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An International, Prospective, Multicenter Evaluation of the Combination of AdvanDx *Staphylococcus Quick*FISH BC with *mecA Xpress*FISH for Detection of Methicillin-Resistant *Staphylococcus aureus* Isolates from Positive Blood Cultures

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Sepsis caused by *Staphylococcus aureus* is a major health problem worldwide. Better outcomes are achieved when rapid diagnosis and determination of methicillin susceptibility enable early optimization of antimicrobial therapy. Eight large clinical laboratories, seven from the United States and one from Scotland, evaluated the combination of the *Staphylococcus Quick*FISH BC and the new *mecA Xpress*FISH assay (both AdvanDx, Woburn, MA, USA) for the detection of methicillin-resistant *S. aureus* in positive blood cultures. Blood cultures flagged as positive by automated blood culture instruments and demonstrating only Gram-positive cocci in clusters on Gram stain were tested by *Quick*FISH, a 20-min assay. If only *S. aureus* was detected, *mecA Xpress*FISH testing followed. The recovered *S. aureus* isolates were tested by cefoxitin disk diffusion as the reference method. The *Quick*FISH assay results were concordant with the routine phenotypic testing methods of the testing laboratories in 1,211/1,221 (99.1%) samples and detected 488/491 *S. aureus* organisms (sensitivity, 99.4%; specificity, 99.6%). Approximately 60% of the samples (730) contained coagulase-negative staphylococci or nonstaphylococci as assessed by the *Quick*FISH assay and were not tested further. The 458 compliant samples positive exclusively for *S. aureus* by the *Quick*FISH assay were tested by the *mecA Xpress*FISH assay, which detected 209 of 211 methicillin-resistant *S. aureus* organisms (sensitivity, 99.1%; specificity, 99.6%). The *mecA Xpress*FISH assay also showed high reproducibility, with 534/540 tests performed by 6 operators over 5 days achieving reproducible results (98.9% agreement). The combination of the *Staphylococcus Quick*FISH BC and *mecA Xpress*FISH assays is sensitive, specific, and reproducible for the detection of methicillin-resistant *S. aureus* and yields complete results in 2 h after the blood culture turns positive.

Members of the genus *Staphylococcus* are the most common organisms isolated from blood cultures, with *Staphylococcus aureus* often being the most common cause of bloodstream infections (BSI) (1). The annual incidence of infection in the United States is approximately 50 per 100,000 population. Methicillin-resistant *S. aureus* (MRSA) leads to significant increases in morbidity and mortality compared to methicillin-susceptible *S. aureus* (MSSA) (2). MRSA infections may be health care associated or community acquired (3). The treatment of MSSA bacteremia with vancomycin may lead to delays in the clearance of the organism (4). Since MRSA represents an epidemiologic concern, patients infected with *S. aureus* may be placed in isolation until the susceptibility to methicillin is ascertained (http://www.cdc.gov/mrsa/healthcare/clinicians/precautions.html). Isolation represents a significant medical expense and may adversely affect patient care (5). The rapid identification of the etiologic agent of bloodstream infection leads to early optimization of antibiotic therapy and improved patient outcomes, including decreased mortality, shorter stays in the intensive care unit, shorter hospital stays, and significant reductions in hospital costs (4, 6–8). The FDA has approved/cleared several nucleic acid-based techniques that expedite the identification of *S. aureus* directly from positive blood culture bottles (http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm#microbial). In addition to AdvanDx, the manufacturers of these assays include BioFire (Salt Lake City, UT), BD GeneOhm (Franklin Lakes, NJ), Nanosphere (Northbrook, IL), and Cepheid (Sunnyvale, CA).

A peptide nucleic acid fluorescence *in situ* hybridization (PNA FISH) kit for the rapid detection of *S. aureus* and coagulase-negative staphylococci (CoNS) was launched in 2003 by AdvanDx. Since then, *S. aureus*/CoNS PNA FISH tests have been used in many clinical microbiology laboratories for the rapid identification of *Staphylococcus* organisms and to distinguish *S. aureus* from CoNS in positive blood cultures. PNA FISH probes consist of peptide backbones to which the nucleic acid bases are attached. Such probes lack the net negative charge common to nucleic acid probes, which facilitates both entry into bacterial cells and tighter

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binding to the RNA sequences that serve as targets for the assays (8–10). Recently, the FDA approved the *S. aureus Quick*FISH BC (QFISH) (AdvanDx) assay, which is similar to its predecessor but takes about 20 min (10). Both the original and QFISH assays accurately distinguish *S. aureus* from CoNS (8, 9). However, neither distinguishes MSSA, which can often be treated with oxacillin and some cephalosporins, from MRSA, which responds to a more limited spectrum of antibiotics, such as vancomycin, daptomycin, ceftaroline, and linezolid, and which may be toxic, expensive, or both. Thus, patients whose cultures exhibit Gram-positive cocci in clusters, suggestive of *S. aureus*, previously had to be treated with an antimicrobial suitable for MRSA until phenotypic susceptibility results became available after about 2 days.

The MRSA phenotype is due to the *mecA* gene, which encodes the altered penicillin-binding protein 2a (PBP-2a) (11). The proportion of MRSA varies with the patient setting but is currently around 50% with both health care-associated and community-acquired disease (3) (http://www.cdc.gov/mrsa/healthcare/clinicians/precautions.html). Phenotypic identification and susceptibility testing require a minimum of 2 days after the culture bottle is flagged as positive. AdvanDx recently designed a PNA-FISH assay for the detection of mRNA encoded by the *S. aureus mecA* gene called *mecA Xpress*FISH (*mecA*X). Part of the data presented here was submitted to the FDA. Eight sites participated in the original study. However, the data from the site that used the Trek system were not submitted to the FDA due to the limited number of samples tested at that site.

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MATERIALS AND METHODS

Laboratories and study design. Seven geographically diverse U.S. microbiology laboratories and one in the United Kingdom participated in this study. Blood cultures were collected and incubated according to each laboratory's standard operating procedure. When a bottle was flagged as positive by the automated blood culture instrument, a Gram stain was performed on the fluid, and any organisms present were identified by the standard automated phenotypic procedures of the laboratory. If the Gram stain showed only Gram-positive cocci in clusters (GPCCL) and the specimen was eligible for enrollment (see below), the residual specimen was deidentified, given a unique study number, and tested by the QFISH assay. Those specimens containing only *S. aureus* by the QFISH assay were next tested by *mecA*X. The human subjects research committee of each institution waived the requirement for informed consent. Prior to initiating the study, the coordinators and technical personnel were trained at each site. Training samples were tested at each site, and a reproducibility study was performed at three sites.

Enrollment criteria and routine testing procedures. A single set of blood cultures was enrolled per patient. Both bottles of that individual set were eligible for enrollment, provided the organisms in each bottle were worked up separately for organism identification. One of three continuously monitored blood culture systems (BacT/Alert, bioMérieux, Durham, NC; Bactec, Becton Dickinson, Franklin Lakes, NJ; or VersaTREK, Thermo Fisher Scientific, Oakwood Village, OH) was used at each of the eight sites that participated in this study. Of the eight sites, five used Bactec, two used BacT/Alert, and one site used VersaTREK automated blood culture systems. Bottles containing charcoal to adsorb antibiotics and the VersaTREK REDOX2 blood culture bottles were not used, as they are not compatible with the QFISH assay, according to the manufacturer's instructions (10).

The sites performed their standard bacterial identification and susceptibility testing according to the manufacturer's instructions for the instrumentation and in accordance with CLSI guidelines for any other testing (12). The automated instrumentation included MicroScan (Siemens Medical Solutions, Deerfield, IL) (2 sites), Phoenix (BD Diagnostics, Sparks, MD) (2 sites), and Vitek (bioMérieux, Durham, NC) (4 sites). Blood culture bottles that were flagged as positive on an automated blood culture device and exhibited only GPCCL by Gram stain were enrolled in the first part of the study. The testing personnel were given a deidentified bottle and the Gram stain results. In addition, they were blinded to any organism identification or antibiotic susceptibility test results that might have already been available. The residual fluid from the bottle was first subjected to testing with the QFISH assay for the rapid identification of *S. aureus* and CoNS, according to the manufacturer's instructions. Briefly, fluorescein-labeled *S. aureus* and Texas Red-labeled CoNS probes are added to a fixed smear prepared from liquid from a positive blood culture bottle. After hybridization and a stringent wash, the slide is coverslipped and observed under a fluorescence microscope (10).

The samples positive for *S. aureus* only by the QFISH assay were then tested with the *mecA*X assay. Polymicrobial specimens were excluded. The samples were stored at room temperature (20 to 23°C) between bottle positivity and testing. Testing was required to be completed within 60 h. Most were completed within 24 h, and only 13 (3.8%) exceeded 48 h. Repeat *mecA*X testing of specimens with discrepant *mecA*X assay results took place within 1 week of bottle positivity.

***mecA Xpress*FISH BC assay.** Positive blood culture bottles that contained only *S. aureus* as assessed by the QFISH assay were tested for the presence of *mecA* by the *mecA*X assay, according to the manufacturer's instructions, which are summarized briefly below. The fluids from the positive bottles were filtered if they contained resin beads (anaerobic bottles). Approximately 10 drops of sample were transferred to a proprietary filter vial, and the plunger was depressed to push the fluid into the vial while retaining the beads. The filtrates and fluids obtained directly from the culture bottles were subsequently treated identically. Because the *mecA*X assay detects *mecA* mRNA, transcription of the gene is first induced (13) by incubating the organism in the presence of cefoxitin (FOX). The *mecA*X kit includes induction tubes containing 0.5 ml of Trypticase soy broth and swabs containing FOX. Prior to induction, a FOX swab is inserted into the broth in the induction tube, swirled to release the antibiotic, and discarded. The induction tubes are inoculated with 250 μl of filtrate or fluid from blood culture bottles positive for *S. aureus* (FOX final concentration, 6.3 μg/ml), mixed, and incubated at 33 to 35°C for 40 to 50 min. *mecA*X testing is carried out immediately.

Proprietary slides containing a sample well and positive- and negative-control wells are heated to 55 ± 1°C. Fluid (10 μl) from the induction tube is added to the center of the sample well and spread. One drop of fixing solution is added and spread evenly throughout the sample well. After the smear becomes visibly dry, 1 drop of *mecA*X probe solution is added to the sample and negative-control wells. The sample well is covered with one coverslip, and the negative- and positive-control wells are covered with another. The slides, still on the heating block, are incubated for 10 to 20 min. The slides are then transferred to a stringent wash solution preheated to and maintained at 57 ± 1°C. The coverslips fall off spontaneously or are removed with forceps. After incubation for 10 to 20 min, the slides are air-dried. One drop of mounting fluid is added to the sample well and another to the negative-control well. Separate coverslips are applied to the sample well and the control wells. The slides are examined with a fluorescence microscope using a filter provided by the manufacturer. *S. aureus* carrying *mecA* fluoresce bright green against a yellowish-brown background, while negative organisms should not stain.

Reference antimicrobial susceptibility testing method. The comparator assay for the *mecA*X assay was FOX disc susceptibility. This was performed at all sites using 30-μg discs, according to Clinical and Laboratory Standards Institute M100-S23 guidelines (12), regardless of the standard procedure generally employed in the laboratory. A zone size of ≥22 mm was interpreted as methicillin susceptible, while ≤21 mm was defined as resistant to methicillin. Discordant FOX and *mecA*X test results were re-

TABLE 1 *Staphylococcus Quick*FISH BC assay performance versus phenotypic methods for identification of *S. aureus*

Routine identification	No. identified by *Staphylococcus Quick*FISH BC assay				Performance (% [no. detected/total no.] [95% CI]) of *Staphylococcus Quick*FISH BC assay	
	S. aureus	CoNS	Other GPCCL	Total	Sensitivity	Specificity[a]
S. aureus	488	3	0	491	99.4 (488/491) (98.2–99.4)	99.6 (730/733) (98.8–99.9)
CoNS	0	682	7	689	99.0 (682/689) (97.9–99.5)	98.1 (529/539) (96.6–99.1)
Other GPCCL	0	0	41	41		100 (41/41) (91.4–100)

[a] For calculation of the specificity and 95% confidence intervals, see Materials and Methods.

solved by repeating the *mec*AX testing. The unresolved discrepant sample was subjected to analysis by the FilmArray blood culture identification system (BioFire, Salt Lake City, UT), according to the manufacturer's instructions.

***mec*AX reproducibility analysis.** A *mec*AX assay reproducibility study was performed by two operators at each of three sites on 5 separate testing days. Three organisms were included (*S. aureus* strains CT-178 [formerly NRS674], [BEI Resources, National Institute of Allergy and Infectious Diseases {NIAID}, NIH], a homogeneous MRSA; ATCC 4330 [ATCC, Gaithersburg, MD], a heterogeneous MRSA; and ATCC 29213, an MSSA). The specimens were prepared at AdvanDx, encoded, and shipped on ice to the testing sites. All contained approximately 1×10^7 to 6×10^7 organisms/ml. Each sample was tested in triplicate by each operator on each testing day.

Data analysis. Sensitivity and specificity were calculated from routine 2 by 2 result tables. The 95% confidence intervals were calculated by the method of Clopper and Pearson (14) using the online calculator at http://statpages.org/confint.html.

RESULTS

***S. aureus Quick*FISH BC assay.** After a Gram stain that revealed only GPCCL, 1,221 residual clinical specimens from 1,082 patients were enrolled in the QFISH assay study. The 1,221 specimens were from Bactec (918 blood culture bottles), BacT/Alert (266 bottles), and VersaTREK (37 bottles). There were 139 paired aerobic and anaerobic blood culture bottles from the same patient included in the final analysis. All the others were single bottles from unique patients. Six cultures were positive for both CoNS and *S. aureus* by the QFISH assay and were included in the analysis of the sensitivity and specificity of the QFISH assay. Of the QFISH results, 1,211 of the 1,221 (99.2%) agreed with the results of standard phenotypic testing (Table 1), with 488/491 *S. aureus* (green fluorescence), 682/689 CoNS (red fluorescence), and 41/41 other GPCCL (no fluorescence). Three false-negative results were obtained for *S. aureus* and 7 for CoNS. Four of the seven CoNS results remained negative on retesting, including one *Staphylococcus simulans*. which is a known limitation of the QFISH assay (data not shown). The remaining three were not retested. The CoNS and "other" categories were combined to calculate a specificity for *S. aureus*, while the *S. aureus* and "other" categories were combined to calculate a specificity for CoNS (Table 1).

***mec*AX testing.** Of the 488 specimens identified by QFISH as containing *S. aureus*, 458 provided evaluable results for the *mec*AX assay. Six specimens fluoresced both red and green, indicating the presence of both *S. aureus* and CoNS. According to protocol, these were not subjected to the *mec*AX evaluation. In addition, 24 specimens were not included in the *mec*AX evaluation due to procedural errors or protocol violations: 2 samples were accidentally skipped for *mec*AX testing, 6 were eliminated due to the *mecA* mRNA induction time being too short or too long, and the first 16 samples from one site were excluded due to an incorrect stringent wash temperature (the same as the hybridization temperature).

The results of the 458 evaluable *S. aureus* specimens that were subjected to both FOX disk diffusion testing and *mec*AX testing are shown in Table 2. FOX disk diffusion revealed 211/458 or 46.1% of the *S. aureus* specimens to be MRSA; of these, 209/211 were *mecA Xpress*FISH positive. Of the 247 MSSA by FOX disk diffusion, 246 were *mec*AX negative. The overall sensitivity and specificity of *mec*AX were 99.1% and 99.6%, respectively.

To resolve the three discrepant results, the *mec*AX testing was repeated. The single false-positive specimen originally exhibited an atypical weak green *mec*AX signal and was negative on repeat testing. One false-negative *mec*AX result (FOX disc, 19 mm) was positive on repeat testing, while the other remained *mec*AX negative (FOX disc, 11 mm). The persistently false-negative specimen was subjected to *mecA* PCR using an FDA-cleared *mecA* PCR assay from a different manufacturer and was found to be *mecA* positive.

***mec*AX reproducibility study.** The tests were performed in triplicate by two operators per site at three sites over 5 days and showed agreement for 534/540 (360/360 *mec*AX-positive and 174/180 *mec*AX-negative) samples (98.9%). The six nonreproducible false-positive results (triplicate determinations of 2 negative samples) were obtained at one site on the same day by the same operator. This is presumed to represent a systematic error, but its nature was not determined.

DISCUSSION

This multicenter study is the first report of the clinical performance of the AdvanDx *mec*AX test. The combination of the *S. aureus* QFISH and *mec*AX tests for the identification of MRSA in blood cultures is rapid, robust, reliable, sensitive, and specific. Our data also revealed high intra- and interlaboratory reproducibility.

TABLE 2 Performance of *mecA Xpress*FISH compared to cefoxitin disk diffusion[a]

*mecA Xpress*FISH result	No. in cefoxitin disk diffusion test found to be[b]:	
	Methicillin resistant (≤21 mm)	Methicillin susceptible (≥22 mm)
Positive	209	1[c]
Negative	2[d]	246

[a] The sensitivity of the assay was 99.1% (209/211) (95% CI, 96.6 to 99.7%), and the specificity was 99.6% (246/247) (95% CI, 97.8 to 99.99).

[b] $n = 458$.

[c] The single false positive (FOX, 28 mm) originally exhibited atypical weakly positive green fluorescence and was negative on repeat testing.

[d] One of the two false negatives (FOX, 19 mm) was positive on repeat *mecA Xpress*FISH testing. The other (FOX, 11 mm) remained negative on repeat *mecA Xpress*FISH testing. That specimen was subjected to PCR for *mecA*, which was positive.

Our results with the QFISH assay parallel those of the multicenter study by Deck et al. (9). In that study, the authors reported sensitivities for detecting *S. aureus* and CoNS of 99.5% and 98.8%, respectively. They also reported that the *Staphylococcus* QFISH assay turnaround time was <30 min and the hands-on time was <5 min. Likewise, Carretto et al. (15) reported 100% sensitivity and specificity for *S. aureus* detection in their single-center evaluation, with minimal impact with respect to modifying laboratory workflow to accommodate testing.

Compared to FOX testing, the *mecA*X test had two false-negative and one atypical, weak, green, and false-positive fluorescent result (sensitivity and specificity, 99.1 and 99.6%, respectively; Table 2). One false-negative and one false-positive result resolved on retesting. The third repeatedly *mecA*X false-negative result was positive for *mec*A by the FilmArray test. We did not investigate possible sequence differences that might have accounted for the *mecA*X assay result that remained falsely negative on repeat testing.

The combination of the *Staphylococcus* QFISH and *mecA*X assays might significantly speed up the diagnosis of MRSA BSI. After the 20-min QFISH assay indicates the presence of *S. aureus*, labs using the *mecA*X assay can determine its susceptibility to methicillin and notify the physician in <2 h. Compared with other rapid tests, the combination of the QFISH and *mecA*X assays has low barriers to implementation. The training is straightforward, and test performance does not require any molecular skills beyond those expected of a competent clinical laboratory technologist. In total, the hands-on time is approximately 15 min. As Carretto et al. (15) point out, the cost of PNA-FISH is two-thirds the cost of some reverse transcription-PCR (RT-PCR) assays. To perform the QFISH and *mecA*X assays, only a slide warmer, water bath, and fluorescence microscope equipped with a dual-band filter are needed. In addition, variable costs for the *mecA*X assay, including fixative, probes, wash solutions, slides, and coverslips, are minimal. This compares very favorably to matrix-assisted laser desorption ionization–time of flight mass spectrometry and nucleic acid amplification, both of which require expensive instruments for test performance. Nucleic acid amplification may also require additional equipment for nucleic acid extraction and perhaps a laboratory designed specifically for DNA amplification with unidirectional workflow and clean rooms.

Many of our laboratories have been successfully using the various generations of *S. aureus* PNA FISH testing for years, and the addition of the *mecA*X assay to the test menu would require little additional effort.

It has already been shown that the implementation of PNA FISH and the more rapid QFISH technologies lead to improved patient outcomes and antibiotic utilization (8, 9). After utilizing QFISH technology to identify *S. aureus*, the detection of methicillin resistance by phenotypic techniques still requires 2 days. Thus, performing the *mecA*X assay immediately after the QFISH test might enhance patient outcomes significantly, providing there is a rapid clinical response to the results. The implementation of an additional test, such as the *mecA*X, into the laboratory workflow depends on clinical considerations, convenience, and cost-effectiveness (including laboratory staffing levels, clinician availability, and communication support). This may include the availability of pharmacy representatives to reinforce rapid and appropriate antibiotic changes. As shown by Holtzman et al. (16), without active antimicrobial stewardship intervention, the implementation of a dual-probe staphylococcal PNA FISH test did not lead to shorter hospital stays or decreased vancomycin use after the identification of CoNS. Whether the implementation of the QFISH and *mecA*X assays will be cost-effective in individual hospitals, especially those that batch their PNA-FISH tests and perform them once per shift (for example), will require cost-benefit analyses and outcome studies.

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