AdvanDx

GNR Traffic Light® PNA FISH®

Escherichia coli, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* Culture Identification Kit

CE

Σ 50

IVD

REF KT011

Intended Use

GNR Traffic Light PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Escherichia coli,* and/or *Klebsiella pneumoniae* and/or *Pseudomonas aeruginosa* on smears from positive blood cultures containing Gram-negative rods observed on Gram stain.

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

The GNR Traffic Light PNA FISH assay is indicated for use as an aid in the diagnosis of *E. coli,* and/or *K. pneumoniae,* and/or *P. aeruginosa* bacteremia.

IVD 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).

GNR Traffic Light PNA FISH is a multicolor fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to specific ribosomal RNA sequences of *E. coli, K. pneumoniae* (including the three subspecies: *pneumoniae, ozaenae* and *rhinosclermatis*) and *P. aeruginosa*.

The test provides rapid (within 90 minutes) identification of *E. coli, K. pneumoniae* and *P. aeruginosa* on smears made from positive blood cultures.

Principle of the Procedure

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

Reagents

GNR Traffic Light PNA FISH is comprised of the following kit components:

GN Fixation Solution	**GN Fixation Solution** 3 mL phosphate-buffered saline with detergent.
GNR Traffic Light PNA	**GNR Traffic Light 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	3 mL photobleaching inhibitor in glycerol.

Precautions

IVD For *in vitro* diagnostic use.

Caution: Federal Law restricts this device to sale by or on the order of a licensed practitioner.

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

Safety Precautions

GNR Traffic Light 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.
GN 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 microscope filters other than those listed in the Section on **Materials Required and Available from AdvanDx**.

Do not use microscope slides other than 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 Water Rinse

Place 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

Follow the blood culture bottle manufacturer's instruction to properly mix the blood culture bottle before smear preparation.

- Place one drop of GN Fixation Solution on a well on the microscope slide.
- 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 – 80 °C, or allow the smears to dry and fix them by methanol-fixation or by flame-fixation.

Test Procedure

Material Provided

GNR Traffic Light® PNA FISH®	KT011

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	AC001
Coverslips	Coverslips, 22 x 22 mm, Thickness: 0.15 mm	AC002
AdvanDx Microscope Filter	Dual Band Filter for use with high pressure mercury vapor arc lamp light sources or equivalent	AC003 AC007
AdvanDx Metal Halide Filter	Dual Band Filter for use with modified mercury vapor arc lamps (metal halide)	AC033
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
GNR Traffic Light Control Slide	GNR Traffic Light Control Slide.	CS011

Contains a Positive Control prepared from a liquid culture of *E. coli*, ATCC# 35218, *K. pneumoniae* ATCC# 13882 and *P. aeruginosa* ATCC# 10145 and a Negative Control prepared from a liquid culture of *K. oxytoca* ATCC# 43086.

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. In a second covered staining dish place 200 ml of water and also preheat. For size of staining dish and volume of Wash Solution please see Procedural Notes.

Hybridization

- Add one drop of GNR Traffic Light 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 30 ± 5 min. at 55 ± 1 °C.

Warm 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 wash solution. Occasionally, coverslips must be pushed off with forceps.

Stringent Wash

- Transfer slides in slide rack to preheated 1x 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 more easily.

Use AdvanDx GNR Traffic Control Slide (CS011). Alternatively, culture each QC strain to achieve a Limit of Detection concentration (e.g. $\geq 10^5$ CFU per mL) before smear preparation by using the continuous monitoring blood culture system available. Follow smear preparation instruction as described above under Specimen Collection and Preparation. Positive Controls *E. coli* ATCC 35218, *K. pneumoniae* ATCC13882 and *P. aeruginosa* ATCC 10145 can be either on separate slides or mixed on a single slide and *Klebsiella oxytoca* ATCC 43086 as the Negative Control on a separate slide. The laboratory prepared 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 of smears prepared for quality control or stored under desiccated conditions.

E. coli must test Green-Positive, *P. aeruginosa* must test Red-Positive and *K. pneumoniae* must test Yellow-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.

Temperature Control:

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

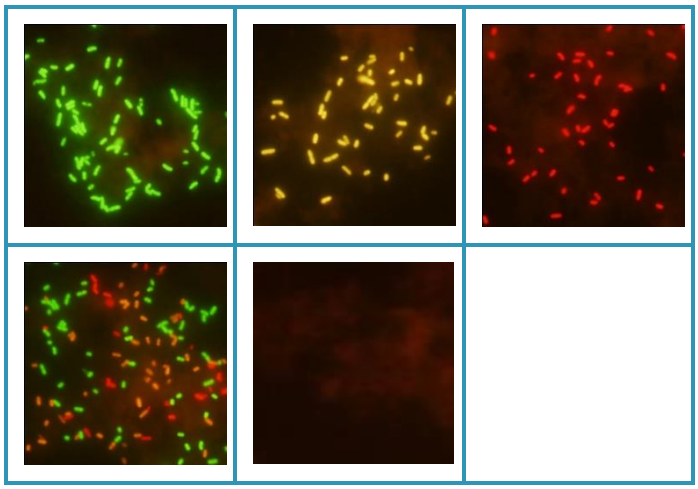
Parallel Testing Using Different PNA FISH Tests: The PNA FISH kits are designed for parallel testing. 60x Wash Solution and Mounting Medium are identical and may be interchanged between different tests.

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

Interpretation of Results

The *Klebsiella pneumoniae* PNA probe does not differentiate between the three subspecies: *pneumoniae, ozaenae* or *rhinoscleromatis.*

Examine slides using a fluorescence microscope with an AdvanDx filter. The smear background appears reddish in color. *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 and *K. pneumoniae* is identified as multiple bright yellow fluorescent rods in multiple fields of view. Gram negative rods that are not *E. coli, K. pneumoniae,* or *P. aeruginosa* appear non-fluorescent.



Representative examples of Green-Positive *E. coli* (top-left); Yellow-Positive *K. pneumoniae* (top-middle); Red-Positive *P. aeruginosa* (top-right); mixture of Green-Positive *E. coli*, Yellow-Positive *K. pneumoniae* and Red-Positive *P. aeruginosa* (bottom-left). Negative test results with reddish background (bottom-middle).

Troubleshooting

- False Positive Control and Sample test results may occur if an AdvanDx Filter 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.
- False Negative results may infrequently occur due to mixed growth or due to error in assay technique.

Please refer to the Precaution and Limitation sections in the product insert or contact AdvanDx.

Limitations

- False Green Positive results will occur with *Shigella spp.* (serogroups A, B, C, and D), *Escherichia albertii* and *Escherichia fergusonii* due to rRNA sequence similarity.
- False Red Positive results will occur with *Brevundimonas diminuta, Herbaspirillum huttiense, Pseudomonas fulva, Acinetobacter radioresistens* and some strains of *Pseudomonas putida.* In clinical studies, of the 4 *Acinetobacter baumannii* tested, 1 produced a red fluorescence result. However, rRNA sequence analysis or analytical study data did not indicate cross-reactivity with *A. baumannii*.
- False Yellow Positive results will occur with *Escherichia vulneris* and *Klebsiella variicola*.
- *P. aeruginosa* with the BACTEC and VersaTREK blood culture systems, and *K. pneumoniae* with VersaTREK blood culture systems were not extensively evaluated during the clinical investigation, and therefore the performance is unknown.
- Clinical studies were conducted using the BACTEC Plus aerobic/anaerobic, BacT/Alert FA/FN, and VersaTREK aerobic blood culture bottles. BACTEC Standard/10 aerobic and BacT/ALERT SA were tested in an internal compatibility study. The performance of the GNR Traffic Light PNA FISH with other blood culture bottle types has not been established.
- There were not sufficient pediatric samples tested to establish the performance data with the GNR Traffic Light PNA FISH.
- False Negative results may infrequently occur due to mixed growth or due to error in assay technique.
- Subculture and isolation of organisms on solid media is needed to differentiate mixed growth.
- False Positive autofluorescence may occur if a standard filter is used instead of an AdvanDx Filter.
- The type and condition of the microscope 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.
- The product has not been validated with specimens other than blood cultures.

Expected Results

The expected *E. coli, K. pneumoniae,* and *P. aeruginosa* positive rates for Gram-negative rod positive blood cultures as determined by the clinical studies are approximately 37%, 22% and 9%, respectively.

Performance Characteristics

Clinical Studies

The performance of GNR Traffic Light PNA FISH has been validated on a total of 358 routine GNR positive blood culture bottles (and 32 spiked samples) from four clinical sites in the U.S. The studies showed 100% sensitivity (135/135) for *E. coli,* 98.7% sensitivity (77/78) for *K. pneumoniae* and 96.9% sensitivity (62/64) for *P. aeruginosa.* The specificity was 97.5% (115/118) for GNR-positive blood culture bottles.

Study	Sensitivity *E. coli*	Sensitivity *K. pneumoniae*	Sensitivity *P. aeruginosa*	Specificity	Blood Culture System
A	35/35	17/17	7/9[3]	35/37[4]	BACTEC
B	31/31	30/30	39/39[6]	36/36	BacT/ALERT
C	48/48	28/28	8/8	29/30[5]	BACTEC
D	21/21	2/3[2]	8/8[1]	15/15	VersaTREK
Total	100% (135/135) 95% CI (97.8-100)	98.7% (77/78) 95% CI (93.1-100)	96.9% (62/64) 95% CI (89.2-99.6)	97.5% (115/118) 95% CI (92.8-99.5)	N=395

1. Includes 4 samples spiked with *P. aeruginosa* clinical isolates.
2. One false negative for *K. pneumoniae* in a mixed culture of *E. coli* and *K. pneumoniae*.
3. Both false negatives were in mixed cultures of *P. aeruginosa* and *K. pneumoniae*.
4. One false green positive *E. cloacae* (negative upon re-test) and one false red positive *A. baumannii*.
5. One false red positive *Acinetobacter radioresistens* in a mixed culture with *E. faecalis*
6. Includes 28 samples spiked with *P. aeruginosa* isolates.

Analytical Sensitivity

The detection limit for *E. coli, K. pneumoniae* and *P. aeruginosa* was 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

The GNR Traffic Light PNA FISH was used to test a total of 163 micro-organisms including 144 Gram-negative strains, 13 Gram-positive organisms, and 6 yeasts. All (19/19) *E. coli* were green-positive, (20/20) *K. pneumoniae* (including the three subspecies: *pneumoniae, ozaenae* and *rhinosclermatis*) were Yellow-Positive, (20/20) *P. aeruginosa* were Red-Positive and 75 other Gram-negative rods were negative. Four *Shigella spp.* (serogroup A, B, C and D), 1 *Escherichia albertii* and 1 *Escherichia fergusonii* cross-reacted producing a green signal. One *Brevundimonas diminuta,* 1 *Herbaspirillum huttiense* and 1 *Acinetobacter radioresistens* cross-reacted producing a red signal. One *Escherichia vulneris* cross-reacted producing a yellow signal. GNR Traffic Light PNA FISH was also tested on 13 Gram-positive bacteria strains and 6 yeast species, all of which yielded negative results.

Reproducibility

A panel of 19 strains on 14 slides was analyzed by GNR Traffic Light 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	45/45	45/45	43/45*	133/135 (98.5%)
Positive Agreement Yellow	45/45	45/45	45/45	135/135 (100%)
Positive Agreement Red	45/45	45/45	45/45	135/135 (100%)
Negative Agreement	36/36	36/36	33/36**	105/108 (97.2%)
Total Agreement	171/171 (100%)	171/171 (100%)	166/171 (97.1%)	508/513 (99.0%)

***One *E. coli* gave a false red color and one gave a false yellow
****One Negative slide produced false green, red and yellow results

Summary of Reproducibility Results by Day Across 3 Sites

	Site 1	Site 2	Site 3	Total
Positive Agreement Green	45/45	45/45	43/45*	133/135 (98.5%)
Positive Agreement Yellow	45/45	45/45	45/45	135/135 (100%)
Positive Agreement Red	45/45	45/45	45/45	135/135 (100%)
Negative Agreement	36/36	36/36	33/36**	105/108 (97.2%)
Total Agreement	171/171 (100%)	171/171 (100%)	166/171 (97.1%)	508/513 (99.0%)

*One *E. coli* gave a false red color and one gave a false yellow
**One Negative slide produced false green, red and yellow results

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.

Definitions

REF	Product code/catalog number	LOT	Batch code
	Consult the instructions for use		Storage temperature limitations
	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

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

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

techsupport@opgen.com **www.OpGen.com**

27 March 2018 **PN1708I-EN**
DCN 26-18