Use of CultureSwab Plus Swabs with Amies Gel Agar for Testing of Naris Specimens with the GeneOhm MRSA Assay[▽]

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The GeneOhm MRSA assay detects nasal colonization with methicillin-resistant *Staphylococcus aureus* (MRSA). We compared the use of seeded swabs with liquid Stuart's medium and that of seeded swabs with Amies gel for the assay. Overall, the swabs with liquid Stuart's medium detected significantly greater numbers of MRSA than the swabs with Amies gel ($P = 0.0003$).

The GeneOhm MRSA assay (BD Diagnostics-GeneOhm, San Diego, CA) is a Food and Drug Administration-cleared test for the direct detection of nasal colonization with methicillin-resistant *Staphylococcus aureus* (MRSA). Many institutions, including ours (Geisinger Medical Center, Danville, PA), selectively or broadly screen newly admitted patients for MRSA nasal colonization to facilitate appropriate isolation procedures for MRSA carriers. Utilized in conjunction with the Smart Cycler instrument (Cepheid, Sunnyvale, CA), the GeneOhm MRSA assay provides rapid real-time results.

The product insert specifies that specimens to be tested with the GeneOhm MRSA assay be collected with a swab system utilizing liquid Stuart's medium, such as the BBL CultureSwab Plus, an Amies gel swab collection device. The purpose of this study was to compare the use of the CultureSwab and that of the CultureSwab Plus to detect MRSA by the GeneOhm MRSA assay.

Preliminary work in our laboratory with the GeneOhm MRSA assay, using seeded swabs, indicated that the sensitivity of the assay was generally between 10^2 and 10^3 CFU/reaction mixture, which is consistent with the limit of detection stated in the product package insert. For this evaluation, starting with a 0.5 McFarland standard of each organism sample, we prepared dilutions with intended final concentrations of 1,000, 5,000, and 10,000 CFU/ml. Actual inoculum concentrations were not verified. For each concentration, a 100- μ l inoculum was pipetted into each of two microtiter wells, yielding intended inoculum concentrations of 100, 500, and 1,000 CFU of each isolate/well. CultureSwab swabs with liquid Stuart's medium and CultureSwab Plus swabs with Amies gel agar medium were inoculated by placing the swab tips into the microtiter wells until the liquid was absorbed. A total of six swabs (two swab types each at three dilutions) for each isolate were inoculated, with intended final inoculum concentrations of 100, 500, and 1,000 CFU/swab. After inoculation, each swab was placed into the tube according to the instructions provided with the device and was held for a minimum of 15 min prior to the assay.

The standard GeneOhm MRSA processing protocol for swabs with liquid Stuart's medium was modified slightly for use with agar gel swabs by the addition of one extra processing step, heating at $95 \pm 2^\circ\text{C}$ for 2 min, as noted below.

Each swab was removed from the holder and placed into the sample buffer tube. The swab stem was broken off into the tube by lifting the swab a few millimeters from the bottom, bending the stem against the tube to break it, and discarding the swab stem. The sample buffer tube was tightly capped and subjected to a vortex at high speed for 1 min. By use of a sterile disposable fine-tip pipette, the entire cell suspension was transferred into a yellow-capped lysis tube. The lysis tubes from the CultureSwab Plus swabs with Amies gel agar medium were then heated at $95 \pm 2^\circ\text{C}$ for 2 min. The lysis tubes from the CultureSwab swabs with liquid Stuart's medium were not subjected to this heating step. All tubes were centrifuged at high speed ($>14,000 \times g$) for 5 min at room temperature. Supernatants were removed using a sterile, disposable fine-tip pipette and discarded. Fifty microliters of sample buffer was added to each lysis tube, and the tubes were tightly closed. The remaining buffer was saved for future possible use. Lysis tubes were subjected to a vortex at high speed for 5 min. The lysis tubes were briefly centrifuged by using the quick-spin button on the centrifuge, holding the button for 8 s, and then releasing. Lysis tubes were held at $95 \pm 2^\circ\text{C}$ for 2 min. The lysis tubes were then removed, placed into a tube holder, and maintained at 2 to 8°C until pipetted into the Smart Cycler reaction tubes.

As a measure of the potential effect of the additional 95°C heating step utilized only for the agar gel swabs, 60 additional MRSA isolates were tested using paired swabs with liquid Stuart's medium. Swabs were inoculated, as previously described, with a target inoculum concentration of 500 CFU/swab. One swab with liquid Stuart's medium was tested using the standard GeneOhm MRSA protocol, while the second swab with liquid Stuart's medium was subjected to the additional 95°C heating step for 2 min according to the exact protocol previously described for the gel agar swabs. The GeneOhm MRSA assay was then performed on the swabs as previously described.

The GeneOhm MRSA assay procedure was performed as indicated in the package insert and training manuals. When a test result indicated inhibition, the testing was repeated with the reserved lysate.

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TABLE 1. Number of positive GeneOhm MRSA test results for each swab type with different quantities of inoculum

Inoculum (CFU)	No. of positive results for ^a :			P value
	All swabs	Swabs with gel-based medium	Swabs with liquid-based medium	
1,000	117	0	3	NS ^b
500	98	4	16	0.007
100	40	15	29	0.03
Total	255	19	48	0.0003

^a The maximum possible number of positive results for each concentration was 120.

^b NS, not statistically significant.

The GeneOhm MRSA assay was performed with three dilutions of 120 recent MRSA isolate specimens inoculated onto the two swabs. As noted in Table 1, there were 274 out of a possible 360 positive test results with the CultureSwab Plus swabs with Amies gel and 303 out of a possible 360 positive test results for the CultureSwab swabs with liquid Stuart's medium ($P = 0.0003$).

Of the 60 MRSA isolates inoculated onto paired swabs with liquid Stuart's medium and tested in parallel with and without the 95°C heating step, 44 of the isolates yielded positive GeneOhm MRSA assay results for both swabs, 8 yielded positive results only for the swab that was heated at 95°C, and 5 yielded positive results only for the swab that was not heated (the P value was not statistically significant).

There were differences in the results related to the inoculum concentration. For the testing with the intended inoculum of 1,000 CFU, both swabs in 117 swab pairs gave positive GeneOhm MRSA results, only the CultureSwab swabs in 3 pairs gave positive results, and none of the pairs had only the CultureSwab Plus swab give a positive result (the P value was not statistically significant). For the testing with the intended inoculum of 500 CFU, both swabs in 98 pairs gave positive GeneOhm MRSA results, only the CultureSwab swabs in 16 pairs gave positive results, and only the CultureSwab Plus swabs in 4 pairs gave positive results ($P = 0.007$). For the testing with the intended inoculum of 100 CFU, both swabs in 40 pairs yielded positive GeneOhm MRSA results, only the CultureSwab swabs in 29 pairs yielded positive results, and only the CultureSwab Plus swabs in 15 pairs yielded positive results ($P = 0.03$).

Both culture and molecular methods have been developed for the detection of MRSA. Molecular methods, when utilized in a real-time format, have the potential to offer more rapid results than culture-based methodologies, particularly if performed directly on patient specimens. When MRSA testing is used to facilitate hospital inpatient isolation policies, the rapid results offered by real-time PCR assays potentially offer more rapid isolation.

Studies describing molecular assays for MRSA developed or validated in-house have been performed previously (3, 6), but the only Food and Drug Administration-cleared assay for the rapid detection of MRSA is the GeneOhm MRSA assay (5, 7). The GeneOhm MRSA assay package insert specifies that a liquid Stuart's medium-based swab such as the BBL CultureSwab collection device be used to collect the specimen for

testing. In our laboratory, we routinely use an Amies gel-based swab transport medium, the BBL CultureSwab Plus. It is clear that the type of swab collection device that is utilized for microbiology testing can impact the performance of certain microbiology tests (1, 2). Specifically, the use of an agar-based transport medium has been shown to inhibit PCRs (4). Consequently, the use of a type of medium (gel based versus liquid) different from what is specified in the package insert for an assay needs to be validated prior to the assay.

In our study, using a slight modification of the protocol for liquid media, we tested 120 samples with three inoculum concentrations using two swab transport devices. Overall, there were more positive test results for the liquid medium than for the gel-based medium. Moreover, the number of positive test results was related to the concentration of the organism sample tested. At higher concentrations, there was no significant difference in detection, but differences at the lower concentrations were noted.

The additional heating step for the agar gel swabs was included because information provided by the manufacturer of the GeneOhm MRSA assay indicated that the presence of chunks of agar may interfere with the assay. The heating step melts the agar, which is then removed by subsequent centrifugation. The results that we obtained by testing MRSA isolates inoculated onto swabs with liquid Stuart's medium suggest that the heating step per se is not responsible for the overall superiority of the performance of the swabs with liquid Stuart's medium to that of the swabs with Amies gel agar medium. We suggest that these differences in results obtained with agar gel swabs are also not due to the inhibition of the PCR per se, as only one specimen demonstrated inhibition. Indeed, this specimen showed no inhibition upon repeat testing. Rather, we suspect that the lower sensitivity with the agar medium was due to the diffusion of the inocula into the medium because of the close proximity of the agar to the swab, in contrast to the liquid medium that comes in contact primarily with the swab tip only. It is not clear that a similar decrease in sensitivity would occur with patient specimens in which the organisms are presumably associated with a cellular matrix. Moreover, the limits of the assay were stressed in this evaluation by intentionally utilizing inocula at concentrations near the detection limit of the assay. Indeed, the concentration of 100 CFU/swab is below the limit of detection of 325 CFU/swab claimed in the package insert for the GeneOhm MRSA assay using liquid Stuart's medium, and 500 CFU/swab is close to the limit of detection.

In conclusion, we have demonstrated that a gel-based transport system can be used for the GeneOhm MRSA assay by following the protocol that we have described here. Using seeded specimens, we have shown that sensitivity is lower for the gel-based than for the liquid-based transport medium, but whether this phenomenon would be observed with patient specimens is unknown.

REFERENCES

1. Bourbeau, P. 2005. Just a swab, you say? Balderdash! Clin. Microbiol. Newsl. 27:19–23.
2. Bourbeau, P. P., and B. J. Heiter. 2003. Evaluation of Copan swabs with liquid transport media for use in the Gen-Probe Group A Strep Direct Test. J. Clin. Microbiol. 41:2686–2689.
3. Fang, H., and G. Hedin. 2003. Rapid screening and identification of methicillin-resistant *Staphylococcus aureus* from clinical samples by selective-broth and real-time PCR assay. J. Clin. Microbiol. 41:2894–2899.
4. Gibb, A. P., and S. Wong. 1998. Inhibition of PCR by agar from bacteriological transport media. J. Clin. Microbiol. 36:275–276.

5. Huletsky, A., R. Giroux, V. Rossbach, M. Gagnon, M. Vaillancourt, M. Bernier, F. Gagnon, K. Truchon, M. Bastien, F. J. Picard, A. van Belkum, M. Ouellette, P. H. Roy, and M. G. Bergeron. 2004. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J. Clin. Microbiol.* **42**:1875–1884.
6. Jonas, D., M. Speck, F. D. Daschner, and H. Grundmann. 2002. Rapid PCR-based identification of methicillin-resistant *Staphylococcus aureus* from screening swabs. *J. Clin. Microbiol.* **40**:1821–1823.
7. Warren, D. K., R. S. Liao, L. R. Merz, M. Eveland, and W. M. Dunne, Jr. 2004. Detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swab specimens by a real-time PCR assay. *J. Clin. Microbiol.* **42**:5578–5581.