

E. coli/*P. aeruginosa* PNA FISH®

Escherichia coli/*Pseudomonas aeruginosa* Culture Identification Kit



REF KT007



Intended Use

E. coli/*P. aeruginosa* PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for identification of *Escherichia coli* and *Pseudomonas aeruginosa* on smears from positive blood cultures containing Gram-negative rods. The *E. coli*/*P. aeruginosa* PNA FISH assay is indicated for use as an aid in the diagnosis of *E. coli* and/or *P. aeruginosa* bacteremia.

Subculturing of positive blood cultures is necessary for susceptibility testing and/or differentiation of mixed growth.

IVD For *in vitro* diagnostic use.

Summary and Explanation

E. coli and *P. aeruginosa* are recognized as causes of both community and hospital acquired bacteremia.

Identification of *E. coli* and *P. aeruginosa* in blood cultures are routinely based on presumptive identification as Gram-negative rods followed by final identification after subculture and biochemical analysis (1).

E. coli/*P. aeruginosa* PNA FISH is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to specific ribosomal RNA sequences of *E. coli* and *P. aeruginosa*.

The test provides rapid (within 1.5 hours) identification of *E. coli* and *P. aeruginosa* on smears made from positive blood cultures.

Principle of the Procedure

A mixture of a fluorescein-labeled, *E. coli* specific PNA probe and a Texas Red-labeled, *P. aeruginosa* specific PNA probe is added to a smear prepared from a culture. Hybridization is performed at 55°C for 30 min. The hybridization is followed by a water rinse at 55°C to remove the cover slips followed by a 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. coli/*P. aeruginosa* PNA FISH is comprised of the following kit components:

GN Fixation Solution

GN Fixation Solution

3 mL phosphate-buffered saline with detergent.

E. coli/*P. aeruginosa* PNA

E. coli/*P. aeruginosa* 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

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

Safety Precautions

The *E. coli*/*P. aeruginosa* PNA contains 30% formamide. May cause harm to the unborn child. Keep out of reach of children. Avoid exposure - obtain special instructions before use. Material Safety Data Sheet is available upon request. Formamide is non-hazardous once diluted into Wash Solution during the wash step.

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 other filters than the Dual Band Filter (AC003).

Do not use other microscope slides 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 Rinse Water

Add 200 mL distilled or deionized water into a Staining Dish.

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. Prepare fresh working strength Wash Solution as required for each run. 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

- Place one drop of GN Fixation Solution on a well on the microscope slide (AC001).
- Transfer 10 µL or a small drop from a ventilation needle of culture to the Fixation Solution and mix gently to emulsify.
- Fix the smears by either heating them for 20 min. at 55°C - 80°C, or allow the smears to dry and fix them by methanol-fixation or by flame-fixation.

Test Procedure

Material Provided

- E. coli/P. aeruginosa* PNA FISH KT007
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

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
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
<i>E. coli/P. aeruginosa</i> Control Slide		CS007

E. coli/P. aeruginosa Control Slide (CS007). Contains a positive control prepared from liquid culture containing a mixture of *E. coli*, ATCC# 11775, and *P. aeruginosa*, ATCC# 10145, and negative control prepared from liquid culture of *K. pneumoniae*, ATCC# 13883.

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 Rinse Water and working strength Wash Solution in two separate Staining Dishes, add cover and start preheating in the water bath (55 ± 1°C). Do not reuse Rinse Water and Wash Solution, but prepare fresh Rinse Water and working strength Wash Solution for each run.

Hybridization

- Add one drop of *E. coli/P. aeruginosa* 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.

Water Rinse

- Transfer slide to slide rack
- Immerse slide in preheated Rinse Water at 55°C for < 1 min to carefully remove the coverslip. Often, the coverslip slides off by gently agitating the slide in the Rinse Water. Occasionally, the coverslip must be pushed off with forceps.

Stringent Wash

- Transfer slide rack to preheated Wash Solution at 55°C.
- 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 AdvanDx *E. coli/P. aeruginosa* Control Slide (CS007) or prepare smears from cultures of laboratory or reference strains of *E. coli* and *P. aeruginosa* as Positive Controls either on separate slides or mixed on one slide and *Klebsiella spp.* as Negative Control as described above under Specimen Collection and Preparation. The smears may be stored for up to 1 month at room temperature. Do not expose smears to high humidity as that will cause the formation of visible crystals which are associated with reduced shelf life. When using an *E. coli/P. aeruginosa* Control Slide (CS007) simply remove slide from pouch and follow the PNA FISH procedure starting with the hybridization step.

E. coli must test green-positive and *P. aeruginosa* must test red-positive in accordance with the Interpretation of Results.

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.

Temperature Control:

It is important that the temperature of the PNA FISH Workstation has reached 55°C prior to starting the hybridization and that Rinse Water and Wash Solution has reached 55°C prior to immersion of the slides. The temperature of the Water Bath should be checked using a thermometer as outside temperature 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.

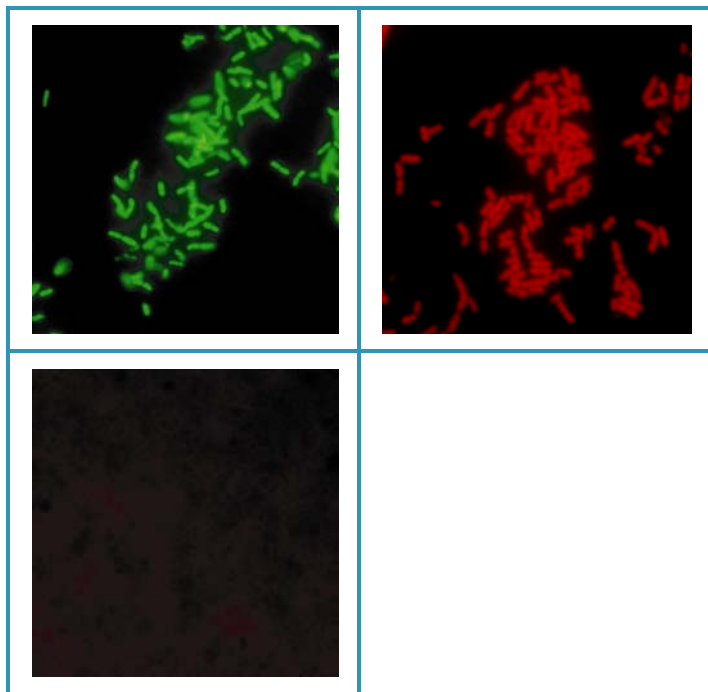
GN Fixation Solution is designed for optimal performance in the identification of Gram-negative bacteria and must not be interchanged with Fixation Solution from other PNA FISH tests for Gram-positive bacteria and yeast.

Major Blood Culture Systems and Bottle Type Compatibility:

The PNA FISH platform is compatible with the major commercially available continuous 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 smear appears in general reddish. *E. coli* is identified as multiple bright green fluorescent rods in multiple fields of view, whereas *P. aeruginosa* is identified as multiple bright red fluorescent rods in multiple fields of view. Other microorganisms appear non-fluorescent.



Representative examples of green-positive *E. coli* (top-left), red-positive *P. aeruginosa* (top-right), and negative (bottom-left) test results.

Definitive identification is pending positive blood subculture, additional microbiological evaluation and antimicrobial susceptibility testing.

Troubleshooting

- False positive Control and Sample test results may occur if the Dual Band Filter (AC003) 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 Precaution and Limitation sections in this product insert or contact AdvanDx.

Limitations

Green positive results will occur with *Shigella* spp. (serogroup A, B, C or D), *Escherichia albertii* and *Escherichia fergusonii* due to sequence similarity.

Red positive results will occur with *Brevundimonas diminuta*, *Herbaspirillum huttiense*, *Pseudomonas nitroreducens*, *Pseudomonas fulva* and *Acinetobacter radioresistens*.

Pure or mixed growth of *K. pneumoniae* may potentially cause false positive results with the *P. aeruginosa* and/or *E. coli* probes. Pediatric samples were not extensively analyzed during the clinical investigation; therefore, the performance of this assay with pediatric samples is unknown.

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

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

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 combined expected *E. coli* and *P. aeruginosa* positive result rate for Gram-negative rods positive blood culture bottles are approximately 37%, and 13%, respectively, but may vary depending on institution and patient population (2). The *E. coli* rates and *P. aeruginosa* rates in the four clinical laboratory studies were in the ranges of 23%-51% and 9%-21%, respectively.

Performance Characteristics

Clinical Studies

A total of 385 routine GNR positive blood culture bottles were included in the five clinical studies, which showed 99.7% (384/385) agreement between *E. coli/P. aeruginosa* PNA FISH and conventional routine methods.

Performance Data for *E. coli/P. aeruginosa* PNA FISH vs. Conventional Methods on GNR-positive Blood Culture Bottles

Study	Sensitivity <i>E. coli</i>	Sensitivity <i>P. aeruginosa</i>	Specificity	Blood Culture System
A	51/51	12/12	54/54	BACTEC
B	51/51	9/9	40/40	BacT/Alert
C	17/17	7/7	51/51	BACTEC
D	32/32	7/8	36/36	BACTEC
E	7/7	4/4	6/6	VersaTREK
Total	100% (158/158) 95% CI (98.1-100)	97.5% (39/40) 95% CI (86.8-99.9)	100% (187/187) 95% CI (98.4-100)	

Analytical Sensitivity

The detection limit for *E. coli* and *P. aeruginosa* were determined to be approximately 10⁵ colony-forming units per mL by serial dilutions of positive cultures. This is consistent with the analytical sensitivity of slide-based staining techniques.

Analytical Specificity

E. coli/P. aeruginosa PNA FISH has also been tested on laboratory and reference strains comprising of 14 *E. coli*, 17 *P. aeruginosa*, 60 additional Gram-negative organisms, 12 Gram-positive organisms and 6 yeasts, representing phylogenetically closely related strains. All (14/14) *E. coli* strains were green-positive and all (17/17) *P. aeruginosa* strains were red-positive. *Shigella spp.* (serogroup A, B, C, or D), *Escherichia albertii* and *Escherichia fergusonii* cross-reacted to create a green signal, *Brevundimonas diminuta*, *Herbaspirillum huttiense*, *Pseudomonas nitroreducens*, and *Pseudomonas fulva* cross-reacted to create a red signal. All other strains were negative.

Interference

A study consisting of 10 Gram-negative rods were tested on four types of blood culture bottles, BD BACTEC, BacTAlert SA and BacTAlert FA, and VersaTREK using heat fixation at 70°C. Fifteen organisms were tested at different heat fixation temperatures (55°C and 80°C) for the interference from charcoal. No interferences were observed.

Reproducibility Studies

A panel of 16 strains was analyzed by *E. coli/P. aeruginosa* PNA FISH in triplicate on three separate days at three separate sites.

Summary of Reproducibility Results by Site Across 3 Days

	Site 1	Site 2	Site 3	Total
Positive Agreement Green	36/36	36/36	36/36	100% (108/108)
Positive Agreement Red	45/45	44/45	45/45	99.3% (134/135)
Negative Agreement	63/63	61/63	63/63	98.9% (187/189)
Total Agreement	100% (144/144)	97.9% (141/144)	100% (144/144)	99.3% (429/432)









Summary of Reproducibility Results by Day Across 3 Sites

	Day 1	Day 2	Day 3	Total
Positive Agreement Green	36/36	36/36	36/36	100% (108/108)
Positive Agreement Red	44/45	45/45	45/45	99.3% (134/135)
Negative Agreement	61/63	63/63	63/63	98.9% (187/189)
Total Agreement	97.9% (141/144)	100% (144/144)	100% (144/144)	99.3% (429/432)

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 D.C.
2. **Karlowsky, J. A., M. E. Jone, D. C. Draghi, C. Thornsberry, D. F. Sahn, and G. A. Volturo.** 2004. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Ann. Clin. Microbiol. Antimicrob.* 3:7.

Definitions

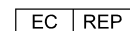
	Product code/catalog number
	Consult the instructions for use
	Contains sufficient for <n> tests
	Manufacturer
	Authorized representative
	Use by
	Batch code
	Storage temperature limitations

Technical Advice and Customer Service

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



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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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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