

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

A. 510(k) Number:

K082068

B. Purpose for Submission:

New submission for SE determination

C. Measurand:

E. coli 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 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):

E. coli PNA FISH® is a qualitative nucleic acid hybridization assay intended for the presumptive identification of *Escherichia coli* on smears from positive blood cultures containing gram-negative rods. The test does not distinguish between *E. coli* and *Shigella* spp. Confirm all positive results with conventional identification methods.

Subculturing of positive blood cultures is necessary to recover organisms for definitive identification and susceptibility testing.

2. Indication(s) for use:

E. coli PNA FISH® is indicated for use as an aid in the diagnosis of *E. coli* 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:

E. coli PNA FISH® is a fluorescence in situ hybridization (FISH) method using PNA probes hybridizing to *E. coli*-specific ribosomal RNA sequences.

PNA FISH is performed directly on smears fixed onto microscope slides. Hybridization is performed at 55°C for 90 min. The coverslip is removed and a post-hybridization wash at 55°C 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). While maintaining the morphology of the cells, presumptive *E. coli* cells show green fluorescence by binding of the fluorescein-labeled PNA probes.

E. coli PNA FISH® is provided in a kit box with the following kit components:

GN Fixation Solution

E. coli PNA

Wash Solution

Mounting Medium

J. Substantial Equivalence Information:

1. Predicate device name(s):

S. aureus PNA FISH™

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

K060099

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
Time to Result	2.5 hours	Same
Interpretation of Results	Qualitative Fluorescence microscope	Same

Differences		
Item	Device	Predicate
Function	Presumptive identification of <i>Escherichia coli</i>	Identification of <i>S. aureus</i>
Control organisms	Pos control: <i>E. coli</i> ATCC 11775 Neg control: <i>P. aeruginosa</i> ATCC 10145	Pos control: <i>S. aureus</i> Neg control: <i>S. epidermidis</i>

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

Not applicable

L. Test Principle:

The PNA FISH® technology uses species-specific peptide nucleic acid (PNA) probes in a fluorescence in situ hybridization format.

A mixture of a fluorescein-labeled *E.coli* specific PNA probe is added to a smear prepared from a positive blood culture. Hybridization is performed at 55°C for 90 minutes. The hybridization is followed by a post-hybridization wash at 55°C 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* 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 an operator at each site performing and reading the assay according to the package insert. The study is acceptable for a qualitative assay as only positive or negative results are provided

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 with expected results.

d. *Detection limit:*

The detection limit was determined to be approximately 10⁵ CFU/mL by serial dilutions of *E. coli* 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 10⁵ CFU/mL to produce a positive result for the *E. coli* PNAFISH® assay.

e. *Analytical reactivity:*

The analytical reactivity study included 21 *E. coli* of which all were correctly identified.

f. *Analytical specificity:*

The analytical specificity of the *E. coli* PNA FISH® was determined by BLAST search and sequence alignments and experimentally by testing well characterized laboratory and reference strains comprising of 21 *E. coli*, 67 Gram-negative organisms, 11 Gram-positive organisms and 5 yeast strains. *Escherichia coli* provided expected positive results. However, *Escherichia albertii*, *Escherichia fergusonii*, and *Shigella* species (serogroup A, B, C, or D) also provided positive results with *E. coli* PNA FISH®. A limitation statement has been included to address the cross reactivity issue.

g. *Interference Study:*

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 indicate that sodium polyanetholesulfonate (SPS) or charcoal does not cause interference with PNA FISH assays.

g. *Assay cut-off:*

Not applicable

2. Comparison studies:

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

A Clinical Study was conducted at five sites in the United States and Europe, directly on blood culture bottles containing Gram negative rods (GNR). A total of 122 GNR-positive blood bottles, from two commercial continuously monitoring blood culture systems (BacT/Alert and BACTEC) were included in the study. Performance results compared to routine identifications, based on conventional methods following subculture, are summarized below.

Table 1 BACTEC - verses Vitek2 (bioMérieux)

Study Site C+E	Sensitivity	Specificity	Total
Positive	27	0	
Negative	1	64	
	96.4% (27/28)	100% (64/64)	92
	95% CI (81.7-99.9)	95% CI (95.4-100)	

Table 2 BACTEC – versus Chromagar and Indol Spot:

Study Site D	Sensitivity	Specificity	Total
Positive	56	1	
Negative	0	50	
	100% (56/56) 95% CI (94.8-100)	98.0% (50/51) 95% CI (89.6-99.9)	107

Table 3 BacT/Alert SA/SN – versus API ID 32E (bioMérieux)

Study Site A	Sensitivity	Specificity	Total
Positive	8	0	
Negative	0	9	
	100% (8/8) 95% CI (68.8-100)	100% (9/9) 95% CI (71.7-100)	17

Table 4 BacT/Alert SA/SN – versus Vitek2 (bioMérieux)

Study Site B	Sensitivity	Specificity	Total
Positive	5	1	
Negative	0	8	
	100% (5/5) 95% CI (54.9-100)	88.9% (8/9) 95% CI (51.8-99.7)	14

Table 5 BacT/Alert FA/FN – API ID32E (bioMérieux)

Study Site A	Sensitivity	Specificity	Total
Positive	6	0	
Negative	0	12	
	100% (6/6) 95% CI (60.7-100)	100% (12/12) 95% CI (77.9-100)	18

Table 6 BacT/Alert FA - Vitek2 (bioMérieux)

Study Site B	Sensitivity	Specificity	Total
Positive	17	0	
Negative	0	10	
	100% (17/17) 95% CI (83.8-100)	100% (10/10) 95% CI (74.1-100)	27

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity:*

Not applicable

b. *Clinical specificity:*

Not applicable

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: green fluorescence

Negative: non fluorescence

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.