

## S. aureus/CNS PNA FISH™

*Staphylococcus aureus*/  
Coagulase-negative staphylococci  
Culture Identification Kit



Cat. No. KT005

50 tests

### Intended Use

*S. aureus*/CNS PNA FISH is intended for identification of *Staphylococcus aureus* and selected other staphylococci on smears 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

*S. aureus* is well-recognized as a leading cause of both community- and hospital-acquired bacteremia, whereas selected other *Staphylococcus* species, commonly referred to as coagulase-negative staphylococci CNS) are common blood culture contaminants.

Both *S. aureus* and CNS in blood cultures are presumptively identified as Gram-positive cocci in clusters; final identification and differentiation must await subculture and biochemical analysis (1).

*S. aureus*/CNS PNA FISH is a fluorescence in situ hybridization (FISH) method using

PNA probes hybridizing to *S. aureus*-specific ribosomal RNA sequences to detect *S. aureus*, and generic, non-*aureus* staphylococci ribosomal RNA sequences, to detect selected other *Staphylococcus* species.

The test provides rapid identification and differentiation of *S. aureus* and CNS on smears made from positive blood cultures.

### Principle of the Procedure

A mixture of a fluorescein-labeled *S. aureus*-specific PNA probe and a rhodamine-labeled PNA probe specific for selected other *Staphylococcus* species 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. *In vitro* diagnostic medical device. Use by. Batch code. Storage temperature limitations.

### Reagent

*S. aureus*/CNS PNA FISH is comprised of the following kit components:

**Fixation Solution** - Fixation Solution  
3 mL phosphate buffered saline with detergent

**S. aureus/CNS PNA** - *S. aureus*/CNS PNA  
1.5 mL PNA probe 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

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 *S. aureus*/CNS 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 (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 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

- S. aureus*/CNS PNA FISH (Cat. No. KT005). Each kit contains sufficient material for testing 50 cultures. 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) (see procedural notes).
- 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).
- S. aureus/CNS Control Slide** - *S. aureus*/CNS Control Slide (Cat. No. CS005). Contains a positive control prepared from liquid culture containing a mixture of *S. aureus*, ATCC# 29213, and *S. epidermidis*, ATCC# 14990; and a negative control prepared from liquid culture of *S. agalactiae*, ATCC# 13813.

#### 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 *S. aureus*/CNS 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 AdvanDx *S. aureus*/CNS Control Slide (Cat. No. CS005) or prepare smears from cultures of laboratory or reference strains of *S. aureus* and *S. epidermidis* as Positive Controls either on separate slides or mixed on one slide and a *Streptococcus* or *Enterococcus* 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 *S. aureus*/CNS Control Slide (Cat. No. CS005), simply remove slide from pouch and follow the PNA FISH procedure starting with the hybridization step.

The performance of the Positive Control has been demonstrated using *S. aureus* and *S. epidermidis* present on the same slide, and separated onto individual positive control slides for each organism.

*S. aureus* must test green-positive and CNS must test red-positive 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:

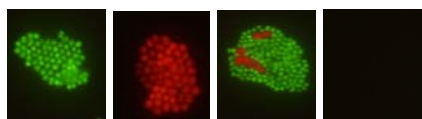
It is important that the temperature of the PNA FISH Workstation has reached 55°C prior to starting the hybridization and that the Wash Solution has reached 55°C prior to immersion of the slides. The temperature of the Water Bath should be checked using a thermometer as outside temperature readings may 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. *S. aureus* is identified as multiple bright green fluorescent clusters of cocci in multiple fields of view. CNS is identified as multiple bright red fluorescent clusters of cocci in multiple fields of view. Non-*Staphylococcus* cells appear non-fluorescent.



Representative examples of green-positive *S. aureus* (left), red-positive CNS (middle-left), mixture of green-positive *S. aureus* and red-positive CNS (middle-right), 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 in this product insert or contact AdvanDx.

## Limitations

The following rare-occurring *Staphylococcus* species are negative by *S. aureus*/CNS PNA FISH: *Staphylococcus caseolyticus*, *Staphylococcus felis* and *Staphylococcus simulans*.

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 and to identify positive blood cultures yielding a negative result.

The product has not been validated with specimens other than blood cultures.

## Expected Results

The combined expected *S. aureus* and CNS positive result rate from Gram-positive cocci positive blood culture bottles is approximately 21% and 55%, respectively, but may vary depending on institution and patient population (2).

## Performance Characteristics

### Clinical Studies

The performance of *S. aureus*/CNS PNA FISH on 275 GPCC and 54 other positive routine blood cultures has been determined at four sites in the United States (MA, RI, PA, and MD) and one site in Denmark and is summarized below where the results were compared to results obtained by subculture and subsequent identification by standard methods (3).

Blood culture Medium	Sensitivity <i>S. aureus</i>	Sensitivity CNS	Specificity	Sites
ESP	100% (23/23)	96.2% (25/26)	100% (26/26)	A
BACTEC	100% (14/14)	100% (39/39)	100% (11/11)	B
BacT/Alert	100% (16/16)	97% (32/33)	100% (7/7)	C
BacT/Alert	100% (15/15)	100% (29/29)	90% (18/20)	D
BACTEC	100% (26/26)	100% (54/54)	-	E
Total	100% (94/94)	98.9% (179/181) <sup>a</sup>	96.9% (62/64) <sup>b</sup>	

<sup>a</sup> 2 false negatives were found, one was positive upon retest and the other was *S. simulans*.

<sup>b</sup> 64 non-staphylococcal species were identified by routine methods comprising micrococci (4), streptococci (19), yeast (4), Gram negative (19), enterococci (5), diptheroids (3), corynebacterium (2), mixed species (6), and no growth (2).

## Analytical Sensitivity

The detection limit for *S. aureus* and *S. epidermidis* were both determined to be approximately 10<sup>5</sup> 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

*S. aureus*/CNS PNA FISH has also been tested on 65 laboratory and reference strains comprising 39 *Staphylococcus* species, and 24 other frequently isolated organisms. All (2/2) *S. aureus* strains were green-positive, and most (35/38) CNS species were red-positive, except *S. caseolyticus*, *S. felis* and *S. simulans* which were negative. Out of 11 fungal species and 13 bacterial species all strains were negative.

## Bibliography

1. **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.
2. **Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahm DF, Volturo GA. 2004.** Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. *Ann Clin Micro and Antibi.* 3(7)
3. **Whitehead V, Castellone T, Chapin KC, Colasante G, Beckwith DG, Stamper P, Carroll KC, Oliveira KM, Gillespie W, Profft Larsen T, Horvat L, Shepard JR, Fiandaca MJ. 2007.** Evaluation of dual color *S. aureus*/CNS PNA FISH for simultaneous identification of *Staphylococcus aureus* and coagulase-negative Staphylococci

directly from positive blood culture bottles. Abstract #C-239, 107<sup>th</sup> Annual Meeting of American Society for Microbiology, Toronto, Canada.

## Technical Advice and Customer Service

For all inquiries please contact AdvanDx or your local distributor.

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