

C. albicans PNA FISH™

Candida albicans
Culture Identification Kit



Cat. No. KT002

50 tests

Intended Use

C. albicans PNA FISH is a qualitative nucleic acid hybridization assay intended for identification of *Candida albicans* from smears made from positive blood cultures.

EU + Canada: For *in vitro* diagnostic use.
US: For research use only. Not for use in diagnostic procedures.

Summary and Explanation

C. albicans is well-recognized as a leading cause of fungemia.

Identification of *C. albicans* in blood cultures is routinely based on presumptive identification as yeasts followed by final identification after subculture and biochemical analysis (1).








C. albicans PNA FISH is a fluorescence in situ hybridization (FISH) method using PNA probes hybridizing to unique *C. albicans*-

specific ribosomal RNA sequences. The test provides rapid identification of *C. albicans* on smears made from positive blood cultures.

Principle of the Procedure

A fluorescein-labeled, *C. albicans*-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 a stringent Wash Solution. 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

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

Fixation Solution - Fixation Solution

3 mL phosphate-buffered saline with detergent

C. albicans PNA - C. albicans PNA

1.5 mL fluorescein-labeled, *C. albicans*-specific PNA probe in hybridization solution. Contain 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

EU + Canada: For *in vitro* diagnostic use.
US: For research use only. Not for use in diagnostic procedures.

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

Safety Precautions

The *C. albicans* 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 Slides (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 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 in a well on the microscope slide.
- Transfer 10 µL or a small drop from a ventilation needle of 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* PNA FISH (Cat. No. KT002). 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 (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).
- C. albicans* Control Slide - *C. albicans* Control Slide (Cat. No. CS002). Contains a positive control prepared from liquid culture of *C. albicans*, ATCC# 18804, and negative control prepared from liquid culture of *C. glabrata*, ATCC# 2001.

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* 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.

Use AdvanDx *C. albicans* Control Slide (Cat. No. CS002) or prepare smears from liquid cultures of laboratory or reference strains of *C. albicans* as a positive control and *C. glabrata* as a 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 *C. albicans* Control Slide (Cat. No. CS002), simply remove slide from pouch and follow the PNA FISH procedure starting with the hybridization step.

C. albicans must test positive and *C. glabrata* must test negative in accordance with the Interpretation of Results below.

Procedural Notes

Preparation of Smears:

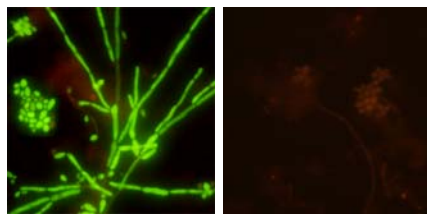
It is recommended to use the same type of fixation (heat, methanol or flame fixation) that is used for Gram-staining. To reduce the reporting time smears for PNA FISH may be prepared in parallel with smears for Gram-staining.

Temperature Control:

It is important that the temperature of the PNA FISH Workstation has reached 55°C prior to starting the hybridization and that 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. Fixation Solution, 60x Wash Solution and Mounting Medium are identical and may be interchanged between different tests.

Interpretation of Results



Examine slides using a fluorescence microscope. The blood culture smear appears in general reddish. *C. albicans* is identified as multiple bright yeast cells in multiple fields of view.

Representative examples of positive (left) 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 Precaution and Limitation sections above or contact AdvanDx.

Limitations

False-positive (green) results may occur with *Candida orthopsilosis*. All other yeast will likely have two or more mismatches and not be reactive.

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.

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 expected *C. albicans* positive result rate from yeast positive blood culture bottles is 25% - 50% (2-4).

Performance Characteristics

Clinical Studies

The performance of *C. albicans* PNA FISH was assessed on routine blood cultures in three separate studies where the results were compared to results obtained by subculture and subsequent identification by standard methods.

Study A. A total of 33 routine blood culture bottles positive for yeasts recovered from both aerobic and anaerobic media (BacT/Alert, bioMérieux) from 1 US (OH) clinical microbiology laboratory were included in the study, which showed 100% (9/9) sensitivity and 100% (24/24) specificity (2).

Routine Identification	<i>C. albicans</i> PNA FISH	
	Positive (n)	Negative (n)
<i>C. albicans</i>	9	0
<i>C. glabrata</i>	0	9
<i>C. parapsilosis</i>	0	6
<i>C. tropicalis</i>	0	1
<i>C. krusei</i>	0	1
<i>C. lusitanae</i>	0	5
<i>Cryptococcus neoformans</i>	0	1
Other yeast	0	1

Study B. A total of 244 routine blood culture bottles positive for yeasts from three different clinical microbiology laboratories from US (OH, NC, MN) were included in the study, which showed 99.0% (96/97) sensitivity and 100% (147/147) specificity (3).

Routine Identification	<i>C. albicans</i> PNA FISH	
	Positive (n)	Negative (n)
<i>C. albicans</i>	93	1 ^a
<i>C. albicans</i> + <i>C. parapsilosis</i>	2	0
<i>C. albicans</i> + <i>C. glabrata</i>	1	0
<i>C. glabrata</i>	0	74
<i>C. glabrata</i> + <i>C. parapsilosis</i>	0	1
<i>C. parapsilosis</i>	0	19 ^b
<i>C. tropicalis</i>	0	21
<i>C. krusei</i>	0	12
<i>C. dubliniensis</i>	0	3 ^c
<i>Cryptococcus neoformans</i>	0	6
<i>Saccharomyces cerevisiae</i>	0	2
<i>S. cerevisiae</i> + <i>C. glabrata</i>	0	1
Other yeast	0	8 ^d

^a *C. albicans* positive by PNA FISH by repeat testing.

^b One *C. parapsilosis* was initially misidentified by routine methods as *C. albicans*.
^c 1 initially misidentified by routine methods as *C. albicans*.
^d 1 each of *Candida guilliermondii*, *Candida kefyr*, *Candida lusitanae*, *Pichia ohmeri*, *Rhodotorula glutinis*, *Fusarium* sp., *Trichosporon* sp., and *Exophiala dermatidis*.

Study C. A total of 57 routine blood culture bottles positive for yeasts recovered from

both aerobic and anaerobic media (BacT/Alert, bioMérieux) from 1 Danish clinical microbiology laboratory were included in the study, which showed 100% (29/29) sensitivity and 100% (28/28) specificity (4).

Routine Identification	<i>C. albicans</i> PNA FISH	
	Positive (n)	Negative (n)
<i>C. albicans</i>	29	0
<i>C. glabrata</i>	0	20
<i>C. tropicalis</i>	0	1
<i>C. guilliermondii</i>	0	2
<i>Rhodotorula glutinis</i>	0	1
<i>Cryptococcus neoformans</i>	0	3
<i>S. cerevisiae</i>	0	1

Analytical Sensitivity

The detection limit for *C. albicans* was determined to be approximately 10⁵ colony-forming units per mL by serial dilutions of a *C. albicans*-positive culture. This is consistent with the analytical sensitivity of slide-based staining techniques (2).

Analytical Specificity

C. albicans PNA FISH has been evaluated using cultures of 24 reference strains representing phylogenetically related yeast species and 148 clinical isolates representing clinically significant yeast species. All (85/85) *C. albicans* strains were positive by *C. albicans* PNA FISH, most (86/86) of the other strains were negative (2), and *Candida orthopsilosis* (1/1) cross reacted to create a green signal.

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.
- Rigby, S., G.W. Procop, G. Haase, D. Wilson, G. Hall, C. Kurtzman, K. Oliveira, S. Von Oy, J.J. Hyldig-Nielsen, J. Coull, H. Stender. 2002. Fluorescence in situ hybridization with peptide nucleic acid probes for rapid identification of *Candida albicans* directly from blood culture bottles. J. Clin. Microbiol. 40:2182-2186.
- Wilson, D.A., M.J. Joyce, L.S. Hall, L.B. Reller, G.D. Roberts, G.S. Hall, B.D. Alexander, G.W. Procop. 2005. Multicenter evaluation of a *Candida albicans* peptide nucleic acid fluorescent in situ hybridization probe for characterization of yeast isolates from blood cultures. J. Clin. Microbiol. 43:2909-12.
- Sogaard M, H. Stender, H.C. Schonheyder. 2005. Direct identification of major blood culture pathogens, including *Pseudomonas aeruginosa* and *Escherichia coli*, by a panel of fluorescence in situ hybridization assays using peptide nucleic acid probes. J. Clin. Microbiol. 2005. 43:1947-9.

Technical Advice and Customer Service

For all inquiries please contact AdvanDx or your local distributor.

AdvanDx, Inc. AdvanDx A/S
10A Roessler Road Bygstubben 11
Woburn, MA 01801 2950 Vedbæk
USA Denmark
Tel: +1 781 376 0009 Tel: +45 45 16 07 99
Fax: +1 781 376 0111 Fax: +45 45 16 07 98

February 18, 2009. Rev. B

PN1590B

Produced under license from Boston Probes, Inc.
The product must not be used for Slide-Based human Cytochemistry, ISH-based Cancer Cytogenetics and Flow Cytometry.