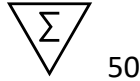


C. albicans/*C. glabrata* PNA FISH®

Candida albicans/*Candida glabrata* Culture Identification Kit



IVD

REF KT006

Intended Use

C. albicans/*C. glabrata* PNA FISH is a fluorescence qualitative nucleic acid hybridization assay intended for identification of *C. albicans* and/or *C. glabrata* on smears made from yeast positive blood cultures.

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

C. albicans/*C. glabrata* PNA FISH is indicated for use as an aid in the diagnosis of *C. albicans* and/or *C. glabrata* 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 (1).

C. albicans/*C. glabrata* PNA FISH is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to specific ribosomal RNA sequences of *C. albicans* and *C. glabrata* (2,3).

Principle of the Procedure

A mixture of a fluorescein-labeled, *C. albicans*-specific PNA probe and a rhodamine-labeled, *C. glabrata*-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 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

C. albicans/*C. glabrata* PNA FISH is comprised of the following kit components:

Fixation Solution

Fixation Solution
3 mL phosphate-buffered saline with detergent.

C. albicans/*C. glabrata* PNA

***C. albicans*/*C. glabrata* 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 fluorescent microscopy.

Safety Precautions

<i>C. albicans</i> / <i>C. glabrata</i> 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 other filters than the Dual Band Filter (AC003 or AC007).

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. 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 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

C. albicans/C. glabrata PNA FISH® KT006

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 or AC007
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
<i>C. albicans/C. glabrata</i> Control Slide	<i>C. albicans/C. glabrata</i> Control Slide.	CS006

C. albicans/C. glabrata Control Slide (CS006). Contains a positive control prepared from liquid culture containing a mixture of *C. albicans*, ATCC# 18804, and *C. glabrata*, ATCC# 2001; and negative control prepared from liquid culture of *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 *C. albicans/C. glabrata* PNA to the well on the microscope slide with the smear.
- Add coverslip. Avoid air bubbles.
- 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 *C. albicans/C. glabrata* Control Slide (CS006) or prepare smears from liquid cultures of laboratory or reference strains of *C. albicans* and *C. glabrata* as Positive Controls either on separate slides or mixed on one slide and *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.

The performance of the Positive Control has been demonstrated using *C. albicans* and *C. glabrata* present on the same slide, and separated onto individual positive control slides for each organism.

C. albicans must test green-positive and *C. glabrata* 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.

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 as instrument temperature readings may not always be accurate.

Parallel Testing Using Different PNA FISH Tests:

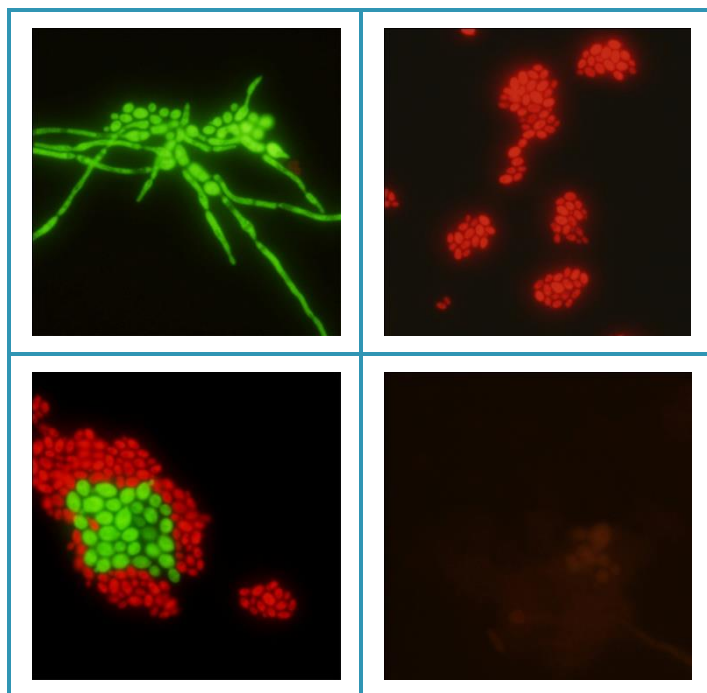
The PNA FISH kits are designed for parallel testing. Fixation Solution, 60x Wash Solution and Mounting Medium are identical and may be interchanged between different tests.

Major Blood Culture Systems and Bottle Type Compatibility:

The PNA FISH platform is compatible with major continuous automated 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* is identified as multiple bright green fluorescent cells in multiple fields of view. *C. glabrata* is identified as multiple bright red fluorescent cells in multiple fields. Yeast cells may appear as buds or pseudohyphae. Definitive identification is pending positive blood subculture, additional microbiological evaluation and antimicrobial susceptibility testing.



Representative examples of green-positive *C. albicans* (top-left), red-positive *C. glabrata* (top-right), mixture of green-positive *C. albicans* and red-positive *C. glabrata* (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

False-positive (green) results may occur with *Candida orthopsilosis* (*C. parapsilosis*).

False-positive (red) results may occur with *Candida nivariensis*, *Candida bracarensis*, and *Kluyveromyces delphensis*. Both *C. nivariensis* and *C. bracarensis* are clinically rare; when encountered, they are likely to be misidentified as *C. glabrata*. *K. delphensis* has not been reported clinically.

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.

Histoplasma capsulatum has not been tested therefore; the performance of the *C. albicans/C. glabrata* PNA FISH with this isolate is unknown.

Candida africana has not been tested therefore; the performance of the *C. albicans/C. glabrata* PNA FISH with this isolate 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.

Isolation on solid media is needed to differentiate mixed growth with other organisms.

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

Expected Results

The *C. albicans* and *C. glabrata* positive result rate from yeast positive blood culture bottles in the clinical trials was approximately 40% and 25%, respectively, however rates may vary depending on institution and patient population.

Performance Characteristics

Clinical Studies

The performance of *C. albicans/C. glabrata* PNA FISH (shortened) versus *C. albicans/C. glabrata* PNA FISH (original) and conventional routine methods has been assessed in four clinical laboratory studies. A total of 126 routine yeast positive blood culture bottles which showed 100% (192/192) agreement between *C. albicans/C. glabrata* PNA FISH (shortened) and *C. albicans/C. glabrata* PNA FISH (original) and 100% (126/126) agreement between *C. albicans/C. glabrata* PNA FISH (original) 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 *C. albicans/C. glabrata* PNA FISH (new) vs. *C. albicans/C. glabrata* PNA FISH (Original) on Yeast-Positive Blood Culture Bottles

Study	Positive Agreement <i>C. albicans</i>	Positive Agreement <i>C. glabrata</i>	Negative Agreement	Blood Culture System
A	100% (21/21)	100% (9/9)	100% (20/20)	BACTEC
B	100% (21/21)	100% (13/13)	100% (16/16)	BacT/ALERT
C	100% (5/5) ¹	100% (6/6) ¹	100% (15/15)	BACTEC
Total	100% (47/47) 95% CI (93.8-100)	100% (28/28) 95% CI (89.9-100)	100% (51/51) 95% CI (94.3-100)	N= 126

¹ One bottle contained both *C. albicans* and *C. glabrata*

Performance Data for *C. albicans/C. glabrata* PNA FISH (new) vs. Routine Identification Methods on Yeast-Positive Blood Culture Bottles

Study	Sensitivity <i>C. albicans</i>	Sensitivity <i>C. glabrata</i>	Specificity	Blood Culture System
A	100% (21/21)	100% (9/9)	100% (20/20)	BACTEC
B	100% (21/21)	100% (13/13)	100% (16/16)	BacT/ALERT

C	100% (5/5) ¹	100% (6/6) ¹	100% (15/15)	BACTEC
Total	100% (47/47) 95% CI (93.8-100)	100% (28/28) 95% CI (89.9-100)	100% (51/51) 95% CI (94.3-100)	N= 126

¹ One bottle contained both *C. albicans* and *C. glabrata*

Analytical Sensitivity

The detection limit for *C. albicans* and *C. glabrata* were both 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

C. albicans/C. glabrata PNA FISH has also been tested on 72 laboratory and reference strains comprising *Candida* species, other closely related species and a variety of other frequently isolated organisms. All (22/22) *C. albicans* strains were green-positive, and all (14/14) *C. glabrata* strains were red-positive. *Candida nivariensis*, *Candida bracarensis* and *Kluyveromyces delphensis* were red positive and two strains of *Candida orthopsilosis* were green-positive. All others (31/31) fungal and bacteria strains were negative.

Reproducibility

A reproducibility study was performed on 10 isolates 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	63/63	63/63	63/63	100% (189/189)
Positive Agreement Red	63/63	63/63	63/63	100% (189/189)
Negative Agreement	63/63	63/63	63/63	100% (189/189)
Total Agreement	100% (189/189)	100% (189/189)	100% (189/189)	100% (567/567)













Summary of Reproducibility Results by Day Across 3 Sites

	Day 1	Day 2	Day 3	Total Agreement
Positive Agreement Green	63/63	63/63	63/63	100% (189/189)
Positive Agreement Red	63/63	63/63	63/63	100% (189/189)
Negative Agreement	63/63	63/63	63/63	100% (189/189)
Total Agreement	100% (189/189)	100% (189/189)	100% (189/189)	100% (567/567)

blood bottles in less than 1.5 hr. American Society of Microbiology. Boston, MA.

- Shepard, J. R., R. M. Addison, B. D. Alexander, P. Della-Latta, M. Gherna, G. Haase, G. Hall, J. K. Johnson, W. Merz, H. Peltroche-Llacsahuanga, H. Stender, R. A. Venezia, D. Wilson, G. W. Procop, F. Wu, and M. J. Fiandaca. 2008. Multicenter evaluation of the *Candida albicans/Candida glabrata* peptide nucleic acid fluorescent *in situ* hybridization method for simultaneous dual-color identification of *Candida albicans* and *Candida glabrata* directly from blood culture bottles. J Clin Microbiol 46:50-55.

Definitions

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

Technical Advice and Customer Service

For all inquiries, please contact OpGen 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.

27 March 2018



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PN1744H-EN
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

Bibliography

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