Candida albicans/Candida glabrata PNA FISH®

C. albicans/C. glabrata PNA FISH® is a fluorescence qualitative nucleic acid hybridization assay intended for identification of \( \text{C. albicans} \) and/or \( \text{C. glabrata} \) 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 \( \text{C. albicans} \) and/or \( \text{C. glabrata} \) fungemia.

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

### Precautions

**IVD** For *in vitro* diagnostic use.

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

### Safety Precautions

<table>
<thead>
<tr>
<th><strong>C. albicans/C. glabrata PNA</strong></th>
<th><strong>Danger</strong></th>
<th><strong>Contains 30% Formamide</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fixation Solution</strong></td>
<td><strong>Warning</strong></td>
<td>Causes serious eye irritation. 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.</td>
</tr>
</tbody>
</table>

### Principle of the Procedure

A mixture of a fluorescein-labeled, \( \text{C. albicans} \)-specific PNA probe and a rhodamine-labeled, \( \text{C. glabrata} \)-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**
  3 mL phosphate-buffered saline with detergent.

- **C. albicans/C. glabrata PNA**
  1.5 mL PNA probes in hybridization solution. Contains 30% formamide.
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.

**Material Provided**
- C. albicans/C. glabrata 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.
- The smear may be stored for up to 1 month at room temperature.

**Test Procedure**

**Material Required and Available from AdvanDx.**
- Microscope Slides
- Coverslips
- Dual Band Filter
- Staining Dish
- PNA FISH Workstation
- Water Bath
- C. albicans/C. glabrata Control Slide
- C. albicans/C. glabrata Control Slide
- C. albicans/C. glabrata Control Slide
- C. albicans/C. glabrata Control Slide
- C. albicans/C. glabrata Control Slide

**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.

### 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 sunlight 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.

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Positive Agreement C. albicans</th>
<th>Positive Agreement C. glabrata</th>
<th>Negative Agreement</th>
<th>Blood Culture System</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100% (21/21)</td>
<td>100% (9/9)</td>
<td>100% (20/20)</td>
<td>BACTEC</td>
</tr>
<tr>
<td>B</td>
<td>100% (21/21)</td>
<td>100% (13/13)</td>
<td>100% (16/16)</td>
<td>BacT/ALERT</td>
</tr>
<tr>
<td>C</td>
<td>100% (5/5)</td>
<td>100% (6/6)</td>
<td>100% (15/15)</td>
<td>BACTEC</td>
</tr>
<tr>
<td>Total</td>
<td>100% (47/47)</td>
<td>100% (28/28)</td>
<td>100% (51/51)</td>
<td>N= 126</td>
</tr>
</tbody>
</table>

95% CI (93.8-100) (95% CI (89.9-100) (94.3-100)

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

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity C. albicans</th>
<th>Sensitivity C. glabrata</th>
<th>Specificity</th>
<th>Blood Culture System</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100% (21/21)</td>
<td>100% (9/9)</td>
<td>100% (20/20)</td>
<td>BACTEC</td>
</tr>
<tr>
<td>B</td>
<td>100% (21/21)</td>
<td>100% (13/13)</td>
<td>100% (16/16)</td>
<td>BacT/ALERT</td>
</tr>
</tbody>
</table>
A reproducibility study was performed on 10 isolates in triplicate on three strains at three separate sites. The results of the reproducibility study; by site across three days of testing at three separate sites.

Analytical Sensitivity

The detection limit for C. albicans and C. glabrata were both determined to be approximately 10^3 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 Kluveromyces 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

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

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

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

### Bibliography

