AdvanDx

S. aureus/CNS PNA FISH®

Staphylococcus aureus/ Coagulase-negative staphylococci Culture Identification Kit

CE

Σ 50

IVD

REF KT005

Intended Use

S. aureus/CNS PNA FISH is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Staphylococcus aureus* and/or selected other *Staphylococcus* species on smears made from positive blood cultures containing Gram-positive cocci in clusters observed on Gram stain.

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

S. aureus/CNS PNA FISH is intended as an aid in the diagnosis of *S. aureus* bacteremia.

IVD For *in vitro* diagnostic use.

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).

The *S. aureus*/CNS PNA FISH is a fluorescence *in situ* hybridization (FISH) method using PNA probes hybridizing to *S. aureus*-specific ribosomal RNA sequences and PNA probes hybridizing to ribosomal RNA of selected other *Staphylococcus* species (CNS).

The test provides rapid identification of *S. aureus* and selected other *Staphylococcus* species (CNS) on smears made from positive blood cultures (5). By selected other staphylococci means all *Staphylococcus* species other than *S. aureus*, except for *S. felis and S. simulans.*

The test provides rapid (within 90 min) identification of *S. aureus* and/or CNS on smears made from positive blood cultures leading to improved patient therapy and management (2,4).

Principle of the Procedure

A fluorescein-labeled, *S. aureus*-specific PNA probes and Texas Red-labeled PNA probes targeting other staphylococci (CNS) is added to a smear prepared from a 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 Wash Solution. Finally, the smear is mounted with Mounting Medium and examined by fluorescence microscopy.

Reagents

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 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 fluorescence microscopy.

Safety Precautions

S. aureus/CNS PNA	 Danger Contains 30% Formamide	May cause harm to the unborn child. Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.
Fixation Solution	 Warning	Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.
60X Wash Solution	 Danger Contains polyethylene glycol octylpheno ether	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.
Mounting Medium	 Warning Propyl 3,4,5-trihydroxybenzoate	May cause an allergic reaction. Avoid exposure - obtain special instructions before use. Safety Data Sheet is available upon request.

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 filters other than the Dual Band Filter (AC003 or AC007).

Do not use microscope slides other 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

- Follow the blood culture system manufacturer's instruction to properly mix the blood culture bottle before smear preparation. Follow established laboratory procedures on sub-culturing of positive blood culture.
- 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.

Test Procedure

Material Provided

S. aureus/CNS PNA FISH®	KT005

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

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 or AC007
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
***S. aureus*/CNS Control Slide**	*S. aureus*/CNS Control Slide.	CS005

Positive Control well contains a mixture of *S. aureus* ATCC#29213, and *S. epidermidis* ATCC#14990, and a Negative Control well contains *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. Use a sterile loop to remove resin beads if needed.
- 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 *S. aureus*/CNS Control Slide (CS005) or prepare smears from liquid cultures of laboratory or reference strains of *S. aureus and S. epidermidis* as Positive Control either on separate slides or mixed on one slide and *Streptococcus agalactiae* 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.

S. aureus must test green-positive, and *S. epidermidis* 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 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.

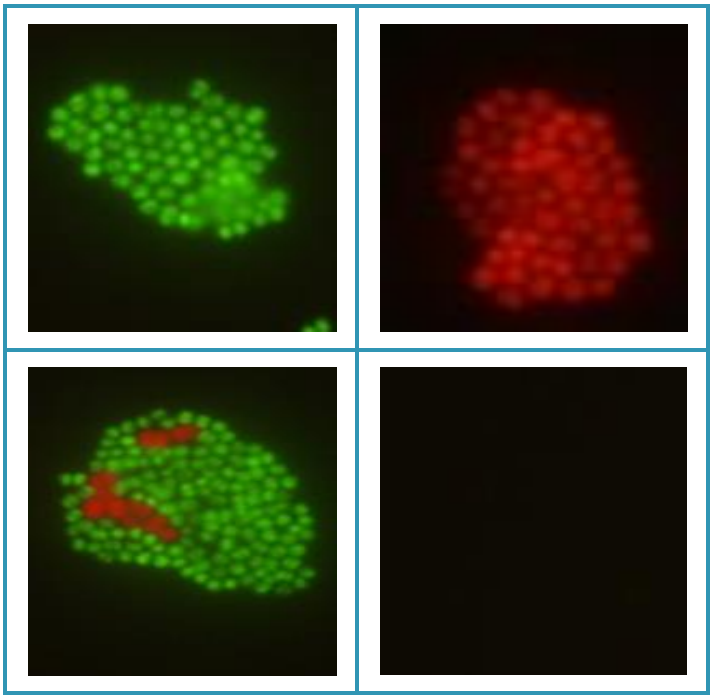
Major Blood Culture Systems and Bottle Type Compatibility:

The PNA FISH platform is compatible with the commercially available continuously monitoring blood culture systems and bottle types, 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. *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.

Definitive identification is pending subculture and additional microbiological evaluation.



Representative examples of green-positive *S. aureus* (top-left), red-positive CNS (top-right), mixture of green-positive *S. aureus* and red-positive CNS (bottom-left), and negative (bottom-right) test results.

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

The following species are negative by *S. aureus*/CNS PNA FISH: *Macrococcus caseolyticus* (formerly *Staphylococcus cohnii* subspp. *cohnii*) ATCC 35662, *Staphylococcus simulans, Staphylococcus felis,* and *Macrococcus equipericus* (formerly *Staphylococcus equipericus*).

The analytical specificity data demonstrated weak green fluorescence with *Candida krusei.*

The clinical data demonstrated false positive-red with *Micrococcus* spp from the VERSA TREK REDOX1 blood culture bottles.

Insufficient pediatric bottles have been tested in clinical studies; therefore, the performance of the *S. aureus*/CNA PNA FISH assay with pediatric bottles is unknown.

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 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 (3).

Performance Characteristics

Clinical Studies

A total of 402 routine GPCC positive blood culture bottles were included in the studies, which showed 99.5% (400/402) agreement between *S. aureus*/CNS PNA FISH and conventional routine methods. These studies included three commercially available, continuously monitoring blood culture systems (BacT/ALERT, bioMérieux, NC, BACTEC, Becton Dickinson, MD and VersaTREK, Magellan Biosciences, OH).

Performance Data for *S. aureus*/CNS PNA FISH (New) vs. Routine Identifications on GPCC-Positive Blood Culture Bottles

Study	*S. aureus*	CNS	Other	Blood Culture System
A	100% (32/32) 95% CI (91.1-100)	100% (67/67) 95% CI (95.6-100)	100% (1/1) 95% CI (5.0 - 100)	BACTEC
B	100% (17/17) 95% CI (83.8-100)	100% (82/82) 95% CI (96.4-100)	100% (4/4) 95% CI (47.3-100)	BacT/ALERT
C	100% (32/32) 95% CI (91.1-100)	100% (65/65) 95% CI (95.5-100)	100% (4/4) 95% CI (47.3-100)	BACTEC
D	100% (32/32) 95% CI (91.9. - 100)	100% (64/64) 95% CI (95.4. - 100)	0% (0/2)[1] 95% CI (0 - 77.6)	VersaTREK

	Sensitivity	Sensitivity	Specificity
Total	100% (113/113) 95% CI (95.1-100)	100% (278/278) 95% CI (98-100)	81.8% (9/11) 95% CI (48.2-97.7)

[1] Two false positive red were *Micrococcus* spp. on cultures

Limit of Detection

The detection limit for *S. aureus* and *S. epidermidis* were both 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.

Analytical Specificity and Sensitivity

S. aureus/CNS PNA FISH (New) has been collectively tested on 150 laboratory and reference strains comprising 43 *S. aureus* strains and an additional 25 Gram-positive organisms, 33 Gram-negative organisms and 6 yeast strains which included phylogenetically related bacteria species and a variety of clinically significant species. All (43/43) *S. aureus* were green positive and 39 selected other staphylococci were red positive; as expected, *Macrococcus caseolyticus* (formerly *Staphylococcus cohnii* subspp. *cohnii*) ATCC 35662, *Staphylococcus simulans, Macrococcus equipericus* (formerly *Staphylococcus equipericus*) and *Staphylococcus felis* were not detected by the CNS PNA probes. *Candida krusei* demonstrated weak green fluorescence, and all (63/63) other strains were negative.

Reproducibility

A reproducibility study was performed using the S. aureus/CNS PNA FISH the results of the reproducibility study are presented; 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
Positive Agreement Green	36/36	36/36	36/36	108/108
Positive Agreement Red	18/18	18/18	18/18	54/54
Negative Agreement	36/36	36/36	36/36	108/108
Total Agreement	100% (90/90)	100% (90/90)	100% (90/90)	270/270

Summary of Reproducibility Results by Day Across 3 Sites

	Day 1	Day 2	Day 3	Total
Positive Agreement Green	36/36	36/36	36/36	108/108
Positive Agreement Red	18/18	18/18	18/18	54/54
Negative Agreement	54/54	54/54	54/54	108/108
Total Agreement	100% (90/90)	100% (90/90)	100% (90/90)	270/270

Bibliography

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3. **Karlowsky J. A., M. E. Jones, D. C. Draghi, C. Thornsberry, D. F. Sahm, and G. A. Volturo.** 2004. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann Clin Micro and Antibiol. **3**(7).
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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		Exclamation Mark
EC REP	Authorized representative		Corrosion
	Use by		Environment

Technical Advice and Customer Service

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