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Performance of the BD GeneOhm MRSA Achromopeptidase Assay for Real-Time PCR Detection of Methicillin-Resistant *Staphylococcus aureus* in Nasal Specimens▽

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We evaluated the BD GeneOhm MRSA achromopeptidase (ACP) assay, which incorporates a new specimen preparation approach. A total of 1,216 leftover nasal samples were tested; using culture as the gold standard, the sensitivity and specificity were 92% and 94.6%, respectively. The new lysis method provides good sensitivity and simplifies specimen preparation.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of health care-associated infections and is responsible for increased hospital stays with high financial cost (7, 19, 24). In fact, by 2005 MRSA had a higher mortality rate than tuberculosis, salmonella infection, influenza, and HIV-AIDS combined within the United States (5). Moreover, Delorme and colleagues recently reported an increase of MRSA infections and disease rate in long-term care facility (LTCF) residents between 2006 and 2007 (6). There has been some success reported in the reduction of invasive infection since 2005, but most of the improvement was related to bloodstream infection and may be related to improved central venous catheter management in acute care hospitals (11). Another recent study has shown that a reduction in the rate of MRSA invasive infection must involve more than a general improvement in basic infection control practice, such as improved hand hygiene, since improved hand hygiene did not lower hospital-acquired MRSA colonization (14).

MRSA detection is often followed by either decolonization or isolation to reduce MRSA prevalence within hospitals and in the community (4, 8, 10). In an elegant crossover study of the impact rapid testing has on MRSA transmission, Hardy and colleagues demonstrated significantly reduced transmission when surveillance testing was done using a real-time PCR assay, as opposed to no significant impact when routine, culture-based testing was utilized by the laboratory (9). Thus, rapid and reliable screening tests for MRSA detection are very important. Real-time PCR assays are fast, reliable, and accurate, which has been useful to reduce the incidence of MRSA disease (19, 20).

Paule and colleagues developed an achromopeptidase (ACP) lysis procedure for high-volume testing and compared its performance with the original BD GeneOhm MRSA lysis kit method (IDI-MRSA assay). The study results demonstrated that the assay performed equally well using both procedures (18). BD Diagnostics subsequently developed an ACP lysis method for use with the BD GeneOhm MRSA kit. The purpose of this current study was to assess the BD GeneOhm MRSA ACP assay for direct detection of MRSA in nasal specimens as part of an application to the U.S. FDA for clearance as an *in vitro* diagnostic device.

Nasal swabs were collected using double-headed BBL culture swabs with liquid Stuart (Becton Dickinson, Sparks, MD) or liquid Amies (Becton Dickinson) transport medium; single-headed Amies gel swabs without charcoal (Becton Dickinson) were also included. Excess, deidentified nasal specimens were used for testing after routine laboratory procedures were completed. The subjects enrolled in this study were from inpatient, outpatient, and nursing home settings. Only one specimen per patient was included. This study was approved by the Institutional Review Board of all three sites.

The BD GeneOhm MRSA ACP assay was performed according to the manufacturer's package insert. Briefly, prior to the test, the dried lysis reagent is reconstituted in lysis diluents, aliquoted, and refrigerated until ready to use. A nasal swab was plated to CHROMagar SA and then broken off into a sample buffer and vortexed for 1 min. Next, 50 μl of eluted sample was transferred into the lysis tube, which contained ACP solution, and vortexed for 5 s. The lysis tube next was incubated for 15 min at 37 ± 2°C and 10 min at 99 ± 2°C. The lysate was then placed in a cold block (4°C) for at least 10 min, and the supernatant was used in real-time PCR. Real-time PCR was performed by using a SmartCycler instrument (Cepheid, Inc., Sunnyvale, CA) with the BD GeneOhm MRSA ACP assay PCR protocol. In order to reduce hands-on processing time, three steps (two centrifugations and one washing) are omitted from this new BD GeneOhm MRSA ACP assay in comparison to the BD GeneOhm MRSA kit method.

A positive control provided in the kit which contains Tris-EDTA buffer, carbohydrate, and >0.001% noninfectious genomic MRSA (ATCC 43300) DNA and a negative control (sample buffer) were included in each run. In addition, external specimen processing controls, MRSA (ATCC strain 43300) and methicillin-susceptible *S. aureus* (ATCC strain 25923),

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TABLE 1. Sensitivity and specificity of the BD GeneOhm MRSA ACP assay compared with culture as a gold standard[a]

BD GeneOhm MRSA ACP assay	No. of samples from all three sites with result by culture		
	Positive	Negative	Total
Positive	172	56	228
Negative	15	973	988
Total	187	1,029	1,216

[a] For the BD GeneOhm MRSA ACP assay, sensitivity was 92.0% (95% confidence interval, 87.1% to 95.4%), specificity was 94.6% (95% confidence interval, 93% to 95.9%), positive predictive value was 75.4%, and negative predictive value was 98.5%.

were included with each run and underwent the entire testing procedure. Prior to the study start and periodically during the study, the work area and all equipment were monitored for MRSA DNA contamination using environmental swabs and the BD GeneOhm MRSA ACP assay.

For the direct culture, one nasal swab was first plated onto CHROMagar SA (BBL, Becton Dickinson) before breaking the swab head into the sample buffer tube for real-time PCR. Plates were incubated at 33 to 35°C for 48 h before finalizing a negative result. After 24 h, the plate was examined, and mauve colonies were subcultured to blood agar (BBL, Becton Dickinson) and incubated for 24 h. *S. aureus* identification was confirmed on pure colonies by performing the Staphyloslide agglutination test (BBL, Becton Dickinson). Methicillin resistance was determined using the cefoxitin disk diffusion test performed according to the manufacturer's instructions and CLSI guidelines.

Enriched culture was performed by adding 1 ml of tryptic soy broth with 6.5% NaCl (BBL, Becton Dickinson) to the sample buffer tube which contained the swab. After 24 to 48 h of incubation, this broth was plated onto CHROMagar SA and blood agar plates and incubated for 24 to 48 h at 33 to 35°C.

Any positive culture was considered a gold standard for this study. A total of 1,216 samples were properly collected, stored, and tested for this investigation. For these specimens, the BD GeneOhm MRSA ACP assay had a sensitivity, specificity, positive predictive value, and negative predictive value of 92%, 94.6%, 75.4%, and 98.5%, respectively. Results shown in Table 1 were calculated by using the chi-square method and/or logistic regression and/or log-linear testing as appropriate. The exact 95% confidence interval (CI) for the sensitivity and specificity was calculated using the *F* distribution method given in Collett and also described by Leemis and Trivedi (3, 13). Median time to positivity for positive culture was 69.33 h and for positive PCR was 2.83 h.

The BD GeneOhm MRSA ACP assay validation included a reproducibility analysis and a discrepancy analysis. A total of 343 specimens were retested from frozen lysate for the repeatability analysis, which included all positive samples, all discordant samples, and 10% of randomly selected negative samples. There was 87.8% agreement between the initial and repeat results and 98.8% agreement for the positive samples, 94.0% agreement for the negative samples, and 52.1% agreement for the discordant samples.

Discrepant analysis was performed to investigate discordant test results between PCR and culture. The PCR was repeated from the frozen lysate, and culture was repeated from the frozen enrichment broth for 71 specimens. Discrepancy was resolved for 32 of 71 samples (8 by culture and 24 by PCR). Of the 39 remaining discordant samples, 31 were found to be falsely positive by PCR and 8 were falsely negative. For calculating the assay sensitivity and specificity, we used the PCR results (as false positive or false negative) based on the initial test results, regardless of the outcome of the discrepancy testing. During this study, 12 specimens out of 1,216 total specimens (1%) were reported unresolved at first occurrence. After repeating from frozen lysates, no specimens remained unresolved.

Expanded active surveillance for MRSA using nasal specimens and a real-time PCR assay for MRSA detection requires sample preparation optimization to facilitate high-volume testing (1, 22). There are several real-time PCR tests developed for detection of MRSA that have good sensitivity and specificity and rapid turnaround time when performed on nasal swabs (2, 12, 18, 25, 27). Importantly, this new specimen preparation method provides results similar to the original testing approach where the sensitivity and specificity were 92.5% and 96.4%, respectively (1a), compared to 92% sensitivity and 94.6% specificity with the ACP assay when both were compared to culture-based recovery of MRSA. Thus, the BD GeneOhm MRSA ACP assay can replace the current BD GeneOhm test because it is easier and faster to perform (due to elimination of the two centrifugation and one washing steps) and has test performance comparable to the original assay (1a). Importantly, no specimens remained unresolved after a freeze-thaw cycle and repeat testing, which suggests an improvement using the new assay (16, 17).

A limitation of our study was that we did not have access to past antibiotic use for all specimens or information regarding prior MRSA colonization/infection, so we could not use this in our discrepancy analysis. Using both nasal culture positives and a history of MRSA has been suggested as the appropriate reference standard for surveillance testing for infection control purposes; this improves both the sensitivity and specificity of molecular testing for MRSA detection (18). Other factors that may contribute to apparent changes in sensitivity between this assay and that of Paule and colleagues are that in the BD GeneOhm MRSA ACP assay the sample is diluted by transferring 50 μl from the sample buffer tube to the lysis tube, which is not done in the Paule ACP assay, where the nasal swab was broken off into a microcentrifuge tube containing 200 μl ACP, briefly vortexed, and then incubated for 15 min at 37 ± 2°C and 5 min at 99 ± 2°C. For the current method, the lysate is then placed in a cold block (4°C) and the supernatant used in real-time PCR. Furthermore, in the new BD ACP assay, bacteria are eluted from the swab, whereas in the ACP assay of Paule and colleagues the MRSA was lysed within the swab. We believe these differences contribute to the apparent change in sensitivity for the new assay procedure.

In summary, the BD GeneOhm MRSA ACP assay is an accurate and rapid test for detection of MRSA colonization with performance comparable to the original method. The reduced number of sample preparation steps shortens operator time required for specimen preparation and thus optimizes use in a high-volume setting and facilitates automation (26).

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