AdvanDx

Candida QuickFISH® BC

Candida Culture Identification Kit

CE

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IVD

REF QFCANBC1-25

US only: RUO *For Research Use Only. The performance characteristics of this product have not been established*

Intended Use

The *Candida QuickFISH* BC is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Candida albicans* and/or *Candida glabrata* and/or *Candida parapsilosis* on smears made from positive blood cultures containing yeasts observed on Gram stain.

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

Candida QuickFISH BC is indicated as an aid in the diagnosis of fungemia due to *Candida albicans* and/or *Candida glabrata* and/or *Candida parapsilosis.*

Summary and Explanation

Candida species are well-recognized as a leading cause of both community and hospital-acquired fungemia with *C. albicans*, *C. glabrata,* and *C. parapsilosis* being among the most commonly isolated yeast species.

Routinely, *C. albicans, C. glabrata,* and *C. parapsilosis* in blood cultures are initially identified as yeast by Gram stain. Final identification and differentiation must await subculture and biochemical analysis (2).

Candida QuickFISH BC is a fluorescence *in situ* hybridization (FISH) assay using PNA probes hybridizing to *Candida* specific ribosomal RNA sequences.

The test provides rapid (20 minutes assay time) identification of *C. albicans*, *C. glabrata*, and *C. parapsilosis* on smears made from positive blood cultures containing yeast. Rapid identification of yeast positive blood cultures supports appropriate antifungal selection and has been shown to reduce antifungal expenditures (1,3-6).

Principle of the Procedure

A mixture of fluorescein-labeled *C. albicans* specific PNA probes, Tamra-labeled *C. glabrata* PNA probes, and fluorescein- labeled and Tamra-labeled *C. parapsilosis* PNA probes is added to a smear prepared from a positive blood culture.

Hybridization is performed at 55° ± 1°C for 15 min. and the smear is examined by fluorescence microscopy.

Reagents

Candida QuickFISH BC is comprised of the following kit components:

***Candida* PNA Blue**	***Candida* PNA Blue** 0.85 mL PNA probes in hybridization. solution. Contains 15% formamide.
***Candida* PNA Yellow**	***Candida* PNA Yellow** 0.85 mL PNA probes in hybridization. solution. Contains 15% formamide.

Precautions

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

Safety Precautions

Candida PNA Blue		May cause harm to the unborn child. Harmful to aquatic life with long lasting effects. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.
Candida PNA Yellow	Danger Contains 15% Formamide	
*Quick*Fix-1	Contains 24% ethanol	Harmful to aquatic life with long lasting effects. Safety Data Sheet is available upon request. Available in the *Quick*FISH Fixation Kit.
*Quick*Fix-2	 Danger Contains 97% methanol	Highly flammable liquid and vapor. Toxic if swallowed. Tonic in contact with skin. Toxic if inhaled. Causes damage to the central nervous system. Safety Data Sheet is available upon request. Available in the *Quick*FISH Fixation Kit.

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.

Be sure to use a new pipette tip and inoculating needle for mixing with each sample.

Do not use microscope filters other than an AdvanDx Filter listed in **Materials Required and Available from AdvanDx**.

Do not use microscope slides other than *Quick*FISH Slides (CS012).

It is important that the AdvanDx SlideStation-10 is level and equilibrated to 55 ± 1°C prior to the test procedure.

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 according to the following instructions:

Store kit components at 2-8°C. Store bottles upright and tighten caps after use. Reagents are supplied ready for use.

*Quick*FISH slides are provided in individually sealed pouches with nitrogen and a desiccant. Store slides at 2-8°C. Slides must be used immediately after breaking pouch seal. Do not use slides after the expiration date.

Specimen Collection and Preparation

Preparation of Smears

Candida QuickFISH BC is not compatible with blood culture media containing charcoal or Versa TREK REDOX 2 blood culture bottles.

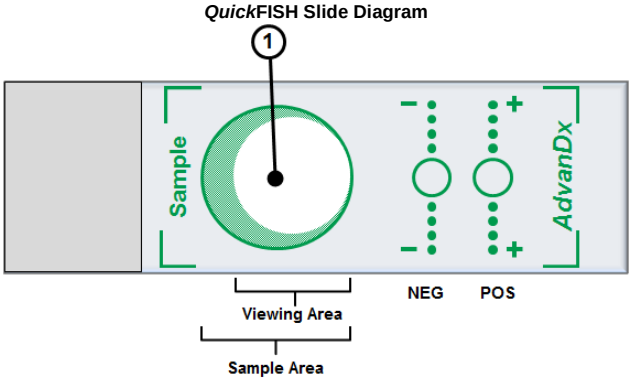
- Follow the blood culture system manufacturer’s instructions to properly mix the blood culture bottle before smear preparation.
- Place slide on SlideStation at 55 ± 1°C. When running multiple samples, ensure slides do not come in contact with each other to avoid contamination.
- Do not allow dropper bottle tip to touch the smear as this may cause cross contamination of material between slides, or cause contamination of the reagent.
- Add 1 or more drops of blood culture sample into a secondary vessel (e.g., microcentrifuge tube).
    - For bottles containing resin beads – Add 10 or more drops of sample to an AdvanDx Filter vial. Do not exceed fill line. Insert the filter

plunger into the vial and push all the way down to remove the resin beads.

- Remove cap of AdvanDx Filter Vial to access sample for smear preparation.

- Ensure the blood culture sample is well mixed. Using the AdvanDx 10 µL Pipette, transfer 10 µL of sample to the center of the sample area of a *Quick*FISH slide. Refer to reference ① in the *Quick*FISH Slide Diagram.
- Immediately place one drop of *Quick*Fix-1 onto the sample and spread evenly throughout sample area with a plastic inoculating needle. Avoid air bubbles.
- Allow the smear to dry (1-3 minutes). Smear must be visibly dry.
- Add two drops of *Quick*Fix-2 to the center of the sample area. Refer to reference ① in the *Quick*FISH Slide Diagram.
- Allow the smear to dry (~1 minute). Smear must be visibly dry.
- Fixed *Quick*FISH smears may be left on the slide warmer at 55 ± 1°C for up to 5 minutes. Prepared smears which are not used within 5 minutes can be kept at room temperature for 1 hour prior to testing or may be stored at 2-8 °C for up to 1 day before testing.

***Quick*FISH Slide Diagram**



Test Procedure

Material Provided

*Candida Quick*FISH BC QFCANBC1-25

Each kit contains sufficient material for 25 tests. Reagents are supplied ready for use. The expiration date of the kit is as indicated on the outer box label.

Material Required and Available from AdvanDx.

Large Coverslips 50 x 24 mm No. 1. AC027

AdvanDx Microscope Filter Dual Band Filter for use with high pressure mercury vapor arc lamp light sources or equivalent AC007

AdvanDx Metal Halide Filter Dual Band Filter for use with modified mercury vapor arc lamps (metal halide) AC033

AdvanDx SlideStation-10 Slide warmer (55 ± 1°C) AC028

***Quick*FISH Coverslip Mixing Station** AC030
Holds up to 3 coverslips for mixing *Candida* PNA Yellow & Blue

AdvanDx 10 µL Pipette 10 µL fixed volume pipette AC029

***Quick*FISH Slide** *Quick*FISH slide with controls* CS012

***Quick*Fix-1** Primary fixation solution* CP0169

***Quick*Fix-2** Secondary fixation solution* CP0170

AdvanDx Filter Vials Filter vials for removal of resin beads AC008

* *Quick*FISH Slide, *Quick*Fix-1, and *Quick*Fix-2 are available in the *Quick*FISH Fixation Kit.

Material Required but Not Provided

- Fluorescence microscope equipped with a 60x or 100x oil objective.
- Immersion oil. Must comply with the microscope objective and be non-fluorescent.
- Venting needle.
- Pipette tips.
- Plastic inoculating needles.

Assay Procedure

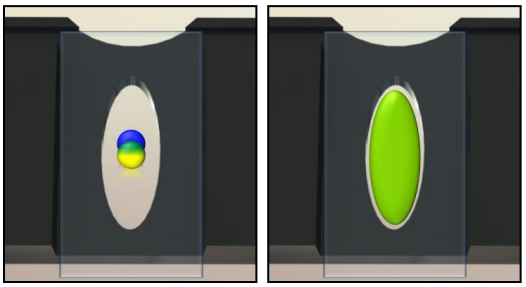
*Quick*FISH smears should be tested immediately following fixation; however, if smears were stored at 2-8°C or room temperature they must be placed on the slide warmer for approximately 5 minutes at 55 ± 1°C before adding the hybridization reagents.

It is important that the AdvanDx SlideStation-10 is level and equilibrated to 55 ± 1°C prior to the test procedure.

Hybridization

- Place a coverslip into one of the *Quick*FISH Coverslip Mixing Template slots. Refer to Diagram #1.
- Invert and hold each bottle and allow a drop to form in the dropper tip before squeezing the bottle to avoid formation of foam in the hybridization mixture.
- Add one drop of *Candida* PNA Blue to the center of the coverslip. Note: the ovoid cutout of the *Quick*FISH Mixing Template slot denotes the center of the coverslip. Place one drop of *Candida* PNA Yellow directly on top of the first drop. Avoid air bubbles. Refer to Diagram #1.
- Thoroughly mix PNA Blue and PNA Yellow together using a plastic inoculating needle until they produce a uniform green color, or no identifiable blue or yellow color remains. Spread lengthwise in order to fill the ovoid template. Refer to Diagram #2.

Diagram #1 **Diagram #2**



- Flip coverslip and apply to slide aligning the edges with the printed border markers on the slide. The coverslip must be placed within the markers. If the coverslip is placed on the white frosted area, the assay may fail due to insufficient flow of reagents.
- Incubate for 15 - 20 min. at 55 ± 1°C.
- Note: Avoid cross contamination of bottles. Replace dropper caps on appropriate bottles.
- Examine slides as described below.

Do not expose the slides to direct sunlight or strong light sources as this may lead to fluorescence bleaching.

Quality Control

Quality control for fluorescent testing should be performed each time testing is performed.

Control material should be tested in accordance with guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Use *Quick*FISH Slides with Controls (CS012).

*Quick*FISH slides are provided in individually sealed pouches with nitrogen and a desiccant. Store slides at 2-8°C. Slides must be used immediately after breaking pouch seal. Do not use slides after expiration date.

The Positive Control will display multiple fluorescent green, red, and yellow yeast cells. The Negative Control will not contain fluorescent cells. Positive (POS, +) and Negative (NEG, -) control wells contain representative organisms for all AdvanDx *Quick*FISH BC kits. Control organisms for other kits may be weakly visible (non-fluorescent) in both the Positive and Negative control wells.

Cell morphology and color may vary between samples and controls due to natural variations.

If the Positive and Negative Controls do not perform in accordance with the Interpretation of Results below, results are invalid and patient results should not be reported.

Locating Controls:
Align the center of the microscope objective with the dots of the POS (+) well on the *Quick*FISH Slide. Move the slide stage forward or backward until the green outline of the well appears in the field of view. Use the fine focus knob to focus on the green well outline (this is the correct focal plane for reading the slide). Move the objective into the central region of the POS Control to view. To view the NEG Control, move the objective laterally into the center of the NEG well. Continue moving laterally to find the viewing area of the sample well.

Procedural Notes

The *Quick*FISH platform is compatible with commercially available continuously monitoring blood culture systems and bottle types except bottle types supplemented with charcoal and the VersaTREK REDOX 2 anaerobic bottle. The bottle types tested were:

Clinically:
BacT/ALERT SA, BACTEC Lytic/10 Anaerobic/F and Plus Aerobic/F

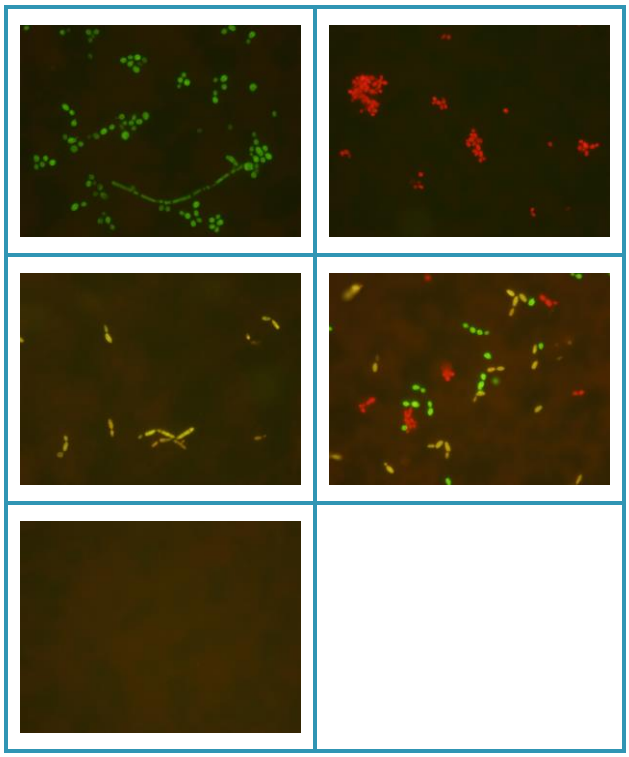
Analytically:

VersaTREK REDOX 1 aerobic, BacT/ALERT SN, BACTEC (Plus Anaerobic/F, Standard/10 Aerobic/F, Standard/10 Anaerobic/F, Peds Plus/F). The clinical performance of these blood culture bottle types with the *Candida Quick*FISH BC has not been established.

Temperature Control:
It is important that the temperature of the SlideStation be maintained at 55 ± 1°C prior to starting the hybridization.

Interpretation of Results
Read slides within 2 hours after hybridization.

Examine slides using a fluorescence microscope. View the sample in the viewing area within the sample area. The smear background may appear reddish in color. *Candida albicans* is identified as multiple bright green fluorescent yeast in multiple fields of view, *Candida glabrata* is identified as multiple bright red fluorescent yeast in multiple fields of view, and *Candida parapsilosis* is identified as multiple bright yellow fluorescent yeast in multiple fields of view. Non-candida appear non-fluorescent. Floating organisms or debris should not be interpreted or confused with positive organisms.



Representative examples of Green-Positive *C. albicans* (top-left), red-positive *C. glabrata* (top-right), Yellow-Positive *C. parapsilosis* (middle-left), mixture of Green-Positive *C. albicans*, Red-Positive *C. glabrata* and Yellow-Positive *C. parapsilosis* (middle-right) and negative (bottom) test results

Troubleshooting

False Positive and/or Negative Control and Sample test results may occur if an AdvanDx Microscope Filter is not used, or by contamination of the specimens.

False Negative Control or Sample test results may occur if AdvanDx *Quick*FISH Slides (CS012) are not used or if the temperature is not accurately controlled during hybridization.

Actual results may vary in brightness and color tone. Refer to Positive and Negative Control wells when scoring slides.

Please refer to the Precautions and Limitations sections in this product insert or contact AdvanDx.

The lid on the SlideStation is not required to be in place for the kit to perform properly.

The assay may be sensitive to small changes in drop volumes of *Candida* PNA Blue and *Candida* PNA Yellow. If foam is dispensed from bottles, DO NOT USE, discard the coverslip and prepare a new one using fresh hybridization reagents.

Limitations

- *C. viswanathii* and *C. stellatoidea* produce False Positive green results.
- *Kluyveromyces delphensis (Nakaseomyces delphensis), C. bracarensis* and *C. nivariensis* produce false positive red results.
- *Candida africana* has not been tested with *Candida Quick*FISH; therefore, the performance with this species has not been established.
- Clinical studies were conducted using the BACTEC Plus Aerobic/F, BACTEC Lytic/10 Anaerobic/F, and BacT/ALERT SA blood culture bottles. The performance of *Candida Quick*FISH BC with other blood culture bottle types has not been established.
- BACTEC Plus Anaerobic/F, BACTEC Peds Plus/F and BacT/ALERT SN bottles were not extensively evaluated during the clinical investigation, and therefore the performance has not been adequately established.
- The performance of VersaTREK REDOX 1 and BACTEC Standard/10 Aerobic/F and Standard/10 Anaerobic/F blood culture bottles was evaluated in an internal compatibility study only. Therefore, the performance is unknown.
- False Positive green autofluorescence may occur if a standard FITC filter is used instead of the AdvanDx Microscope 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 and to identify positive blood cultures yielding a negative FISH result.
- The product has not been validated with specimens other than blood cultures.

Expected Results

The clinical study population of yeast-positive blood culture bottles was derived from 7 healthcare centers in the United States and included 102 blood cultures from 100 patients. The *C. albicans, C. glabrata* and *C. parapsilosis* positive result rates were 34%, 34% and 13% respectively. Other yeast-positive organisms were identified in 19% of the samples.

The rates presented are a percentage of unique patient blood cultures (multiple samples from the same patient and spiked samples were not included) as identified by routine methods as a percent of the total number of all species identified in the studies.

Rates of positive and negative species results obtained with *Candida Quick*FISH BC may vary depending on institution and patient population.

Performance Characteristics

The performance of *Candida Quick*FISH BC versus routine laboratory methods has been assessed in a multicenter study including seven clinical laboratories. A total of 102 routine *Candida* (yeast) positive blood culture bottles from 100 patients and 81 contrived (spiked) samples were included in the studies, which showed 99.5% agreement between *Candida Quick*FISH BC and conventional routine methods. These studies included two commercially available, continuously monitoring blood culture systems (BacT/ALERT, bioMérieux, NC and BACTEC, Becton Dickinson, NJ). Bottles were stored at room temperature after Gram stain and prior to *Quick*FISH testing. Bottles over 48 hours old were excluded from the study. The data is presented below:

		Routine Identification			
		C. albicans	***C. glabrata***	***C. parapsilosis***	**Other**
Candida QuickFISH	***C. albicans***	55	0	0	0
	C. glabrata	0	54	0	0
	C. parapsilosis	0	0	30	0
	Negative	0	0	1[1]	43
		Positive Percent Agreement 100% (55/55) 95% CI (93.5-100)	**Positive Percent Agreement** 100% (54/54) 95% CI (93.4-100)	**Positive Percent Agreement** 96.8% (30/31) 95% CI (83.8-99.4)	**Negative Percent Agreement** 100% (43/43) 95%CI (91.8-100)

[1]One false negative was yellow upon repeat testing (spiked sample)

Additional testing was performed at AdvanDx on sixty (60) isolates of twenty (20) each for *C. albicans, C. glabrata* and *C. parapsilosis*. There was 100% Agreement. The following Table is presented below:

		Routine Identification			Total
		C. albicans	***C. glabrata***	***C. parapsilosis***	
Candida QuickFISH	***C. albicans***	20	0	0	20
	C. glabrata	0	20	0	20
	C. parapsilosis	0	0	20	20
	Negative	0	0	0	60
		Positive Percent Agreement 100% (20/20) 95% CI (83.9-100)	**Positive Percent Agreement** 100% (20/20) 95% CI (83.9-100)	**Positive Percent Agreement** 100% (20/20) 95% CI (83.9-100)	**Positive Percent Agreement** 100% (60/60) 95% CI (94-100)

Limit of Detection
The analytical sensitivity of *Candida Quick*FISH BC as measured as the detection limit of the *Candida Quick*FISH BC assay for *C. albicans*, *C. glabrata* and *C. parapsilosis* was determined to be approximately 5.0 x10^5 CFU/mL by serial dilutions.

Analytical Specificity and Sensitivity (inclusivity)
*Candida Quick*FISH BC was tested on 67 reference and clinical laboratory strains including 8 strains of *C. albicans*, 6 strains of *C. glabrata*, and 6 strains of *C. parapsilosis* which included 2 strains of *C. orthopsilosis* and 1 strain of *C. metapsilosis*. All 8 strains of *C. albicans* tested green positive. All 6 strains of *C. glabrata* tested red positive. All 3 strains of *C. parapsilosis* tested yellow positive. Both strains of *C. orthopsilosis* and 1 strain of *C. metapsilosis* produced negative results.

Testing included 34 strains of other yeast. Twenty-eight of these produced the expected negative results. One strain of *C. viswanathii* and 2 strains of *C. stellatoidea* (formerly *C. albicans*) produced positive green results. One strain of *Kluyveromyces delphensis (Nakaseomyces delphensis*), 1 strain of *C. bracarensis* (*Candida glabrata*) and 1 strain of *C. nivariensis* (*C. glabrata*) produced positive red results.

Reproducibility
A reproducibility study was performed with *Candida Quick*FISH BC by two independent operators who were blinded to the identification of the organisms. The results are presented below:

	Day 1	**Day 2**	**Day 3**	**Total**
Positive Agreement Green	15/15	15/15	15/15	**45/45**
Positive Agreement Red	15/15	15/15	15/15	**45/45**
Positive Agreement Yellow	15/15	15/15	15/15	**45/45**
Negative Agreement	15/15	15/15	15/15	**45/45**
Total Agreement	**100% 60/60**	**100% 60/60**	**100% 60/60**	**100% 180/180**

Bibliography

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3. Della-Latte, P., S. Whittier, and F. Wu. 2008. Impact of Rapid Identification of C. albicans and C. glabrata Directly from Blood Cultures using PNA FISH Technology on Selection of Antifungal Therapy. Poster #P1382. European Congress of Clinical Microbiology and Infectious Disease, Barcelona, Spain.
4. Forrest, G., K. Johnson, and R. Venezia. 2009. Sustained Effect of Peptide Nucleic Acid Fluorescent in-situ Hybridization (PNA FISH) on Antimicrobial Utilization and Costs. Poster# D-787. Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco.
5. Forrest, G., M. Kent, and R. Venezia. 2008. Evaluation of the Candida albicans/glabrata (CAG) Peptide Nucleic Acid Fluorescence In-situ Hybridization (PNA FISH) Test on Patient Management. Poster# M-707. Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, D.C.
6. Forrest, G., K. Mankes, E. Jabra-Rizk, E. Weekes, J. Johnson, D. Lincalis, and R. Venezia. 2006. Peptide nucleic acid fluorescence in situ hybridization-based identification of Candida albicans and its impact on mortality and antifungal therapy costs. J. Clin. Microbiol. 44:3381-3383.

Definitions

REF	Product code/catalog number	LOT	Batch code
	Consult the instructions for use		Storage temperature limitations
	Contains sufficient for <n> tests		Health Hazard
	Manufacturer		Skull and Crossbones
EC REP	Authorized Representative		Flame
	Use by		

Technical Advice and Customer Service

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