510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K092393

B. Purpose for Submission:

SE determination for a modification of the assay procedure for *EK/P. aeruginosa* 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:

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, JSZ

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. F*urther 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.c*oli/Klebsiella pneumoniae* 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:

The *EK/P. aeruginosa* PNA FISH is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to *E.coli,* fluorescein labeled *K. pneumoniae* specific PNA probe and a Texas Red labeled *P. aeruginosa* specific PNA probe. The test is performed directly on smears fixed onto a microscope slide prepared from a positive blood culture. Hybridization is performed at 55°C for 30 minutes. The hybridization is followed by a water rinse at 55°C to remove the cover slips, followed by a wash at 55°C 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):

   EK/P. aeruginosa PNA FISH

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

   K081433

3. Comparison with predicate:

	Similarities	
Technology	Fluorescence In Situ Hybridization (FISH) using protein nucleic acid (PNA) probe	Same
Sample	Positive blood culture	Same
PNA Probes	Fluorescein-labeled *EK/P. aer* 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:

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 55^0C for 30 minutes the hybridization is followed by a water rinse at 55°C to remove the cover slips followed by a wash at 55°C 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:

The detection limit was determined to be approximately 10^5 CFU/mL by serial dilutions of *E.coli, K. pneumoniae,* and *P. aeruginosa* 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 *EK/P aeruginosa* PNA FISH™ assay.

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 (19) *E.coli*, (12) *K. pneumoniae,* (20) *P. aeruginosa*, (51) other additional Gram negative organisms, (13) Gram positive organisms, and (6) yeasts representing phylogenetically closely related organisms and a variety of clinically significant organisms. All (19/19) *E.coli* strains and (12/12*) K. pneumoniae* showed green-positive fluorescence and all (20/20) *P. aeruginosa* strains showed red-positive. *Shigella spp* (serogroup A, B, or D), *Escherichia albertii* and *Escherichia fergusonii* cross reacted to create a green signal. *Brevundimonas diminuta, Herbaspirillum huttiense, Pseudomonas nitroreducens,* and *Pseudomonas fuva* cross-reacted to create a red signal. All other strains 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 *EK/P aeruginosa* PNA FISH (New) versus *EK/P aeruginosa* PNA FISH, was compared to conventional routine methods. A total of 368 blood culture bottles with Gram negative rods (GNR) 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 *EK/P. aeruginosa* PNA FISH (New) vs. *EK/P. aeruginosa* PNA FISH (K081433).

Study	Positive Agreement E.coli/K. pneumoniae	Positive Agreement P. aerugnosa	Negative Agreement	Blood Culture System
A	65/65	12/12*	42/42	BACTEC
B	69/69	9/9	22/22	BacT/Alert
C	34/34	7/7	34/34	BACTEC
D	47/47	7/7	22/22	BACTEC
Total	100% (215/215) 95%CI (98.6-100)	100% (35/35) 95% CI (91.8-100)	100% (120/120) 95% CI (97.5-100)	N=370

*2 mix P. aeruginosa and K. pneumoniae samples

Performance Data for *EK/P. aeruginosa* PNA FISH (New) vs Conventional Methods.

Study	Sensitivity *E.coli/K. pneumoniae*	Sensitivity *P. aeruginosa*	Specificity	Blood Culture System
A	65/65	12/12*	42/42	BACTEC
B	69/69	9/9	22/22	BacT/Alert
C	34/34	7/7	34/34	BACTEC
D	47/47	7/8	21/21	BACTEC
Total	100% (215/215) 95%CI (98.6-100)	97.2% (35/36) 95% CI (85.5-99.9)	100% (120/120) 95% CI (97.5-100	N=370

*2 mix P. aeruginosa and K. pneumoniae samples

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The expected *E coli/K. pneumoniae* results are multiple bright green fluorescent rods in multiple fields. *P. aeruginosa* cells are multiple bright red fluorescent rods in multiple fields. In the studies conducted, *E coli, K. pneumoniae* and *P. aeruginosa* had positive result rates for Gram negative rods with positive blood culture bottles of approximately 37%, 18% and 13%, respectively. This may differ depending on the patient population and the institution.

N. Proposed Labeling:

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

O. Conclusion:

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