E. faecalis/OE PNA FISH®

Enterococcus faecalis/Other enterococci Culture Identification Kit

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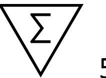
 50

IVD

REF KT003

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

IVD For *in vitro* diagnostic use.

Summary and Explanation

In recent years, enterococci have emerged as important causes of nosocomial and community infections.

Identification of enterococci in blood cultures is routinely based on presumptive identification as Gram-positive cocci in pairs or chains (GPCPC) followed by final identification after subculture and biochemical analysis (1).

E. faecalis/OE PNA FISH is a multicolor fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to *E. faecalis*-specific ribosomal RNA sequences and to ribosomal RNA sequences of other *Enterococcus* species.

The test provides rapid identification of *E. faecalis* and selected other enterococci (OE) on smears made from positive blood cultures.

Principle of the Procedure

A mixture of a fluorescein-labeled *E. faecalis*-specific PNA probe and a rhodamine-labeled PNA probe specific for other enterococci (OE) is added to a smear prepared from a positive blood culture. Hybridization is performed at 55°C for 30 min. 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.

Reagents

E. faecalis/OE PNA FISH is comprised of the following kit components:

Fixation Solution	**Fixation Solution** 3 mL phosphate-buffered saline with detergent.
***E. faecalis*/OE PNA**	***E. faecalis*/OE PNA** 1.5 mL PNA probes in hybridization. solution. Contains 30% formamide.
60x Wash Solution	**60x Wash Solution** 50 mL Tris-buffered saline with detergent
Mounting Medium	**Mounting Medium** 3 mL photobleaching inhibitor in glycerol.

Precautions

IVD For *in vitro* diagnostic use.

For professional use only, by personnel trained in laboratory techniques and experienced in fluorescence microscopy.

Safety Precautions

E. faecalis/OE PNA	Danger Contains 30% Formamide	May cause harm to the unborn child. Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.
Fixation Solution	Warning	Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.
60X Wash Solution	Danger Contains polyethylene glycol octylpheno ether	Causes skin irritation. Causes serious eye damage. Toxic to aquatic life with long lasting effects. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.
Mounting Medium	Warning Propyl 3,4,5-trihydroxybenzoate	May cause an allergic reaction. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.

Establish precautions against microbiological hazards.

Do not eat, drink, smoke, apply cosmetics, store or prepare foods within the designated work area.

Dispose of reagents in accordance with federal, state and local regulations.

Technical Precautions

Reagents must not be used after the expiration dates printed on the labels.

Reagents are provided at fixed concentrations. Assay performance may be affected if the reagents are modified in any way or are not stored under the recommended conditions as detailed in “Storage of Kit Components”.

Avoid microbial contamination of reagents.

Avoid any cross-contamination of samples and reagents, as this may give rise to erroneous results.

Do not allow dropper bottle tip to touch the smear as this may cause cross contamination of material between slides, or cause contamination of the reagent.

Do not use filters other than the Dual Band Filter (AC003 or AC007).

Do not use microscope slides other than the Microscope Slides (AC001).

It is important that the microscope is functioning properly. Make sure that the microscope bulb is correctly adjusted and has not aged beyond its specified lifetime.

Storage and Preparation of Kit Components

To ensure optimal kit performance, it is important that kit components are stored and prepared according to the following instructions:

Storage

Store kit components at 2-8°C. Place kit components at room temperature prior to use and return the kit components to 2-8°C after use.

Preparation of Wash Solution

Prepare working strength Wash Solution by adding 4 mL of 60x Wash Solution followed by 240 mL of fresh deionized or distilled water directly to the Staining Dish. Store remaining concentrate at 2-8°C.

Preparation of Mounting Medium

The Mounting Medium should be left at room temperature for at least 5 min. before use.

Specimen Collection and Preparation

Preparation of Smears

- Follow the blood culture system manufacturer's instruction to properly mix the blood culture bottle before smear preparation. Follow established laboratory procedures on sub-culturing of positive blood culture.
- Place one drop of Fixation Solution on a well on the microscope slide.
- Transfer 10 µL or a small drop from a ventilation needle of a blood culture to the Fixation Solution and mix gently to emulsify.
- Fix the smears by either heating them for 20 min. at 55-80°C or allow the smears to dry and fix them by methanol-fixation or by flame-fixation.

Test Procedure

Material Provided

E. faecalis/OE PNA FISH® KT003

Each kit contains sufficient material for 50 tests. Reagents are supplied ready for use except where indicated. The expiration date of the kit is as indicated on the outer box label.

Material Required and Available from AdvanDx

Item	Description	Code
Microscope Slides	1-well microscope slides.	AC001
Coverslips	Coverslips, 22 x 22 mm, Thickness: 0.15 mm.	AC002
Dual Band Filter	Dual band filter.	AC003 or AC007
Staining Dish	Staining dish with cover and slide holder.	AC004
PNA FISH Workstation	Slide warmer (55 ± 1°C).	AC005
Water Bath	Water Bath (55 ± 1°C).	AC006
***E. faecalis*/OE Control Slide**	*E. faecalis*/OE Control Slide.	CS003

Contains a positive control prepared from liquid culture containing a mixture of *E. faecalis,* ATCC 19433, and *E. faecium,* ATCC 19434; and a negative control prepared from liquid culture of *S. agalactiae,* ATCC 13813.

Material Required but Not Provided

- Water, deionized or distilled.
- Fluorescence microscope equipped with a 60x or 100x oil objective.
- Immersion oil. Must comply with the microscope objective and be non-fluorescent.

Assay Procedure

All steps are performed at room temperature unless otherwise stated.

Before starting the assay procedure, prepare working strength Wash Solution in the Staining Dish, add cover and start preheating in the water bath (55 ± 1°C). Do not reuse Wash Solution, but prepare fresh working strength Wash Solution for each run.

Hybridization

- Add one drop of *E. faecalis*/OE PNA to the well on the microscope slide with the smear.
- Add coverslip. Avoid air bubbles. Use a sterile loop to remove resin beads if needed.
- Incubate for 30 ± 5 min. at 55 ± 1°C.

Stringent Wash

- Immerse slide in preheated Wash Solution at 55°C and carefully remove the coverslip. Often, the coverslip slides off by gently agitating the slide in the Wash Solution. Occasionally, the coverslip must be pushed off with forceps.
- Incubate for 30 ± 5 min. at 55 ± 1°C.
- Allow the slide to air dry

Mounting

- Add one drop of Mounting Medium to the smear.
- Add coverslip. Avoid air bubbles.
- Examine slide as described below within 2 hours.
- Do not expose the slides to direct sun light or other strong light sources as this may lead to fluorescence quenching.

Quality Control

Control material should be tested in accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations, including controls grown in liquid media.

Quality control for fluorescent testing should be done each time testing is performed. The QC results should be able to monitor for appropriate testing conditions, particularly those affecting hybridization stringency and cell wall penetration, since PNA methodology is designed to optimize cell wall penetration.

Use *E. faecalis/*OE Control Slide (CS003) or prepare smears from liquid cultures of laboratory or reference strains of *E. faecalis* and *E. faecium* as Positive Control either on separate slides or mixed on one slide and *Staphylococcus* spp or *Streptococcus* spp as Negative Control as described above under Preparation of Smears, Specimen Collection and Preparation. The smears may be stored for up to 1 month at room temperature. When using an AdvanDx *E. faecalis*/OE Control Slide (CS003), simply remove slide from pouch and follow the PNA FISH procedure starting with the hybridization step.

The performance of the Positive Control has been demonstrated by using *E. faecium* and *E. faecalis* present on the same slide, and separated onto individual positive control slides for each organism.

E. faecalis must test green-positive and *E. faecium* must test red-positive in accordance with the Interpretation of Results below.

Procedural Notes

Preparation of Smears:
It is recommended to use the same type of fixation (heat, methanol or flame fixation) that is used for Gram-staining. To reduce the reporting time, smears for PNA FISH may be prepared in parallel with smears for Gram-staining.

Note: Fixation Solution is designed for optimal performance in the identification of Gram-positive bacteria and Yeast and must not be interchanged with GN Fixation Solution from other PNA FISH tests for Gram-negative bacteria.

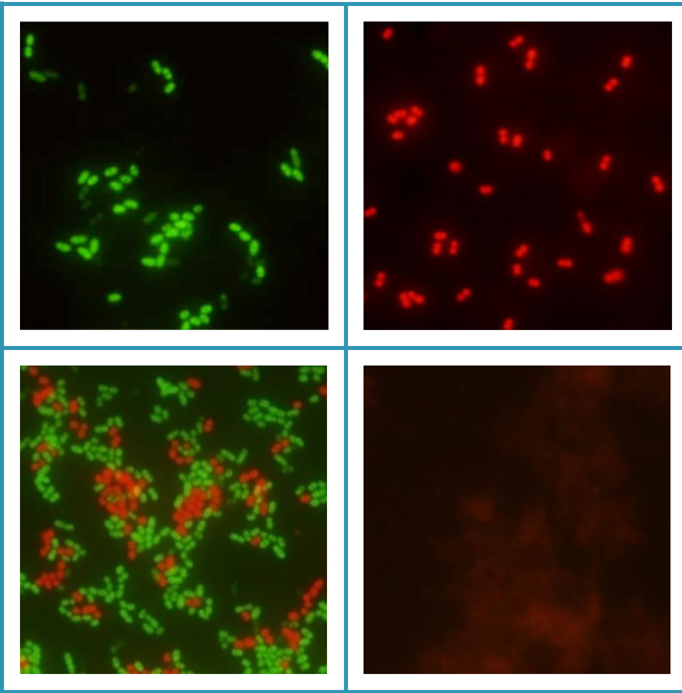
Temperature Control:
It is important that the temperature of the PNA FISH Workstation has reached 55°C prior to starting the hybridization and that Wash Solution has reached 55°C prior to immersion of the slides. The temperature of the Water Bath should be checked by using a thermometer as instrument readings may not always be accurate.

Parallel Testing Using Different PNA FISH Tests:
The PNA FISH kits are designed for parallel testing. 60x Wash Solution and Mounting Medium are identical and may be interchanged between different tests.

Major Blood Culture Systems and Bottle Type Compatibility:
The PNA FISH platform is compatible with the commercially available continuously monitoring blood culture systems and bottle types, including those which are supplemented with charcoal, resins and/or sodium polyanetholesulfonate.

Interpretation of Results

Examine slides using a fluorescence microscope. The blood culture smear appears in general reddish. *E. faecalis* is identified as multiple bright green fluorescent cocci in multiple fields of view, whereas other enterococci (OE) are identified as multiple bright red fluorescent cocci in multiple fields of view. Definitive identification is pending subculture and additional microbiological evaluation. Non-enterococci appear non-fluorescent.



Representative examples of green-positive *E. faecalis* (top-left), red-positive OE (top-right), mixture of green-positive *E. faecalis* and red-positive OE (bottom-left) and negative (bottom-right) test results.

Troubleshooting

False positive Control and Sample test results may occur if the Dual Band Filter (AC003 or AC007) is not used, or by contamination of the specimens.

False negative Control or Sample test results may occur if AdvanDx Microscope Slides (AC001) are not used or if the temperature is not accurately controlled during hybridization and washing.

Please refer to the Precautions and Limitations sections in this product insert or contact AdvanDx.

Limitations

Some rare-occurring *Enterococcus* species are not detected by the PNA probe hybridizing to other *Enterococcus* species, such as *E. asinii, E. dispar, E. haemoperoxidus, E. cecorum, E. columbae, E. saccharolyticus, E. solitarius* and *E. sulfurous*.

E. moraviensis is identified as *E. faecalis* due to sequence identity (2). Some strains of *Streptococcus anginosus* produce a false green positive fluorescence due to sequence similarities.

VersaTREK Blood Culture bottles were not evaluated during the clinical studies. The performance to detect *E. faecalis* and other *Enterococcus* spp. with VersaTREK blood culture bottles is unknown.

False positive green autofluorescence may occur if a standard FITC filter is used instead of the Dual Band Filter (AC003 or AC007).

False negative results may infrequently occur due to mixed growth or due to error in assay technique.

The type and condition of the instrumentation used will influence the visual appearance of the image obtained. The fluorescence may vary due to the type of microscope employed, the light source and the level of rRNA in the cells. Each laboratory should establish its own criteria for reading the results using appropriate controls.

Isolation on solid media is needed to differentiate mixed growth with other organisms and to identify positive blood cultures yielding a negative result

The product has not been validated with specimens other than blood cultures.

Expected Results

The expected *E. faecalis* positive result rate from GPCPC positive blood culture bottles is 40-50% whereas other enterococci account for 15-25% depending on institutional and patient population (2-6).

Performance Characteristics

Clinical Studies

The performance of *E. faecalis*/OE PNA FISH using the modified, shortened assay procedure verses the original assay procedure has been assessed in three (3) clinical laboratory studies.

A total of 152 routine GPCPC-positive blood culture bottles were included in the studies. The results showed 100% (152/152), 95% CI (98.1-100), agreement between results obtained with shortened and with the original assay procedure for *E. faecalis*/OE PNA FISH. These included two commercially available, continuously monitoring blood culture systems (BacT/ALERT, bioMérieux, NC; BACTEC, Becton Dickinson, MD)

Agreement Data for *E. faecalis*/OE PNA FISH with Shortened Assay Procedure (30 minutes hybridization, and without 5-10 minutes ethanol treatment) vs. Original Assay Procedure on GPCPC-Positive Blood Culture Bottles

Study	Positive Agreement (Green)	Positive Agreement (Red)	Negative Agreement	Blood Culture System Used in Study
A	100% (19/19)	100% (13/13)	100% (18/18)	BACTEC

B	100% (9/9)	100% (12/12)	100% (31/31)	BacT/ALERT
C	100% (13/13)	100% (8/8)	100% (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)	

Analytical Sensitivity
The detection limit for *E. faecalis* was determined to be approximately 10^5 colony-forming units per mL by serial dilutions of an *E. faecalis*-positive culture. This is consistent with the analytical sensitivity of slide-based staining techniques.
Analytical Specificity
E. faecalis/OE PNA FISH has been evaluated on 33 selected *Enterococcus* species, and correctly identified *E. faecalis* (11/11) green-positive and most (18/22) other enterococci were red-positive.

E. faecalis/OE PNA FISH assay has been tested on 78 laboratory and reference strains comprising 11 *E. faecalis*, 22 other enterococci, 17 Gram negative organisms, 21 Gram positive organisms and 7 yeasts representing phylogenetically closely related organisms and a variety of clinically significant organisms.

Bibliography

1. **Baron, E. J.** 1998. Processing and interpretation of blood cultures, chap. 2.3. In: H.D. Isenberg (Ed.) Essential procedures for clinical microbiology, ASM Press, Washington DC.
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3. **Chapin, K.C., M. C. Musgnug, K. Oliveira, P. C. DeGirolami, J. Dakos, J. T. Johansen, G. W. Procop, D. Wilson, E. Padilla, V. Gonzalez, and H. Stender.** 2003. Multicenter evaluation of *E. faecalis* PNA FISH for rapid identification and differentiation of *Enterococcus faecalis* and other *Enterococcus* species directly from positive blood cultures. Abstract # C-163, 103rd Annual Meeting of American Society for Microbiology, Washington DC.
4. **Rausei, V., E. Youssef, and M. Morgan.** 2005. Direct identification of *Staphylococcus aureus*, *Enterococcus faecalis*, and *Candida albicans* from blood cultures using fluorescence in situ hybridization with nucleic acid probes. Abstract # V-002, 104th Annual Meeting of American Society for Microbiology, New Orleans, LA.
5. **Novak-Weekley, S. M., M. LaForga, L. Carey, and L. Frazier.** 2006. Validation of *S. aureus* PNA FISH, *E. faecalis* PNA FISH & *C. albicans* PNA FISH for Rapid Identification of Positive Blood Culture Bottles. Abstract #C-007, 106th Annual Meeting of American Society for Microbiology, Orlando, FL.
6. **Johnson, J. K., A. A. Roberts, G. N. Forrest, D. P. Lincalis, and R. A. Venezia.** 2006. Rapid Identification of *Enterococcus* Species in Positive Blood Cultures with Therapeutic Implications. Abstract #C-147, 106th Annual Meeting of American Society for Microbiology, Orlando, FL.

Definitions

Symbol	Meaning	Symbol	Meaning
REF	Product code/catalog number	LOT	Batch code
	Consult the instructions for use		Storage temperature limitations
Σ	Contains sufficient for <n> tests		Health Hazard
	Manufacturer		Exclamation Mark
EC REP	Authorized representative		Corrosion
	Use by		Environment

Technical Advice and Customer Service

For all inquiries, please contact OpGen or your local distributor.

OpGen, Inc.
708 Quince Orchard Rd
Gaithersburg, MD 20878
USA

Tel: +1 301 869 9683
Fax: +1 301 869 9684

techsupport@opgen.com

EC REP
OpGen A/S
Gungevej 2, 2. Sal
2650 Hvidovre
Denmark

Tel: +45 45 16 07 99
Fax: +45 45 16 07 98

www.OpGen.com

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The product must not be used for Slide-Based human Cytochemistry, ISH-based Cancer Cytogenetics and Flow Cytometry.

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DCN 26-18

Purchase of this kit licenses its use under Patent numbers: US 5,985,563; US 5,888,733; US 6,395,474; US 6,357,163; US 5,539,082; US 7,223,833; EP 862,650; EP 804,456