

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k060099

B. Purpose for Submission:

New product *Staphylococcus aureus* PNA FISH™

C. Measurand:

S. aureus specific 16S ribosomal RNA

D. Type of Test:

Fluorescent In Situ Hybridization (FISH) using protein nucleic acid (PNA) probes

E. Applicant:

AdvanDx, Inc.

F. Proprietary and Established Names:

S. aureus PNA FISH™, *Staphylococcus aureus* Culture Identification Kit

G. Regulatory Information:

1. Regulation section:

21 CFR Part 866.3700 – *Staphylococcus aureus* serological reagents

2. Classification:

Class I

3. Product code:

NXX

4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use(s):

S. aureus PNA FISH is a qualitative nucleic acid hybridization assay intended for presumptive identification of *Staphylococcus aureus* from blood cultures.

2. Indication(s) for use:

This submission is for the new product *S. aureus* PNA FISH, which is a qualitative nucleic acid hybridization assay, intended for the presumptive identification of *Staphylococcus aureus* from blood cultures containing Gram-positive cocci in clusters (GPCC).

3. Special conditions for use statement(s):

Prescription use only.

4. Special instrument requirements:

AdvanDx Teflon-coated Microscope Slides. A fluorescent microscope equipped with an AdvanDx Dual Band Filter.

I. Device Description

The *S. aureus* PNA FISH Culture Identification Kit contains a 3 mL bottle of fixation solution, a 1.5 mL bottle of fluorescein-labeled PNA probe in hybridization solution, a 50 mL bottle of concentrated wash solution, which must be diluted prior to use, and a 3 mL bottle of mounting medium. The one-well, Teflon-coated microscope slides, glass coverslips and the external quality control organism slides are sold separately. User-prepared quality control organism slides are also acceptable. After processing, the slides must be examined within two hours by using a fluorescent microscope equipped with a dual band filter.

J. Substantial Equivalence Information:

1. Predicate device name(s):

a. AccuProbe *S. aureus* Culture Identification Test

2. Predicate K number(s):

a. K902213

3. Comparison with predicate(s):

| Similarities | | |
|--------------|---|-----------|
| Item | Device | Predicate |
| Intended Use | Intended for the <i>in vitro</i> identification of <i>S. aureus</i> | Same |
| Organism | Blood cultures with GPCC | Same |

| Differences | | |
|-----------------------------|--|---|
| Item | Device | Predicate |
| | PNA FISH | AccuProbe |
| Technology Method | Fluorescence in situ hybridization | Hybridization protection assay |
| Time to result | 2.5 hours from time of smear preparation | 18 – 24 hours following positive blood culture detection (e.g. 0 – 5 days) |
| Sample | Smear of blood cultures | Colonies grown on solid media culture, isolated from liquid blood culture media |
| Control organisms | <i>S. aureus</i> and <i>S. epidermidis</i> | <i>S. aureus</i> and <i>S. epidermidis</i> |
| Mechanism of identification | <i>S. aureus</i> specific 16S rRNA | <i>S. aureus</i> specific 16S rRNA |
| Interpretation of results | Qualitative fluorescent microscopy | Quantitative luminometer |

K. Standard/Guidance Document Referenced (if applicable):
Not applicable

L. Test Principle:

One drop of fluorescein-labeled, *S. aureus*-specific PNA probe is added to a methanol, heat, or flame fixed smear, prepared from liquid blood culture media with GPCC. Hybridization is performed during a 90 +/- 5 minute incubation at 55 +/- 1° C, in an incubator or on a slide warmer. The slide is examined by fluorescent microscopy within two hours of staining. *S. aureus* is presumptively identified as multiple bright green fluorescent clusters of cocci in multiple fields on a reddish background, whereas non-*S. aureus* cells will not fluoresce.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

The performance data were generated by multiple, geographically distinct, clinical laboratories. Each laboratory compared the device results to corresponding results obtained by standard culture identification procedures. These laboratories reported the sensitivity and specificity results in peer reviewed literature articles, and during presentations performed at professional meetings (posters).

a. **Precision/Reproducibility:**

Inter-laboratory and Intra-laboratory testing demonstrated >95% reproducibility. The ten isolate study described in the guidance document was used. (10 organisms tested 3 times on 3 days at 3 sites.)

b. **Linearity/assay reportable range:**

Not applicable

- c. **Traceability, Stability, Expected values (controls, calibrators, or methods):**
The recommended QC isolates, *S. aureus* ATCC #29213 and *S. epidermidis* ATCC #14990, provided by the manufacturer as External Control Slides, or as user-prepared slides from stock or liquid blood cultures, are recommended for testing in parallel for each batch of tests performed. At one testing (Spain) site the *S. aureus* PNA FISH user-prepared organism control slides were tested a sufficient number of times to demonstrate that the device can produce acceptable quality control results >95% of the time (285/285).

The stability of the *S. aureus* PNA FISH reagents were evaluated using real-time data. The sponsor periodically removed samples from five lots of *S. aureus* PNA FISH reagent from storage for testing. The analytical fluorescence performance and a functional performance of each lot were tested 5 times over 30 months after the manufacturing date. The fluorescent dye on the probe is light sensitive and is considered the least stable component of the kit. There was no change in performance for at least 24 months when stored at 2 - 8° C and protected from light, as indicated in the product labeling.

- d. **Detection limit:**

The claimed detection limit for *S. aureus* in blood cultures was determined to be approximately 1×10^5 colony forming units (CFU) per mL by serial dilutions of a *S. aureus* positive culture. This is consistent with the analytical sensitivity of slide-based staining techniques and is not limited by the test itself, but rather by the general requirement for 1×10^5 CFU/mL for interpretation by standard light microscopy.

- e. **Analytical specificity:**

Specificity of *S. aureus* PNA FISH probe was evaluated using cultures of 17 reference strains and 48 clinical isolates, methicillin-resistant and methicillin-susceptible *S. aureus*, coagulase-negative *Staphylococcus* species, and other clinically relevant and phylogenetically related bacterium, and yeast species. The analytical specificity for *S. aureus* strains positive by PNA FISH was 100% (17/17), and 96% (46/48) of the other strains were negative. Of these two false-positive strains, one was *S. schleiferi* and the other one was one of five *Stomatococcus* strains.

The target sequence is unique for *S. aureus*. False positive results with *S. schleiferi* may occur due to a single base mismatch. All other gram positive bacteria will likely have two or more mismatches and not be reactive. Other non-*S. aureus* species may potentially cause false-positive results.

- f. **Assay cut-off:**

Not applicable

2. **Comparison studies:**

- a. **Method comparison of device to AccuProbe, as the reference method:**

The *S. aureus* PNA FISH™, *Staphylococcus aureus* Culture Identification Kit was compared to the AccuProbe *S. aureus* Culture Identification Test (K902213) hybridization protection method. PNA tests were performed on 157 isolates on

GPCC isolated from the ESP Blood Culture System. The results are presented in the table below. The data demonstrates substantial equivalence to the predicate for the presumptive identification of *S. aureus* isolated from blood cultures with GPCC.

| N=157 | Sensitivity | Specificity | Blood Culture System Evaluated |
|------------------------------|-------------------------|--------------------------|---------------------------------------|
| <i>S. aureus</i> PNA FISH | 98.6% (68/69) | 100.0% (88/88) | ESP |

AccuProbe = AccuProbe *S. aureus* Culture Identification Test (Gen-Probe, San Diego, CA)

ESP = ESP Blood culture system (Trek Diagnostics, Cleveland, OH)

The performance of *S. aureus* PNA FISH was evaluated using three different automated blood culture media systems at multiple sites. The data demonstrated that the *S. aureus* PNA FISH™ can be used with major blood culture systems, and results from testing are similar to results obtained by conventional methods. The results are displayed in the table below.

Automated blood culture system media evaluation comparing device results to results obtained by subculture and subsequent identification by standard methods, by site

| Blood Culture System Evaluated | <i>S. aureus</i> Prevalence Rate | Sensitivity | Specificity | Study |
|---------------------------------------|---|---------------------------|---------------------------|--------------|
| BacT/Alert | 42.5% (37/87) | 97.3% (36/37) | 100% (50/50) | Site A |
| ESP | 32.8% (57/174) | 100% (57/57) | 99.2% (115/117) | Site B |
| BACTEC | 34.2% (68/199) | 98.5% (67/68) | 98.5% (129/131) | Site B |
| BacT/Alert | 39.0% (74/190) | 100% (74/74) | 99.1% (115/116) | Site B |
| BacT/Alert | 44.9% (128/285) | 100% (128/128) | 99.4% (156/157) | Site C |
| BacT/Alert | 30.4% (153/503) | 98.7% (151/153) | 100% (350/350) | Site D |
| Total | 36.0% (517/1438) | 99.2% (513/517) | 99.5% (916/921) | |

BacT/Alert = BacT/Alert Blood culture system (bioMérieux, Durham, NC)

BACTEC = BACTEC Blood culture system (Becton Dickenson, Sparks, MD)

ESP = ESP Blood culture system (Trek Diagnostics, Cleveland, OH)

b. Matrix comparison:

Not applicable

3. Clinical studies:

- a. ***Clinical Sensitivity:***
Not applicable
 - b. ***Clinical specificity:***
Not applicable
 - c. ***Other clinical supportive data (when a. and b. are not applicable):***
Not applicable
4. **Clinical cut-off:**
Not applicable
5. **Expected values/Reference range:**
The expected *S. aureus* positive result rate from GPCC positive blood culture bottles is 30% - 43%, depending on institutional and patient population.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

