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

A. 510(k) Number:

K083074

B. Purpose for Submission:

Modify the assay procedure of *E. faecalis*/OE PNA FISH. The specific modifications are: A reduction of the hybridization step from 90 minutes to 30 minutes; removal of the 5- 10 minutes ethanol step during smear preparation.

C. Measurand:

E. faecalis and other enterococci (OE) 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. faecalis/OE PNA FISH™

G. Regulatory Information:

1. Regulation section:

   866.3740

2. Classification:

   Class I

3. Product code:

   OAH

4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use(s):

   E. faecalis/OE PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Enterococcus faecalis* and the detection of selected other enterococci (OE) on smears made from positive blood cultures containing Gram-positive cocci in pairs and chains observed on Gram stain. Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.

2. Indication(s) for use:

   E. faecalis/OE PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Enterococcus faecalis* and the detection of selected other enterococci (OE) on smears made from positive blood cultures containing Gram-positive cocci in pairs and chains observed on Gram stain. Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.

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 a fluorescein-labeled, *E. faecalis*-specific PNA probe and a rhodamine-labeled PNA probe specific for OE is added to a smear prepared from a blood culture. Hybridization is performed at 55ºC for 30 minutes. The hybridization is followed by a post-hybridization wash at 55ºC for 30 min with a stringent Wash Solution. Finally, the smear is mounted with Mounting Medium and examined by fluorescence microscopy.

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
PNA Probes	Fluorescein-labeled *E. faecalis* specific PNA probe and rhodamine-labeled specific for other enterococci	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 a fluorescein-labeled, *E. faecalis*-specific PNA probe and a rhodamine-labeled PNA probe specific for OE is added to a smear prepared from a blood culture. Hybridization is performed at 55ºC for 30 minutes. The hybridization is followed by a post-hybridization 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. faecalis*/OE PNA FISH assay was performed by using ten reference Gram positive cocci, once per day with positive and

negative controls, over a period of three days at three different sites, by at least two different operators 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 on each day of testing. 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. faecalis* and *E. faecium* positive cultures. 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 *E. faecalis*/OE PNA FISH™ assay.

e. Analytical specificity:

The modified assay procedure was tested and compared to the original assay procedure. *E. faecalis*/OE PNA FISH has been evaluated on (11) *E. faecalis*, and (22) *Enterococcus* spp, not *faecalis*; (17) Gram negative organisms, (21) Gram positive organisms and (7) yeasts representing phylogenetically closely related organisms and a variety of clinically significant organisms. All eleven *E. faecalis* showed green fluorescence in both procedures; *E. cecorum, E. columbae*, and *E. dispar* were negative (no fluorescence) and *E. saccharolyticus* showed yellow fluorescence in both procedures.

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.

b. Matrix comparison:

Not applicable

3. Clinical studies:

Clinical Study was conducted at three sites, directly on blood culture bottles

containing Gram positive cocci in pairs and chains (GPCPC). A total of 152 GPCPC-positive blood bottles, from two commercial continuously monitoring blood culture systems (BacT/Alert and BACTEC) were included in the study. A total of (50) BACTEC Standard 10A Aerobic and (50) BACTEC Standard Anaerobic, (23) BacT/Alert SA and (29) BacT/Alert SN blood culture bottles were tested. Performance results of the modified assay procedure (i.e. 30 minutes hybridization, prepared smears not treated with ethanol) compare to the original assay procedure was summarized below.

Study	Positive Agreement (Green) *E. faecalis*	Positive Agreement (Red) Other *Enterococci*	Negative Agreement	Blood Culture System
A	100% (19/19)	100% (11/11)	100% (18/18)	BACTEC
B	100% (9/9)	100% (12/12)	96.6% (31/31)	BacT/Alert
C	100% (13/13)	92.3% (8/8)	93.1% (29/29)	BACTEC
Total	100% (41/41) 95% CI (93.0 - 100)	100% (33/33) 95% CI (91.3 - 100)	100% (78/78) 95% CI (96.2 - 100)	

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:

E. faecalis cells: bright green fluorescent cocci in multiple fields

Enterococcus spp, not *E. faecalis* cells: bright red fluorescent cocci in multiple

fields

The expected positive rate from positive blood culture bottles is 40-50% for *E. faecalis* and 15-25% for other Enterococci.

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.