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

A. 510(k) Number:

K081309

B. Purpose for Submission:

To determine substantial equivalence of the device for the identification of *E. coli* and/or *P. aeruginosa* on smears from positive blood cultures containing Gram-negative rods.

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(s):

   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 intended for use in conjunction with positive blood subcultures as an aid in the identification of *E.coli* and/or *P. aeruginosa.*

2. Indication(s) 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 intended for use in conjunction with positive blood subcultures as an aid in the identification of *E.coli* and/or *P. aeruginosa.*

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, *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 90 min. The hybridization is followed by a rinse step to remove the cover slip and a post-hybridization wash at 55ºC for 30 min. with a stringent wash solution to remove unbound PNA probe. The smear is finally mounted with Mounting Medium for examination with fluorescence microscopy (Dual Band Filter). *E. coli* cells show green fluorescence whereas *P. aeruginosa* cells show red fluorescence.

J. Substantial Equivalence Information:

1. Predicate device name(s):

E. faecalis/OE PNA FISH

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

   K063127

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
Interpretation of Results	Qualitative Fluorescence microscope	Same

Differences		
Item	Device	Predicate
Function	Identification of *Escherichia coli* and *Pseudomonas aeruginosa*	Identification of *E. faecalis* and other *Enterococci*
Control organisms	Pos control: *E. coli* and *P. aeruginosa* Neg control: *Klebsiella spp*	Pos control: *E. faecalis* and *E. faecium* Neg control: *Streptococcus spp*
PNA Probes	*E. coli* and *P. aeruginosa*	*E. faecalis* and other *Enterococci*

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

Non applicable

L. Test Principle:

A mixture of a fluorescein-labeled, *E.coli* specific PNA probe and a Texas Red-label, *P. aeruginosa* specific PNA probe is added to a smear prepared from a positive blood culture. Hybridization is performed at 55ºC for 90 minutes. The hybridization is followed by a water rinse at 55ºC to remove the cover slips followed by a 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 *E coli/ P. aeruginosa* PNA FISH assay was performed by using ten reference isolates of Gram negative rods, once per day with positive and negative controls, over a period of three days at three different sites, by one operator 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. 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:*

The analytical specificity of the *E. coli/P. aeruginosa* PNA FISH™ was determined by BLAST search and sequence alignments and experimentally by testing of well characterized laboratory and reference strains comprising of 14 *E. coli* and 17 *P. aeruginosa*, 76 additional Gram negative, 12 Gram positive organisms and 7 yeasts. All *E. coli* and *P. aeruginosa* were correctly identified. *Brevundimonas diminuta, Herbaspirillum huttiense, Pseudomonas nitroreducens and Pseudomonas fulva* cross reacted to create a red signal; *Shigella* spp, (serogroup A, B, C, or D), *Escherichia fergusonii* and *Escherichia albertii* cross reacted to create a green signal. All other strains were negative.

Interference

A study consisting of 15 Gram negative rods were tested on BACTEC Plus blood culture bottles for the interference of resin. No interferences were observed. Current peer-reviewed publications indicated that sodium polyanetholesulfonate (SPS) or charcoal does not cause interference with PNA FISH assays.

f. Assay cut-off:

Not applicable

2. Comparison studies:

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

Performance results compare to routine identifications, based on conventional methods following subculture.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Clinical Study was conducted at four sites in the United States and Europe, directly on blood culture bottles containing Gram negative rods (GNR). A total of 240 GNR-positive blood bottles, from two commercial continuously monitoring blood culture systems (BacT/Alert and BACTEC) were included in the study. Performance results compare to routine identifications, based on conventional methods following subculture, was summarized below.

Study	Sensitivity *E. coli* (Green)	Sensitivity *P. aeruginosa* (Red)	Specificity	Blood Culture System
A	100% (14/14) 95%CI (80.7-100)	100% (11/11) 95%CI (76.2-100)	100% (25/25) 95%CI (88.7-100)	BacT/Alert
B	100% (35/35) 95%CI (91.8-100)	100% (9/9) 95%CI (71.7-100)	96.6% (28/29) 95%CI (92.2- 99.9)	BacT/Alert
C	100% (20/20) 95%CI (86.1-100)	92.3% (12/13) 95%CI (64.0-99.8)	93.1% (27/29) 95%CI (77.7-99.2)	BACTEC
D	100% (32/32) 95%CI (91.1-100)	100% (2/2) 95%CI (22.4-100)	100% (21/21) 95%CI (86.7-100)	BacT/Alert
Total	100% (101/101) 95% CI (97.1 - 100)	97.1% (34/35) 95% CI (85.1 - 99.9)	97.1% (101/104) 95% CI (91.8 - 99.4)	

*b. Clinical specificity**:***

Refer to 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:

E. coli cells: green fluorescence

P. aeruginosa cells: red fluorescence

The expected positive rate from positive blood culture bottles is 37% for *E.coli* and 13% for *P. aeruginosa*.

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