An Evaluation of the AdvanDx *Staphylococcus aureus*/CNS PNA FISH™ Assay

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PURPOSE: A study was conducted to compare the *S. aureus*/CNS PNA FISH™ Culture Identification Kit (AdvanDx, Woburn MA) to standard microbiology identification methods for presumptive identification of *S. aureus* and coagulase-negative staphylococcus (CNS) in positive blood cultures.

MATERIALS AND METHODS: Blood cultures (n=301) that signaled positive on the BacT/Alert™ 3D (bioMerieux, Durham NC) automated blood culture system and had gram-positive cocci in clusters on Gram stain were processed using standard microbiology methods and were analyzed with the *S. aureus*/CNS PNA FISH™ assay. The *S. aureus*/CNS PNA FISH™ assay was performed in accordance with the manufacturer's instructions.

RESULTS: Overall agreement was 96.7%. Sensitivity for *S. aureus* was 96.5% (83/86). Specificity for *S. aureus* was 100% (215/215). Sensitivity for CNS was 96.6% (201/208). Specificity for CNS was 96.8% (90/93). Three cultures reported as *S. aureus* were identified as CNS by *S. aureus*/CNS PNA FISH™. Three cultures that reported only *S. aureus* were positive for both *S. aureus* and CNS by *S. aureus*/CNS PNA FISH™. One *S. viridans* was identified as CNS by *S. aureus*/CNS PNA FISH™. Seven cultures that reported CNS were negative by *S. aureus*/CNS PNA FISH™.

CONCLUSION: When used in conjunction with Gram stain and culture the *S.aureus*/CNS PNA FISH™ assay may be beneficial in terms of reducing time to correct therapy, thereby decreasing mortality and costs associated with *S. aureus* bacteremia.

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ABBREVIATIONS: CNS = coagulase negative staphylococci; FISH = fluorescent in situ hybridization; PNA = peptide nucleic acid.

INDEX TERMS: blood culture; PNA FISH; *S. aureus*.

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Septicemia is one of the leading causes of death in the United States.[1] *Staphylococcus aureus* infections, including *S. aureus* septicemia, place economic and personal burdens on the healthcare system in terms of increased morbidity, mortality, length of hospital stay, and associated costs.[2-6] While coagulase negative staphylococcus (CNS) is the organism most commonly isolated from positive blood cultures it is often a contaminant.[7-9] Both *S. aureus* and CNS are seen as gram-positive cocci in clusters (GPCC) on a Gram stained smear and this is typically the first report that a clinician receives regarding a positive blood culture. Differentiation of contamination from true bacteremia is important in

terms of treatment and there are differing opinions, even among infectious diseases experts, about the best strategy to follow.[10-13] Equally important is the differentiation of *S. aureus* and CNS in cases of true bacteremia. Concerns about the overuse or misuse of vancomycin must be weighed against the need for proper antimicrobial treatment of methicillin-resistant *S. aureus* (MRSA) bacteremia. Information supplied by the clinical microbiology laboratory to clinicians plays an important role in decision making. Traditional microbiology methods of identification involving subculture to solid media and biochemical testing take one to two days for an identification of *S. aureus* to be confirmed during which time the decision must be made whether or not to start empiric therapy. Evaluation of rapid methods for identification of *S. aureus* directly from blood culture bottles, including real-time polymerase chain reaction (PCR), peptide nucleic acid fluorescent in situ hybridization (PNA FISH), PNA FISH combined with flow cytometry, tube coagulase, and the API RAPIDEC staph system (bioMerieux, Durham NC), have been reported in the literature.[14-18] Rapid testing and providing a presumptive identification to the clinician within hours of positivity have the potential to improve management of *S aureus* bacteremia by decreasing the time to appropriate therapy.[19] *S. aureus*/CNS PNA FISH™ (AdvanDX, Woburn MA) is a FISH method that utilizes PNA probes specific for *S. aureus* and non-aureus 16S ribosomal RNA sequences. The test is performed on smears made directly from positive blood culture bottles and results are available in approximately three hours. The inclusion of probes for both *S. aureus* and CNS allows not only differentiation of the two organisms but also serves as a backup for Gram stain interpretation since nonstaphylococcal isolates which may be misinterpreted as GPCC will not stain with the *S. aureus*/CNS PNA FISH™ probes. This study evaluated the performance of the *S. aureus*/CNS PNA FISH™ assay when compared to traditional microbiology methods of identification.

METHODS

Blood cultures collected as standard of care and submitted to two clinical microbiology laboratories were incubated on BacT/Alert™ 3D (bioMerieux, Durham NC) automated blood culture systems. Blood culture bottle types were BacT/Alert™ PF, BacT/Alert™ SA 40 mL, and BacT/Alert™ SN 40 mL. Cultures that signaled positive on the BacT/Alert™ 3D were Gram stained and were included in the study if the Gram stain was reported as GPCC (n=294). Several blood cultures positive with non-staphylococcal isolates (n=7) were included in the study to evaluate the specificity of the *S. aureus*/CNS PNA FISH™ assay. Isolates were identified by standard microbiology techniques. Briefly, broth from positive bottles was subcultured to trypticase soy agar with 5% sheep's blood (TSA II, BBL, Becton-Dickinson and Company, Sparks MD) and the plates were incubated at 35±1°C in 5% CO_2. Following overnight incubation a catalase test (hydrogen peroxide, Brite-Lite, AmerisourceBergen, Valley Forge PA) and a Staphaurex (Remel, Lenexa KS) test were performed on all isolates exhibiting typical staphylococcal morphology. Isolates that were catalase positive and Staphaurex positive were identified as *S. aureus*. Isolates that were catalase positive and Staphaurex negative were identified as CNS. Each positive bottle was also analyzed by *S. aureus*/CNS PNA FISH™ for presumptive identification of *S. aureus* or CNS. The *S. aureus*/CNS PNA FISH™ slides were batched and transported to a separate research lab for analysis. The manufacturer's instructions for the *S. aureus*/CNS PNA FISH™ assay were followed. Briefly, a drop of positive blood culture broth was mixed with a drop of fixation solution on the microscope slide provided with the kit. The slides were air dried and then fixed by dipping them in methanol for three to five seconds and allowing them to air dry again. The slides were then immersed in 80% ethanol for 10 minutes and air dried again. One drop of *S. aureus*/CNS PNA hybridization solution (PNA reagent), containing a mixture of a fluorescein-labeled *S. aureus* specific PNA probe and a rhodamine-labeled PNA probe specific for non-aureus staphylococci, was added to the smear after which the slides were coverslipped and incubated at 55 ± 1°C for 90 ± 5 minutes in the PNA FISH Workstation (AdvanDx). The slides were immersed in preheated (55 ± 1°C) wash solution, gently agitated to remove the coverslips, and then incubated in the wash solution at 55 ± 1°C for 30 ± 5 minutes. The slides were air dried and then one drop of mounting media and a coverslip were added to each slide. The slides were examined for fluorescence within two hours using a Nikon Optiphot microscope with an EXFO X-Cite 120 Fluorescence Illumination System (Photonic Solutions, Inc, Mississauga Ontario, Canada) and a 100X oil objective. The microscope was equipped with a FITC/Texas Red dual band filter (AdvanDx, Woburn MA). *S. aureus* appeared as green fluorescence, CNS appeared as red fluorescence, and nonstaphylococcus isolates did not fluoresce. A positive control slide containing both *S. aureus* and CNS and a negative control slide were included on each run. The identifications obtained by the standard microbiology methods and the *S. aureus*/CNS PNA FISH™ assay were compared.

RESULTS

Overall agreement between the two methods was 96.7% (291/301). Sensitivity, specificity, positive predictive value, and negative predictive value were calculated for *S. aureus* and CNS. See Table 1. Three isolates identified as *S. aureus* by standard testing methods were identified as CNS by *S. aureus*/CNS PNA FISH™. Seven cultures that were reported as positive for CNS using the standard testing methods for identification were negative by *S. aureus*/CNS PNA FISH™. For three cultures in the study *S. aureus* was the only isolate reported following standard testing methods but both *S. aureus* and CNS were seen on the *S. aureus*/CNS PNA FISH™ smears. Isolates identified as *Enterococcus faecalis*, Group B Streptococcus, *Micrococcus* sp., and *Candida albicans* by standard testing methods were negative when analyzed by the *S. aureus*/CNS PNA FISH™ assay. There were two cultures included in the study that were identified as *Streptococcus viridans* by standard testing methods. One of the *S. viridans* cultures was negative by *S. aureus*/CNS PNA FISH™ and the other was identified as CNS by *S. aureus*/CNS PNA FISH™.

DISCUSSION

Historically, and in contrast to other sections of the clinical laboratory, there have been few "stat" procedures in the microbiology laboratory. That situation is changing due to the advancement of molecular techniques and the availability of molecular diagnostic assays. Having the ability to provide rapid accurate diagnostic information to clinicians is a goal of most clinical microbiology laboratories. The *S. aureus*/CNS PNA FISH™ assay is available on the market in kit format and enables the microbiology laboratory to provide rapid presumptive identification of *S. aureus* and CNS. With the exception of methanol and 80% ethanol all reagents needed to complete the assay are included in the kit so reagent preparation time, including dilution of the wash buffer, is minimal. Slide preparation was easily integrated into our normal routine for handling positive blood culture bottles. We found it best to prepare and read the Gram stain and then prepare the PNA/FISH slide if necessary. However, AdvanDx is marketing PNA/FISH assays for other organisms and, with the exception of the organism specific PNA reagent, all kit components are interchangeable. Currently kits for *E. faecalis*/Other Enterococcus and *C. albicans*/*C. glabrata* are available. As more kits are introduced it may become more time effective to prepare the Gram stain and PNA FISH slides at the same time and then select the correct PNA FISH assay to perform based on the Gram stain result. Even though slide preparation is easily integrated into the daily routine the *S. aureus*/CNS PNA FISH™ assay is not an "on demand" test like the Gram stain. The actual hands-on time to perform the *S. aureus*/CNS PNA FISH™ assay is approximately 12-15 minutes. But because of the drying and incubation periods the total time from slide preparation to reading for fluorescence is approximately three hours. For practical reasons slides need to be batched, thereby eliminating the PNA FISH assay from a true "stat" procedure. Still, the decrease in time from 24 hours to 3 hours from time of positivity to presumptive identification could have a significant impact on patient care. The PNA FISH Workstation will accommodate up to 30 slides for the 90 minute incubation. Depending on the laboratory workload one or more batches of slides could be assayed during the day.

The *S. aureus* green fluorescence was very intense and easily read on every *S. aureus*/CNS PNA FISH™ slide that was positive for *S. aureus*. The red fluorescence for the CNS was variable in our study, ranging from a vivid red that was easily distinguished to a dull red that could barely be differentiated from the reddish background. Perhaps the fluorescence variability contributed to the fact that we had seven CNS culture positive specimens that were *S. aureus*/CNS PNA FISH™ negative. It is possible that the cells were there but the fluorescence could not be seen. The poor fluorescence was not confined to single runs and did not correlate with the number of cells present in the smear or a particular type of blood culture bottle. We made certain that the entire surface of the blood smear

Table 1. Calculated sensitivity, specificity, positive predictive value, and negative predictive value

	S. aureus	CNS
Sensitivity	96.5% (83/86)	96.6% (201/208)
Specificity	100% (215/215)	96.8% (90/93)
Positive predictive value	100% (83/83)	98.6% (201/204)
Negative predictive value	98.5% (215/218)	92.8% (90/97)

was covered with the PNA reagent and that bubbles were not present under the coverslip during the hybridization period so we feel confident that each slide had an equal exposure to the PNA reagent. One shortcoming of this study is that not all of the CNS isolates were identified to species level. If we had this information we may have been able to correlate the poor staining with certain species of CNS. However, in this study, we could not identify a factor that contributed to the poor fluorescence seen on some of the CNS slides.

Unfortunately we were unable to resolve the ten discrepancies in this comparison study. None of the blood culture bottles and only one of the isolates were available for retesting. All ten of the *S. aureus*/CNS PNA FISH™ slides were reexamined and the reported results were confirmed. The one available isolate was retested and confirmed as *S. aureus.* Since the corresponding *S. aureus*/CNS PNA FISH™ slide for this isolate was reexamined and confirmed as CNS the discrepancy remains unresolved.

Overall we found the *S. aureus*/CNS PNA FISH™ assay easy to perform and interpret. The inclusion of the assay in the routine workup of positive blood cultures that have GPCC on Gram stain could provide information to clinicians that would be useful in determining appropriate antimicrobial therapy thereby decreasing morbidity, mortality, length of hospital stay, and associated costs. However, the fact that we had three *S. aureus* positive cultures that were identified as CNS by *S. aureus*/CNS PNA FISH™ emphasizes that the *S. aureus*/CNS PNA FISH™ assay should be used as an adjunct to traditional microbiology testing methods and not replace them.

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