# 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.

---

## 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.

---

## Precautions

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 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.

---

## Reagents

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

- **Fixation Solution**: 3 mL phosphate-buffered saline with detergent.
- **C. albicans PNA**: 1.5 mL PNA probes in hybridization solution. Contains 30% formamide.
- **60x Wash Solution**: 50 mL Tris-buffered saline with detergent.
- **Mounting Medium**: 3 mL photobleaching inhibitor in glycerol.

---

**CE**

**REF** KT002

**Σ** 50
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®

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.**

<table>
<thead>
<tr>
<th>Material Required</th>
<th>Material Available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscope Slides</td>
<td>AC001</td>
</tr>
<tr>
<td>Coverslips</td>
<td>AC002 or AC007</td>
</tr>
<tr>
<td>Dual Band Filter</td>
<td>AC003</td>
</tr>
<tr>
<td>Staining Dish</td>
<td>AC004</td>
</tr>
<tr>
<td>PNA FISH Workstation</td>
<td>AC005</td>
</tr>
<tr>
<td>Water Bath</td>
<td>AC006</td>
</tr>
<tr>
<td><em>C. albicans</em> Control Slide</td>
<td>CS002</td>
</tr>
</tbody>
</table>

**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 polyethylenesulfonate.

Interpretation of Results
Examine slides using a fluorescence microscope. The blood culture smear
appears in general reddish. C. albicans is identified as multiple bright
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 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 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

<table>
<thead>
<tr>
<th>Study</th>
<th>Positive Agreement C. albicans</th>
<th>Negative Agreement</th>
<th>Blood Culture System</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15/15</td>
<td>14/14</td>
<td>BacT/Alert</td>
</tr>
<tr>
<td>B</td>
<td>4/4</td>
<td>31/31</td>
<td>BacT/Alert</td>
</tr>
<tr>
<td>C</td>
<td>12/12</td>
<td>39/39</td>
<td>BACTEC</td>
</tr>
<tr>
<td>Total</td>
<td>100% (31/31)</td>
<td>100% (84/84)</td>
<td>N= 115</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity C. albicans</th>
<th>Specificity</th>
<th>Blood Culture System</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15/15</td>
<td>14/14</td>
<td>BacT/Alert</td>
</tr>
<tr>
<td>B</td>
<td>4/4</td>
<td>31/31</td>
<td>BacT/Alert</td>
</tr>
<tr>
<td>C</td>
<td>12/12</td>
<td>39/39</td>
<td>BACTEC</td>
</tr>
<tr>
<td>Total</td>
<td>100% (31/31)</td>
<td>100% (84/84)</td>
<td>N= 115</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Site</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Total Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Agreement</td>
<td>54/54</td>
<td>54/54</td>
<td>54/54</td>
<td>100% (162/162)</td>
</tr>
<tr>
<td>Negative Agreement</td>
<td>63/63</td>
<td>63/63</td>
<td>63/63</td>
<td>100% (189/189)</td>
</tr>
<tr>
<td>Total Agreement</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100% (351/351)</td>
</tr>
</tbody>
</table>

### Summary of Reproducibility Results by Day Across 3 Sites

<table>
<thead>
<tr>
<th>Day</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Total Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Agreement</td>
<td>54/54</td>
<td>54/54</td>
<td>54/54</td>
<td>100% (162/162)</td>
</tr>
<tr>
<td>Negative Agreement</td>
<td>63/63</td>
<td>63/63</td>
<td>63/63</td>
<td>100% (189/189)</td>
</tr>
<tr>
<td>Total Agreement</td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td>100% (351/351)</td>
</tr>
</tbody>
</table>

### Bibliography


### Definitions

<table>
<thead>
<tr>
<th>REF</th>
<th>Product code/catalog number</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>Consult the instructions for use</td>
</tr>
<tr>
<td>X</td>
<td>Contains sufficient for &lt;n&gt; tests</td>
</tr>
<tr>
<td>M</td>
<td>Manufacturer</td>
</tr>
<tr>
<td>P</td>
<td>Authorized representative</td>
</tr>
<tr>
<td>H</td>
<td>Use by</td>
</tr>
<tr>
<td>g</td>
<td>Batch code</td>
</tr>
<tr>
<td>l</td>
<td>Storage temperature limitations</td>
</tr>
</tbody>
</table>

### Technical Advice and Customer Service

For all inquiries, please contact AdvanDx or your local distributor.

AdvanDx, Inc.  
400 TradeCenter  
Woburn, MA 01801  
USA  
Tel: +1 781 376 0009  
Fax: +1 781 376 0111

AdvanDx A/S  
Bygstubben 11  
2950 Vedbæk  
Denmark  
Tel: +45 45 16 07 99  
Fax: +45 45 16 07 98

technical@advandx.com  
www.PNA-FISH.com

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.

10 August 2012  
PN1743. Rev. C