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Performance of the BD GeneOhm Methicillin-Resistant *Staphylococcus aureus* Test before and during High-Volume Clinical Use▽

Suzanne M. Paule,[1]* Donna M. Hacek,[1] Bridget Kufner,[1] Karine Truchon,[2] Richard B. Thomson, Jr.,[1,3] Karen L. Kaul,[1,4] Ari Robicsek,[3,4] and Lance R. Peterson[1,3,4]

Departments of Pathology and Laboratory Medicine[1] and Medicine,[3] Evanston Northwestern Healthcare, Evanston, and Feinberg School of Medicine, Northwestern University, Chicago,[4] Illinois, and BD Diagnostics, San Diego, California[2]

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We evaluated the use of the BD GeneOhm MRSA real-time PCR assay (BD Diagnostics, San Diego, CA) for the detection of nasal colonization with methicillin-resistant *Staphylococcus aureus* (MRSA). The initial evaluation consisted of 403 paired nasal swabs and was done using the specimen preparation provided with the kit and an in-house lysis method that was specifically developed to accommodate large-volume testing using a minimal amount of personnel time. One swab was placed in an achromopeptidase (ACP) lysis solution, and the other was first used for culture and then prepared according to the kit protocol. PCR was performed on both lysates, and results were compared to those for culture. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the PCR assay were 98%, 96%, 77%, and 99.7% with the kit lysate and 98%, 95%, 75%, and 99.7% with the ACP lysate (*P*, not significant), respectively. The second evaluation was done after implementation of all-admission surveillance using PCR with ACP lysis and a sampling of 1,107 PCR-negative samples and 215 PCR-positive samples that were confirmed by culture. The results of this sampling showed an NPV of 99.9% and a PPV of 73.5% (prevalence, 6%), consistent with our initial findings. The BD GeneOhm MRSA assay is an accurate and rapid way to detect MRSA nasal colonization. When one is dealing with large specimen numbers, the ACP lysis method offers easier processing without negatively affecting the sensitivity or specificity of the PCR assay.

Methicillin-resistant *Staphylococcus aureus* (MRSA) continues to increase in prevalence within U.S. hospitals and in the community (13, 26). Furthermore, the burden of MRSA in U.S. healthcare organizations is often underestimated due to the significant prevalence of unrecognized, asymptomatic colonization (20). The recognized ecologic niche of *S. aureus* is the anterior nares (30). Studies have shown that approximately 25 to 30% of the population is colonized with *S. aureus* and that 0.2 to 7% is colonized with MRSA (17, 22, 30). Nasal colonization with MRSA can serve as a reservoir for transmission and is also considered a risk for subsequent infection (4, 7, 9).

While the optimal method for controlling MRSA is currently unproven and continues to be debated, largely due to the fact that there is no well-designed randomized trial assessing the impact of active surveillance, many (including the Centers for Disease Control and Prevention [CDC]) advocate active surveillance for MRSA carriers as an option to decrease spread in a setting where MRSA infection rates are not decreasing (3, 24). Culture-based detection of MRSA with traditional media requires 48 to 96 h for results (12, 21). A combination of molecular methods with culture decreases the time to results to about 24 to 40 h (15). In contrast, the BD GeneOhm MRSA real-time PCR assay, formerly called the IDI-MRSA assay (BD Diagnostics, San Diego, CA), offers rapid identification of MRSA-colonized patients, in as little as 2 h (3, 29). Also, this real-time PCR method has recently been compared to plating of samples onto agar, and if any test (including PCR) was assumed to be a true positive, the sensitivity of culture was 62% for direct plating, increasing to 85% with broth enrichment, while the sensitivity of PCR was 95% (31). The prompt and sensitive detection of MRSA carrier status can allow for several infection control benefits. First, a MRSA-colonized patient can be placed in contact isolation earlier and thus decrease the chance for nosocomial transmission (19). Second, the patient can take a decolonization regime to potentially reduce the likelihood of a subsequent MRSA infection. Third, earlier identification of MRSA-negative patients can minimize the number of isolation days for facilities practicing preemptive isolation. Finally, the greater sensitivity of PCR compared to culture identifies more patients colonized with MRSA.

Evanston Northwestern Healthcare, to enhance infection control, deployed a universal MRSA surveillance program, testing all 40,000 annual admissions, on 1 August 2005; this was the first program of its kind in North America. The purpose of our work was to validate the alternative lysis procedure we developed (to facilitate the processing of 100 to150 nasal swabs a day by a single laboratory worker) for use with the BD GeneOhm MRSA real-time PCR assay in this initiative and to evaluate the overall performance of the PCR test during high-volume clinical use.

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* Corresponding author. Mailing address: Evanston Northwestern Healthcare, Department of Pathology and Laboratory Medicine, 2650 Ridge Avenue, Evanston, IL 60201. Phone: (847) 570-2034. Fax: (847) 733-5314. E-mail: spaule@enh.org.

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MATERIALS AND METHODS

Patient population and specimen collection. Evanston Northwestern Healthcare is a three-hospital, 850-bed academic organization in the northern suburbs of Chicago, IL, that is affiliated with Northwestern University's Feinberg School of Medicine. Nasal samples were collected with premoistened (using Amies medium in the transport container) double-headed rayon-tipped swabs (CultureSwab; BBL, Becton Dickinson, Sparks, MD); both swabs were rubbed inside the anterior nares, first on one side and then on the other, yielding a paired swab sample.

Initial investigation comparing the kit lysis procedure with our in-house achromopeptidase lysis method. On 22 separate days from May to July 2005, nasal specimens collected from patients before orthopedic surgery or upon admission to the intensive care unit were analyzed by culture and the BD GeneOhm MRSA real-time PCR test by using two lysis methods.

High-volume clinical-use investigation using the in-house achromopeptidase lysis method. Universal surveillance for MRSA began on 1 August 2005. Nasal specimens were collected for all admissions and tested by the BD GeneOhm MRSA real-time PCR test using our achromopeptidase lysis method. On 12 separate days from November to December 2005 and on 20 separate days from March to May 2006, all real-time PCR-negative specimens were cultured. On consecutive days from 5 May through 22 May 2006 and 13 November through 30 November 2006, all real-time PCR-positive specimens were cultured.

Culture for MRSA. One of the paired swabs from each nasal specimen was plated onto Columbia-colistin-nalidixic acid agar (CNA) with 5% sheep blood (Remel, Inc., Lenexa, KS) and incubated under 5% CO_2 at 35°C for 24 to 48 h. For PCR-positive and CNA culture-negative samples, broth enrichment for additional *S. aureus* detection was performed. Swabs were stored refrigerated until they were incubated in 1 ml of thioglycolate medium without an indicator (Thio; BBL) or in 5 ml of tryptic soy broth with 6.5% NaCl (Remel) for 24 and 48 h at 35°C; then they were plated onto CNA. *S. aureus* was identified by colony morphology and a Staphaurex latex agglutination test (Murex Biotech Limited, Dartford, Kent, United Kingdom). Methicillin resistance was determined by testing colonies for the presence of the *mecA* gene specifically by an in-house real-time PCR test (18).

Kit lysis procedure. Samples were processed by following the BD GeneOhm MRSA assay protocol. Briefly, after plating, the swab was broken off into a tube containing sample buffer and was vortexed for 1 min. The entire cell suspension was then transferred to a lysis tube and centrifuged for 5 min. The supernatant was removed, and 50 μl of fresh sample buffer was added. Then the sample was vortexed for 5 min, followed by a brief centrifugation. Lastly, the samples were incubated at 95°C for 2 min. The samples were placed at 4°C, and the supernatant was used directly in real-time PCR. After PCR, the samples were stored at −20°C.

Achromopeptidase lysis procedure. The second swab from the nasal specimen was broken off into a screw-top microcentrifuge tube containing 200 μl of 1 U/μl achromopeptidase in 1× TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA; Sigma-Aldrich Co., St. Louis, MO). Swabs in the achromopeptidase solution were vortexed for 5 to 10 s, and the sample was incubated at 37°C for 15 min. Then samples were incubated at 99°C for 5 min. Only samples tested before 1 August 2005 were centrifuged at >10,000 × *g* for 1 min (17); this step was subsequently removed, because it was unnecessary (data not shown). The samples were placed at 4°C, and the free fluid around the swab was used directly in real-time PCR.

Real-time PCR procedure. Real-time PCRs were performed by following the manufacturer's protocol. Each master mix tube was hydrated with 225 μl of diluent. A 25-μl aliquot of the master mix was pipetted into SmartCycler reaction tubes, and 2.8 μl of lysate was added. Real-time PCR was carried out using the SmartCycler instrument (Cepheid, Inc., Sunnyvale, CA) with the BD GeneOhm MRSA assay PCR protocol. A positive control (supplied with the kit) and a negative control (kit sample buffer or achromopeptidase solution) were included in each run. During the high-volume clinical testing, all specimen lysates were stored at 4°C, and any with an unresolved result had the PCR repeated during the final run of the day on these stored samples.

The PCR reagents contained in the BD GeneOhm MRSA assay kit were updated by GeneOhm Sciences in early 2006. The proprietary changes were validated by the company at external reference laboratories. After 20 February 2006, all testing was performed with the new (current) PCR reagents.

In vitro testing comparison of lysis methods. Tenfold serial dilutions of clinically isolated MRSA strains were spiked directly into achromopeptidase lysis tubes and kit lysis tubes. Additionally, 10-fold serial dilutions of MRSA in 1× TE buffer were spiked directly onto swabs in 10- or 20-μl aliquots and compared in order to determine the limit of detection from actual swabs for both Copan single swabs (kit recommended) and our selected double-headed swabs with both lysis methods.

The amount of time required for each lysis method was determined by documenting the time taken to process patient nasal swabs from the point just after they were broken into the tube until the specimens were ready for PCR. The incubation times were subtracted from this total so as to determine the hands-on processing time. Two separate technologists, familiar with the kit method, were self-timed as they processed two different batches of 14 samples.

Statistical analysis was done using the chi-square test. This retrospective assessment of the assay method performance was approved by the institutional review board of Evanston Northwestern Healthcare.

RESULTS

In vitro testing before patient analysis found the real-time BD GeneOhm MRSA PCR assay to have an analytic sensitivity of approximately 2 CFU/PCR for both lysis methods. Tenfold serial dilutions of MRSA spiked directly onto swabs yielded a sensitivity of approximately 300 CFU/swab for both single swabs and double-headed swabs with both lysis methods (data not shown).

The time required for processing of 14 samples was 35 min for the kit lysis method and 23 min for the achromopeptidase lysis method. After the incubation time was subtracted, the total hands-on time was calculated at 22 min for the kit lysis and 3 min for the achromopeptidase method (see Table 2).

Initial investigation comparing the kit lysis procedure with our in-house achromopeptidase lysis method. A total of 403 nasal specimens were randomly selected from testing done between May and July 2005 where culture was compared to real-time PCR using both lysis methods. Directly plated or Thio broth-enriched culture identified 50 specimens (12.4%) with MRSA. PCR using the kit lysate compared to culture yielded 48 swabs positive for MRSA by both methods, 336 negative by both methods, 1 positive by culture only, 14 positive by PCR only, and 4 unresolved (0.99%) by PCR. The PCR assay gives definitive results with no need for interpretation. A sample's result can be positive for MRSA, negative for MRSA with a valid internal control, or unresolved (internal-control failure), which generally indicates some amount of PCR inhibition. Nine specimens (2.2%) had an initial PCR result of unresolved. For those samples, the PCRs were repeated using the frozen lysate as recommended in the kit directions. On repeat PCR, five samples gave negative results and four samples remained unresolved, one of which contained MRSA in culture. Specimens with a repeated unresolved PCR result were excluded from statistical analysis. The sensitivity, specificity, and positive and negative predictive values (PPV and NPV, respectively) are shown in Table 1. For 6 of the 14 samples that were positive by PCR only, the culture grew no *S. aureus* but the patients had a recent history of MRSA, which consisted of MRSA colonization or infection within the prior year. This provided revised performance characteristics when these six samples were considered true positives (Table 1).

Real-time PCR using the achromopeptidase lysate compared to culture yielded 47 swabs positive for MRSA by both methods, 317 negative by both methods, 1 positive by culture only, 16 positive by PCR only, and 22 unresolved (5.5%) by PCR. Unresolved results were initially obtained for 47 samples (11.7%), and PCRs were repeated using the frozen lysate. On

TABLE 1. Sensitivity, specificity, PPV, and NPV of the BD GeneOhm MRSA real-time PCR assay using two different lysis methods[a]

Lysis method (prevalence [%])	Value (% [95% CI])			
	Sensitivity	Specificity	PPV	NPV
Kit lysis (12.3)	98.0 (87.8–99.9)	96.0 (93.2–97.7)	77.4 (64.7–86.7)	99.7 (98.1–100)
Kit lysis with MRSA history (14.2)	98.2 (89.0–99.9)	97.7 (95.3–98.9)	87.1 (75.6–93.9)	99.7 (98.1–100)
Achromopeptidase lysis (12.6)	97.9 (87.5–99.9)	95.2 (92.2–97.1)	74.6 (61.8–84.4)	99.7 (98.0–100)
Achromopeptidase lysis with MRSA history (14.7)	98.2 (89.2–99.9)	97.5 (95.0–98.9)	87.3 (76.0–94.0)	99.7 (98.0–100)

[a] The performances of the two lysis methods were assessed by comparison to culture. For both kit lysis and achromopeptidase lysis, true-positive samples were those from which MRSA was isolated in culture. For either lysis method with MRSA history, true-positive samples included both those from which MRSA was isolated in culture and those from patients with a history of MRSA colonization or infection.

repeat PCR, 1 sample was positive (the culture grew MRSA), 24 samples were negative, and 22 remained unresolved, of which 2 had MRSA identified in culture. The sensitivity, specificity, PPV, and NPV are shown in Table 1. For 8 of the 16 samples that were positive by PCR only, the culture grew no *S. aureus* but the patients had a recent history of MRSA, which gave revised performance characteristics when these 8 samples were considered true positives (Table 1).

With both lysis methods, the same four samples were found MRSA positive by PCR and methicillin-susceptible *S. aureus* (MSSA) positive by culture. Three of these four samples had isolates available for additional testing, and all three were confirmed by colony analysis to be BD GeneOhm MRSA PCR positive and *mecA* negative, representing definitive false-positive PCR results.

High-volume clinical-use investigation using the in-house achromopeptidase lysis method. (i) PCR-negative specimens. Between November and December 2005 and between March and May 2006, a total of 1,198 specimens were tested by the PCR assay; of these, 1,107 negative samples were cultured to confirm the absence of MRSA. Among those specimens, culture recovered MRSA from only one sample, which gives an NPV of 99.9% (95% confidence interval [95% CI], 99.4 to 100%).

(ii) PCR-positive specimens. During May 2006 and November 2006, a combined total of 3,903 nasal specimens were tested by the PCR assay. Of these, the 215 PCR-positive samples were cultured for a thorough investigation to confirm the presence of MRSA. Since results for the investigation during the two time periods did not differ, the data were combined for this report. A total of 119 samples grew MRSA on the CNA plate; 15 samples had MRSA recovered from the tryptic soy broth with 6.5% NaCl only; 32 samples grew MSSA on the CNA plate or in broth; and 49 samples were negative for *S. aureus* both on the CNA plate and in broth. For the samples that grew MSSA in culture, the isolated colonies were tested by the PCR assay; 16 were PCR positive, and 16 tested negative. There was one patient whose nasal specimen grew an MSSA isolate that was PCR negative but who had MRSA recovered from an abscess collected on the same day. Of the culture-negative samples, 31 were from patients who had received some antistaphylococcal antibiotics within the prior month and 23 were from patients with a history of MRSA within the last year. Thus, from this analysis, there were 134 samples with culture-confirmed MRSA and 24 samples from patients with a MRSA history; these combined yield 158 true positives, corresponding to a PPV of 73.5% (95% CI, 67 to 79.2%) for the PCR assay.

Calculation of the sensitivity of culture for detection of patients harboring MRSA using this data set of 158 true positives found direct plating to be 75.3% sensitive (95% CI, 67.7 to 81.7%), whereas addition of broth enrichment improved the sensitivity to 84.8% (95% CI, 78.0 to 89.8%). The sensitivity of the BD GeneOhm MRSA test for detecting a patient needing contact isolation for MRSA was superior to that of either culture method ($P \leq 0.001$), and broth enrichment was superior to direct agar plating ($P \leq 0.05$) for detecting persons harboring MRSA.

(iii) Unresolved PCR specimens. The unresolved rate was calculated for all tests performed during the first year of the universal surveillance program using the PCR assay with the achromopeptidase method. During clinical use, 35,935 tests were performed, and the initial unresolved rate was 2.6%, which fell to 0.53% after retesting of the refrigerated specimen lysates on the last run of each day.

DISCUSSION

Many reports have dealt with the risks of MRSA colonization and infection and the benefits of active surveillance (8, 9), and these considerations are part of the new CDC guideline for the management of multidrug-resistant organisms (24). The "search-and-destroy" method used in The Netherlands has allowed that country to maintain a MRSA prevalence in staphylococcal infections of <1% (27). That program is multifaceted, and one aspect includes presumptive isolation until active surveillance culture results are negative for MRSA colonization (2). The theoretical benefits of using a real-time PCR method instead of culture are the rapid turnaround of results (suggesting the ability to wait a few hours for the MRSA colonization status and to then isolate only MRSA-positive patients) and the capacity to increase the sensitivity of detection (17, 19). These have recently been shown to be significantly beneficial by Cunningham and colleagues, who demonstrated a reduction in MRSA transmission incidence from 13.9/1,000 patient days using phenotypic (culture-based) MRSA surveillance to 4.9/1,000 patient days with PCR screening (3). Our study has shown that the BD GeneOhm MRSA real-time PCR assay, using the lysis method modification described here, is a sensitive and specific approach to achieving these benefits that is efficient to deploy in a setting of high-volume use.

TABLE 2. Comparison of time, ease of use, and cost to process 14 samples

Parameter	Kit lysis	Achromopeptidase lysis
Breaking swab into tube		
Time	Same	Same
Reagents	Included	$5.04
Hands-on time (min)	22	3
Labor cost	$11.00	$1.50
Incubation time (min)	13	20
Total time (min)	35	23
Setup of PCR	Same	Same

The use of nasal specimens and a real-time PCR assay allows procedural optimization in sample preparation, which permits high-volume testing. An alternative sample lysis method was designed and compared to the available kit method; the differences in hands-on time, ease of use, and cost, aspects critical to implementation in most clinical laboratories, were clearly evident (Table 2). When both lysis methods showed equivalent sensitivities and specificities (Table 1), the easier achromopeptidase method was selected for use in our universal surveillance program, where it has remained robust in over 1 year of use. Table 2 shows the difference in time required to perform either lysis method when 14 samples, the maximum number for one SmartCycler instrument, are processed. What it does not fully demonstrate is the extra time effect and labor cost for a larger number of samples. The total time saved by using the achromopeptidase method when one is processing 14 samples is 12 min, but that savings increases to 17 min for 28 samples and more than 2 h for 98 samples, with the majority of the difference in hands-on labor. Our optimization permits one technologist to perform 120 to 150 tests in a single 8-h shift and adds only $0.36 to the cost per test for the extra supplies. In addition to our alternate lysis method, there is another assay for MRSA nasal surveillance that simplifies specimen preparation, the Xpert MRSA test (Cepheid, Inc.), recently cleared by the FDA, which increases the options for surveillance programs.

Of interest was the fact that during the initial validation, the rate at which we obtained unresolved results (indicating inhibition of the PCR) was 11.7% with achromopeptidase lysis. After PCR was repeated with the same lysate, the unresolved rate was 5.5%, which is consistent with rate shown in the product insert. However, during the first month of all-admission surveillance, the mean unresolved rate was 4.5%, and after 7 months it decreased to 3.5%. The remainder of the first year demonstrated a 1.7% unresolved rate, with 80% of unresolved samples yielding a valid result upon repeat PCR. There were no major changes to the lysis procedure that would account for the decrease in the rate of unresolved results. Possible explanations are (i) the possibility that the technologists performing the test were slower in the beginning and with increased experience were able to start the PCR setup and process the reactions in the instrument in a shorter period and (ii) the introduction of the updated PCR reagents. During the past 12 months, our unresolved rate (after one repeat of initially unresolved tests) has remained under 1%. Others who used the BD GeneOhm MRSA assay with the kit lysis method have published rates of 4.7% and 6.4%, suggesting an improvement with the achromopeptidase method (14, 16). In daily testing, a specimen with an unresolved result has the PCR repeated once. If it remains unresolved, the specimen is plated and culture is used to determine the presence of MRSA.

Our PCR validation included a thorough investigation for MRSA on discordant results. If the PCR results or culture did not agree, the PCR was repeated and a broth enrichment was added to the culture. Even after broth enrichment, there were still samples that were PCR positive and culture negative. These samples can be calculated as false-positive PCR results or potentially false-negative cultures. For PCR-positive samples with MSSA in culture, testing of the colonies by PCR identified 19 samples that were positive (3 in the initial investigation and 16 during high-volume use). These most likely represent the previously described staphylococcal cassette chromosome *mec* (SCC*mec*) remnant (6, 10). SCC*mec* contains the *mecA* gene and is a mobile element; the right insertion junction of this cassette in the *S. aureus* chromosome is the target of the PCR assay. In some cases, *S. aureus* has a partial excision of SCC*mec* and no longer has the *mecA* gene but still has some of the junction section. These MSSA isolates are misidentified as MRSA and are true PCR false positives. For PCR-positive samples that are culture negative (either no growth of any *S. aureus* or growth of MSSA colonies that are not PCR positive), additional information is necessary to assess their classification. Therefore, patients with these results had their medical records examined to determine if they had a history of MRSA colonization or infection. The majority of people colonized with MRSA remain colonized for years (23), so we believe that previous specimens positive for MRSA are good evidence that these patients carry this pathogen and that a positive molecular test for MRSA in their noses represents a valid result for a MRSA surveillance program. Since nasal colonization with *S. aureus* serves as a source for the spread of the organism to other parts of the body and antedates bacteremia as well as nonbacteremic infection (11, 28), it is most likely that these patients truly harbored the microbe, even if it was not cultivatable in the nares at the time of our sampling; perhaps this represents another benefit of molecular testing over traditional culture techniques.

Although the recognized ecologic niche of *S. aureus* is the anterior nares (30), there is a question of whether to sample other body sites, such as the groin, axilla, or skin, in addition to the nose or whether the nose alone is sufficient for MRSA surveillance. Increased effort and cost would be associated with testing of multiple sites. Studies have shown that the sensitivity for detection of MRSA is 90% for nose-only specimens, 88% for the nose and groin combined, and 76.5% for skin or other superficial sites (1, 5), suggesting that a sample of the nose alone is adequate. Our own data indicated that nasal surveillance captured >90% of MRSA colonization (25), and thus, we adopted nasal surveillance as our plan for MRSA control.

Since any MRSA surveillance program has the objective of detecting the most persons harboring MRSA, the goal is to have a sensitive test that detects MRSA carriage with a high NPV. Our results indicating the suboptimal performance of conventional testing compared to real-time PCR, particularly without prior broth enrichment before plating onto agar, are supported by the recent publication by Na-

himana and colleagues, who found a sensitivity of 47 to 65% with direct plating to four chromogenic medium products compared to 79 to 95% when prior broth enrichment was included (12). This report did not include PCR testing, and our data further suggest that agar-based surveillance remains less sensitive than molecular amplification even when broth enrichment is included. More recently, using PCR as a comparison, Wren and colleagues also found this, with direct plating to agar having a sensitivity of only 62%, which increased to 85% when broth enrichment before plating was employed (31).

A limitation of our study is the procedure for testing for PPV and NPV during high-volume clinical use, which was done not simultaneously but rather sequentially. However, the facts that we have included a very large number of samples in our testing assessment and that performance has remained constant during a period of >17 months suggest that the results presented are reliable.

By using the achromopeptidase lysis method with the BD GeneOhm MRSA real-time PCR assay, a universal surveillance program to screen all admissions for MRSA was possible in a health care organization with more than 40,000 annual admissions, which translates to more than 100 specimens per day. The performance of the PCR assay was evaluated during this high-volume testing to confirm its reliability, and we found that the assay performed at least as well as in our original evaluation. Our results with the BD GeneOhm MRSA assay show that it is an accurate and rapid way to detect MRSA colonization. When one is dealing with large numbers of specimens, the achromopeptidase lysis method offers easier processing than the kit-recommended method without negatively affecting PCR sensitivity or specificity.

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