

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K121991

B. Purpose for Submission:

To obtain 510(k) SE determination for the *Enterococcus QuickFISH* BC Identification Kit for the identification of *Enterococcus faecalis* and other selected enterococci from positive blood cultures.

C. Measurand:

Enterococcus faecalis-specific ribosomal RNA sequences and ribosomal RNA sequences specific to other selected *Enterococcus* species

D. Type of Test:

Qualitative test using fluorescence *In Situ* nucleic acid hybridization (FISH) with peptide nucleic acid (PNA) probes

E. Applicant:

AdvanDx Inc.

F. Proprietary and Established Names:

Enterococcus QuickFISH BC

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OAH	Class I	866.3740 Streptococcus spp. serological reagents	Microbiology (83)

H. Intended Use:

1. Intended use(s):

Enterococcus QuickFISH BC is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Enterococcus faecalis* and/or the detection of selected other enterococci on smears prepared from positive blood cultures containing gram positive cocci in pairs and chains observed on Gram stain.

Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing and/or differentiation of mixed growth.

Enterococcus QuickFISH BC is indicated in an aid in the diagnosis of bacteremia caused by enterococci.

2. Indication(s) for use:

Enterococcus QuickFISH BC is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Enterococcus faecalis* and/or the detection of selected other enterococci on smears prepared from positive blood cultures containing gram positive cocci in pairs and chains observed on Gram stain.

Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing and/or differentiation of mixed growth.

Enterococcus QuickFISH BC is indicated in an aid in the diagnosis of bacteremia caused by enterococci.

3. Special conditions for use statement(s):

For prescription use.

4. Special instrument requirements:

QuickFISH slides with controls (AdvanDx CS012)

AdvanDx SlideStation 10 (AdvanDx AC028)

Dual Band Microscope Filter (AdvanDx AC007)

Additional equipment necessary for performance of the assay includes a fluorescent microscope equipped with a 60X or 100X-oil objective.

I. Device Description:

Enterococcus QuickFISH BC is a fluorescence *in situ* hybridization (FISH) assay which uses a PNA probe to hybridize to *E. faecalis*-specific ribosomal RNA sequences, and a PNA probe to hybridize to ribosomal RNA of selected other *Enterococcus* species. The test provides rapid (20 minutes) identification of *E. faecalis* and other selected enterococci on smears made from positive blood cultures.

J. Substantial Equivalence Information:

1. Predicate device name(s):
E. faecalis/OE PNA FISH
2. Predicate 510(k) number(s):
K083074
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	<i>Enterococcus QuickFISH BC</i>	<i>E. faecalis</i> /OE PNA FISH (K083074)
Intended Use	<p><i>Enterococcus QuickFISH BC</i> is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of <i>Enterococcus faecalis</i> and/or the detection of selected other enterococci on smears prepared from positive blood cultures containing gram positive cocci in pairs and chains observed on Gram stain.</p> <p>Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing and/or differentiation of mixed growth.</p> <p><i>Enterococcus QuickFISH BC</i> is indicated in an aid in the diagnosis of bacteremia caused by enterococci.</p>	<p><i>E. faecalis</i>/OE PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of <i>Enterococcus faecalis</i> 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.</p> <p>Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.</p>

Similarities		
Item	Device	Predicate
	<i>Enterococcus QuickFISH BC</i>	<i>E. faecalis</i> /OE PNA FISH (K083074)
Indication for Use	<p><i>Enterococcus QuickFISH BC</i> is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of <i>Enterococcus faecalis</i> and/or the detection of selected other enterococci on smears prepared from positive blood cultures containing gram positive cocci in pairs and chains observed on Gram stain.</p> <p>Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing and/or differentiation of mixed growth.</p> <p><i>Enterococcus QuickFISH BC</i> is indicated in an aid in the diagnosis of bacteremia caused by enterococci.</p>	<p><i>E. faecalis</i>/OE PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of <i>Enterococcus faecalis</i> 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.</p> <p>Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.</p>
Technology	Fluorescence <i>in situ</i> hybridization using PNA probes	same
Sample type	Positive blood cultures from standard automated blood culture device	same
Interpretation of	Qualitative	same

Similarities		
Item	Device	Predicate
	<i>Enterococcus QuickFISH BC</i>	<i>E. faecalis</i> /OE PNA FISH (K083074)
Results	fluorescence microscopy	

Differences		
Item	<i>Enterococcus QuickFISH BC</i>	<i>E. faecalis</i> /OE PNA FISH (K083074)
Fixation Reagents	Fixation Two fixation solutions (<i>QuickFix</i> 1,2), at 55° C	One solution, at room temperature
Probe Reagents	PNA probes in 2 solutions: 1) <i>Enterococcus</i> PNA Blue contains 4 quenching probes 2) <i>Enterococcus</i> PNA Yellow contains 1 PNA probe for <i>E. faecalis</i> and 1 PNA probe for selected other enterococci	2 PNA probes in a single solution: one PNA probe for <i>E. faecalis</i> and 2 PNA probes for selected other enterococci
Wash Reagent	No wash solution	Wash solution with Tris, NaCl and Triton X-100
Mounting Reagent	Not needed	3 mL photobleaching inhibitor in glycerol
Hybridization Time	15-20 minutes	30 minutes
Time To Result	20 minutes	1.5 hours
Assay Total Time	20-25 minutes	1.5 hours
Assay controls	Positive and negative controls included on each slide	Positive and negative controls prepared separately

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

Not applicable

L. Test Principle:

The *QuickFISH* technology uses species-specific peptide nucleic acid (PNA) probes in a fluorescence *in situ* hybridization format. The probes are specific to rRNA targets which are present in high numbers in bacterial cells. The assay is performed directly on smears prepared from blood cultures that have been shown by Gram stain to contain gram-positive cocci in pairs and chains. A mixture of a fluorescein-labeled *E. faecalis*-specific PNA probe and a Tamra-labeled PNA probe targeting selected other enterococci is added to the smear. The probe mixture also contains quencher-labeled PNA probes that serve to bind unreacted fluorescent labeled probes to suppress unwanted signal. Hybridization is performed at 55° C for 15-20 minutes; smear is then ready for examination by fluorescent microscopy. While maintaining their cell morphology, *E. faecalis* or selected other *Enterococcus* species cells become fluorescent by the specific binding of the fluorophore-labeled PNA probes. The fluorescence microscopy is performed using a dual band microscope filter at 60 to 100X magnification. Using fluorescent microscopy, *E. faecalis* will appear as green cells and other selected enterococci will appear as red cells.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

A reproducibility study was performed using 14 isolates including *E. faecalis* (5 isolates – green positive), *E. faecium* (2 isolates – red positive), one isolate each of *E. gallinarum*, *E. casseliflavus*, and *E. raffinosus* (red positive), and one isolate each of *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Streptococcus agalactiae*, and *Streptococcus pyogenes* (red and green negative). Reproducibility was > 95%.

b. *Linearity/assay reportable range:*

Not applicable

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

Controls

Each *Enterococcus QuickFISH* BC test slide contains both positive and negative controls ensuring that controls are run with each patient specimen. The slides are provided in individually sealed pouches with nitrogen and a desiccant. Slides are stored at 2-8° C and must be used immediately after breaking the pouch seal and prior to the expiration date. The controls of the *Enterococcus QuickFISH* BC assay slides contain the following mixtures of organisms specific for the *Enterococcus* test:

Positive Controls	Expected Result
<i>E. faecalis</i> ATCC 29212	Green Positive
<i>E. faecium</i> ATCC 27270	Red Positive
Negative Controls	Expected Result
<i>Micrococcus luteus</i> ATCC 10240	No fluorescence
<i>Cryptococcus neoformans</i> ATCC 204092	No fluorescence
<i>Klebsiella oxytoca</i> ATCC43086	No fluorescence
<i>Streptococcus pyogenes</i> ATCC 12384	No fluorescence

Fixed Smear Stability

The stability of the slides prepared for the *Enterococcus QuickFISH* BC assay were analyzed under a variety of conditions to support the labeling claim that fixed smears can be kept at room temperature for 1 hour prior to initiation of testing by the *Enterococcus QuickFISH* assay or stored at 2 – 8° C for up to one day before initiation of testing. The evaluation included 6 strains of *E. faecalis*, 2 strains of *E. faecium*, 4 strains of other enterococcal species, 1 strain of *Staphylococcus* and 3 species of streptococci (not enterococci). Slides were prepared for each strain and read at time zero and 5 minutes at 55° C; 5, 15 and 30 minutes at room temperature; and at 4, 18 and 24 hours at 2 - 8° C. Controls were run with each test and gave the expected results.

The data demonstrated that fixed *Enterococcus QuickFISH* smears were stable at 55° C for 5 minutes, at room temperature for 1 hour, and for 24 hours at 2-8° C.

d. Detection limit:

The detection limit for the *Enterococcus QuickFISH* was determined to be approximately 1-2 X 10⁵ CFU/mL. Half-log serial dilutions of aliquots from positive blood cultures containing *E. faecalis* and *E. faecium* were prepared using blood/culture media. Aliquots of each dilution were plated in triplicate to determine the average concentration of organisms in each aliquot (CFU/mL); each aliquot was tested using the *Enterococcus QuickFISH* BC assay.

Co-Infection Studies

Co-infection studies were performed for the *Enterococcus QuickFISH* BC assay using growth from BacT/ALERT SA blood culture bottles with sterile human blood added. The concentration of the target organism (*E. faecalis*) was tested at or near the LoD while the competing organisms were introduced at increasing 10-fold concentrations (*E. faecium* 2.0 X 10³ to 2.0 X 10¹⁰; *Streptococcus mitis*, 6.4 X 10² to 6.4 X 10⁹; *Escherichia coli*, 8.0 X 10³ to 8.0 X 10¹⁰ and *Candida albicans*, 1.2 X 10¹ to 1.2 x 10⁷). Results demonstrated that the target organism was detected in the presence of even high concentrations of competing organisms.

Analytical Sensitivity

The sensitivity of the *Enterococcus QuickFISH* was tested with a variety of *Enterococcus* species. All 16 *Enterococcus faecalis* isolates tested gave the expected green positive result. Three species of *Enterococcus* gave false green positive results: *Enterococcus caccae*, *Enterococcus haemoperoxidus* and *Enterococcus moraviensis*. Fourteen species of *Enterococcus* gave red positive results and 7 species gave negative results with both the green and red probe. See tables below.

The following table lists the *Enterococcus* species that gave green positive results:

Species	Strain ID	<i>Enterococcus QuickFISH BC</i>
<i>Enterococcus faecalis</i>	ATCC 51299	Green
<i>Enterococcus faecalis</i>	NCTC 775	Green
<i>Enterococcus faecalis</i>	ATCC 19433	Green
<i>Enterococcus faecalis</i>	NCIMB 13280	Green
<i>Enterococcus faecalis</i>	Clinical isolate	Green
<i>Enterococcus faecalis</i>	Clinical isolate	Green
<i>Enterococcus faecalis</i>	ATCC 29212	Green
<i>Enterococcus faecalis</i>	ATCC 49533	Green
<i>Enterococcus faecalis</i>	ATCC 14506	Green
<i>Enterococcus faecalis</i>	ATCC 51188	Green
<i>Enterococcus faecalis</i>	ATCC 7080	Green
<i>Enterococcus faecalis</i>	ATCC 49532	Green
<i>Enterococcus faecalis</i>	ATCC 49452	Green
<i>Enterococcus faecalis</i>	ATCC 33186	Green
<i>Enterococcus faecalis</i>	ATCC 13280	Green
<i>Enterococcus faecalis</i>	NCTC 13379	Green

The following table lists the *Enterococcus* species that gave false green positive results:

Species	Strain ID	<i>Enterococcus QuickFISH BC</i>
<i>Enterococcus caccae</i>	ATCC BAA-1240	Green
<i>Enterococcus haemoperoxidus</i>	ATCC BAA-382	Green
<i>Enterococcus moraviensis</i>	ATCC BAA-383	Green

The following table lists the *Enterococcus* species that gave red positive results:

Species	Strain ID	<i>Enterococcus QuickFISH BC</i>
<i>Enterococcus faecium</i>	ATCC 27270	Red
<i>Enterococcus faecium</i>	ATCC 35667	Red
<i>Enterococcus faecium</i>	ATCC 51559	Red
<i>Enterococcus faecium</i>	ATCC 19434	Red
<i>Enterococcus faecium</i>	ATCC 49224	Red
<i>Enterococcus faecium</i>	ATCC BAA-472	Red
<i>Enterococcus faecium</i>	ATCC 51858	Red
<i>Enterococcus faecium</i>	ATCC 6569	Red
<i>Enterococcus flavescens</i>	ATCC 49996	Red
<i>Enterococcus avium</i>	ATCC 49463	Red
<i>Enterococcus casseliflavus</i>	ATCC25788	Red
<i>Enterococcus durans</i>	ATCC 6056	Red
<i>Enterococcus gallinarum</i>	ATCC 49573	Red
<i>Enterococcus gilvus</i>	ATCC BAA-350	Red
<i>Enterococcus hirae</i>	ATCC 8043	Red
<i>Enterococcus hirae</i>	ATCC 49135	Red
<i>Enterococcus malodoratus</i>	ATCC 43197	Red
<i>Enterococcus mundtii</i>	ATCC 43187	Red
<i>Enterococcus phoeniculicola</i>	ATCC BAA-412	Red
<i>Enterococcus raffinosus</i>	ATCC 49464	Red
<i>Enterococcus ratti</i>	ATCC 700914	Red
<i>Enterococcus villorum</i>	ATCC 700913	Red

The following table lists the *Enterococcus* species that gave negative results with both the green and red probes:

Species	Strain ID	<i>Enterococcus QuickFISH BC</i>
<i>Enterococcus asini</i>	ATCC700915	Negative
<i>Enterococcus cecorum</i>	ATCC BAA-597	Negative
<i>Enterococcus columbae</i>	ATCC 51263	Negative
<i>Enterococcus dispar</i>	ATCC51266	Negative
<i>Enterococcus pallens</i>	ATCC BAA-351	Negative
<i>Enterococcus saccharolyticus</i>	ATCC 43076	Negative
<i>Enterococcus sulfureus</i>	ATCC 49903	Negative

e. *Analytical specificity:*

The specificity of the *Enterococcus QuickFISH* BC assay was determined using a panel of 76 gram positive (non-enterococci) and gram negative organisms and 6 *Candida* species. All isolates tested negative with the assay with the exception of *Granicatella adiacens* which gave a fluorescent orange signal. In addition weak green signals were seen with *Enterobacter cloacae*, *Proteus mirabilis*, *Granicatella elegans* and 2 strains of *Streptococcus anginosus*.

f. *Assay cut-off:*

Not applicable

g. *Media Interference Studies*

A media compatibility study was performed using the following blood culture bottle types: BacT/Alert (SA and SN), BACTEC (Lytic 10, Aerobic Plus, Anaerobic Plus, PEDS Plus, Standard 10 Aerobic, Standard Anaerobic) and VersaTREK REDOX-1 Aerobic. Organisms used included 6 strains of *E. faecalis*, 2 strains of *E. faecium* as well as 4 strains of additional *Enterococcus* species expected to give positive results, one strain of *Staphylococcus epidermidis* and 3 *Streptococcus* species. Results showed the *Enterococcus QuickFISH* to be compatible with all of the above mentioned blood culture bottle types.

The *Enterococcus QuickFISH* is not compatible with blood culture media containing charcoal or with VersaTREK REDOX 2 blood culture bottles.

h. *Validation studies:*

Not applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Not Applicable

3. Clinical studies:

The performance of the *Enterococcus QuickFISH* BC assay was compared to results obtained from routine identification methods for the identification of enterococci from blood cultures. The clinical studies were performed at 5 clinical laboratory sites in the U.S. A total of 244 blood culture bottles growing gram positive cocci in pairs and chains (as assessed by Gram stain) from 244 patients were included in the study. One blood culture contained a mixture of 2 species of *Enterococcus* (one red positive and one green positive) to provide a total of 245 test results. Blood cultures were performed using two commercially available, continuously monitoring blood

culture systems, BacT/ALERT (BioMérieux) and BACTEC (Becton Dickinson and Co). The identification comparator methods included BD Phoenix Automated Microbiology System (1 site), Siemens Microscan (1 site) and BioMérieux VITEK (3 sites). The positive agreement for *E. faecalis* was 100% (70/70). For other enterococci the positive agreement was 97.5% (39/40). Negative percent agreement was 100% (135/135).

Performance Data for *Enterococcus QuickFISH* BC vs. Reference Identification Methods on Blood Cultures Positive with Gram-Positive Cocci in Pairs and Chains (all sites)

<i>Enterococcus QuickFISH</i> BC	Reference Method		
	<i>E. faecalis</i>	Other Enterococci	Other (non enterococci) ³
<i>E. faecalis</i>	70	0	0
Other Enterococci	0	39	0
Negative	0	1 ¹	135
Total	Positive Percent Agreement 100% (70/70) ² 95% CI (94.8 – 100)	Positive Percent Agreement 97.5% (39/40) ² 95% CI (87.5 – 99.6)	Negative Percent Agreement 100% (135/135) 95% CI (97.2 – 100)

¹ One false negative sample (tested 1 hour and 15 minutes from the time of Gram stain) was a mixed culture comprised of *E. faecium*, methicillin resistant *Staphylococcus aureus* and *Klebsiella pneumoniae*. Repeat testing one week later was weak red positive.

² Includes 1 mixed culture comprised of *E. faecalis*, *E. gallinarum* and *Serratia marcescens*

³ Other organisms included: *Streptococcus* species (unspeciated Alpha hemolytic streptococci, unspeciated Beta hemolytic streptococci, *S. agalactiae*, *S. anginosus*, *S. constellatus*, *S. gallolyticus*, *S. gordonii*, *S. intermedius*, *S. mitis*, *S. mutans*, *S. oralis*, *S. parasanguinis*, *S. pneumoniae*, *S. pyogenes*, *S. salivarius*, *S. sanguinis*, *S. viridans*), *Staphylococcus* species (*S. aureus*, *S. capitis*, *S. epidermidis*, *S. hominis*, and other unspeciated coagulase negative staphylococci), *Lactococcus lactis*, *Lactococcus raffinolactis*, *Leuconostoc spp*, *Granulicatella adiacens*, unspeciated anaerobic gram positive cocci, and gram negative rods in mixed culture with gram positive cocci in pairs and chains.

In the clinical studies, bottles were stored at room temperature after Gram stain

and before *Enterococcus QuickFISH* testing. The time between Gram stain and preparation of the *Enterococcus QuickFISH* slides varied from less than 2 hours to greater than 48 hours. There was only one test discrepancy in the study (1/244). This sample was from a mixed culture and was tested within 2 hours of Gram stain.

Performance Data for *Enterococcus QuickFISH* BC vs. Reference Identification Methods by Blood Culture Bottle Types (all samples)

Bottle Type	Positive Percent Agreement <i>E. faecalis</i>	Positive Percent Agreement other enterococci	Negative Percent Agreement
Total BACTEC (BACTEC Plus Aerobic and BACTEC Lytic/10 Anaerobic)	100% (42/42) 95% CI (91.6 – 100)	95.8% (23/24) 95% CI (79.8 – 99.3)	100% (74/74) 95% CI (95.1 – 100)
Total BacT/ALERT (BacT/ALERT SA Aerobic and BacT/ALERT SN Anaerobic)	100% (23/23) 95% CI (85.7 – 100)	100% (13/13) 95% CI (77.2 – 100)	100% (45/45) 95% CI (92.1 – 100)

a. *Clinical Sensitivity:*

See table above

b. *Clinical specificity:*

See 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:

Enterococcus faecalis: multiple bright green fluorescent cocci in multiple fields of view.

Selected other enterococci: multiple bright red fluorescent cocci in multiple fields of view.

Non-enterococci and species of enterococci not identified by this assay appear non-fluorescent.

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.