

EK/P. aeruginosa PNA FISH

Escherichia coli + *Klebsiella pneumoniae*
Pseudomonas aeruginosa

Culture Identification Kit



Cat. No. KT008 50 tests

Intended Use

EK/P. *aeruginosa* PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for identification of *Escherichia coli*/K. *pneumoniae* and *Pseudomonas aeruginosa* on smears from positive blood cultures containing Gram-negative rods. The test does not distinguish between *E. coli* and *K. pneumoniae*. Further testing is needed to differentiate *E. coli* and *K. pneumoniae*. The EK/P. *aeruginosa* PNA FISH assay is indicated for use in conjunction with positive blood subcultures as an aid in the identification of *E. coli*/K. *pneumoniae*, and/or *P. aeruginosa*.

For *in vitro* diagnostic use.

Summary and Explanation

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

Identification of *E. coli*, *K. pneumoniae* 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).

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

The test provides rapid (within 3 hours) identification of *E. coli* + *K. pneumoniae* and *P. aeruginosa* on smears made from positive blood cultures. The test does not distinguish between *E. coli* and *K. pneumoniae*.

Principle of the Procedure

A mixture of a fluorescein-labeled, *E. coli* and *K. pneumoniae* specific PNA probe and a Texas Red labeled, *P. aeruginosa* specific PNA probe is added to a smear prepared from a positive blood culture containing Gram negative rods by Gram stain. Hybridization is performed at 55°C for 90 min. The hybridization is followed by a post-hybridization rinse in water at 55°C to remove the cover slips followed by a wash in 1x Wash solution at 55°C for 30 min. Finally, the smear is mounted with Mounting Medium and examined by fluorescence microscopy.

Definitions

	Product code/catalogue number.
	Consult the instructions for use.
	Contains sufficient for <N> tests.
	Manufactured by.
	Authorized representative.
	<i>In vitro</i> diagnostic medical device.
	Use by.
	Batch code.
	Storage temperature limitations.

Reagent

EK/P. *aeruginosa* PNA FISH is comprised of the following kit components:

GN Fixation Solution - GN Fixation Solution
3 mL phosphate-buffered saline with detergent for use.

EK/P. aeruginosa PNA -
EK/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 *in vitro* diagnostic use.

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

Safety Precautions

The EK/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 (Cat. No. AC003).

Do not use other microscope slides than the Microscope Slide (Cat. No. 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, place dish in 55°C water bath.

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 (Cat. No. AC001).
- Transfer 10 µL or a small drop from a ventilation needle of culture to the GN Fixation Solution and mix gently to emulsify.
- Fix the smears by either heating them for 20 min. at 55°C or 80°C or allow the smears to dry and fix them by methanol-fixation or by flame-fixation.
- Immerse the slide in 80% or 96% ethanol for 5-10 min and leave to air-dry until the smear is dry (approximately 10 min.).

Test Procedure

Material Provided

EK/P. *aeruginosa* PNA FISH (Cat. No. KT008). Each kit contains sufficient material for testing 50 cultures. 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 (100 pcs.), (Cat. No. AC001).
- Coverslips** - Coverslips, (100 pcs.) 22 x 22 mm, Thickness: 0.15 mm (Cat. No. AC002).
- Dual Band Filter** - Dual band filter (Cat. No. AC003).
- Staining Dish** - Staining dish with cover and slide holder (Cat. No. AC004).
- PNA FISH Workstation** - Slide warmer (55 ± 1°C). (Cat. No. AC005).
- Water Bath** - Water Bath (55 ± 1°C). (Cat. No. AC006).

- EK/P. aeruginosa Control Slide** - EK/P. *aeruginosa* Control Slide (Cat. No. CS008). Contains a positive control prepared from a mixture of liquid culture of *E. coli*, ATCC# 11775, and *P. aeruginosa*, ATCC# 10145, and negative control prepared from liquid culture of *K. oxytoca*, ATCC# 13182.

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 EK/P. *aeruginosa* PNA to the well on the microscope slide with the smear.
- Add coverslip. Avoid air bubbles. Use sterile loop to remove resin beads if needed.
- Incubate for 90 ± 5 min. at 55 ± 1°C.

Water Rinse

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

Stringent Wash

- Transfer slide rack to preheated Wash Solution at 55°C ± 1°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 EK/*P. aeruginosa* Control Slide (Cat. No. CS008) or prepare smears from cultures of laboratory or reference strains of *E. coli* ATCC 35218 and *P. aeruginosa* ATCC 10145 as Positive Controls either on separate slides or mixed on one slide and *Klebsiella oxytoca* ATCC 43086 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 crystals which are associated with reduced shelf life. When using an AdvanDx EK/*P. aeruginosa* Control Slide (Cat. No. CS008), 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 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.

Temperature Control:

It is important that the temperature of the Slide warmer/Incubator has reached 55°C prior to starting the hybridization and that the 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.

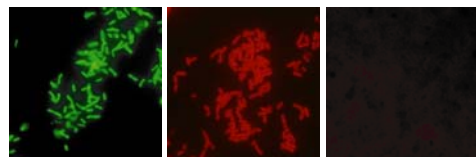
Major Blood Culture Systems and Bottle Type Compatibility:

The PNA FISH platform is compatible with all 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 fluorescent microscope. The smear appears in general reddish. *E. coli* and *K. pneumoniae* are 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.

Definitive identification is pending subculture and additional microbiological evaluation and antimicrobial susceptibility testing. The test does not distinguish between *E. coli* and *K. pneumoniae*. Other microorganisms appear non-fluorescent.



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

Troubleshooting

- False positive Control and Sample test results may occur if the Dual Band Filter (Cat. No. AC003) is not used, or by contamination of the specimens.
- False negative Control or Sample test results may occur if AdvanDx Microscope Slides (Cat. No. 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 the product insert or contact AdvanDx.

Limitations

Green false-positive results may occur with *Shigella spp.*, (serogroup A, B, C or D),

Escherichia albertii and *Escherichia fergusonii* due to sequence similarity.

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

The *K. pneumoniae* probe is specific for *K. pneumoniae*, including the biotype *K. ozaenae* of the *K. pneumoniae* complex, *K. variicola*, and the closely related *Enterobacter aerogenes*.

In the event of co-infection, the accuracy of this device for detecting *P. aeruginosa* at the LoD (10^5 CFU/mL) concentration in the presence of *E. coli* and/or *K. pneumoniae* at the concentration higher than ($>$) 10^7 CFU/mL has not been established and therefore unknown.

Growth of *Pseudomonas putida* may potentially cause false positive results with the *E. coli*/*K. pneumoniae* probes.

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.

Clinical Studies were conducted using the BacTec and BacT/Alert blood culture instruments only. Therefore the performance of this assay with other blood culture systems is not known. Fifteen Versa Trek Blood culture bottles were tested in an internal analytical study.

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.

Pediatric samples were not extensively analyzed during the clinical investigation; therefore, the performance of this assay with pediatric samples is unknown.

Isolation on solid media is needed to differentiate mixed growth with other organisms and identification is needed to differentiate *E. coli* and *K. pneumoniae*.

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

Expected Results

The expected *E. coli*, *K. pneumoniae*, and *P. aeruginosa* positive result rate for Gram-negative rods positive blood culture bottles are approximately 37%, 18% and 13%, respectively, but may vary depending on institution and patient population (4). The *E. coli*, *K. pneumoniae* and *P. aeruginosa* rates in the four clinical laboratory studies were in the ranges of 28%-58% and 4%-22%, respectively (2, 3).

Performance Characteristics

Clinical Studies

The performance of EK/*P. aeruginosa* PNA FISH on 240 blood culture bottles containing Gram-negative rods has been determined at four clinical microbiology laboratories in the United States and Europe are summarized below where the results were compared to results obtained by subculture and subsequent identification by standard methods (2, 3).

Study	Sensitivity EK	Sensitivity <i>P. aeruginosa</i>	Specificity	Blood Culture System
A	100% (26/26) 95% CI (89.1-100)	100% (11/11) 95% CI (76.2-100)	92.3% (12/13) 95% CI (64.0-100)	BacT/Alert
B	100% (47/47) 95% CI (93.8-100)	88.9% (8/9) 95% CI (51.8-99.7)	100% (18/18) 95% CI (84.7-100)	BacT/Alert
C	100% (31/31) 95% CI (90.8-100)	92.3% (12/13) 95% CI (64.0-99.8)	94.4% (17/18) 95% CI (72.9-99.9)	BACTEC
D	100% (40/40) 95% CI (92.8-100)	100% (2/2) 95% CI (22.4-100)	100% (13/13) 95% CI (79.4-100)	BacT/Alert
Total	100% (144/144) 95% CI (97.9-100)	94.3% (33/35) 95% CI (80.8-99.3)	95.9% (60/62) 95% CI (88.8-99.6)	

Analytical Sensitivity

The detection limit for *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were determined to be approximately 10^5 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

EK/*P. aeruginosa* PNA FISH has also been tested on laboratory and reference strains comprising of Gram-negative spp., 22 *E. coli*, 24 *P. aeruginosa*, 17 *K. pneumoniae*, 74 additional Gram negative organisms, 13 Gram positive organisms and 7 yeasts representing phylogenetically closely related species and a variety of clinically significant species. All (22/22) *E. coli* and all (17/17) *K. pneumoniae* strains were green-positive. All (24/24) *P. aeruginosa* strains were red-positive. *Escherichia albertii*, *Escherichia fergusonii*, and *Shigella spp.* (serogroup A, B, C or D cross

reacted to create a green signal; *Brevundimonas diminuta*, *Herbaspirillum huttiense*, *Pseudomonas nitroreducens*, and *Pseudomonas fulva* were red-positive and *Herbaspirillum huttiense* and *Pseudomonas nitroreducens* cross-reacted to create a red signal. All other strains were negative.

Bibliography

- 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.
- Fiandaca, M. J., Tilahun, Y., Torpey, D. J. III, Waga, M., Pieters, E., Ruge, D., and Sandin, R. 2007. Evaluation of *E. coli*/*P. aeruginosa* PNA FISH™ and EK/*P. aeruginosa* PNA FISH™; two Dual Color Assays for Simultaneous Identification of Gram Negative Rods Directly from Positive Blood Culture Bottles. Abstract #C-275, 107th Annual Meeting of American Society for Microbiology, Toronto, Canada.
- Hansen, D.S., Leerbeck, L., Fiandaca, M.J., and Bruun, B. 2008. Evaluation of *E. coli*/*P. aeruginosa* PNA FISH™ and EK/*P. aeruginosa* PNA FISH™; two dual colour assays for identification of Gram-negative rods directly from positive blood culture bottles. Abstract #P-1992, 18th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain.
- Karlowsky, J. A., Jone, M. E., Draghi, D. C., Thornsberry, C., Sahm, D. F., and Volturo, G. A. 2004. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann. of Clin. Micro. and Antimicro. 3:7

Technical Advice and Customer Service

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