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

A. 510(k) Number:

K091827

B. Purpose for Submission:

To obtain a SE determination for a modification of the assay procedure for *S. aureus* PNA FISH. The specific modifications are: elimination of the 5- 10 minutes ethanol step in smear preparation; a reduction of the hybridization time from 90 minutes to 30 minutes

C. Measurand:

S. aureus specific ribosomal RNA sequences

D. Type of Test:

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

E. Applicant:

AdvanDx, Inc

F. Proprietary and Established Names:

AdvanDx *S. aureus* PNA FISH Culture Identification Kit

G. Regulatory Information:

1. Regulation section:

   866.3700

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 the identification of *S. aureus* on smears made from positive blood cultures containing Gram-positive cocci in clusters observed on Gram stain. Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.

   S. aureus PNA FISH is intended as an aid in the diagnosis of *S. aureus* bacteremia.

2. Indication(s) for use:

   S. aureus PNA FISH is a qualitative nucleic acid hybridization assay intended for the identification of *S. aureus* on smears made from positive blood cultures containing Gram-positive cocci in clusters observed on Gram stain. Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.

   S. aureus PNA FISH is intended as an aid in the diagnosis of *S. aureus* bacteremia.

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Dual Band Filter (Cat. No. AC003)

   Microscope Slides (Cat. No. AC001)

I. Device Description:

The *S. aureus* PNA FISH is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to *S. aureus*-specific ribosomal RNA (rRNA) sequences to detect *S. aureus*. PNA FISH is performed directly on smears fixed onto microscope slides. The fluorescein-labeled PNA probes are added to a smear prepared from a positive blood culture. Hybridization is performed at 55ºC for 30 minutes. The hybridization is followed by a post-hybridization wash at 55ºC for 30 min with a stringent Wash Solution to remove unbound PNA probe. Finally, the smear is

mounted with Mounting Medium and examined by fluorescence microscopy.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   S. aureus PNA FISH

2. Predicate 510(k) number(s):

   K060099

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Technology	Fluorescence In Situ Hybridization (FISH) using protein nucleic acid (PNA) probe	Same
Sample	Positive blood culture	Same
PNA Probes	Fluorescein-labeled *S. aureus* specific PNA probe	Same

Differences		
Item	Device	Predicate
Fixed smear treatment	None	Ethanol for 10 minutes and air dried
Hybridization at 55ºC	30 minutes	90 minutes

K. Standard/Guidance Document Referenced (if applicable):

Non applicable

L. Test Principle:

The PNA FISH technology uses species-specific peptide nucleic acid (PNA) probes in a fluorescence *in situ* hybridization format. rRNA sequencing is able to detect differences between closely related species. Each microorganism harbors several thousand rRNA molecules in sufficient concentration to allow individual cells to be detected and identified by fluorescent-labeled probes. The fluorescein-labeled PNA probes are added to a smear prepared from a blood culture. Hybridization is performed at 55ºC for 30 minutes. The hybridization is followed by a post-hybridization wash at 55ºC for 30 min with a stringent Wash Solution. Finally, the smear is mounted with Mounting Medium and examined by fluorescence microscopy.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

      A reproducibility study for *S. aureus* PNA FISH assay was performed by using ten reference Gram positive cocci, in triplicates with positive and negative controls, over a period of three days at three different sites, by at least two different operators at each site. Results showed 100% precision and reproducibility between and within sites.

   b. *Linearity/assay reportable range:*

      Not applicable

   c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

      Positive and negative control slides were performed at each testing site on each day of testing. All results were as expected.

   d. *Detection limit:*

      The detection limit was determined to be approximately 10^5 CFU/mL by serial dilutions of *S. aureus* cultures. The average number of colonies per mL (CFU/mL) was calculated from three plates. The data sets showed a minimum of 10^5 CFU/mL to produce a positive result for the *S. aureus* PNA FISH™ assay.

   e. *Analytical specificity:*

      The modified assay procedure was tested and compared to the original assay procedure. *S. aureus* PNA FISH was evaluated on (46) *S. aureus*, (66) other additional Gram positive organisms (i.e. 42 *Staphylococcus* spp); (32) Gram negative organisms, and (5) yeasts representing phylogenetically closely related organisms and a variety of clinically significant organisms. All (46) *S. aureus* showed green fluorescence in both procedures; (2) *S. schleiferi* showed weak positive results and all other (100) organisms were negative.

   f. *Assay cut-off:*

      Not applicable

2. Comparison studies:

   a. *Method comparison of device to conventional methods, as the reference method:*

The modified assay procedure was compared to the original assay procedure and the conventional culture methods.

b. Matrix comparison:

Not applicable

3. Clinical studies:

The Clinical Study was conducted at three sites: the performance of *S. aureus* PNA FISH (New) was compared to *S. aureus* PNA FISH, and to conventional routine methods. A total of 208 blood culture bottles with Gram positive cocci in clusters (GPCC) were included in the studies.

Performance results of the modified, shortened assay procedure (i.e. 30 minutes hybridization, prepared smears not treated with ethanol) compared to the original assay procedure, and compared to the conventional methods are summarized below.

Performance Data for *S. aureus* PNA FISH (Shortened) vs. *S. aureus* PNA FISH (Original)

Study	Positive Agreement	Negative Agreement	Blood Culture System
A	100% (15/15) 95% CI (81.9-100)	100% (35/35) 95% CI (91.8-100)	BacT/ALERT
B	96.9% (32/33) 95% CI (84.2-99.9)	100% (70/70) 95% CI (95.8-100)	VersaTREK
C	100% (13/13) 95% CI (79.4-100)	100% (42/42) 95% CI (93.1-100)	BACTEC
Total	98.4% (60/61) 95% CI (91.2-100)	100% (147/147) 95% CI (98.0-100)	

Performance Data for *S. aureus* PNA FISH (New) vs. Conventional Methods

Study	Sensitivity	Specificity	Blood Culture System
A	100% (15/15) 95% CI (81.9-100)	100% (35/35) 95% CI (91.8-100)	BacT/ALERT
B	100% (32/32) 95% CI (84.2-99.9)	100% (71/71) 95% CI (95.8-100)	VersaTREK
C	100% (13/13) 95% CI (79.4-100)	100% (42/42) 95% CI (93.1-100)	BACTEC
Total	100% (60/60) 95% CI (95.1-100)	100% (148/148) 95% CI (98-100)	

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:

S. aureus cells: bright green fluorescent cocci in multiple fields

The expected positive rate from positive blood culture bottles is 30%- 43% for *S. aureus*

N. Proposed Labeling:

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

O. Conclusion:

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