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

A. 510(k) Number:

K092236

B. Purpose for Submission:

To obtain a substantial equivalence determination for a modification of the assay procedure for the *E. coli* and/or *P. aeruginosa* PNA FISH. The specific modifications are: elimination of the 5- 10 minutes ethanol step in smear preparation and a reduction of the hybridization time from 90 minutes to 30 minutes.

C. Measurand:

E. coli and *P. aeruginosa* 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:

E. coli/P. aeruginosa PNA FISH™

G. Regulatory Information:

1. Regulation section: 866.2660

2. Classification: Class I

3. Product code: JSS

4. Panel: 83-Microbiology

H. Intended Use:

1. Intended use:

   Escherichia coli/Pseudomonas aeruginosa PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for identification of *Escherichia coli* and *Pseudomonas aeruginosa* on smears from positive blood cultures containing Gram-negative rods. The *E. coli*/*P. aeruginosa* PNA FISH assay is indicated for use as an aid in the diagnosis of *E. coli* and/or *P. aeruginosa* bacteremia.

   Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.

2. Indications for use:

   Escherichia coli/Pseudomonas aeruginosa PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for identification of *Escherichia coli* and *Pseudomonas aeruginosa* on smears from positive blood cultures containing Gram-negative rods. The *E. coli*/*P. aeruginosa* PNA FISH assay is indicated for use as an aid in the diagnosis of *E. coli* and/or *P. aeruginosa* bacteremia.

   Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.

3. Special conditions for use statement:

   Prescription use only

4. Special instrument requirements:

   Dual Band Filter (Cat. No. AC003)
   Microscope Slides (Cat. No. AC001)

I. Device Description:

The *Escherichia coli/Pseudomonas aeruginosa* PNA FISH is a multicolor, qualitative, nucleic acid hybridization assay intended for identification of E. coli and P. aeruginosa on smears made from positive blood culture. This new proposed model of the assay, purported to provide rapid (within 1.5 hours) identification, consists of the following reagents:

- GN Fixation Solution
  3 mL phosphate-buffered saline

- *E. coli/P. aeruginosa* PNA
  1.5 mL fluorescein-labeled, *E. coli*/*P. aeruginosa* specific PNA probes in hybridization solution. Contains 30% foramide

- 60X Wash Solution
  50 mL Tris-buffered saline with detergent

- Mounting Medium
  3 mL photobleaching inhibitor in glycerol

Materials required but not provided:

- Water, deionized or distilled
- Fluorescence microscope equipped with a 60x or 100x oil objective lens
- Immersion oil. Must comply with the microscope objective and be non- fluorescent

J. Substantial Equivalence Information:

1. Predicate device name:

   E. coli/P. aeruginosa PNA FISH™

2. Predicate 510(k) number:

   K081309

3. Comparison with predicate:

Similarities		
Item	**Device (K092236)**	**Predicate (K081309)**
Function	Identification of *E. coli* and *P. aeruginosa*	Same
Technology	Fluorescence In Situ Hybridization (FISH) using protein nucleic acid (PNA) probe	Same
Sample Type	Positive Blood Cultures	Same
Controls	*E. coli, P. aeruginosa and Klebsiella spp.*	Same
Interpretation of Results	Qualitative Fluorescence microscope	Same
PNA Probes	Eco 16S06-Flu Pse23S32-TXR	Same

Differences		
Item	**Device (K092236)**	**Predicate (K081309)**
Fixed smear treatment	None	Ethanol for 10 minutes and air dried
Hybridization Time	30 minutes	90 minutes
Time to Result	1.5 hours	2.5 hours

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

Not applicable

L. Test Principle:

A mixture of fluorescein-labeled, *E. coli* specific PNA probe and a Texas Red labeled, *P. aeruginosa* specific PNA probe is added to a smear prepared from a positive blood culture. Hybridization is performed at 55ºC for 30 minutes. The hybridization is followed by a rinse step to remove the cover slip followed by a wash at 55ºC for 30 minutes with a stringent wash solution to remove unbound PNA probe. Subsequently, the smear is mounted with Mounting Medium and examined by fluorescence microscopy.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Reproducibility:
   A Reproducibility study for *E coli/ P. aeruginosa* PNA FISH assay was performed by using 16 reference isolates of Gram negative rods, once per day with positive and negative controls, over a period of three days at three different sites. Each batch was run independently by at least two blinded operators at each site.

   Results showed > 97.9 % reproducibility between and within sites. This is acceptable for this type device.

   b. Linearity/assay reportable range:

   Not applicable

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

   Quality Control

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

   d. Detection limit:

   The detection limit was determined to be approximately 10^5 CFU/mL by serial dilutions of *E. coli* or *P. aeruginosa* positive cultures. Serial dilutions of exponentially growing cultures were prepared. Then 0.01 mL was plated on growth

media and 0.01 mL was used for preparation of smears. The smears were run through PNA FISH and scored positive or negative. The following day, colonies were counted on the plates and the average number of colonies per dilution was calculated. The data sets showed a minimum of 10^5 CFU/mL to produce a positive result for the *E. coli/P. aeruginosa* PNA FISH™ assay.

e. Analytical specificity:

E. coli/P. aeruginosa PNA FISH has also been tested on laboratory and reference strains comprising of 14 *E. coli*, 17 *P. aeruginosa*, 60 additional Gram negative organisms, 12 Gram positive organisms and 6 yeasts, representing phylogenetically closely related strains. All (14/14) *E. coli* strains were green-positive and all (17/17) *P. aeruginosa* strains were red-positive. *Shigella spp.* (serogroup A, B, C, or D), *Escherichia albertii* and *Escherichia fergusonii* cross-reacted to create a green signal, *Brevundimonas diminuta, Herbaspirillum huttiense, Pseudomonas nitroreducens,* and *Pseudomonas fulva* cross-reacted to create a red signal. All other strains were negative.

Interference

A study consisting of 10 Gram negative rods were tested on four types of blood culture bottles, BD BACTEC, BacTAlert SA and BacTAlert FA, and VersaTREK for Heat Fixation at 70^0. Fifteen organisms were tested at 55^0 and 80^0 for at for the interference from charcoal and different temperatures for Heat Fixation. No interferences were observed.

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:

a. Clinical Sensitivity:

The performance of *E. coli/P. aeruginosa* PNA FISH (new procedure) versus

E. coli/P. aeruginosa PNA FISH (K081309), along with conventional routine methods, was assessed in five clinical laboratory studies. A total of 385 blood culture bottles with Gram negative rods were included in the studies. Performance results were as follows:

Performance data *E. coli/P. aeruginosa* PNA FISH (proposed device) versus Routine Conventional Methods on GNR-positive Blood Culture Bottles

Study	Sensitivity *E. coli*	Sensitivity *P. aeruginosa*	Specificity	Blood Culture System
A	51/51	12/12	54/54	BACTEC
B	51/51	9/9	40/40	BacT/Alert
C	17/17	7/7	51/51	BACTEC
D	32/32	7/8	36/36	BACTEC
E	7/7	4/4	6/6	VersaTREK
Total	100% (158/158) 95% CI (98.0-100)	97.5% (39/40) 95% CI (87-99.9)	100% (187/187) 95% CI (98.4-100)	

b. Clinical specificity

Refer to table in section 3a.

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:

Positive *E. coli* cells - green fluorescence
Positive *P. aeruginosa* cells - red fluorescence

The expected positive rates from positive blood culture bottles for *E.coli* and *P. aeruginosa* are 37% and 13%, respectively.

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 substantial equivalence decision.