Multicenter Evaluation of the *Staphylococcus QuickFISH* Method for Simultaneous Identification of *Staphylococcus aureus* and Coagulase Negative Staphylococci Directly from Blood Culture Bottles in less than Thirty Minutes.

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Abstract

A novel rapid PNA FISH method, *Staphylococcus QuickFISH*, for the direct detection of *Staphylococcus* species from positive blood culture bottles was evaluated in a multicenter clinical study. The method utilizes a microscope slide with pre-deposited positive and negative control organisms and a self-reporting 15 minute hybridization step which eliminates the need for a wash step. Five clinical laboratories tested 722 positive blood culture bottles containing gram-positive cocci in clusters. The sensitivities for detection of *S. aureus* and coagulase-negative staphylococci (CoNS) were 99.5% (217/218) and 98.8% (487/493) respectively, and the combined specificity of the assay was 89.5% (17/19). The combined positive and negative predictive values of the assay were 99.7% (696/698), and 70.8% (17/24) respectively. Studies were also performed on spiked cultures to establish the specificity and performance sensitivity of the method. *Staphylococcus QuickFISH* has a turn-around-time (TAT) of <30 minutes and a hands-on-time (HOT) of <5 minutes. The ease and speed of the method has the potential to improve the accuracy of therapeutic intervention by providing *S. aureus*/CoNS identification simultaneously with Gram stain results.
Introduction

Staphylococci are the organisms most frequently isolated from blood cultures with *Staphylococcus aureus* being the most frequently isolated pathogen in true bacteremia (20, 22). Each year in the United States, more than 300,000 patients contract *S. aureus* infections leading to more than 12,000 deaths, 2.7 million excess days of hospitalization and close to $9.5 billion in excess hospital charges (11, 8).

CoNS are the predominant organisms isolated from positive blood cultures (7); 60-80% of which are the result of contamination of the sample during the blood draw due to inadequate antiseptic techniques (21, 2, 17). Discrimination between *S. aureus* and CoNS has significant clinical value, allowing clinicians to differentiate between likely contamination and true bacteremia, thereby enabling more informed and appropriate patient management decisions (1, 2). The proven value of this information creates a need for a rapid, accurate and simple method to identify and distinguish between *S. aureus*, CoNS, and other gram-positive cocci in clusters (GPCC) in the clinical microbiology lab.

There are a number of methods currently used for identification of staphylococci in clinical samples, varying greatly in accuracy, speed and cost. The tube coagulase test detects the presence of free coagulase directly from blood culture with a high specificity (>99%), but sensitivities range from 62-100% depending on the duration and dilution method (19, 9). The principal disadvantage of latex agglutination coagulase tests, traditional growth-dependent culture methods, or automated microbroth identification systems is the prolonged turn-around-time (TAT) to results. In contrast, rapid molecular techniques such as PNA FISH® provide results in <2 hours. Assays
that generate identifications directly from positive blood cultures in real time are likely to have significant clinical impact and become mainstream diagnostic tools of clinical microbiology laboratories.

The PNA FISH method has been used in clinical microbiology labs for over 10 years for identification of a variety of organisms (12, 18, 13). In clinical evaluations, the PNA FISH method has demonstrated sensitivities and specificities ranging from 96 to 100% (6, 15, 16). Other studies have measured the value of PNA FISH in preventing overuse of antibiotics and unnecessary hospitalization (5, 10). Unfortunately, clinical laboratories have incentives to batch PNA FISH testing to minimize the total number of control samples and maximize labor efficiency, which creates delays in reporting results and prevents the full realization of the value of the method for the hospital and for the patient (3, 4). In response to this need, a second generation assay, QuickFISH, was developed, which implements several innovations designed to simplify the method and decrease TAT.

Like PNA FISH, QuickFISH takes advantage of the highly specific and rapid binding properties of peptide nucleic acid (PNA) probes to detect species-specific rRNA sequences. Where PNA FISH incorporates a 30 minute hybridization step and a 30 minute wash step to remove unbound fluorescent probe, QuickFISH introduces a novel self-reporting probe design which enables a 15 minute hybridization without the need for a wash step. Elimination of the wash in QuickFISH is achieved by quenching excess PNA fluorescence through formation of stable hybrids with complementary quencher-labeled sequences. Another QuickFISH innovation is an improved slide design incorporating built-in positive and negative controls on the same slide as the sample.
Fixed organisms are pre-deposited into control wells and serve as controls for the hybridization step of the assay. The presence of controls on each sample slide reduces the number of slides a technologist needs to handle and track to process a sample and eliminates the need for the laboratory to grow and maintain control organisms.

The *Staphylococcus* QuickFISH method is designed to rapidly differentiate *S. aureus* from other *Staphylococcus* species directly from positive blood culture bottles (the method does not differentiate MRSA from MSSA). The purpose of this study was to determine whether the *Staphylococcus* QuickFISH method is robust and specific for identification of *S. aureus* and CoNS in the clinical microbiology laboratory setting.

**Materials and Methods**

**Clinical study**

Samples were tested and results recorded in five hospital microbiology laboratories located in New York, Pennsylvania, Virginia, California and Indiana. Each site received the same materials and training. All sites worked under a protocol that included blinding and discrepant resolution methods approved by their institutional review boards. Study samples were classified as leftover clinical material.

**Clinical Specimens**
A total of 722 GPCC-positive blood culture bottles from five clinical laboratories were included in the study. Two sites used BacT/ALERT system (bioMérieux, Durham, NC) blood culture bottles (N= 236) and the other three sites used BACTEC systems (Becton, Dickenson & Co, Sparks, MD) (N= 486). Samples from all available BacT/ALERT and BACTEC blood culture bottle types were included in the study, except BacT/ALERT PF, FA and FN FAN (prior to initiation of the studies, BacT/ALERT bottles containing charcoal were found to interfere with the QuickFISH method). BACTEC samples included 203 Lytic Anaerobic, 230 Plus Aerobic, 34 Peds Plus, and 19 Plus Anaerobic bottles; BacT/ALERT samples included 101 SN (anaerobic) and 135 SA (aerobic) bottles. Bottles that signaled positive by the automated blood culture monitoring instrument and showed GPCC upon Gram stain were enrolled in the study.

**Routine Identification of Bacteria**

Routine identification methods varied across the participating clinical laboratories. Of the three sites using BACTEC blood culture systems, one site used *S. aureus*/CNS PNA FISH (AdvanDx) combined with conventional subculture and BD Phoenix (BD Diagnostic Systems, Sparks, MD) for speciation of the CoNS isolates; the second site used Xpert MRSA/SA (Cepheid), with subculture followed by Staphaurex latex agglutination (Remel) if the Xpert MRSA/SA was negative; the third site also used Xpert MRSA/SA and conventional subculture as well as MicroScan (Siemens Healthcare). The BacT/ALERT sites employed *S. aureus*/CNS PNA FISH, conventional
subculture and VITEK or VITEK 2 (bioMérieux, Durham, NC) as routine laboratory identification methods.

Non-Staphylococcus-GPCC Spiked Blood Cultures

There is a low prevalence of GPCC other than *S. aureus* and CoNS species in blood culture samples (22). In order to broaden the number of organisms with a GPCC morphotype, additional testing was performed on spiked clinical isolates, including *Aerococcus*, *Gemella*, *Kocuria*, *Micrococcus*, *Rothia*, *Granulicatella*, and *Lactococcus* species. All species tested were preserved strains isolated at the institution that performed the testing with the exception of *Micrococcus luteus* ATCC 4698, which was purchased from ATCC (Manassas, VA). Isolates were inoculated into aerobic and anaerobic BacT/ALERT and BACTEC blood culture bottles that had failed to signal growth after incubation for seven days. Approximately 0.5 mL of bacteria suspended in 0.9% saline, initially equivalent to a 0.5 McFarland standard then diluted 1:10, was inoculated into a blood culture bottle. The blood culture bottle was returned to the automated blood culture machine and incubated until the instrument reported a positive signal. One smear was prepared from each blood culture bottle. Cultures were assumed to contain the spiked bacteria and were not re-identified.

Reference Strain Spiked Blood Cultures
A wide variety of microorganisms including non-GPCC organisms were screened to examine the specificity and performance sensitivity of the method. The rationale for expansion of screening beyond the GPCC morphotype was twofold. In the clinical microbiology lab there is an expectation of a low rate of Gram stain error (14), which requires the specificity of the method to be independent of the Gram stain result. Additionally, there is also a low prevalence of polymicrobial blood cultures (22) with a likelihood of one strain being the GPCC morphotype, therefore there is the potential for non-GPCC organisms to be found in a sample which will be tested by the Staphylococcus QuickFISH method.

Simulated blood culture samples were prepared at AdvanDx for 116 reference strains and 26 preserved clinical isolates (142 samples in total) including 40 S. aureus strains, 34 strains of other Staphylococcus species as well as other gram-positive and gram-negative bacteria and yeasts. Thirty-two of the 44 recognized species of staphylococci were represented in the study. Samples were cultured and Gram stained with species identifications blinded from the operator. BacT/ALERT SA bottles were inoculated with 8 mL of sterile human donor blood; 3 mL aliquots were transferred into sterile test tubes. Each tube was inoculated with one to two colonies selected from agar plates for each strain and incubated appropriately for growth. Gram stains were performed on all samples to verify adequate growth.

Staphylococcus QuickFISH Method
Fixation Solutions (QuickFix-1 and QuickFix-2), Hybridization Solutions 
(Staphylococcus PNA Blue and Staphylococcus PNA Yellow), QuickFISH Slides and 
coverslips were provided by AdvanDx. All reagents were provided in dropper bottles. 
Instrumentation included a digitally controlled heat block, and a fluorescence 
microscope equipped with a custom dual band filter and 60 or 100X oil-immersion 
objective. AdvanDx filter vials were provided to sites using BACTEC Plus blood culture 
bottles and were used to eliminate media resin from the sample. Processed slides were 
examined by fluorescence microscopy.

**Preparation of Smears**

QuickFISH slides were placed on a heat block at 55±1°C. A small volume (<0.5 
mL) of each blood culture sample was transferred to a secondary vessel (e.g. 
microcentrifuge tube) via an aspirating needle. Ten microliters of sample was 
transferred from the secondary vessel to the center of the QuickFISH slide sample area. 
One drop of QuickFix-1 was immediately added on top of the 10 μL sample and the 
mixture was spread evenly throughout the sample area with an inoculating needle. The 
slide remained on the slide warmer until the smear was visibly dry (1-3 minutes). Two 
drops of QuickFix-2 were added to the center of the sample area and allowed to dry (<1 
minute).

**Hybridization and Scoring**
The hybridization reagent is a two part mixture which is separately prepared for each slide prior to use. For each fixed sample, one drop of *Staphylococcus* PNA Blue was applied to the center of a 25 x 50 mm coverslip, followed by one drop of *Staphylococcus* PNA Yellow. The blue and yellow hybridization reagents were thoroughly mixed with an inoculating needle until a uniform green color was observed (approximately 5-10 seconds/coverslip). Coverslips were inverted and applied to the *QuickFISH* slides to simultaneously cover the control wells and the fixed samples with mixed hybridization reagents. Hybridization was performed for 15 minutes on a heat block at 55±1°C. Slides were transferred immediately to a fluorescence microscope for scoring. The presence of multiple bright green fluorescent cocci in multiple fields of view indicated a *S. aureus*-positive sample, red fluorescent cocci indicated a CoNS-positive sample, and no fluorescence indicated a negative sample.

**Results**

**Clinical study**

Sensitivities of 99.5% (217/218) for *S. aureus*, and 98.8% (487/493) for CoNS were obtained; the combined specificity for the assay was 89.5% (17/19). The combined positive and negative predictive values of the assay were 99.7% (696/698), and 70.8% (17/24) respectively. In total 722 clinical samples were tested; 8 specimens were positive for both green and red fluorescent cocci. The results of the clinical evaluation are displayed in Table 1. The amount of time which passed between the bottle signaling positive, and the initiation of testing by the *Staphylococcus QuickFISH*
method was recorded for 537 samples. Thirteen percent of samples (73/537) were tested within 2 hours, 47% (254/537) within 24 hours, and 97% (522/537) within 48 hours.

Two hundred eleven samples were identified as *S. aureus*, of which 41 were further described as methicillin resistant or methicillin susceptible (18, MRSA; 23, MSSA). Four hundred eighty-five samples were identified as non-*S. aureus* staphylococci of which 185 were identified to the species level: whereas 302 were simply identified as “CoNS”. Sixteen different *Staphylococcus* species were identified in total. Only 18 samples (2.5%) included in the clinical study contained non-*staphylococcal* species, of these, only 12 samples (1.7%) contained GPCC phenotypes (*Micrococcus* and *Kocuria*).

**Non-*Staphylococcus*-GPCC Spiked Blood Cultures**

Twenty-nine seeded blood cultures representing 21 separate GPCC-positive, non-*staphylococcal* strains were included in a supplementary study performed at two of the clinical sites; the data are displayed in Table 2. All samples in the seeded culture experiment tested negative, including 11 strains of *Micrococcus*.

**Reference Strain Spiked Blood Cultures**

Blinded screening of simulated blood culture samples inoculated with reference strains was performed. All *S. aureus* samples produced a green positive result (15
samples produced a red positive result except S. simulans and S. felis. A variety of other organisms were screened and all had negative test results. Samples included less common GPCC genera (12), species identified as gram-positive cocci in pairs in chains (11), gram-positive bacilli (5), yeast (7), gram-negative cocci (1), gram-negative bacilli (23), gram-variable bacilli (6), gram-negative coccobacilli (2), and gram-variable coccobacilli (1). Full screening data is available in Table 3 (in supplementary material available on line).

Discussion

Our study presents the first description of a next generation PNA FISH technology, *Staphylococcus QuickFISH*. The method provides a presumptive identification of *S. aureus* and classifies most non-*S. aureus* staphylococci as CoNS. Clinical trial results demonstrate high sensitivity and specificity for detection of staphylococci in blood cultures and rapid and easy discrimination of *S. aureus* and CoNS.

The study’s discrepant resolution protocol required reevaluation of routine identifications and retesting of discrepant samples. Eleven discrepant results were reported, including two misidentifications (upon re-examination of the routine identifications, the *Staphylococcus QuickFISH* results were determined to be correct). Two false positive and seven false negative scores were recorded. One sample was scored as a dual positive (green/red) by *Staphylococcus QuickFISH* but identified only...
as *S. aureus* by routine methods; this sample was not available to retest and was scored as a CoNS false positive. Another discrepant sample was determined to be a *Micrococcus* species by conventional methods but produced a green positive result by *Staphylococcus QuickFISH*. This result did not repeat upon retest and was recorded as a *S. aureus* false positive. A sample identified as *S. aureus* by routine methods was scored as a red positive by initial *Staphylococcus QuickFISH* testing, the sample is recorded as *S. aureus* false negative; however, the expected green *QuickFISH* result was confirmed upon retest. Six CoNS false negative results were recorded; two samples produced the correct red result upon retest, the other four samples were identified as *S. simulans*. All discrepant results were reported from sites using BacT/ALERT systems only. No patterns emerged in the discrepant results to suggest a consistent assay failure mode. The rare number and varied type of discrepant results and reconciliation of discrepancies upon retest support the conclusion that these results were due to human error and not related to the assay itself.

*S. simulans* and *S. felis* were the only CoNS species tested that produced a negative result in the reference strain screening experiment. This limitation was predicted by sequence alignment of the probes used in the test. Four negative results were recorded at clinical sites for samples identified as *S. simulans* by Vitek or Vitek-2. No clinical samples were identified as *S. felis*. The four *S. simulans* false negative results represent 0.81% (4/493) of all non-*S. aureus* staphylococci. These four *S. simulans* positive bottles occurred on separate days and appear to be independent with no clear clinical relevance. Since *S. simulans* produces an expected negative result, it
could be interpreted as a true negative instead of a false negative. Recalculation of the CoNS sensitivity, excluding *S. simulans*, increases the value to 99.6% (487/489).

Reference strain screening data of spiked simulated blood cultures expanded the breadth of organisms evaluated. Forty strains of *S. aureus*, (15 strains and 25 clinical isolates) and 34 non-*S. aureus* staphylococci, representing 31 different species were included in the study. Eleven strains of gram-positive cocci in pairs and chains, 5 strains of gram-positive bacilli, and 12 non-staphylococci GPCC strains all produced the expected negative result. The results predict a high specificity and analytic sensitivity of the method performed on true samples containing phylogenetically diverse organisms.

Although this study included a large number of clinical samples, a limitation of the study was the small number of true negative samples encountered during the trial. Despite GPCCs being the most common morphotype identified in blood cultures (8), the vast majority of study isolates were staphylococci and only eighteen were non-staphylococci GPCC. Non-staphylococci entered into the study as GPCC included 11 *Micrococcus* spp., 1 *Kocuria varians*, 2 *Enterococcus* spp., 2 *Peptostreptococcus* spp., 1 alpha-hemolytic *Streptococcus*, and 1 *Streptococcus parasanguinis*. Spiked negative blood cultures of 29 organisms were tested at two clinical labs to augment the variety of GPCC genera in the study. All clinical spiked isolates of non-staphylococci GPCC tested negative by *Staphylococcus Quick* FISH. These data demonstrate the broad specificity of the *Staphylococcus Quick* FISH assay. The addition of results from the seeded samples increased the specificity of the assay from 89.5% to 95.8% (46/48) demonstrating performance similar to PNA FISH (96-100% (3, 6, 12, 15, 16)).
The results of this study, although not a direct comparison to PNA FISH, demonstrate sensitivity and specificity values comparable to previous reports on PNA FISH assays (15, 16). The *Staphylococcus* *Quick*FISH method eliminates the wash step, integrates controls and shortens the fixation and hybridization steps, thus reducing HOT and TAT as compared to PNA FISH. The integration of the *Staphylococcus* *Quick*FISH method into the lab workflow will hasten availability of species identification, providing a greater potential clinical impact than previously obtained by the PNA FISH method.

As GPCC are the most commonly encountered bacterial morphotype in blood cultures, laboratorians and clinicians are often challenged with determining whether the organism is a true pathogen or a culture contaminant. *Staphylococcus* *Quick*FISH will enable a clinical microbiology laboratory to accurately identify *S. aureus* from CoNS in blood cultures in <30 minutes. The rapid presumptive identification of CoNS with the *Quick*FISH method provides valuable information regarding a possible contamination. On the other hand, a *S. aureus*-positive result confirms to the clinician that the organism represents a true infection which requires further testing to determine the strain's susceptibility profile (MRSA or MSSA). The method has the potential to revolutionize standard testing and reporting protocols for blood cultures, as it will provide laboratories with the ability to report Gram stain and species identification for gram positive cocci at the same time. *Staphylococcus* *Quick*FISH has the potential to significantly impact individual patient outcomes and overall BSI treatment costs by providing physicians with the information to allow earlier implementation of targeted antibiotic therapy.
Table 1. Performance of the *Staphylococcus* QuickFISH method with 722 blood cultures positive for gram-positive cocci in clusters tested at five hospital laboratories. Routine identification methods were compared to QuickFISH results.

<table>
<thead>
<tr>
<th>Routine Identification (#samples)</th>
<th><em>S. aureus</em></th>
<th>CoNS</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> (211)</td>
<td>209</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td><em>S. aureus</em> + CoNS (8)*</td>
<td>8</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>CoNS (302)</td>
<td>-</td>
<td>301</td>
<td>1</td>
</tr>
<tr>
<td><em>S. auricularis</em> (2)</td>
<td>-</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td><em>S. capitis</em> (11)</td>
<td>-</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td><em>S. caprae</em> (4)</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
<tr>
<td><em>S. epidermidis</em> (129)</td>
<td>-</td>
<td>129</td>
<td>-</td>
</tr>
<tr>
<td><em>S. haemolyticus</em> (5)</td>
<td>-</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td><em>S. hominis</em> (20)</td>
<td>-</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td><em>S. hyicus</em> (1)</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>S. intermedius</em> (1)</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>S. lugdunensis</em> (1)</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>S. saccharolyticus</em> (1)</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>S. simulans</em> (4)</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><em>S. schleiferi</em> (1)</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>S. warneri</em> (1)</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td><em>S. xylosus</em> (1)</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Streptococcus spp. (2)</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>--------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Micrococcus spp. (10)</strong></td>
<td>1</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td><strong>Kocuria varians (1)</strong></td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><strong>Enterococcus spp. (2)</strong></td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><strong>Peptostreptococcus spp. (2)</strong></td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

* Indicates 1 sample identified as *S. aureus* + *S. epidermidis*, 1 as *S. aureus* and *S. saprophyticus*, and 1 as MSSA and *S. epidermidis*. 
Table 2. Performance of the *Staphylococcus* *QuickFISH* method with 29 blood cultures inoculated with non-staphylococcal gram-positive cocci in clusters tested at two hospital laboratories.

<table>
<thead>
<tr>
<th>Routine Identification (# strains, # samples)</th>
<th>Blood Culture System</th>
<th>Bottle*</th>
<th>S. aureus</th>
<th>CoNS</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aerococcus urinae</em> (1,2)</td>
<td>BacT/ALERT</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Aerococcus viridans</em> (1,2)</td>
<td>BacT/ALERT</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Gemella spp.</em> (1,2)</td>
<td>BACTEC</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Granulicatella adiacens</em> (1,2)</td>
<td>BacT/ALERT</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Kocuria</em> spp. (2,2)</td>
<td>BacT/ALERT</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Lactococcus garvieae</em> (1,2)</td>
<td>BacT/ALERT</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>M. luteus ATCC 4698</em> (1,1)</td>
<td>BacT/ALERT</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><em>Micrococcus</em> spp. (4,4)</td>
<td>BacT/ALERT</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><em>Micrococcus</em> spp. (2,4)</td>
<td>BacT/ALERT</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><em>Micrococcus</em> spp. (4,4)</td>
<td>BACTEC</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td><em>Rothia mucilaginosa</em> (1,2)</td>
<td>BACTEC</td>
<td>A</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td><em>Rothia</em> spp. (2,2)</td>
<td>BacT/ALERT</td>
<td>B</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
</tbody>
</table>

* A= Aerobic bottle only; B= Both aerobic and anaerobic bottles
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