C. albicans PNA FISH®

Candida albicans
Culture Identification Kit

KT002

50

Intended Use

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

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

C. albicans PNA FISH is indicated for use as an aid in the diagnosis of *C. albicans* fungemia.

IVD For *in vitro* diagnostic use.

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 yeast followed by final identification after subculture and biochemical analysis (2).

C. albicans PNA FISH® is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to unique *C. albicans* specific ribosomal RNA sequences (4,5,6).

Rapid identification of *Candida albicans* from yeast-positive blood cultures using *C. albicans* PNA FISH supports appropriate antifungal selection and has been shown to reduce anti fungal expenditures (1,3).

Principle of the Procedure

A fluorescein-labeled, *C. albicans* specific PNA probes is added to a smear prepared from a yeast positive blood 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.

Reagents

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

Label	Component
Fixation Solution	**Fixation Solution** 3 mL phosphate-buffered saline with detergent.
***C. albicans* PNA**	***C. albicans* 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
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 on 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 (AC003*).*

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 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® 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.

Item	Description	Code
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
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
***C. albicans* Control Slide**	*C. albicans* Control Slide.	CS002

Positive Control well contains mixture of *C. albicans* ATCC#18804; and Negative Control well contains *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.

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* Control Slide (CS002) or prepare smears from liquid cultures of laboratory or reference strains of *C. albicans* 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 (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.

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.

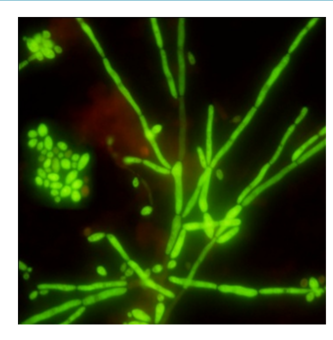
Note: Fixation Solution is designed for optimal performance in the identification of Gram-positive bacteria and Yeast and must not be interchanged with GN Fixation Solution from other PNA FISH tests for Gram-negative bacteria.

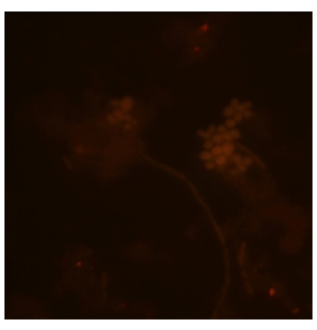
Temperature Control:
It is important that the temperature of the PNA FISH Workstation has reached 55°C prior to starting the hybridization and that Water Bath Solution has reached 55°C prior to immersion of the slides. The temperature of the Water Bath should be checked using a thermometer as outside 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.

Major Blood Culture Systems and Bottle Type Compatibility:
The PNA FISH platform is compatible with major commercially available continuous 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* is identified as multiple bright green fluorescent cells in multiple fields of view. Yeast cells may appear as buds or pseudohyphae.





Representative examples of positive (left), and negative (right) test results.

Definitive identification is pending positive blood subculture, additional microbiological evaluation and antimicrobial susceptibility testing.

Troubleshooting

- False positive Control and Sample test results may occur if the Dual Band Filter (AC003) 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 parapsilosis* (*C. 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 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.

Histoplasma capsulatum has not been tested with *C. albicans* PNA FISH; therefore, the performance with this species has not been established.

Expected Results

The expected *C. albicans* positive result rate from yeast positive blood culture bottles is 25% -50%.

Performance Characteristics

Clinical Studies
The performance of *C. albicans* PNA FISH (Shortened) versus *C. albicans* PNA FISH (Original) and versus conventional routine methods has been assessed in three clinical laboratory studies.

A total of 115 routine positive blood culture bottles were included in the studies, which showed 100% (115/115) agreement between *C. albicans* PNA FISH (Shortened) and *C. albicans* PNA FISH (Original) and 100% (115/115) agreement between *C. albicans* PNA FISH (Shortened) and conventional routine methods. These studies included two commercially available, continuously monitoring blood culture systems (BacT/ALERT, bioMérieux, NC and BACTEC, Becton Dickinson, MD). The performance data of *C. albicans* PNA FISH (Shortened) vs. *C. albicans* PNA FISH (Original) is presented and the performance data *C. albicans* PNA FISH (Shortened) versus the clinical sites' routine identification methods is presented.

Performance Data for *C. albicans* PNA FISH (Shortened) vs. *C. albicans* PNA FISH (Original) on Yeast-Positive Blood Culture Bottles

Study	Positive Agreement *C. albicans*	Negative Agreement	Blood Culture System
A	15/15	14/14	BacT/Alert
B	4/4	31/31	BacT/Alert
C	12/12	39/39	BACTEC
Total	100% (31/31) 95% CI (90.8-100)	100% (84/84) 95% CI (96.5-100)	N= 115

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

Study	Sensitivity *C. albicans*	Specificity	Blood Culture System
A	15/15	14/14	BacT/Alert
B	4/4	31/31	BacT/Alert
C	12/12	39/39	BACTEC
Total	100% (31/31) 95% CI (90.8-100)	100% (84/84) 95% CI (96.5-100)	N= 115

Analytical Sensitivity
The detection limit for *C. albicans* 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 (2).

Analytical Specificity
C. albicans PNA FISH has been evaluated using on 65 laboratory and reference strains representing phylogenetically related yeast species comprising 59 fungal strains, and 6 other frequently isolated organisms. All (22/22) *C. albicans* strains were positive, and the remaining (43/43) fungal and bacteria strains were negative.

Reproducibility
A reproducibility study was performed on 13 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	54/54	54/54	54/54	100% (162/162)
Negative Agreement	63/63	63/63	63/63	100% (189/189)
Total Agreement	100% (117/117)	100% (117/117)	100% (117/117)	100% (351/351)

Summary of Reproducibility Results by Day Across 3 Sites

	Day 1	Day 2	Day 3	Total Agreement
Positive Agreement	54/54	54/54	54/54	100% (162/162)
Negative Agreement	63/63	63/63	63/63	100% (189/189)
Total Agreement	100% (117/117)	100% (117/117)	100% (117/117)	100% (351/351)

Bibliography

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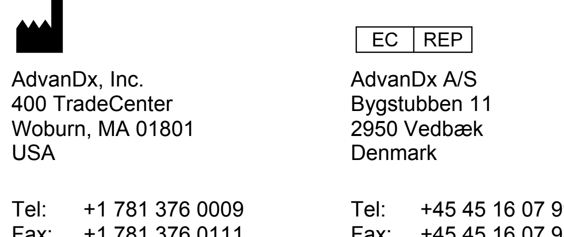
Definitions

Symbol	Definition
REF	Product code/catalog number
	Consult the instructions for use
Σ	Contains sufficient for <n> tests
	Manufacturer
EC REP	Authorized representative
	Use by
LOT	Batch code
	Storage temperature limitations

Technical Advice and Customer Service

For all inquiries, please contact AdvanDx 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.

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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