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Multicenter Evaluation of the LightCycler Methicillin-Resistant *Staphylococcus aureus* (MRSA) Advanced Test as a Rapid Method for Detection of MRSA in Nasal Surveillance Swabs▽

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The rate of methicillin-resistant *Staphylococcus aureus* (MRSA) infection continues to rise in many health care settings. Rapid detection of MRSA colonization followed by appropriate isolation can reduce transmission and infection. We compared the performance of the new Roche LightCycler MRSA advanced test to that of the BD GeneOhm MRSA test and culture. Double-headed swabs were used to collect anterior nasal specimens from each subject. For both tests, DNA was extracted and real-time PCR was performed according to manufacturer's instructions. For culture, one swab of the pair was plated directly to CHROMagar MRSA. The swab paired with the BD GeneOhm MRSA test was also placed into an enrichment broth and then plated to CHROMagar MRSA. Colonies resembling staphylococci were confirmed as *S. aureus* by standard methods. Discrepant specimens had further testing with additional attempts to grow MRSA as well as sample amplicon sequencing. Agreement between results for the two swabs was 99.3% for those with valid results. A total of 1,402 specimens were tested using direct culture detection of MRSA as the gold standard; 187 were culture positive for MRSA. The LightCycler MRSA advanced test had relative sensitivity and specificity of 95.2% (95% confidence interval [CI]: 91.1% to 97.8%) and 96.4% (95% CI: 95.2% to 97.4%), respectively. The BD GeneOhm assay had relative sensitivity and specificity of 95.7% (95% CI: 91.7% to 98.1%) and 91.7% (95% CI: 90.0% to 93.2%), respectively. Following discrepancy analysis, the relative sensitivities of the LightCycler MRSA advanced test and the BD GeneOhm MRSA assay were 92.2 and 93.2%, respectively; relative specificities were 98.9 and 94.2%, respectively. Specificity was significantly better ($P < 0.001$) with the LightCycler MRSA advanced test. The sensitivity of direct culture was 80.4%. The LightCycler MRSA advanced test is a useful tool for sensitive and rapid detection of MRSA nasal colonization.

Worldwide, the number of health care-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) infections is a focus of medical and public concern (9, 26). MRSA was first isolated in the United States in 1968. By 2003, nearly 60% of *S. aureus* isolates recovered from patients in intensive care units (ICUs) were MRSA (25). Similar increases have been observed worldwide, with MRSA accounting for increasing rates of *S. aureus* bacteremia in areas of Canada, the United Kingdom, and Europe (16, 17, 29). With 20% of invasive MRSA infections resulting in death, the failure to reduce HA-MRSA poses a serious threat to patients' health (15). In the United States, approximately 126,000 hospitalizations each year are related to MRSA infection and are associated with high morbidity and mortality rates, prolonged hospital stays, increased cost, and greater use of medical and personnel resources (8, 23). During 2005, it was estimated that invasive MRSA infections in the United States resulted in nearly 19,000 deaths, a result higher than that for HIV/AIDS (15).

Notably, it appears that MRSA does not replace the methicillin-susceptible strains of *S. aureus* (MSSA) as a cause of disease but rather adds to the total burden of *S. aureus* infection in the population (1, 12). Patients colonized with MRSA have an increased risk of developing MRSA disease (7, 11). Thus, minimizing the spread of MRSA to reduce new colonization is one way to lower MRSA infection. Importantly, Hacek and colleagues have recently shown that lowering the number of clinical MRSA isolates reduces the overall number of *S. aureus* clinical isolates recovered from hospitalized patients (10).

The importance of rapidly detecting nasal carriers in a MRSA control program has recently been described (22). Robicsek and colleagues have suggested that after a patient with MRSA is admitted, the percentage of potential isolation days captured has a major impact on the success or failure of a MRSA control program (24); the factors that determine this percentage of potential isolation days captured are the patient population tested, the sensitivity of the laboratory testing, and the speed of result determination (19). Thus, rapid and accurate determination of MRSA colonization can be a critical

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factor for the success of any MRSA control program. The purpose of reporting this multicenter study is to compare the new Roche LightCycler MRSA advanced test to the BD GeneOhm MRSA test (an FDA-cleared diagnostic assay for rapid detection of MRSA) and culture for rapid detection of MRSA found in surveillance nasal swabs.

MATERIALS AND METHODS

Patient population and testing site characteristics. Patient samples were tested at five sites across the United States. For collection, one double-headed swab (double-headed BBL Culture Swab Liquid Stuart; Becton-Dickinson, Sparks, Maryland) was inserted into the nostril and rotated against the mucosa five times; the same procedure was then repeated for the second nostril. The swab was transported to the laboratory in a transport container. The subjects were from hospital intensive care and nonintensive care units, nursing homes/extended-care facilities, dialysis units, and medical staff. All subjects were ≥2 years of age and provided written informed consent to participate in the study if required by the local institutional review board. Exclusion criteria included (i) antibiotic therapy, either systemic or topical, that is active against MRSA colonization (rifampin, mupirocin, or clindamycin) on the day of sample collection or in the previous 7 days, (ii) previous enrollment in the study, and (iii) contraindications to nasal sampling according to the institution's policies. This study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines and regulations of the U.S. Food and Drug Administration.

Analytical performance of the LightCycler MRSA advanced test. The analytical sensitivity of the LightCycler MRSA advanced test was determined for three common swab types using defined suspensions of MRSA cultures comprising staphylococcal cassette chromosome *mec* (SCC*mec*) types I to V spanning a range of 100 to 400 CFU/swab. All suspensions were tested in replicates of 15 to 60. The limit of detection (LoD) was defined as the lowest number of MRSA CFU/swab that could be reproducibly distinguished from negative samples with 95% confidence. A total of 100 MSSA strains, 221 coagulase-negative staphylococcus strains (117 methicillin resistant, 104 methicillin sensitive), and 85 bacterial, viral, and fungal microorganisms were obtained from the American Type Culture Collection, the German Collection of Microorganisms and Cell Cultures (DSMZ), and clinical laboratories to determine analytical specificity. All strains had been kept as frozen stocks and were grown in culture. The LightCycler MRSA advanced test was performed at densities of 10^4 to 10^5 CFU per lysis tube and at densities of 1,000 to 10,000 copies of DNA (viral cultures). Reproducibility of results obtained with the LightCycler MRSA advanced test was assessed using a 12-member panel of MRSA strain ATCC 43300 diluted to densities of 0, 300, and 800 CFU/swab (negative, weak positive, and positive, respectively). Methicillin-sensitive *S. epidermidis* (ATCC 14990) was included in each panel at a constant density of 2.6×10^6 to achieve an appropriate sample matrix. Two operators at each site performed one run each per day for 5 days on each of three reagent lots.

Clinical experimental design. Nasal swab specimens were tested by the LightCycler MRSA advanced test, and the results were compared to those obtained by the (i) BD GeneOhm MRSA assay (Becton Dickinson, San Diego, CA), (ii) directly plated culture on CHROMagar MRSA medium (directly plated culture; BD BBL CHROMagar MRSA; Becton Dickinson, Sparks, MD), and (iii) broth-enriched culture containing 6.5% NaCl (Trypticase soy broth; Remel, Lenexa, KS) followed by culture on CHROMagar MRSA (enrichment culture). All five study sites performed all tests on site. For testing, the double-headed swab was separated, with one swab head processed for directly plated culture and the LightCycler MRSA advanced test. The other swab head was used for directly plated culture on a separate CHROMagar MRSA and processing with BD GeneOhm MRSA assay followed by incubation in broth enrichment culture. Time stamps on a workflow log sheet were used to document the start and finish times for sample preparation, master mix preparation, and PCR preparation of the LightCycler MRSA advanced test and the BD GeneOhm MRSA assay. Similarly, time stamps on a workflow log sheet were used to document the time from streaking/inoculation of the sample to the final reading for directly plated and enrichment culture. Results for all samples included in the study are presented as mean time to result for each technique.

Molecular methods. Both the LightCycler MRSA advanced test and BD GeneOhm MRSA assay were performed according to the manufacturer's instructions. Both assays target the integration site of the SCC*mec* cassette into the *S. aureus* chromosome. Briefly, the LightCycler MRSA advanced test is performed on the LightCycler 2.0 instrument and has been validated with three swab transport media (Liquid Stuart, Amies gel with or without charcoal). The test uses swab extraction and mechanical lysis for specimen preparation (using the MagNA Lyser instrument), real-time PCR amplification of MRSA DNA, and fluorogenic target-specific hybridization probes for detection of amplified DNA. Each LightCycler MRSA advanced test reaction mixture contains an internal control to detect specimen inhibition and to monitor reagent integrity. The internal control is a plasmid amplified with primers used for amplifying the MRSA target. A commercial lyophilized external control (KWIK-STIK MRSA; MicroBioLogics, Inc., St. Cloud, MN) was used as the positive control in each MagNA Lyser run; external controls were performed with each sample preparation run during the clinical study. An internal positive control is provided in the assay, and a sterile swab serves as a negative control.

Culture methods. Both swab heads were directly streaked onto CHROMagar MRSA medium before the swab head was added into the sample preparation buffer of the respective molecular assays. CHROMagar MRSA was incubated for up to 48 ± 4 h at 35 to 37°C. Mauve colonies observed after 24 h of incubation were considered positive for MRSA; positive results obtained after 48 h were confirmed by coagulase testing and the presence of characteristic staphylococcal Gram-staining morphology. One swab head was also incubated in Trypticase soy broth (TSB; enrichment culture) by adding 1 ml of TSB to the BD GeneOhm MRSA assay sample buffer tube and swab following removal of the cell suspension during sample preparation. Following incubation for 18 to 24 h at 35 to 37°C, the broth was streaked onto another CHROMagar MRSA plate, and the plate was incubated for 24 to 48 h at 35 to 37°C as described above.

Discrepancy analysis. A thorough discrepancy analysis, including culture-based methods and molecular assays, was performed at two central laboratories on all samples that gave discordant results between the LightCycler MRSA advanced test and directly plated culture and/or the BD GeneOhm assay. Culture and PCR-based tests were performed at NorthShore University HealthSystem (NorthShore), while sequencing was performed at Roche Molecular Diagnostics. Based on the predefined algorithm developed before the study began, discrepant results were analyzed at NorthShore as follows: (i) lysates were reanalyzed with the LightCycler MRSA advanced test; (ii) broth-enriched samples were plated to CHROMagar MRSA, sheep blood agar, colistin, and nalidixic acid (CNA) agar and into TSB; (iii) recovered suspect staphylococcal isolates were characterized by coagulase testing and *fem*PCR (20), and testing for methicillin resistance was done using disk diffusion (5, 6) and *mecA* PCR (21); and (iv) *S. aureus* colonies were directly tested with the LightCycler MRSA advanced test. For the molecular testing, sequence analysis of discrepant samples was performed at Roche Molecular Diagnostics. Genomic DNA was extracted from isolates recovered at the study sites, from isolates recovered during the enrichment broth testing at NorthShore (to confirm the results of culture), and from the original broth used to enrich patient samples, even if no organism was recovered. The goal was to detect MRSA-specific DNA sequences in these three sample sets. PCR was performed using primers that flank the LightCycler MRSA advanced test target region (the right-extremity [RE] attachment site for SCC*mec*): the left-extremity [LE] attachment site for SCC*mec*, the intact *orfX* (without the SCC*mec*), the *mecA* gene, and the internal transcribed spacer (ITS) region to generate amplicons for sequencing (13). Sequencing was carried out using the ABI BigDye Terminator version 3.1 chemistry and the Applied Biosystems 3730 DNA analyzer. The resulting sequences were analyzed to determine any mismatches to the LightCycler MRSA advanced test primers and probes that may account for the discrepant results. The presence or absence of MRSA was presumed based on sequences from the RE and LE attachment sides or the *mecA* and ITS regions. Sequences from the RE region or from the left-hand side indicated the presence of MRSA; the presence of *mecA* sequences plus the ITS region indicating *S. aureus* was used to presume the presence of MRSA. If the intact *orfX* was present, the presence of MSSA was presumed. In the discrepancy analysis, the detection of MRSA-specific sequences from the RE and LE regions in conjunction with a positive *mecA* PCR and/or a negative *attB* sequence was considered positive and overruled a negative result by culture methods.

Combined results of enriched culture and molecular analysis were used to determine the true status of positivity (presence of MRSA and/or MRSA DNA) in all discrepant samples.

Statistical analysis. The percentage of correct results and the associated exact 95% confidence intervals (Clopper-Pearson) for each panel of the reproducibility panel were calculated by lot, testing site/instrument, operator, and day. The positive and negative percent agreements (here designated sensitivity and specificity, respectively) of the LightCycler MRSA advanced test with respect to the BD GeneOhm MRSA assay and directly plated CHROMagar MRSA were calculated overall and by site. Statistical significance was determined by the chi-square test. Any P of ≤0.05 was considered statistically significant.

TABLE 1. Relative sensitivity and specificity of the LightCycler MRSA advanced test and the BD GeneOhm MRSA assay compared to those of directly plated culture[a]

Site	Relative sensitivity		Relative specificity	
	LightCycler MRSA advanced	BD GeneOhm MRSA	LightCycler MRSA advanced	BD GeneOhm MRSA
1	91.4 (32/35; 76.9–98.2)	91.4 (32/35; 76.9–98.2)	97.5[b] (197/202; 94.3–99.2)	92.0[b] (184/200; 87.3–95.4)
2	95.8 (46/48; 85.7–99.5)	95.8 (46/48; 85.7–99.5)	95.9[c] (301/314; 93.0–97.8)	81.8[c] (257/314; 77.1–86.0)
3	100.0 (13/13; 75.3–100.0)	100.0 (13/13; 75.3–100.0)	97.6 (245/251; 94.9–99.1)	96.8 (243/251; 93.8–98.6)
4	95.0 (76/80; 87.7–98.6)	96.3 (77/80; 89.4–99.2)	93.9 (185/197; 89.6–96.8)	93.4 (183/196; 88.9–96.4)
5	100.0 (11/11; 71.5–100.0)	100.0 (11/11; 71.5–100.0)	96.8 (243/251; 93.8–98.6)	97.2 (243/250; 94.3–98.9)
Total	95.2 (178/187; 91.1–97.8)	95.7 (179/187; 91.7–98.1)	96.4[c] (1,171/1,215; 95.2–97.4)	91.7[c] (1,110/1,211; 90.0–93.2)

[a] Results are shown as percentage (no. of results/total no. of samples; 95% CI [%]). There were 1,402 valid LightCycler MRSA advanced test results and 1,398 valid BD GeneOhm MRSA test results.
[b] Difference between relative specificity results is $P = 0.024$.
[c] Difference between relative specificity results is $P < 0.001$.

RESULTS

Analytical performance. Using the complete workflow, including swab extraction, amplification, and detection, the LightCycler MRSA advanced test detected 240 CFU MRSA/swab in three swab collection media (Liquid Stuart, Amies gel without charcoal, and Amies gel with charcoal) with 95% confidence. The panel of 85 species of bacterial, viral, and fungal microorganisms, including typical commensals as well as pathogens in the upper and lower airways, were correctly identified as MRSA negative. There was no positive signal for MRSA with 221 coagulase-negative staphylococci and 100 MSSA isolates obtained from clinical laboratories. There was little variability across lot, site, operator, or day for the negative, weak-positive, and positive samples. The overall percentage of correct results was 99.6% (807/810); when an apparent transposition of two samples at one site positioned next to each other in the same run was excluded, the overall percentage of correct results was 99.9% (809/810).

Clinical performance. A total of 1,402 specimens were collected from eligible subjects enrolled at the five clinical study sites. In total, 63.9% of subjects were aged 21 to 65 years, and 34.3% of subjects were aged >65 years. Most specimens were collected from subjects in a hospital non-ICU setting (71.8%), followed by specimens from nursing homes/extended-care facilities (18.2%), medical staff (5.2%), and hospital ICU patients (4.8%). Agreement between the two swab pairs collected for this testing was 99.3% among those with valid results. The overall positivity rate as determined by directly plated culture was 13.3% (14.8, 13.3, 4.9, 28.9, and 4.2% for sites 1 to 5, respectively).

The sensitivity and specificity for the LightCycler MRSA advanced test and the BD GeneOhm MRSA assay compared to directly plated culture are presented in Table 1. Positive percent agreement (relative sensitivity) with directly plated culture for the LightCycler MRSA advanced test and the BD GeneOhm MRSA assay did not differ significantly. There was little variation in the positive percent agreement between individual sites (91.4 to 100%). However, the negative percent agreement (relative specificity) with directly plated culture for the LightCycler MRSA advanced test and the BD GeneOhm MRSA assay showed significantly lower performance for the BD GeneOhm MRSA assay than for the LightCycler MRSA advanced test ($P < 0.001$). This was due to the results at two sites (sites 1 and 2).

The positive percent (relative sensitivity) and negative percent (relative specificity) agreement results between the LightCycler MRSA advanced test, BD GeneOhm MRSA assay, and directly plated culture compared to enrichment culture are presented in Table 2. The negative percent agreement (relative specificity) with enrichment culture also was lower for the BD GeneOhm MRSA test than for the LightCycler MRSA advanced test, again due to the results at sites 1 and 2.

Table 3 shows a three-way comparison of results obtained in 1,398 specimens that yielded valid results with the LightCycler MRSA advanced test, the BD GeneOhm MRSA assay, and directly plated culture. All samples that revealed discordant results between the LightCycler MRSA advanced test and directly plated culture or discordant results between the LightCycler MRSA advanced test and the BD GeneOhm MRSA assay (but showed concordant results by directly plated culture for the two swab heads) underwent discrepancy analysis ($n = 124$). Discrepancy analysis using highly sensitive culture methods and molecular techniques (PCR and sequencing) revealed the presence of MRSA in 30 of 43 samples (12 by culture and 18 by sequence analysis of isolates or broth-enriched patient samples) that gave positive results in the LightCycler MRSA advanced test but were negative by directly plated culture; the presence of MRSA was also confirmed in 4 out of 5 samples that gave negative results in the LightCycler MRSA advanced test and were positive by directly plated culture, indicating 4 true false-negative results. Of interest, MRSA was confirmed in only 13 of 76 samples (6 by culture and 7 by sequence analysis of the amplified product) that were positive in the BD GeneOhm MRSA assay but gave negative results in the LightCycler MRSA advanced test and directly plated culture. Among 13 samples that gave "false-positive" results in the LightCycler MRSA advanced test, MSSA was detected in 4 cases; for the 63 samples that gave "false-positive" results in the BD GeneOhm MRSA assay, MSSA was detected in 6 cases. Table 4 contains the performance characteristics of the three detection methods using the results of the discrepancy analysis.

Ten (0.7%) specimens tested gave invalid results in the LightCycler MRSA advanced test due to internal control fail-

TABLE 2. Relative sensitivity and specificity of the LightCycler MRSA advanced test, the BD GeneOhm MRSA assay, and directly plated culture compared to those of enrichment culture

Site	Relative sensitivity[f]			Relative specificity		
	LightCycler MRSA advanced	BD GeneOhm MRSA	Directly plated culture	LightCycler MRSA advanced	BD GeneOhm MRSA	Directly plated culture
1	83.3 (30/36; 67.2–93.6)	83.3 (30/36; 67.2–93.6)	86.1 (31/36; 70.5–95.3)	96.5[a] (194/201; 93.0–98.6)	91.0[a,b] (181/199; 86.1–94.6)	98.0[b] (197/201; 95.0–99.5)
2	87.9 (51/58; 76.7–95.0)	86.2 (50/58; 74.6–93.9)	81.0 (47/58; 68.6–90.1)	97.3[c] (289/297; 94.8–98.8)	82.5[c,d] (245/297; 77.7–86.6)	99.7[d] (296/297; 98.1–100.0)
3	81.3 (13/16; 54.4–96.0)	81.3 (13/16; 54.4–96.0)	81.3 (13/16; 54.4–96.0)	97.6[b] (242/248; 94.8–99.1)	96.8[b] (240/248; 93.7–98.6)	100.0[b] (248/248; 98.5–100.0)
4	97.5 (79/81; 91.4–99.7)	96.3 (78/81; 89.6–99.2)	95.1 (77/81; 87.8–98.6)	95.4 (187/196; 91.5–97.9)	93.8[b] (183/195; 89.5–96.8)	98.5[b] (193/196; 95.6–99.7)
5	78.6 (11/14; 49.2–95.3)	78.6 (11/14; 49.2–95.3)	78.6 (11/14; 49.2–95.3)	96.8[b] (240/248; 93.7–98.6)	97.2[b] (240/247; 94.2–98.9)	100.0[b] (248/248; 98.5–100.0)
Total	89.8 (184/205; 84.8–93.5)	88.8 (182/205; 83.6–92.8)	87.3 (179/205; 82.0–91.5)	96.8[c,e] (1,152/1,190; 95.6–97.7)	91.8[c,d] (1,089/1,186; 90.1–93.3)	99.3[d,e] (1,182/1,190; 98.7–99.7)

[a] Difference between relative specificity results is $P = 0.037$.
[b] Difference between relative specificity results is $P < 0.05$.
[c] Difference between relative specificity results is $P < 0.001$.
[d] Difference between relative specificity results is $P < 0.001$.
[e] Difference between relative specificity results is $P < 0.001$.
[f] Results are shown as percentage (no. of results/total no. of samples; 95% CI [%]).

TABLE 3. Three-way comparison of results obtained in the LightCycler MRSA advanced test, BD GeneOhm MRSA assay, and directly plated culture

Result with:		No. with result with directly plated culture		Total
LightCycler MRSA advanced	BD GeneOhm MRSA	Positive	Negative	
Positive	Positive	175	25	200
Positive	Negative	3	19	22
Negative	Positive	4	76	80
Negative	Negative	5	1,091	1,096
Total	Total	187	1,211	1,398[a]

[a] The 1,398/1,402 samples that had valid results for the LightCycler MRSA advanced test, the BD GeneOhm MRSA test, and directly plated culture are included in this table.

ure; seven (0.5%) remained invalid upon retesting following a freeze-thaw cycle. The BD GeneOhm MRSA assay generated "unresolved" results in 32 (2.3%) samples tested; five (0.4%) remained "unresolved" following retesting.

The technical hands-on time (for the molecular diagnostic tests) as well as the mean time to result for the LightCycler MRSA advanced test, the BD GeneOhm MRSA assay, and directly plated culture is presented in Table 5. For an average batch size of 25 samples, technologist hands-on time was 41 min for the LightCycler MRSA advanced test, while it took 75 min to process samples with the BD assay (Table 5).

DISCUSSION

Nasal colonies of MRSA have recently been confirmed as the important site for performing colonization surveillance (2). Several new commercial molecular tests developed for use in detecting MRSA colonization during a MRSA control program have been evaluated, and all appear to provide high sensitivity, good specificity, and the potential for rapid reporting of nasal colonization (4, 14, 18, 27, 28). The new LightCycler MRSA advanced test is another of these assays with rapid detection time and high sensitivity. Compared with the BD GeneOhm MRSA assay, this new test also appears to have improved test specificity. High sensitivity of any MRSA surveillance test is desirable since the goal of a MRSA program is to rapidly detect all those colonized with this potential pathogen, even if nasal detection results from identifying DNA no longer associated with viable organisms in the nares. However, improved specificity can be particularly important when performing a large number of assays in a population with a modest level of asymptomatic MRSA colonization to minimize false-positive test results that can lead to inappropriate patient isolation. It is of interest that all tests had lower sensitivity than enrichment using broth (Table 2). However, as reported by Paule and colleagues, when testing is compared to culture plus a history of MRSA, the sensitivity of PCR increases and that of culture decreases (19). We did not have access to history at all sites and thus could not use such information in our discrepancy analysis. Because of that limitation, we used sequence analysis, which demonstrated the enhanced sensitivity of molecular testing as expected (Table 4). Also, the new test required less labor time than the comparator assay and per-

TABLE 4. Test performance of the LightCycler MRSA advanced test, the BD GeneOhm MRSA assay, and directly plated culture compared to final results determined after discrepancy analysis[a]

Test	No. of samples				Result[e]			
	True positive	False positive	False negative	True negative	Sensitivity	Specificity	Positive predictive value	Negative predictive value
Roche LightCycler	202	13	17	1,153	92.2[b] (87.9–95.4)	98.9[c,d] (98.1–99.4)	94.0 (89.9–96.7)	98.5 (97.7–99.2)
BD GeneOhm	204	68	15	1,098	93.2[b] (89.0–96.1)	94.2[c,d] (92.7–95.4)	75.0 (69.4–80.0)	98.7 (97.8–99.2)
Direct CHROMagar culture	176	1	43	1,165	80.4[b] (74.5–85.4)	99.9[d] (99.5–100)	99.4 (96.9–100)	96.4 (95.2–97.4)

[a] In this analysis there were determined to be 219 true-positive swabs for MRSA out of 1,385 samples. To equitably compare the molecular diagnostic tests, only samples that had concordant results on the two directly plated cultures (one with each real-time PCR assay) were used in this analysis.
[b] $P \leq 0.0005$ between the real-time PCR tests and culture.
[c] $P \leq 0.0001$ between the two real-time PCR tests.
[d] $P \leq 0.0032$ between the two real-time PCR test and culture.
[e] Results are shown as percentage (95% CI [%]). CI, exact 95% confidence interval (Clopper-Pearson).

formed as well as culture: approximately 1.5 min of technologist time per sample for the Roche PCR assay compared to 3 min for the BD GeneOhm MRSA assay and 1 to 2 min for direct culture (Table 5).

An additional novel technique used for this investigation was the application of amplification and sequencing as part of the discrepancy analysis investigation. This was done to determine that a positive signal from the PCR test actually detected the target of the assay. Two sites were able to review the records of the patients with positive PCR results and negative cultures (Durham VA Medical Center, Durham, NC, and NorthShore University HealthSystem, Evanston, IL). From these two sites, we detected 14 true-positive assays by sequence analysis (9 at Durham VAMC and 5 at NorthShore) and 11 true-positive patients by history of a positive culture for MRSA (8 at Durham VAMC and 3 at NorthShore). Since there is no current guidance on when a patient with prior MRSA colonization or disease is considered "free" of this pathogen (25), the goal of any surveillance program is to detect those patients most likely positive for MRSA (current as well as prior positive persons) so the affected patient can be placed into appropriate isolation (18, 19). Thus, determining who is or was positive for MRSA seems a reasonable gold standard for determining the performance of any MRSA surveillance test in a final (e.g., discrepancy) analysis. The novel approach we used, of sequencing products amplified by PCR from isolates plus broth-enriched patient samples, proved to be a good surrogate instead of using a history of MRSA in determining the rate of likely true-positive tests when the real-time PCR test was positive and the accompanying direct culture was negative or grew susceptible *S. aureus*. The results using this novel analysis approach that are presented in Table 4 demonstrate that the new test performed very well in this study.

TABLE 5. Mean processing time and time to result for the LightCycler MRSA advanced test, BD GeneOhm MRSA assay, and directly plated culture for an average test batch of 25 samples[a]

Time for assay/test	Mean time ± SD
LightCycler MRSA advanced	
Lysate prepn	22.1 ± 10.4 min
Working master mix prepn	4.3 ± 3.9 min
PCR prepn	14.6 ± 6.1 min
Amplification and detection	77.6 ± 6.4 min
Total time per run	118.5 ± 17.2 min
Total hands-on time per run	41 min
BD GeneOhm MRSA	
Lysate prepn	56.6 ± 24.5 min
PCR prepn	18.8 ± 7.3 min
Amplification and detection	61.8 ± 3.2 min
Total time per run	137.2 ± 28.7 min
Total hands-on time per run	75 min
Directly plated culture	
24-h reading	24.4 ± 1.7 h
48-h reading	48.7 ± 7.2 h

[a] Results for PCR assays are presented as mean time ± SD/run. prepn, preparation. The hands-on time for performing culture has been reported at 1 to 2 min per sample for direct plating, with an additional minute when broth enrichment is employed (18).

Our paper has limitations. The testing was performed by dedicated technologists at each site, so that the results imply only what can be expected in a setting of high-volume clinical use. Also, the finding of significantly improved specificity is very important, but it was largely due to the testing results at two of our five clinical trial sites and thus needs to be confirmed by additional published experience. This may indicate that differences in local or regional epidemiology can affect geographic MRSA strain types (8), a possibility which has not been well studied in the United States. Finally, there has been concern over the potential for MRSA to mutate sequences utilized as binding sites for primers and probes, thus avoiding detection by molecular diagnostic assays (3); however, this has not emerged as a significant problem to date when these tests have been used in MRSA control programs (24).

In summary, we have demonstrated that the new LightCycler MRSA advanced test performed with sensitivity equal to that of the BD GeneOhm MRSA assay, and both outperformed the sensitivity and time to reporting of directly plated culture. The LightCycler MRSA advanced test appeared to have improved specificity compared to the BD GeneOhm MRSA assay. Also, the new real-time PCR assay we evaluated in this paper had hands-on technologist processing time comparable to that of directly plated culture and thus can provide very rapid results with no increase in laboratory personnel. Most importantly, analysis of the recent literature indicates that only tests permitting capture of >70% of potential MRSA patient isolation days will provide a positive impact for a MRSA control program within 1 year from its implementation in a setting of modest MRSA colonization (22); only the new

real-time PCR assays have the capability in sensitivity and result-reporting time to offer that result.

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