

Comparison of the BD Max Methicillin-Resistant *Staphylococcus aureus* (MRSA) Assay and the BD GeneOhm MRSA Achromopeptidase Assay with Direct- and Enriched-Culture Techniques Using Clinical Specimens for Detection of MRSA

Alexander H. Dalpke, Marjeta Hofko, and Stefan Zimmermann

Department of Infectious Diseases, Medical Microbiology and Hygiene, University Hospital Heidelberg, Heidelberg, Germany

We evaluated the new, fully automated molecular BD Max methicillin-resistant *Staphylococcus aureus* (MRSA) assay for detection of methicillin-resistant *S. aureus* in a low-prevalence (4.1%) setting. Sensitivity, specificity, and positive and negative predictive values were 93.9%, 99.2%, 83.8%, and 99.7%, respectively. The assay reported fewer unresolved results than the BD GeneOhm MRSA ACP assay.

Molecular tests for the rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) (10) are used in routine screening programs (6, 24, 25). Despite intrinsic limitations due to SCCmec variability (4, 14, 22, 23), they are considered an important cornerstone in preventing spread of MRSA in health care facilities (2, 7, 12). Implementation of MRSA screening programs in hospitals demands greater automation to manage the increased volume of tests (24, 25). The BD Max system (Becton, Dickinson Diagnostics, Sparks, MD) is a new, fully automated assay system for commercial and user-developed *in vitro* molecular diagnostic tests. It combines cell lysis, nucleic acid extraction, PCR setup, amplification, and detection in a single machine, thereby facilitating use of molecular tests. The aim of this study was to evaluate the BD Max MRSA assay, compared with the widely used BD GeneOhm MRSA achromopeptidase (ACP) assay (5, 11, 17, 18, 20), using direct and enriched culture as the reference method for detection of MRSA.

The study was conducted at the 2,000-bed tertiary care University Hospital Heidelberg from October 2011 to January 2012. Screening swabs (BBL CultureSwab, liquid Stuart; BD) collected from patients admitted to intermediate and intensive care units, from patients admitted from external hospitals, and from surgical patients with wound infections were used. The primary specimen was nasal (91.2%; $n = 734$) as approved for the test, but perianal (3.7%; $n = 30$), wound (3.2%; $n = 26$), and some other swabs were also included. Eight hundred five swabs from 690 individual patients were analyzed by using the BD GeneOhm MRSA ACP assay, BD Max MRSA test, and direct and enrichment culture. Swabs were first placed in 600 μ l BD GeneOhm MRSA ACP sample buffer and vortexed for 1 min. Ninety microliters was used for the BD GeneOhm MRSA ACP assay run on a SmartCycler II PCR system (Cepheid, Sunnyvale, CA). For the new BD Max MRSA assay, 200 μ l of the ACP sample buffer was inoculated into the BD Max sample buffer tube. Tubes were loaded into a rack containing the BD Max MRSA unitized reagent strips, extraction and master mix reagents. The BD Max executes the entire test in a fully automated mode. Each day an external positive control (90 μ l of the hydrated BD GeneOhm MRSA positive control) was included. A negative water control was tested on a weekly basis. Unresolved samples from both molecular tests were reanalyzed once from the sample buffer tube. From the remaining ACP sample buffer, 100

μ l was removed and directly streaked onto cefoxitin-containing BBL CHROMagar MRSA agar plates (BD), which were inspected after 24 h and 48 h. Moreover, 100 μ l of the sample buffer was inoculated into 5 ml of Trypticase soy broth–6.5% sodium chloride (BD) for overnight enrichment of *S. aureus*, followed by plating. Mauve colonies were confirmed by latex agglutination (Pastorex StaphPlus; Bio-Rad, Marbes-la-Coquette, France), growth on DNase and Oxa-screen plates (BD), and in-house PCR for *mecA* and *femB* (13, 15).

As a reference method, we used direct and enrichment culture, which identified 33/805 samples (4.1%) as positive for MRSA in either one or both of the assays. Detection rates were in the range of those of a previous study (15). Of the 33 positive specimens, all were positive with enrichment, but only 29 (88%) were positive with the direct culture, confirming the superiority of enrichment detection (18, 23).

The BD GeneOhm MRSA ACP test initially reported 34/805 samples as unresolved. Retesting resolved 29 of the tests. The unresolved rate of 4.2% is higher than reported (17) or indicated in the packaging insert but in the range that has been observed by others (1, 16). Only 11 of the initial BD Max MRSA tests (1.4%) were unresolved, and all were resolved with retesting (difference between tests, $P < 0.01$, two-sided Fisher's exact test).

The analysis of both molecular tests (Table 1) revealed that the BD Max MRSA assay correctly reported 31 positive and 766 negative samples, and the BD GeneOhm MRSA ACP assay reported 30 positive and 755 negative samples. An additional six culture-negative specimens were positive in the BD Max MRSA assay, and 13 culture-negative specimens were positive in the BD GeneOhm MRSA ACP assay. Sensitivity and specificity were calculated as 93.9% and 99.2% for the BD Max MRSA assay and 93.8% and

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Address correspondence to Alexander H. Dalpke, alexander.dalpke@med.uni-heidelberg.de.

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TABLE 1 Assay characteristics

Assay	No. of samples with result ^a				Sensitivity (%) ^b	Specificity (%) ^b	PPV (%) ^b	NPV (%) ^b
	TP	FP	FN	TN				
BD MAX MRSA	31	6	2	766	93.9 [79.8, 99.3]	99.2 [98.3, 99.7]	83.8 [68.0, 93.8]	99.7 [99.1, 100]
BD GeneOhm MRSA ACP ^c	30	13	2	755	93.8 [79.2, 99.2]	98.3 [97.1, 99.1]	69.8 [53.9, 82.8]	99.7 [99.1, 100]

^a Results obtained in comparison to culture. TP, true positive; FP, false positive; FN, false negative; TN, true negative.

^b Ninety-five percent confidence interval is shown in brackets.

^c Five samples that remained unresolved were excluded from the summarizing analysis. Including those samples as “negatives” accounts for 90.9%, 98.3%, 69.8%, and 99.6% for sensitivity, specificity, PPV, and NPV, respectively.

98.3% for the BD GeneOhm MRSA ACP assay. The observed differences in sensitivity and specificity between the two molecular assays were not statistically significant. The test characteristics of the BD Max MRSA assay are in the range of what has been observed before for the BD GeneOhm MRSA assay (3, 8, 11, 20) and the ACP assay (17–19). Comparison of the two molecular assays gave a Cohen's kappa of 0.816, indicating good agreement.

The BD Max MRSA assay produced four positive test results that were negative or unresolved in the BD GeneOhm MRSA ACP assay. Two samples were confirmed as positive by culture. In contrast, the BD GeneOhm MRSA assay gave 10 additional positive results that were negative in the BD Max MRSA assay. Of those, only one was positive by culture; thus, the BD GeneOhm MRSA assay produced more false-positive results than the BD Max. Positive predictive value (PPV) was 83.8% for the BD Max MRSA assay and 69.8% for the BD GeneOhm MRSA ACP assay ($P = 0.19$, two-sided Fisher's exact test). The PPV for the BD GeneOhm MRSA ACP assay is within the range that has been observed before (3, 8, 17–21); the slightly higher PPV for the new BD Max MRSA assay might help to avoid unnecessary infection control measures, especially in low-prevalence settings. Negative predictive values (NPVs) were high for both assays (99.7%). It has to be acknowledged that data were obtained in only one geographical region in a study population with low MRSA prevalence.

Test characteristics listed in Table 1 are based on culture as a gold standard. Four of the culture-negative specimens were positive by both molecular assays, and those samples showed mean threshold cycles (C_T) in the close range of true-positive test results, for which we observed a C_{Tmean} of 34.0 and a C_{Tmean} of 27 (BD GeneOhm MRSA ACP and BD Max MRSA, respectively). Despite documented false-positive results of MRSA PCR assays due to SCCmec remnants (4, 14, 22), alternative possibilities are that PCR in some cases might be superior to culture, as suggested by others (9), or PCR-amplified DNA from dead organisms.

Labor intensiveness and the need for technical expertise are important restriction variables when implementing molecular MRSA screening. The BD GeneOhm MRSA ACP assay delivered results after 110 min for an 8-sample series and required approximately 25 to 30 min of handling. The BD Max MRSA test needed 140 min; however, hands-on time was reduced to 10 to 15 min, and only two interventions were required. Slightly higher reagent costs for the BD Max MRSA assay can therefore be compensated by reduced labor costs.

This study shows that within a routine clinical setting in a population with low MRSA prevalence, the fully automated BD Max MRSA assay and the established BD GeneOhm MRSA ACP assay have similar sensitivity and specificity characteristics. The BD Max MRSA assay produced fewer unresolved results, had fewer false-

positive results, and showed reduced handling requirements, thereby facilitating the use of this molecular assay.

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We declare no conflict of interest.

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