AdvanDx

Yeast Traffic Light® PNA FISH®

Candida albicans* + *Candida parapsilosis
Candida tropicalis
Candida glabrata* + *Candida krusei
Culture Identification Kit

CE

Σ 50

IVD

REF KT009

Intended Use

Yeast Traffic Light PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Candida albicans* and/or *Candida parapsilosis,* identification of *Candida tropicalis,* and identification of *Candida glabrata* and/or *Candida krusei* on smears made from positive blood cultures containing yeasts observed on Gram stain or other microbiological stains. The test does not distinguish between *C. albicans* and *C. parapsilosis*. The test does not distinguish between *C. glabrata* and *C. krusei.*

Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, differentiation between *C. albicans* and *C. parapsilosis,* differentiation between *C. glabrata* and *C. krusei,* and/or differentiation of mixed growth.

Yeast Traffic Light PNA FISH is indicated for use as an aid in the diagnosis of *C. albicans* and/or *C. parapsilosis, C. tropicalis,* and *C. glabrata* and/or *C. krusei* fungemia.

IVD For *in vitro* diagnostic use.

Summary and Explanation

Candida species are a leading cause of both community- and hospital-acquired fungemia.

Identification of *Candida* species in blood cultures is routinely based on presumptive identification as yeast followed by final identification after subculture and biochemical analysis (2).

Yeast Traffic Light PNA FISH is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to specific ribosomal RNA sequences of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* and *C. krusei* (4).

The test provides rapid (within 90 minutes) identification of *C. albicans* + *C. parapsilosis*, *C. tropicalis*, and *C. glabrata* + *C. krusei* on smears made from positive blood cultures. Rapid identification of yeast positive blood cultures supports appropriate antifungal selection and has been shown to reduce antifungal expenditures. (1,3,5-7).

Principle of the Procedure

A mixture of fluorescein and rhodamine-labeled PNA probes specific to *C. albicans, C. parapsilosis, C. tropicalis, C. glabrata,* and *C. krusei* is added to a smear prepared from a 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 Wash Solution. Finally, the smear is mounted with Mounting Medium and examined by fluorescence microscopy.

Reagents

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

Fixation Solution	**Fixation Solution** 3 mL phosphate-buffered saline with detergent.
Yeast Traffic Light PNA	**Yeast 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	**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, only, by personnel trained in laboratory techniques and experienced in fluorescence microscopy.

Safety Precautions

Yeast 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.
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 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 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 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 Fixation Solution on a well on the microscope slide.
- 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 55-80 °C or allow the smears to dry and fix them by methanol-fixation or by flame-fixation.

Test Procedure

Material Provided

Yeast Traffic Light® PNA FISH® KT009

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

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 PNA FISH Workstation (55 ± 1 °C). AC005

Water Bath Water Bath (55 ± 1 °C). AC006

Yeast Traffic Light Control Slide Yeast Traffic Light Control Slide. CS009

Positive Control well contains mixture of *C. albicans* ATCC #18804, *C. tropicalis* ATTC #750, and *C. glabrata* ATTC #2001; and Negative Control well contains *S. cerevisiae* ATCC #18824.

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 Yeast 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.

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 Yeast Traffic Light Control Slide (CS009) or prepare smears from liquid cultures of laboratory or reference strains of *C. albicans* or *C. parapsilosis, C. glabrata* or *C. krusei* and *C. tropicalis* as Positive Controls either on separate slides or mixed on one slide and other yeast species, for example *Saccharomyces cerevisiae*, as Negative Control as described above under Specimen Collection and Preparation. The smears may be stored for up to 1 month at room temperature. When using an AdvanDx Yeast Traffic Light Control Slide (CS009), simply remove slide from pouch and follow the PNA FISH procedure starting with the hybridization step.

C. albicans and *C. parapsilosis* must test Green-Positive, *C. glabrata* and *C. krusei* must test Red-Positive, and *C. tropicalis* 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. 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 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:

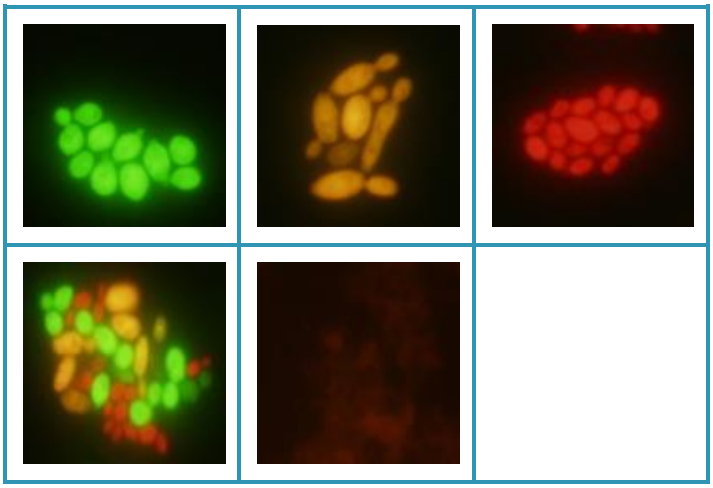
The PNA FISH kits are designed for parallel testing. 60x Wash Solution and Mounting Medium are identical and may be interchanged between different tests.

Major Blood Culture Systems and Bottle Type Compatibility:

The PNA FISH platform is compatible with the major commercially available continuously monitoring blood culture systems, 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. *C. albicans* and *C. parapsilosis* are identified as multiple bright green fluorescent cells in multiple fields of view. The test does not distinguish between *C. albicans* and *C. parapsilosis*. *C. tropicalis* is identified as multiple bright yellow fluorescent cells in multiple fields of view. *C. glabrata* and *C. krusei* are identified as multiple bright red fluorescent cells in multiple fields of view. The test does not distinguish between *C. glabrata* and *C. krusei.* Yeast cells may appear as buds or pseudohyphae.



Representative examples of green-positive *C. albicans* (top-left), yellow-positive *C. tropicalis* (top-middle), red-positive *C. glabrata* (top-right), mixture of green-positive *C. albicans*, red-positive *C. glabrata* and yellow-positive *C. tropicalis* (bottom-left) and negative (bottom-middle) test results.

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.

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

Limitations

Isolation on solid media is needed for identification between *C. albicans* and *C. parapsilosis*, between *C. glabrata* and *C. krusei*, and differentiation of mixed growth with other organisms.

False Positive Red results may occur with *Candida nivariensis* and *Candida bracarensis,* which are closely related to *Candida glabrata,* and *Kluyveromyces delphensis;* all due to sequence similarity.

False Positive Green results may occur with *Candida orthopsilosis and Candida metapsilosis,* a recently described species, closely related to *C. parapsilosis,* due to sequence similarity.

False Positive Yellow results may occur with *Candida sojae* due to sequence similarity.

Histoplasma capsulatum has not been tested; therefore, the performance of Yeast Traffic Light PNA FISH with this isolate is unknown.

Candida africana has not been tested; therefore, the performance of Yeast Traffic Light PNA FISH with this isolate is unknown.

VersaTrek Blood Culture bottles were not evaluated during clinical studies. All VersaTrek Blood culture data were based on internal analytical study.

C. tropicalis with pediatric samples, and *C. glabrata*/*C. krusei* with the BACTEC PEDS Plus/F bottles were not extensively evaluated during the clinical investigation, and therefore the performance is unknown.

False Positive autofluorescence may occur if a standard filter is used instead of an AdvanDx Filter.

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.

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.

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

Expected Results

The expected *C. albicans* + *C. parapsilosis, C. tropicalis* and *C. glabrata* + *C. krusei* positive result rate from yeast positive blood culture bottles is approximately 50%, 9%, and 31% respectively, based on AdvanDx clinical studies, but may vary depending on institution and patient population.

Performance Characteristics

Clinical Studies

The performance of Yeast Traffic Light PNA FISH (New) versus Yeast Traffic Light PNA FISH (Original) and versus conventional routine methods has been assessed in three clinical laboratory studies.

A total of 114 prospective and 41 seeded clinical yeast positive blood culture bottles were included in the studies, which showed 100% (158/158) agreement between shortened and original Yeast Traffic Light PNA FISH procedure and 99.7% (157/158) agreement between Yeast Traffic Light PNA FISH (New) and conventional routine methods. These studies included two commercially available, continuously monitoring blood culture systems (BacT/ALERT, bioMérieux, NC and BACTEC, Becton Dickinson, MD).

Performance Data for Yeast Traffic Light PNA FISH (New) vs. Yeast Traffic Light PNA FISH (Original) on Yeast-positive Blood Culture Bottles

Study	Positive Agreement *C. albicans*/ *C. parapsilosis*	Positive Agreement *C. tropicalis*	Positive Agreement *C. glabrata*/ *C. krusei*	Negative Agreement	Blood Culture System
A	100% (20/20)[1]	100% (1/1)	100% (4/4)[1]	100% (4/4)	BacT/Alert
B	100% (11/11)	100% (5/5)	100% (17/17)	100% (2/2)	BacT/Alert
C	100% (28/28)[2]	100% (25/25)	100% (25/25)[2]	100% (16/16)	BACTEC
Total	100% (59/59) 95% CI (95.1-100)	100% (31/31) 95% CI (90.8-100)	100% (46/46) 95% CI (93.7-100)	100% (22/22) 95% CI (87.3-100)	N= 158

[1] Includes 1 mixed culture: *C. albicans*/*C. glabrata* [2] Includes 2 mixed cultures: *C. krusei*/*C. parapsilosis*

Performance Data for Yeast Traffic Light PNA FISH (New) vs. Routine Identification Methods on Yeast-positive Blood Culture Bottles

Study	Sensitivity *C. albicans*/ *C. parapsilosis*	Sensitivity *C. tropicalis*	Sensitivity *C. glabrata*/ *C. krusei*	Specificity	Blood Culture System
A	100% (20/20)[1]	100% (1/1)	100% (4/4)[1]	100% (4/4)	BacT/Alert
B	100% (11/11)	100% (5/5)	100% (17/17)	100% (2/2)	BacT/Alert
C	100% (28/28)[2]	100% (25/25)	93.3% (25/26)[2,3]	100% (15/15)	BACTEC
Total	100% (59/59) 95% CI (95.1-100)	100% (31/31) 95% CI (90.8-100)	97.9% (46/47) 95% CI (88.7-99.6)	100% (21/21) 95% CI (86.7-100)	N= 158

[1] Includes 1 mixed culture: *C. albicans*/*C. glabrata* [2]Includes 2 mixed cultures: C. krusei/C. parapsilosis [3] One C. glabrata was missed by both Yeast Traffic Light PNA FISH (New) and Yeast Traffic Light (K080719) with the BACTEC PEDS Plus/F bottle

Analytical Sensitivity

The detection limit for *C. albicans, C. parapsilosis, C. glabrata, C. krusei* and *C. tropicalis* were all 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

Yeast Traffic Light PNA FISH has been tested on 75 laboratory and reference strains comprising *Candida* species and other closely related yeasts species and a variety of other frequently isolated organisms.

- All (10/10) *C. albicans*, including 2 *C. stellatoidae*, a variant of *C. albicans*, and (4/4) *C. parapsilosis* strains were green-positive.
- Both (2/2) *C. tropicalis* were yellow-positive.
- All (6/6) *C. glabrata*, (1/1) *Issatchenkia orientalis* (telemorph of *C. krusei*) and (3/3) *C. krusei* strains were red-positive.
- *Candida nivariensis*, *Candida bracarensis* and *Kluyveromyces delphensis* cross-reacted to create a red signal. *Candida orthopsilosis* (3/3) and *Candida metapsilosis* cross-reacted to create a green signal. One of two strains of *Candida sojae* cross-reacted to create a yellow signal. All other (27/27) fungal and (13/13) bacteria strains were negative.

Reproducibility

A reproducibility study was performed on 19 isolates (13 slides) in triplicate on three separate days at three separate sites. The following tables present the results of the reproducibility study; by site across three days of testing and by day across the three sites, respectively.

Summary of Reproducibility Results by Site Across 3 Days

	Site 1	Site 2	Site 3	Total Agreement
Positive Agreement Green	54/54	54/54	54/54	162/162 (100%)
Positive Agreement Yellow	54/54	54/54	54/54	162/162 (100%)
Positive Agreement Red	27/27	27/34	27/27	81/88 (92.0%)
Negative Agreement	36/36	36/36	36/36	108/108 (100%)
Total Agreement	171/171 (100%)	171/178 (96.1%)	171/171 (100%)	513/520 (98.7%)

Summary of Reproducibility Results by Day Across 3 Sites

	Day 1	Day 2	Day 3	Total Agreement
Positive Agreement Green	54/54	54/54	54/54	162/162 (100%)
Positive Agreement Yellow	54/54	54/54	54/54	162/162 (100%)
Positive Agreement Red	27/30	27/29	27/29	81/88 (92.0%)
Negative Agreement	36/36	36/36	36/36	108/108 (100%)
Total Agreement	171/174 (98.3%)	171/173 (98.8%)	171/173 (98.8%)	513/520 (98.7%)

Bibliography

1. **Alexander, B., E. Ashley, L. Reller, and S. Reed.** 2006. Cost savings with implementation of PNA FISH testing for identification of *Candida albicans* in blood cultures. Diagn. Microbiol. Infect. Dis. 54:277-282.
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6. **Forrest, G., M. Kent, and R. Venezia.** 2008. Evaluation of the *Candida albicans/glabrata* (CAG) Peptide Nucleic Acid Fluorescence In-situ Hybridization (PNA FISH) Test on Patient Management. Poster# M-707. Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, D.C.
7. **Forrest, G., K. Mankes, E. Jabra-Rizk, E. Weekes, J. Johnson, D. Lincalis, and R. Venezia.** 2006. Peptide nucleic acid fluorescence *in situ* hybridization-based identification of *Candida albicans* and its impact on mortality and antifungal therapy costs. J. Clin. Microbiol. 44:3381-3383.

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

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30 April 2020 **PN1766I-EN**
DCR 20-0034