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

A. 510(k) Number:

k063127

B. Purpose for Submission:

New product *E. faecalis* PNA FISH™

C. Measurand:

Enterococcus faecalis specific 16 S ribosomal RNA and selected other enterococci-specific 16S ribosomal RNA

D. Type of Test:

Fluorescent In Situ Hybridization (FISH) using protein nucleic acid (PNA) probes

E. Applicant:

AdvanDx, Inc.

F. Proprietary and Established Names:

E. faecalis PNA FISH™, *Enterococcus faecalis* Culture Identification Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR Part 866.3740 – Streptococcus spp. serological reagents

2. Classification:

   Class I

3. Product code:
   OAH - FISH (fluorescent in situ hybridization) kit, protein nucleic acid, *Enterococcus faecalis*

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   E. faecalis PNA FISH™ is a multicolor qualitative nucleic acid hybridization assay intended for the identification of *Enterococcus faecalis* and selected other enterococci from blood cultures.

2. Indication(s) for use:

   E. faecalis PNA FISH™ is a qualitative nucleic acid hybridization assay intended for identification of *Enterococcus faecalis* and selected other enterococci (OE) from blood cultures. *E. faecalis* PNA FISH™ is indicated for testing positive blood cultures with Gram-positive cocci in pairs and chains.

3. Special conditions for use statement(s):

   Prescription use only.

   Limitations: The following organisms are not detected by the PNA probe hybridizing to selected other enterococci: *Enterococcus asinii, E. dispar, E. haemoperoxidus, E. raffinosus, E. saccharolyticus, E. solitarius* and *E. sulfurous.*

   Enterococcus moraviensis is identified as *Enterococcus faecalis* due to sequence identity.

4. Special instrument requirements:

   AdvanDx Teflon-coated Microscope Slides. A fluorescent microscope equipped with an AdvanDx Dual Band Filter.

I. Device Description

The *E. faecalis* PNA FISH™ Culture Identification Kit contains a 3 mL bottle of fixation solution, a 1.5 mL bottle of fluorescein-labeled and rhodamine-labeled PNA probe in hybridization solution, a 50 mL bottle of concentrated wash solution, which must be diluted prior to use, and a 3 mL bottle of mounting medium. The one-well, Teflon-coated microscope slides are sold separately. User- prepared quality control organism slides are acceptable. After processing, the slides must be examined within two hours by using a fluorescent microscope equipped with a dual band filter available from the manufacturer.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   a. Conventional Methods-similar Indication for Use
   b. Gen Probe AccuProbe Enterococcus – similar Indication For Use
   c. *S. aureus* PNA FISH – similar technology

2. Predicate K number(s):
    a. K895103
    b. K060099
    Note: Pre-amendment devices were also referenced.

3. Comparison with predicate(s):

Item	Test Device	Pre-Amendment	Primary Predicate	Secondary Predicate
Product Name	*E. faecalis* PNA FISH	Conventional Routine Methods	Accuprobe Enterococcus	*S. aureus* PNA FISH
Intended Use	Identification of *Enterococcus faecalis* and selected other enterococci	Identification of enterococci from colonies isolated on solid media	Detection and identification of *Enterococcus* species	Identification of *Staphylococcus aureus*
Technology Method	Fluorescence in situ hybridization	Phenotypic, Biochemical	DNA probe hybridization	Fluorescence in situ hybridization
Time to result	2.5 hours from time of smear preparation	1 – 2 days, including subculture	18 - 24 hours including subculture	2.5 hours from time of smear preparation
Sample	Smear of blood cultures	Colonies isolated on solid media	Isolated cultures	Smear of blood cultures
Control organisms	Positive Controls: *E. faecalis,* and *E. faecium* Negative Controls: *Streptococcus* or *Staphylococcus*	Test dependent	*E. faecalis* and *S. bovis*	*S. aureus* and *S. epidermidis*
Mechanism of identification	*E. faecalis* specific 16S rRNA and also other enterococci - specific 16S rRNA	Test dependent	DNA capture and probe which utilizes nucleic acid hybridization	*S. aureus* specific 16 S rRNA
Interpretation of results	Qualitative fluorescent microscopy	Qualitative-Test dependent	Quantitative luminometer	Qualitative fluorescent microscopy

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

Not applicable

L. Test Principle:

Add one drop of *E. faecalis*-specific PNA probe (which contains both fluorescein-labeled *E. faecalis* PNA probe and rhodamine-labeled other *Enterococcus* spp. probe) to a methanol, heat, or flame fixed smear, prepared from liquid blood culture media with Gram-positive cocci in pairs or chains. Hybridization is performed during a 90 +/- 5 minutes incubation at 55 +/- 1° C, in an incubator or on a slide warmer. The slide is examined by fluorescent microscopy within two hours of staining. *Enterococcus faecalis* is identified as multiple bright green fluorescent cocci in multiple fields on a dark reddish background. Selected other *Enterococcus* species are identified as multiple bright red fluorescent cocci on a dark red background. Non-*Enterococcus* cells will not fluoresce.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

The performance data were generated by multiple, geographically distinct, clinical laboratories. Each laboratory compared the device results to corresponding results obtained by standard culture identification procedures. These laboratories reported the sensitivity and specificity results in peer reviewed literature articles, and during presentations performed at professional meetings (posters).

a. ***Precision/Reproducibility:***

Inter-laboratory and Intra-laboratory (one internal site and two external sites) testing demonstrated >95% reproducibility and precision. The ten isolate study was used (10 organisms tested 3 times on 3 days at 3 sites.).

b. ***Linearity/assay reportable range:***

Not applicable

c. ***Traceability, Stability, Expected values (controls, calibrators, or methods):***

The recommended Positive Control QC isolates -- *Enterococcus faecalis* and *Enterococcus faecium* -- may be combined onto one slide, or prepared as separate slides. Negative Control QC isolates can be either *Staphylococcus* species or *Streptococcus* species. User-prepared slides from stock or liquid blood cultures are recommended for testing in parallel for each batch of tests performed.

Liquid culture organism control slide testing was performed at three sites (one internal and two external sites) a sufficient number of times to demonstrate that the device can produce acceptable quality control results >95% of the time.

The stability of the *E. faecalis* PNA FISH reagents were evaluated using real-time data. The sponsor periodically removed samples from five lots of *E. faecalis* PNA FISH reagent from storage for testing. Both the fluorescein and the rhodamine

fluorescence over time were evaluated. The analytical fluorescence performance and a functional performance of each lot were tested 5 times over 30 months after the manufacturing date. The fluorescent dyes on the probes are light sensitive and are considered the least stable components of the kit. There was no change in performance for at least 24 months when stored at 2 - 8° C and protected from light, as indicated in the product labeling. This study is on-going and data are being collected to potentially extend the shelf life.

d. ***Detection limit:***

The claimed detection limit for *E. faecalis* in blood cultures was determined to be approximately 1 x 10^5 colony forming units (CFU) per mL by serial dilutions of an *E. faecalis* positive culture. This is consistent with the analytical sensitivity of slide-based staining techniques and is not limited by the test itself, but rather by the general requirement for 1 x 10^5 CFU/mL for interpretation by standard light microscopy.

e. ***Analytical specificity:***

Specificity of *E. faecalis* PNA FISH probe was evaluated using cultures of 16 selected *Enterococcus* spp. reference strains, excluding species listed under the Limitations section. The results are presented in the table below.

Species	**PNA FISH result**
Enterococcus faecalis	*E. faecalis*
Enterococcus faecium	Other Enterococci
E. avium, E casseliflavus, E. cecorum, E. columbae, E. durans, E. flavescens, E. gallinarium, E. hirae, E. malodoratus, E. mundtii, E. porcinus (reclassified as E. villorum), E. pseudoavium, E. rattii. E. villorum	Other Enterococci

The analytical specificity by organism group is *E. faecalis* at 100% (1/1) and for other Enterococci is 100% (15/15).

E. faecalis PNA FISH has also been evaluated on 24 reference strains representing Gram-positive cocci, other bacteria and yeast species. The analytical specificity for *E. faecalis* strains positive by PNA FISH was *E. faecalis* (3/3) 100%, selected other enterococci (6/6) 100% and the other species (15/15) were negative.

A BLAST search and Sequence Alignments search of the GeneBank nr-database (www.nim.nih.gov/blast) showed that the target sequence matched exactly for 61 *E. faecalis* 16S rRNA sequences stored in the database. The *E. faecalis* PNA probe and the other *Enterococcus* species PNA probe target ribosomal sequences, which are well-suited for the design of species-specific probes.

f. ***Assay cut-off:***

Not applicable

2. Comparison studies:

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

The performance of *E. faecalis* PNA FISH was evaluated using three different automated blood culture media systems at multiple sites. The data demonstrates that the *E. faecalis* PNA FISH™ is compatible with three major blood culture systems, and results from testing are comparable to results obtained by conventional methods. The results are displayed in the table below.

Automated blood culture system media evaluation comparing device results to results obtained by subculture and subsequent identification by standard methods, by study site

Blood Culture System Evaluated	Sensitivity E. faecalis	Sensitivity Other Enterococci	Specificity	Study
ESP	100% (26/26)	93.3% (14/15)	92.3% (12/13)	A
BACTEC, ESP, BacT/Alert	92.9% (26/28)	92.9% (13/14)	100% (79/79)	B
BACTEC	100% (16/16)	N/A (0/0)	100% 4/4	C
BacT/Alert	100% 14/14	N/A[1]	N/A[1]	D
BACTEC	100% (43/43)	100% (29/29)	100% (32/32)	E
Total	98.4% (125/127)	96.6% (56/58)	99.2% (127/128)	

BacT/Alert = BacT/Alert Blood culture system (bioMérieux, Durham, NC)

BACTEC = BACTEC Blood culture system (Becton Dickenson, Sparks, MD)

ESP = ESP Blood culture system (Trek Diagnostics, Cleveland, OH)

N/A[1] Data on 41 non-*E.faecalis* were reported as 100% specificity, but were not differentiated between other enterococci and non-enterococci.

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:

The expected *E. faecalis* positive result rate from positive blood culture bottles showing Gram-positive cocci in pairs and chains is 40% - 50%, where as selected other enterococci account for 15% - 25%, depending on institutional and patient population.

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.