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

A. 510(k) Number:

K081433

B. Purpose for Submission:

Request substantial equivalence for a new device

C. Measurand:

E. coli + *Klebsiella pneumoniae* 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:

EK/*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):

   EK/*P. aeruginosa* PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for identification of *Escherichia coli* /*Klebsiella pneumoniae* and *Pseudomonas aeruginosa* on smears from positive blood cultures containing Gram-negative rods. The test does not distinguish between *E. coli* and *K. pneumoniae*. Further testing is needed to differentiate *E. coli* and *K. pneumoniae*. The EK/*P. aeruginosa* PNA FISH assay is indicated for use in conjunction with positive blood subcultures as an aid in the identification of *E. coli*/*Klebsiella pneumoniae,* and/or *P. aeruginosa.*

2. Indication(s) for use:

   EK/*P. aeruginosa* PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for identification of *Escherichia coli* /*Klebsiella pneumoniae* and *Pseudomonas aeruginosa* on smears from positive blood cultures containing Gram-negative rods. The test does not distinguish between *E. coli* and *K. pneumoniae*. Further testing is needed to differentiate *E. coli* and *K. pneumoniae*. The EK/*P. aeruginosa* PNA FISH assay is indicated for use in conjunction with positive blood subcultures as an aid in the identification of *E. coli*/*Klebsiella pneumoniae,* and/or *P. aeruginosa.*

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Fluorescence microscope with Dual Band Filter (Cat. No. AC003) and Microscope Slides (Cat. No. AC001)

I. Device Description:

A mixture of fluorescein-labeled, *E. coli*, a fluorescein-labeled *K. pneumoniae* 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 water rinse at 55ºC to remove the cover slips followed by a wash at 55ºC for 30 min. with a stringent wash solution. The smear is finally mounted with Mounting Medium for examination with fluorescence microscopy (Dual Band Filter). *E. coli* and *K. pneumoniae* are bright green fluorescent rods whereas *P. aeruginosa* are bright red fluorescent rods.

The test does not distinguish between *E. coli* and *K. pneumoniae*.

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/K. pneumoniae* and *Pseudomonas aeruginosa*	Identification of *E. faecalis* and other *Enterococci*
Control organisms	Pos control: *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 10145 Neg control: *Klebsiella oxytoca* ATCC 43086	Pos control: *E. faecalis* and *E. faecium* Neg control: *Streptococcus spp*
PNA Probes	*E. coli* + *P. aeruginosa* and *P. aeruginosa*	*E. faecalis* and other *Enterococci*

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

Non applicable

L. Test Principle:

A mixture of fluorescein-labeled, *E. coli*, a fluorescein-labeled *K. pneumoniae* 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 minutes 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 EK/*P. aeruginosa* PNA FISH assay was performed by using ten reference 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:

10^5CFU/mL

e. Analytical specificity:

The analytical specificity of the EK/*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 22 *E. coli,* 17 *K. pneumoniae* and 24 *P. aeruginosa,* 74 additional Gram negative, including anaerobes, 13 Gram positive organisms and 7 yeasts. All *E. coli, K. pneumoniae* 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.

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 routineVitek2 identifications, following subculture.

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Clinical Studies were 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, following subculture, was summarized below.

Performance Data for EK/*P. aeruginosa* PNA FISH

Study	Sensitivity EK	Sensitivity *P. aeruginosa*	Specificity	Blood Culture System
A	100% (26/26) 95% CI (89.1-100)	100% (11/11) 95% CI (76.2-100)	92.3% (12/13) 95% CI (64.0-99.8)	BacT/Alert
B	100% (47/47) 95% CI (93.8-100)	88.9% (8/9) 95% CI (51.8-99.7)	100% (18/18) 95% CI (84.7-100)	BacT/Alert
C	100% (31/31) 95% CI (90.8-100)	92.3% (12/13) 95% CI (64.0-99.8)	94.4% (17/18) 95% CI (72.7-99.9)	BACTEC
D	100% (40/40) 95% CI (92.8-100)	100% (2/2) 95% CI (22.4-100)	100% (13/13) 95% CI (79.4-100)	BacT/Alert
Total	100% (144/144) 95% CI (97.9-100)	94.3% (33/35) 95% CI (80.8-99.3)	95.9% (60/62) 95% CI (88.8-99.6)	

*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/K. pneumoniae cells: multiple bright green fluorescent rods in multiple. fields

P. aeruginosa cells: multiple bright red fluorescent rods in multiple fields.

The expected *E. coli*, *K. pneumoniae* and *P. aeruginosa* positive result rate for Gram-negative rods positive blood culture bottles are approximately 37%, 18% 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 a substantial equivalence decision.