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Evaluation of the BD Max MRSA *XT* Assay for Use with Different Swab Types

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We evaluated the performance of the BD Max MRSA *XT* assay for use with different swab types. The 90% detection rates (95% confidence intervals) were 387 (97 to 1,551), 877 (238 to 3,230), 986 (183 to 5,287), 1,292 (328 to 5,078), 2,400 (426 to 13,518), and 5,848 (622 to 55,021) CFU/swab for Liquid Stuart, Liquid Amies, dry, Amies Gel without charcoal, ESwab collection, and Amies gel with charcoal swabs (Becton Dickinson), respectively. Amies Gel without charcoal, ESwab collection, and Amies gel with charcoal swabs had a tendency to be less sensitive, but none of the differences was statistically significant.

The BD Max MRSA *XT* kit (BD, Quebec, Canada) allows detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA from nasal swabs in patients at risk for nasal colonization. The assay is used with the fully automated BD Max instrument (BD, Sparks, MD), which combines nucleic acid extraction, PCR setup, and PCR. The kit uses real-time PCR and fluorigenic hybridization probes to detect *mecA* or *mecC* together with staphylococcal cassette chromosome integration (SCC*mec*)-*orfX*. It is an improved MRSA assay that, by the combined detection of both targets, increases specificity (1, 2). The previous BD Max MRSA assay has been tested in clinical settings and proved to be a sensitive assay for the identification of MRSA carriers (3, 4). Rapid molecular detection of MRSA has been introduced into clinical screening programs (5, 6) and is considered an important means of early infection control to prevent the spread of MRSA (7, 8). So far the assay has been approved for use only with liquid Stuart medium transport swabs (BBL CultureSwab Liquid Stuart [BD], Venturi Transystem Swab Liquid Stuart [Copan Diagnostics, Murrieta, CA]). Clearly, different swabs can affect the performance of molecular assays (9). Recent data indicate that ESwab collection systems are also compatible with the BD Max MRSA assay (10). We therefore intended to examine the analytical sensitivity of the BD Max MRSA *XT* assay with six different swab types that are commonly used in hospitals in Germany.

We compared the following BBL CultureSwabs (BD): (i) Liquid Stuart (catalog no. 220099), (ii) Liquid Amies (220093), (iii) dry (220115), (iv) Amies gel without charcoal (22016), (v) Amies gel with charcoal (220121), and (vi) BD ESwab collection kit (220245). We evaluated the performance of the assays by mimicking the sampling process as follows: a 0.5 McFarland standard bacterial suspension was prepared from MRSA strain NCTC10442 (SCC*mec* type I), and then 1:4 dilutions from 6.25E4 to 2.44E2 CFU/ml were prepared. In this study, 100-μl aliquots were plated on blood agar, counted after 24 h of incubation, and used for CFU calculations. A 50-μl aliquot of each of five 1:4 dilutions was pipetted into an Eppendorf tube. The various swabs were placed into the Eppendorf tubes and left there until the suspension was completely absorbed. After soaking, the swabs were added back to the transport system for 20 min to allow them to be in contact with the different transport media. Afterwards, the swabs were added to the BD Max sample buffer tube as recommended. For ESwabs, 200 μl of the 1-ml liquid volume (1/5) was used. Five bacterial concentrations (corresponding to 1,376 down to 5 CFU/swab, 1:4 dilutions) were tested in eight replicates for each swab. A higher concentration with 12,500 CFU/swab was tested in 4 replicates in a second round. From the obtained data, we calculated the limit of detection (LoD) for a MRSA-positive result by simultaneously fitted 2-parameter log-logistic models with lower limits at 0 and upper limits at 1 estimated by nonlinear least squares. The bacterial loads required for the various detection rates were determined, and the 90% detection rates, including the Delta method-based confidence intervals, are reported. The statistical analysis was performed in the R language and environment for statistical computing, version 3.1.1 (11). For all statistical models and tests, the R package drc, version 2.3-96, was used, which is especially suited for the analysis of data from multiple dose-response curves (12).

We furthermore analyzed threshold cycle (C_T) values for SCC*mec-orfX* amplification obtained from using the BD Max MRSA (1st-generation) assay within our routine diagnostics and from a previous study (4). Sampling was performed using BBL CultureSwabs Liquid Stuart (BD), and all PCR-positive samples underwent culture confirmation on BBL CHROMagar MRSA plates (BD). From the PCR-positive samples, we selected 49 culture-positive and 33 culture-negative samples that were analyzed for C_T distribution.

With the serially diluted MRSA suspensions, all the swabs were tested in eight replicates. To illustrate the model-based limits of detection, we plotted the number of CFU soaked per swab against the MRSA detection rate (Fig. 1A). The detection curves showed slightly different slopes. Graphical assessments of model fit indicated that the specified log-logistic models provide very good fits to the underlying data. We calculated the 90% detection rate as the

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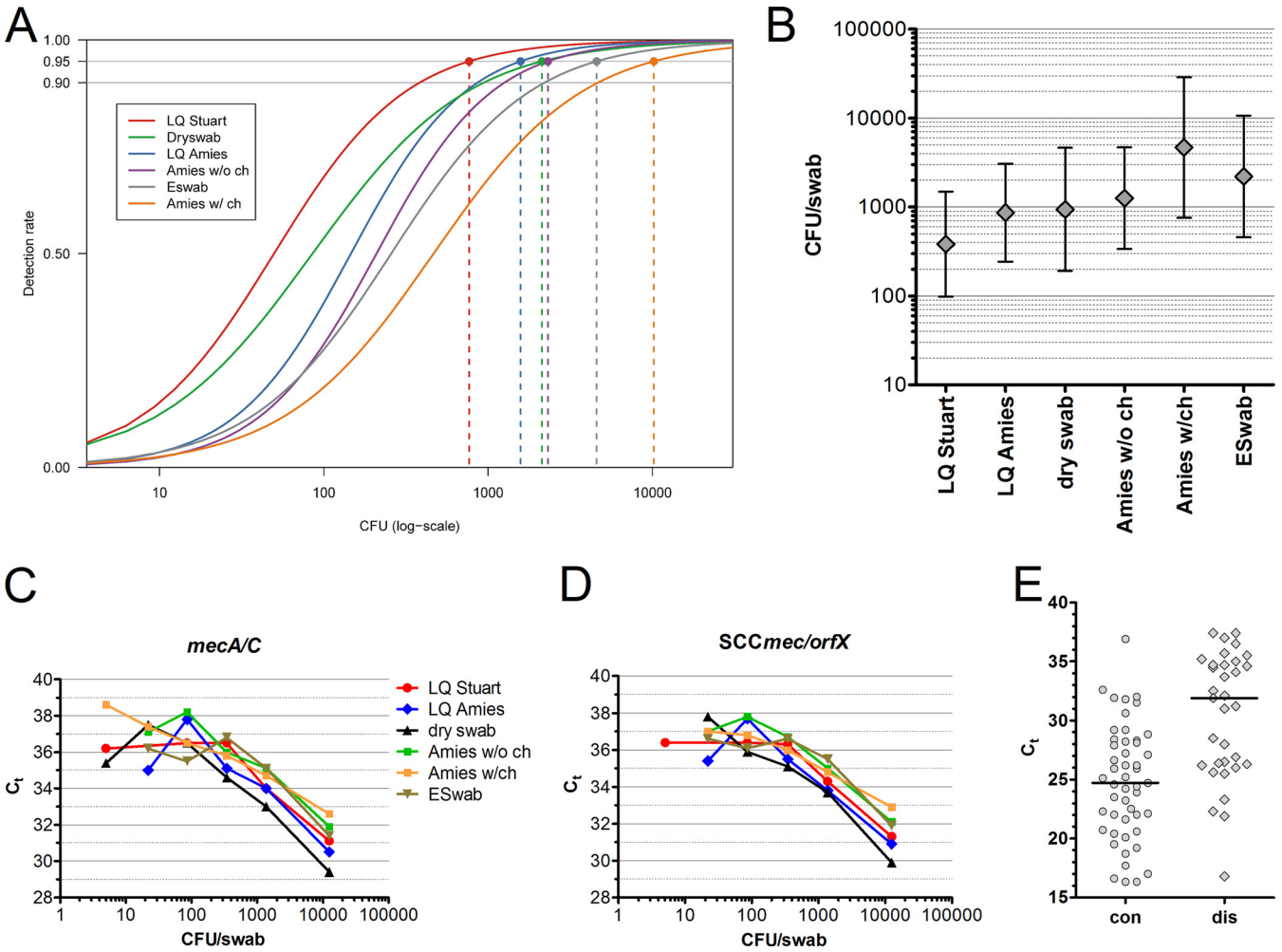

FIG 1 (A) Two-parameter log-logistic model-based curves for the frequency of positive MRSA results in dependency of the bacterial input per swab calculated for six different swab types. Shown are the limits of detection for 95% detection rate. The 90% detection level is also indicated. (B) Estimates for the LoD for the 90% detection rate and 95% confidence intervals for the various swabs. (C and D) Real-time PCR C_T values for serial dilutions of MRSA and detection of *mecA* or *mecC* and SCC*mec-orfX* using the indicated swab types. (E) Distribution of C_T values for SCC*mec-orfX* obtained with the BD Max MRSA assay in a clinical routine setting for samples that were confirmed by culture (concordant [con]) or were culture negative (discordant [dis]). Random selection of 49 concordant and 33 discordant results. Medians are shown. LQ, liquid; w/o ch, without charcoal; w/ch, with charcoal.

LoD. The estimates (95% confidence intervals) were 387 (97 to 1,551), 877 (238 to 3,230), 986 (183 to 5,287), 1292 (328 to 5,078), 2,400 (426 to 13,518), and 5,848 (622 to 55,021) CFU/swab for Liquid Stuart, Liquid Amies, dry, Amies gel without charcoal, ESwab collection, and Amies gel with charcoal swabs, respectively (Fig. 1B). The 95% detection rates were 767, 1,576, 2,127, 2,311, 4,575, and 10,196 CFU/swab, respectively. Although the 90% LoD varied, none of the differences was statistically significant at the 5% level in *t* tests based on the ratios of 90% detection rates. We also tested for differences in the 80% and 95% detection rates, which were also not significant. Liquid Stuart swabs gave the best results. Amies gel without charcoal, ESwab collection, and Amies gel with charcoal swabs ($P = 0.07$, Amies gel with charcoal versus Liquid Stuart) showed slightly worse analytical sensitivities, in that order. Compared to their liquid counterparts, gel-based swabs performed slightly worse in the assay. Similarly, it was shown that Amies gel swabs detected MRSA colonization in the GeneOhm MRSA assay less frequently than Liquid Stuart swabs (13). The BD Max MRSA *XT* assay showed no inhibition of the internal control with any of the swab systems. Thus, the BD Max system gave robust results despite the inhibitory effects that have been reported for agar-based transport systems in molecular tests (14). For Liquid Stuart swabs, the LoD was slightly below the published LoD in the product insert (SCC*mec* type I, 84 [95% confidence interval, 49 to 142]) (15) but well in the range of the first-generation BD Max MRSA assay results (SCC*mec* type I, 645 [95% confidence interval, 314 to 1,326]) (16). For ESwab medium, a reduced sensitivity was assumed, as only one-fifth (200 μl) of the medium was tested. Recently, the suitability of the ESwab collection system was shown for use with the automated BD Max MRSA assay and the GeneXpert MRSA assay (10). In that study, 200 μl of the ESwab transport medium was used for inoculation, but it was also shown that 500 μl might increase sensitivity, which then was in the range of published data. Another option would be to centrifuge the ESwab liquid, remove some supernatant, and then add the whole remaining sample to the inoculation

tube. With the increased availability of automated streaking systems, the usage of fluid transport systems as shown here for the ESwab collection system is of increasing importance.

To analyze whether the differences in the LoD might impact clinical diagnoses, we thought to compare the obtained C_T values from this study with data from our routine clinical application of the BD Max MRSA assay. Threshold cycle (C_T) values for *mecA* or *mecC* and SCC*mec-orfX* amplification are shown in Fig. 1C and D. At higher CFU loads, the amplification showed good efficiency, but below the 90% LoD, the C_T values are out of linear correlation with CFU loads and thus become more variable. In general, above the LoD, C_T values for the individual swabs were <34. We then selected PCR-positive clinical samples for which the results of an additional overnight culture on chromogenic agar were obtained (no enrichment culture data were available). We compared distribution of the C_T values for SCC*mec-orfX* in culture-positive (concordant) and culture-negative samples (discordant) (Fig. 1E). The median C_T value of the concordant samples was 24.7, whereas the discordant samples had a median C_T value of 31.9. Among the samples confirmed by culture, all but one had a C_T value of <34. Similar differences in C_T values have been reported for the GeneXpert system (25.6 versus 31.4) (17) and the BD GeneOhm MRSA assay (30.6 versus 37.3) (18), indicating that the bacterial loads in culture-negative samples are lower than those in culture-positive samples. The clinical significance of these results remains to be determined. For PCR-positive but culture-negative samples, no difference (18) as well as a slightly increased risk for subsequent cultural MRSA positivity (19) has been observed. For nasal swabs, a geometric mean MRSA colony count of 794 CFU/swab was reported (20). However, the recovery rate for conventional swabs is low, probably <10% (21, 22). Thus, the real input load, which we indicated and used in this study, is much higher, and the data cannot be compared directly. Altogether, the data suggest that for all of the tested swabs, the varying LoD is of limited clinical concern.

Moreover, for samples with a concentration near the LOD, PCR assays that make use of the combined interpretation of two targets (SCC*mec-orfX* and *mecA* or *mecC*) might give falsely discordant results, even if both PCRs have the same efficiency. Thus, two targets with an individual probability for a positive result of 0.9 at the 90% LoD will give a positive test result for the combined interpretation with a probability of only 0.81. We observed this in 13/264 samples (4.9%). In those cases, one of the two targets was negative and resulted in a MRSA-negative interpretation. This fact and the observed C_T distribution bring into question the clinical usefulness of low-positive test results and suggest the need for a threshold value (from our data, a C_T value of 34 is a suitable threshold).

Altogether, the results of this study indicate that the fully automated BD Max MRSA *XT* assay, in addition to the approved Liquid Stuart swabs, can be used with Liquid Amies, dry, Amies gel, and ESwab collection systems. The data from clinical usage do not indicate that slight differences in the LoD are of clinical relevance, yet they point toward cautious interpretation of test results around the limit of detection.

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