

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

**A. 510(k) Number:**

K092166

**B. Purpose for Submission:**

To determine substantial equivalence of the device for the identification of *S. aureus* and/or selected other coagulase negative *Staphylococcus* species on smears from positive blood cultures containing Gram positive cocci in clusters.

**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*/CNS PNA FISH Culture Identification Kit

**G. Regulatory Information:**

1. Regulation section:

866.3700

2. Classification:

Class I

3. Product code:

NXX

4. Panel:

**H. Intended Use:**

1. Intended use:

*S. aureus*/CNS PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *S. aureus* and/or selected other *Staphylococcus* species 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*/CNS PNA FISH is intended as an aid in the diagnosis of *S. aureus* bacteremia.

2. Indications for use:

*S. aureus*/CNS PNA FISH is a qualitative nucleic acid hybridization assay intended for the identification of *S. aureus* and/or selected other *Staphylococcus* species 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*/CNS 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:**

A mixture of fluorescein-labeled, *S. aureus* specific PNA probe and a Texas Red labeled PNA targeting other *Staphylococci* (CNS) is added to a smear prepared from a positive blood culture. 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 55°C for 30 minutes. The hybridization is followed by a post-hybridization wash at 55°C for 30 minutes 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

K091827

3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Technology	Fluorescence In Situ Hybridization (FISH) using protein nucleic acid (PNA) probe	Same
Sample	Positive blood culture	Same
Interpretation of Results	Qualitative Fluorescence microscope	Same

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
PNA Probes	Fluorescein-labeled <i>S. aureus</i> specific PNA probe and Texas Red labeled PNA targeting other <i>Staphylococci</i> (CNS)	Fluorescein-labeled <i>S. aureus</i> specific PNA probe
Time to result	1.5 hrs.	2.5 hrs.

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

Non applicable

**L. Test Principle:**

A mixture of fluorescein-labeled, *S. aureus* specific PNA probe and a Texas Red labeled PNA targeting other *Staphylococci* (CNS) is added to a smear prepared from a positive blood culture. Hybridization is performed at 55°C for 30 minutes. The hybridization is followed by a post-hybridization wash at 55°C for 30 minutes with a stringent Wash Solution to remove unbound PNA probe. Finally, the smear is mounted with Mounting Medium and examined by fluorescence microscopy. While maintaining the morphology of the cells, *S. aureus* and selected other *Staphylococcus*

species (CNS) cells become fluorescent by specific binding of the fluorophore-labeled PNA probes.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

A reproducibility study for *S. aureus*/CNS PNA FISH assay, using new procedure, 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. Results were as expected for this type device.

d. *Detection limit:*

The detection limit was determined to be approximately  $10^5$  CFU/mL by serial dilutions of *S. aureus* and *S. epidermidis* 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*/CNS PNA FISH™ assay.

e. *Analytical specificity:*

The new assay procedure was tested and compared to the original assay procedure. All 43 *S. aureus* showed positive-green results, 39 CNS showed positive-red results. Additionally, results indicated that *S. aureus*/CNS PNA FISH is negative (no positive-red) for *S. felis*, *S. simulans*, *Micrococcus casseolyticus* (formerly *Staph cohnii* subspp. *cohnii*), and *Micrococcus equipericus* (formerly *Staph equipericus*).

Thirty-three Gram negative organisms, 25 Gram positive organisms and six yeast isolates were tested with the new procedure, with *Candida krusei* demonstrated weak green fluorescence, and the other 63 non-*Staphylococcus* organisms were negative.

f. *Assay cut-off:*

Not applicable

2. Comparison studies:

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

Not applicable

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

A total of 402 (Gram positive cocci in clusters) positive blood culture bottles at four sites were included in the studies, and showed 99.5% (400/402) overall agreement between *S. aureus*/CNS PNA FISH and conventional routine methods. The performance data are shown at the table below.

a. *Clinical Sensitivity:*

Study	<i>S. aureus</i>	CNS	Other	Blood Culture System
A	100% (32/32) 95% CI (91.1-100)	100% (67/67) 95% CI (95.6-100)	100% (1/1) 95% CI (1.0-100)	BACTEC
B	100% (17/17) 95% CI (83.8-100)	100% (82/82) 95% CI (96.4-100)	100% (4/4) 95% CI (47.3-100)	BacT/ALERT
C	100% (32/32) 95% CI (91.1-100)	100% (65/65) 95% CI (95.5-100)	100% (4/4) 95% CI (47.3-100)	BACTEC
D	96.9% (32/32) 95% CI (84.2-99.9)	98.4% (64/64) 95% CI (81.7-99.9)	0% (0/2)* 95% CI (0-52.7)	VersaTREK
<b>Total</b>	<b>Sensitivity</b> 100 % (113/113) 95% CI (97.4-100)	<b>Sensitivity</b> 100 % (278/278) 95% CI (98.9-100)	<b>Specificity</b> 81.8 % (9/11) 95% CI (48.2-97.7)	

\* Two false positive-red were *Micrococcus* spp.

b. *Clinical specificity:*

See Table above.

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: multiple bright green fluorescent clusters of cocci in multiple fields

CNS cells: multiple bright red fluorescent clusters of cocci in multiple fields

The expected *S. aureus* and CNS positive result rate from Gram positive cocci positive blood culture bottles is approximately 21% and 55%, respectively, but may vary depending on institution and patient population.

**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.