staphylococcus aureus*; PCR, polymerase chain reaction.

* Values in parentheses are for mauve or pink colonies that were confirmed to be MRSA. The differences in performance for the combined CHROMagar MRSA and MRSASelect results when comparing direct plating and broth enrichment were significant at $P = .0024$.

[†] Mauve colonies visible on a CHROMagar MRSA sample plate that may or may not have been MRSA but, nevertheless, corresponded to a sample identified as a true positive.

[‡] Mauve colonies visible on a CHROMagar MRSA sample plate that were confirmed to be *S aureus* and may or may not have been MRSA but, nevertheless, corresponded to a sample identified as a true-positive.

[§] Pink colonies visible on an MRSASelect sample plate that may or may not have been MRSA but, nevertheless, corresponded to a sample identified as a true-positive.

The sensitivity and specificity of culture, based on recovery of 171 confirmed MRSA from any culture, are given in Table 2. In the sample population, there were 15 PCR-positive–culture-negative samples from patients with a history of MRSA; combining these with culture positives produced 186 true-positive surveillance samples (using a definition that captures all persons who would be considered appropriate for placing into contact isolation to prevent the spread of MRSA). The data for culture compared with these 186 true MRSA positives are given in Table 3.

Direct Plating

The manufacturers' guidelines for culture interpretation (Table 1) were compared with the 171 true MRSA culture-positive samples. Direct plating to CHROMagar MRSA at 24 hours identified 146 mauve colonies that correlated with true culture positives, producing a sensitivity of 85.4% and specificity of 99.4%. At 48 hours, the sensitivity was 87.7% and specificity was 100.0% (Table 2). MRSASelect at 24 hours also identified 146 pink colonies that correlated with true culture positives for a sensitivity of 85.4% and a specificity of 97.5% (Table 2). Incubation of MRSASelect samples for 48 hours identified 1 MRSA sample that was missed by all other methods except the CNA.

Compared with the 186 true MRSA-positive samples using the second criterion, the sensitivity and specificity for CHROMagar MRSA with direct plating were 78.5% and 99.4% at 24 hours and 80.6% and 100.0% at 48 hours, respectively (Table 3). For MRSASelect with direct plating, the sensitivity and specificity were 78.5% and 97.4% (Table 3).

Broth Enrichment

Broth enrichment before plating on either chromogenic agar improved the sensitivity (Tables 2 and 3). With the manufacturers' interpretation compared with the 171 true MRSA culture-positive samples (reference standard 1), broth-enriched CHROMagar MRSA at 24 hours identified 160 samples with mauve colonies that correlated with true culture positives for a sensitivity of 93.6% and specificity of 99.7%. At 48 hours, the sensitivity was 94.2% and specificity was 99.7% (Table 2). For MRSASelect, there were 167 samples with pink colonies that correlated with true culture positives and 29 samples with pink colonies that were not true culture positives, generating a sensitivity of 97.7% with a specificity of 91.0% (Table 2).

Compared with the 186 true-positive samples using reference standard 2, broth-enriched CHROMagar MRSA was 86.0% sensitive and 99.7% specific at 24 hours and was 86.6% sensitive and 99.7% specific at 48 hours (Table 3). For MRSASelect, broth enrichment yielded a sensitivity of 90.3% and specificity of 91.6% using this measurement standard (Table 3).

Table 4
Predictive Values of the BD GeneOhm MRSA Real-Time PCR Assay Directly From Swab Samples and From Broth Enrichment Compared to 171 True Culture Positives Defined as Samples With MRSA Isolated From Any Culture and 186 True-Positives Defined as Sample True Culture Positives and a PCR-Positive Result From a Patient History of MRSA Within the Prior Year*

	True Culture Positives (n = 171)		True MSRA Positives (n = 186)	
	Direct Swab	Broth	Direct Swab	Broth
True-positive	170	170	185	172
False-negative	1	1	1	14
False-positive	77	27	62	25
True-negative	247	297	247	284
Positive predictive value (%)	68.8	86.3	74.9	87.3
Negative predictive value (%)	99.6	99.7	99.6	95.3

MRSA, methicillin-resistant *Staphylococcus aureus*; PCR, polymerase chain reaction.
* The prevalence of MRSA in the population tested was 5.5%.

Real-Time PCR

The prevalence of MRSA during the study period was 5.5%. The results for the performance of swab and enrichment broth real-time PCR are given in Table 4. Of the 495 specimens, there was only 1 sample that grew MRSA (in broth enrichment) that was negative by PCR directly on the swab specimen.

Labor and Turnaround Time

The labor involved for only the interpretation of culture was documented for 100 specimens and then averaged for a per sample value. The labor calculations for the 24-hour interpretation were 30 seconds per sample for direct plating and broth enriched with both media. For CHROMagar MRSA, the 48-hour interpretation added an additional 30 seconds with direct plating and an extra 1 minute per sample with broth enrichment, not including the time to inoculate and streak the media or the added time to subculture from the broth or the additional day of broth incubation. The hands-on labor time to completely perform real-time PCR was approximately 1.5 minutes per sample.

The turnaround time depends upon the laboratory workflow. Assuming the manufacturers' instructions for product incubation were carefully followed, for any laboratory using the agar-based tests with batch processing or only first-shift interpretation, an additional time of nearly 24 hours can delay the reporting for CHROMagar MRSA (assuming a full 24-hour incubation is used) and can result in nearly an 18-hour delay for MRSASelect. In our centralized microbiology laboratory, the mean turnaround time for PCR testing is approximately 15 hours from collection to reported result.⁸

Discussion

The identification of MRSA carriers is influenced by the method used, which can differ in speed, sensitivity, and specificity. Factors that impact culture-based detection of MRSA include selective vs nonselective media, broth enrichment, and salt content. In this study, we found that the sensitivity of detecting persons considered positive from an infection control perspective was about 79% to 81% when performing direct plating to the most commonly used chromogenic agar media and 86% to 90% if broth enrichment was used. Previously, we found 98% sensitivity when using a real-time PCR assay to detect MRSA colonization.⁶ It is important to note that from a laboratory practice standpoint, a 2007 ClinMicroNet survey of 88 laboratories through Europe and North America found that of the 39 laboratories (44%) using chromogenic agar for surveillance, 37 (95%) of them only used direct plating and no broth enrichment.⁹ The survey also found that 13 laboratories (15%) used direct plating to mannitol salt agar (MSA) for surveillance activities, 16 (18%) were using a commercial real-time PCR assay, 10 (11%) were performing a variety of other tests (mainly culture-based), and 10 (11%) were not performing any surveillance for MRSA.⁹ Our report suggests that approximately 20% of potentially MRSA-positive persons are missed by most microbiology laboratories simply because the detection system used is direct plating to an agar-based platform.

There was only 1 sample that grew MRSA that was negative by PCR, giving a negative predictive value for PCR on swab specimens of 99.6%. The BD GeneOhm MRSA assay performance with nasal swabs was 98.2% sensitive and 97.5% specific based on our previous evaluations and remains more than 98% sensitive and specific.⁶ The positive and negative predictive values of this assay were 87.3% and 99.7%, respectively, in a prevalence of 12.4% MRSA and 73.5% and 99.9%, respectively, in a MRSA prevalence setting of 5.5%.⁶ During this examination, the prevalence of MRSA in the general sample population from which we selected study specimens was 5.5% and the positive and negative predictive values (Table 4) were equivalent to our previous results.

Chromogenic media have been developed for selective and more rapid identification of MRSA, and the performance characteristics have been determined in several studies, with varying results and reference standards for comparison. Most of these media still require 48 hours of incubation before a negative result can be reported. Early investigations compared the performance of various media using growth of MRSA on at least 1 test agar as the reference standard.

In a study that comprised 4 clinical centers in the United States, trypticase soy agar with 5% sheep blood agar (TSA; BBL, BD) was compared with CHROMagar MRSA for 2,015 nasal specimens. The sensitivity for detection of MRSA on TSA was 86.9% and for CHROMagar MRSA was 95.2%.¹⁰

A prospective evaluation of MSA-oxacillin (Oxoid, Ottawa, Canada), MSA-cefoxitin (Oxoid), CHROMagar MRSA, and MRSASelect using 1,243 nasal and 882 perineal swabs found a total of 111 specimens that were identified as positive for MRSA. The sensitivity and specificity, respectively, for CHROMagar MRSA were 82.9% and 99.1%, and for MRSASelect were 97.3% and 99.8%.¹¹

A similar media comparison of 666 screening swabs plated onto MSA, MRSASelect, oxacillin resistance screening agar base (ORSAB; Oxoid), and an in-house medium found MRSASelect to have the highest sensitivity of 99%, and no broth enrichment was used for any of the solid media.¹² Another evaluation of ORSAB, CHROMagar MRSA, and *S aureus* ID (bioMérieux, La Balme Les Grottes, France) supplemented with various antimicrobials tested 747 swabs from several body sites. The sensitivity and specificity of CHROMagar MRSA were 59% and 99.3% at 24 hours and 72% and 99.3% at 48 hours, respectively.¹³ Of interest, this study included a collection of common European MRSA types; 2 (5%) of 37 MRSA strains did not grow on CHROMagar MRSA when tested at low inoculum (approximately 100 colony-forming units per spot).¹³

A large evaluation of 6,199 surveillance samples from multiple body sites that compared MRSASelect with MSA containing 8 µg/mL cefoxitin found MRSASelect to have a sensitivity of 93% and specificity of 99.5% at 18 to 24 hours and a sensitivity of 98% and specificity of 92% after 48 hours in this population with a MRSA prevalence of 3%.¹⁴

It is important to realize that using growth of MRSA only directly plated to solid agar as a reference standard for these studies resulted in a range of sensitivity from 59% to 99%. Most of these past estimates are likely inflated because no broth enrichment and no amplification test were included for detection of MRSA.

An evaluation of chromogenic media that also included a broth-enrichment comparison was performed by Nahimana and colleagues¹⁵ for MRSA-ID (bioMérieux), CHROMagar MRSA, MRSASelect, and ORSAB media. Of 466 surveillance specimens, 102 grew MRSA on at least one of the media. For direct plating incubated 16 to 18 hours, the sensitivity was 51% for MRSA-ID, 59% for CHROMagar MRSA, 47% for ORSAB, and 65% for MRSASelect. The sensitivities increased to 82%, 75%, 67%, and 80%, respectively, after 42 hours. Enrichment in *m-Staphylococcus* broth further increased the sensitivities to 93% for MRSA-ID, 95% for CHROMagar MRSA, and 79% for ORSAB; MRSASelect was not tested using enrichment.¹⁵

Another interesting study compared direct plating to MRSA-ID and CHROMagar MRSA with ORSAB with and without broth enrichment.¹⁶ The investigators found the sensitivity of ORSAB increased by 13% (to 70%) with broth enrichment at 24 hours, but there was no significant difference

between any of the media at 48 hours with sensitivities at 77% or 73%.¹⁶

A recent evaluation of chromID MRSA (bioMérieux), MRSA-Screen (Oxoid), and MRSASelect, with and without broth enrichment, using 1,002 screening swab specimens found the sensitivity of all media to be less than 50% when directly inoculated plates were incubated for 18 hours.¹⁷ The sensitivity increased to 75%, 81%, and 72%, respectively, after 42 hours, and to 85% following broth enrichment for all media.¹⁷ The specificity for the 3 agars ranged from 76% to 100% at a MRSA prevalence of 6.8% in the population studied.¹⁷

These reports demonstrate the usefulness of broth enrichment for enhancing sensitivity when using agar as a method for detecting MRSA in surveillance swabs and the importance of this step in establishing a reliable gold standard in test evaluation studies.

Another series of reports included a PCR test as part of the investigation. Wren and colleagues¹⁸ compared the BD GeneOhm MRSA PCR assay to culture with direct plating on MSA containing 4 mg/L of oxacillin and to prior enrichment in nutrient broth supplemented with 4% NaCl.¹⁸ They reported a sensitivity of 95% for the BD GeneOhm MRSA assay, whereas the sensitivity of culture was 62% for direct plating that increased to 85% with broth enrichment.¹⁸ In a different report, a 9.5% higher yield was obtained by the BD GeneOhm MRSA assay when compared to CHROMagar MRSA media for 286 nasal specimens with initial discordant culture-negative and PCR-positive results resolved by performing an overnight enrichment in brain-heart infusion broth.¹⁹ The sensitivity, specificity, and positive and negative predictive values of the BD GeneOhm MRSA assay were 100%, 98.6%, 95.8%, and 100%, respectively.¹⁹ Farley and colleagues²⁰ compared the BD GeneOhm MRSA to CHROMagar MRSA in a sample of 602 nasal swabs from a high-risk community population. By using broth enrichment in TSB containing 6.5% NaCl, they found the sensitivity and specificity of PCR to be 89% and 91.7%, respectively.²⁰ Since growth on chromogenic medium was considered the reference standard, no performance analysis of the CHROMagar MRSA was possible. Last, a study that evaluated 2 molecular methods and 3 culture media for the detection of MRSA determined that the BD GeneOhm MRSA assay was the most sensitive method for the detection of MRSA from surveillance swabs.²¹ For 101 nasal specimens, the molecular tests gave sensitivities of 94% for BD GeneOhm MRSA and 70% for GenoType MRSA Direct (Hain Lifescience, Nehren, Germany), whereas culture-based methods had sensitivities of 72% for MRSA ID, 68% for MRSASelect, and 75% for CHROMagar MRSA (CHROMagar Microbiology, Paris, France) after 24 hours.²¹ These studies found the agar-based testing to have a sensitivity ranging from 62% to 91% and real-time PCR ranging from 89% to 100%, consistent with the findings in our report.

In addition to performance, laboratory selection of a new assay depends on cost and labor requirements. The analysis of labor time for culture of 100 specimens was for only the plate growth interpretation and did not include the time required to inoculate and streak the plate media or inoculate and subculture the broth media. The resulting time was compared with labor time for PCR, which included all steps necessary to produce a result. The 0.5 minute to 1.5 minutes to read a culture was equivalent to the 1.5 minutes for PCR, with PCR possibly having an advantage based on the limitations of our culture observation.

As for the cost, the manufacturer's suggested retail price for CHROMagar MRSA was \$6.70 per plate and for MRSASelect was \$8 per plate plus the additional expense of \$3.23 per tube for TSB when broth enrichment is used. The manufacturer's suggested retail price for the BD GeneOhm MRSA PCR assay was \$25.50 per sample. The actual price depends on the health care organization and any discounts that can be based on test volume or total number of products purchased from the vendor. The 2008 clinical diagnostic laboratory fee schedule (<http://www.cms.hhs.gov/ClinicalLabFeeSched/>) for the American Medical Association *Current Procedural Terminology* (CPT) codes listed the national limit for reimbursement of a screening culture (CPT 87081) as \$9.26 and MRSA detection by amplification (CPT 87641) as \$49.04. With labor time neutral, the use of broth-enriched MRSASelect would be \$11.23 for media supplies (reimbursed at \$9.26) and would provide a sensitivity of 90.3% with a 48-hour time to result compared with PCR at \$25.50 (reimbursed at \$49.04) with a sensitivity of 98.2% and results possibly available in 2 hours.

We observed several other interesting findings. First was the number of mauve or pink colonies growing on the chromogenic media that were not MRSA. This was the case for samples that were eventually classified as true-positives and false-positives. The specificity of MRSASelect after enrichment was the lowest of the methods and indicated that definitive identification should be performed on "suspicious" colonies whenever this approach is used. A second observation was that there were specimens that grew MRSA on direct plating only. Broth enrichment increased detection, but not to 100%, indicating the need for direct and broth-enrichment culture, which adds to the overall cost and turnaround time for testing. Although the product insert for MRSASelect at the time we performed this evaluation indicated an incubation of 24 hours and a requirement for confirmatory testing for plates incubated more than 28 hours, direct plating to MRSASelect for 48 hours recovered MRSA from 1 sample that was not identified by any other chromogenic method.

There were potential limitations to our study because we selected a convenience set of samples to enrich for MRSA-positives as opposed to consecutive samples. This restricted

the data analysis for PCR to positive and negative predictive values. Although we calculated the sensitivity and specificity of chromogenic culture methods, our protocol did not allow us to calculate these values for PCR; selection of samples for inclusion on the basis of their results using a given test (here, 250 each of positive and negative PCR tests) influences the calculated sensitivity and specificity for that test. Had we not done this, testing of approximately 5,000 specimens would have been required to capture 250 positives, which was beyond the capacity of this project. Also, we had already documented the sensitivity and specificity of the real-time PCR test we used, so there was no need to repeat that work. Finally, we chose to perform broth enrichment using a non-salt supplemented enrichment broth. Staphylococci are believed to have high salt tolerances, and it is generally recommended to use a media with 6.5% or 7.5% NaCl to increase the sensitivity of culture.²² However, Bruins et al²² reported that for 29% of the MRSA strains they tested growth was inhibited by an NaCl concentration higher than 2.5%. Based on this report and similar observations in our laboratory, the broth enrichment method selected for this evaluation did not include supplemental NaCl.

We defined the optimal gold (reference) standard for a true-positive as a sample with confirmed MRSA isolated from any culture or a PCR-positive result from a patient with a history of MRSA recovered from any source within the prior year. Having a clinical culture positive for MRSA in the past year is strongly associated with MRSA colonization, and the majority of people colonized with MRSA remain colonized for a prolonged period.²³ The Centers for Disease Control and Prevention offers no current guidance as to when someone can be considered “free” of MRSA²⁴; therefore, patients who have been previously positive for MRSA can reasonably be considered true-positives when a PCR test indicates the presence of MRSA in the setting of a surveillance program for MRSA control.

We are only beginning to understand the factors that lead to success or failure of an active surveillance program as part of a MRSA control plan. One key element is the percentage of isolation time that is captured by a MRSA surveillance program.^{8,25} In the setting of an active MRSA surveillance program, the goal is to detect every patient harboring MRSA and rapidly place them into contact isolation, thereby minimizing the number of unisolated patient days. We have previously found that in a setting of modest MRSA colonization prevalence (eg, 5%-6% of patients harboring MRSA), capturing 33% of the MRSA isolation opportunity days was insufficient to cause a lowering of MRSA disease throughout our 3-hospital system.⁸ Robicsek and colleagues⁸ and Peterson et al²⁶ determined that real-time PCR captured most of the MRSA isolation days, which resulted in a dramatic reduction in MRSA disease and an elimination of nearly \$1.2 million in medical expenditures during the program’s first year. This

suggests that a successful MRSA control program can be of medical and economic benefit to a health care system. Of note is the fact that direct plating of nasal swab specimens to chromogenic media would capture roughly 60% of the potential MRSA isolation days.²⁵ Thus, approximately 40% of MRSA isolation days could be missed when using direct plating to chromogenic media, even if performing all-admission universal surveillance.

The impact of this lower proportion of MRSA carrier isolation owing to the diminished sensitivity and longer time to results for the chromogenic media testing approach is not yet known. Of interest in this regard is a report by Harbarth and colleagues²⁷ who did not realize any reduction in MRSA disease when using a rapid assay with a median time to results reporting of 23 hours until they implemented preemptive isolation. This finding suggests that there is a critical time for results reporting if one is to realize a positive impact in lowering MRSA disease unless isolating all patients on admission and not removing the isolation practice until testing is complete (eg, preemptive isolation).

The success of an active MRSA surveillance program depends on multiple variables, one being the laboratory test used. Unidentified MRSA-colonized patients are a source for the spread of MRSA. Once the target population has been selected for surveillance, the assay’s sensitivity will be the major determinant of what percentage of unidentified carriers become known and placed into contact isolation. The higher the yield and better speed the assay has for detecting MRSA colonization, the greater is the likelihood for a successful outcome of the program. Therefore, the performance of laboratory tests must be considered when developing a MRSA surveillance program. Direct plating, the most commonly used method for MRSA surveillance, to CHROMagar MRSA and MRSASelect chromogenic media has inferior sensitivity compared with using a broth-enrichment step ($P < .001$), but enrichment lengthens the reporting time by a full day. Real-time PCR is the only current method with a sensitivity approaching 100% and reliable reporting of all tests in less than 24 hours.⁶ Using a PCR-based method for detection of MRSA colonization allows for a labor-neutral, more timely, and more sensitive identification of persons harboring MRSA.

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