

Staphylococcus QuickFISH™ BC

Staphylococcus aureus/ Coagulase-negative Staphylococci Culture Identification Kit



50

IVD

REF QFSTABC1-50

Intended Use

The *Staphylococcus* QuickFISH BC is a multicolor, qualitative nucleic acid hybridization assay intended for the identification of *Staphylococcus aureus* and/or coagulase-negative staphylococci commonly isolated from human blood cultures, on smears prepared from positive blood cultures containing gram-positive cocci in clusters observed on Gram stain.

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

Staphylococcus QuickFISH BC is indicated as an aid in the diagnosis of *S. aureus* bacteremia and/or coagulase-negative staphylococci commonly isolated from human blood cultures.

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 other *Staphylococcus* species, commonly isolated from blood culture and generally referred to as coagulase-negative staphylococci (CoNS) are common blood culture contaminants.

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

Staphylococcus QuickFISH BC is a fluorescence *in situ* hybridization (FISH) assay using PNA probes hybridizing to *S. aureus*-specific ribosomal RNA sequences and PNA probes hybridizing to ribosomal RNA of other CoNS.

The test provides rapid (20 minutes assay time) identification of *S. aureus* and CoNS on smears made from positive blood cultures containing GPCC leading to improved patient therapy and management (2,4).

Principle of the Procedure

A mixture of fluorescein-labeled, *S. aureus*-specific probes and Tamra-labeled PNA probes targeting select other CoNS is added to a smear prepared from a positive blood culture.

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

Reagents

Staphylococcus QuickFISH BC is comprised of the following kit components:

Staphylococcus PNA Blue

Staphylococcus PNA Blue

1.5 mL PNA probes in hybridization solution. Contains 15% formamide.

Staphylococcus PNA Yellow **Staphylococcus PNA Yellow**

1.5 mL PNA probes in hybridization solution. Contains 15% formamide.

Precautions

IVD For *in vitro* diagnostic use.

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

Safety Precautions

Staphylococcus PNA Blue and *Staphylococcus* PNA Yellow contain 15% 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 upon request.

QuickFix-1 contains 24% ethanol: Irritating to eyes and skin. Vapors may cause drowsiness and dizziness. Material Safety Data Sheet is available upon request. Available in the QuickFISH Fixation Kit.

QuickFix-2 contains 97% methanol: Highly flammable. Toxic by inhalation, skin contact and if swallowed. Material Safety Data Sheet is available upon request. Available in the QuickFISH 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 the AdvanDx Microscope Filter (AC007).

Do not use microscope slides other than QuickFISH 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.

QuickFISH 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.

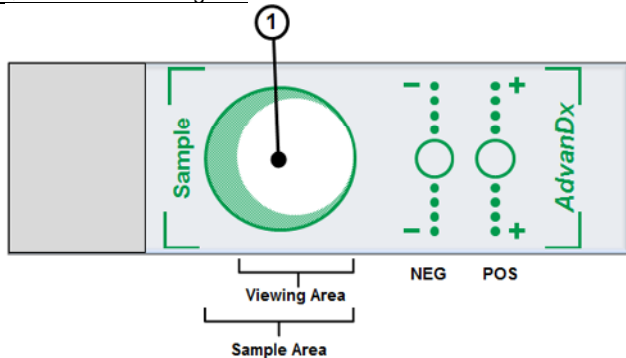
Specimen Collection and Preparation

Preparation of Smears

Staphylococcus QuickFISH BC is not compatible with blood culture media containing charcoal or VersaTREK 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 $55 \pm 1^\circ\text{C}$. 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 QuickFISH slide. Refer to reference ① in QuickFISH Slide Diagram.
- Immediately place one drop of QuickFix-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 QuickFix-2 to the center of the sample area. Refer to reference ① in QuickFISH Slide Diagram.
- Allow the smear to dry (~1 minute). Smear must be visibly dry.
- Fixed QuickFISH smears may be left on the slide warmer at $55 \pm 1^\circ\text{C}$ 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 $2-8^\circ\text{C}$ for up to 1 day before testing.

QuickFISH Slide Diagram:



Test Procedure

Material Provided

Staphylococcus QuickFISH BC

QFSTABC1-50

Each kit contains sufficient material for 50 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 AdvanDx Dual Band Filter. AC007

AdvanDx SlideStation-10 Slide warmer ($55 \pm 1^\circ\text{C}$). AC028

QuickFISH Coverslip Mixing Station AC030

Holds up to 3 coverslips for mixing *Staphylococcus* PNA Yellow & Blue

AdvanDx 10 μL Pipette 10 μL fixed volume pipette. AC029

QuickFISH Slide QuickFISH slide with controls. CS012

QuickFix-1 Primary fixation solution* CP0169

QuickFix-2 Secondary fixation solution* CP0170

AdvanDx Filter Vials AC008

* QuickFISH Slide, QuickFix-1, and QuickFix-2 are available in the QuickFISH 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

QuickFISH smears should be tested immediately following fixation; however, if smears were stored at $2-8^\circ\text{C}$ or room temperature they must be placed on the slide warmer for approximately 5 minutes at $55 \pm 1^\circ\text{C}$ before adding the hybridization reagents.

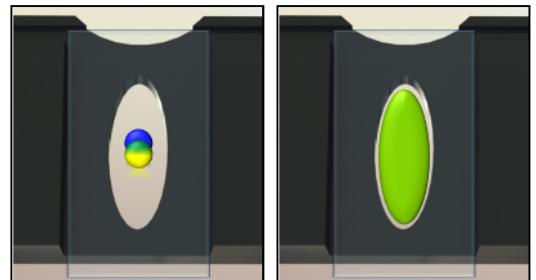
It is important that the AdvanDx SlideStation-10 is level and equilibrated to $55 \pm 1^\circ\text{C}$ prior to the test procedure.

Hybridization

- Place a coverslip into one of the QuickFISH 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 *Staphylococcus* PNA Blue to the center of the coverslip. Note: the ovoid cutout of the QuickFISH Mixing Template slot denotes the center of the coverslip. Place one drop of *Staphylococcus* 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 $55 \pm 1^\circ\text{C}$.

- 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 QuickFISH Slides with controls (CS012).

QuickFISH 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 and red cocci in clusters, the Negative control will not contain fluorescent red or green cells. Positive (POS, +) and Negative (NEG, -) control wells contain representative organisms for all AdvanDx QuickFISH BC kits. Control organisms for other kits may be weakly visible (non-fluorescent) in both the Positive and Negative control wells.

Cell morphology 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 QuickFISH 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

Major Blood Culture Systems and Bottle Type Compatibility:

The QuickFISH 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:

BacT/Alert (SA, SN)

BACTEC (Lytic 10 anaerobic, Aerobic plus, Anaerobic plus, PEDS Plus, Standard 10 aerobic, Standard anaerobic)

VersaTREK REDOX 1 aerobic

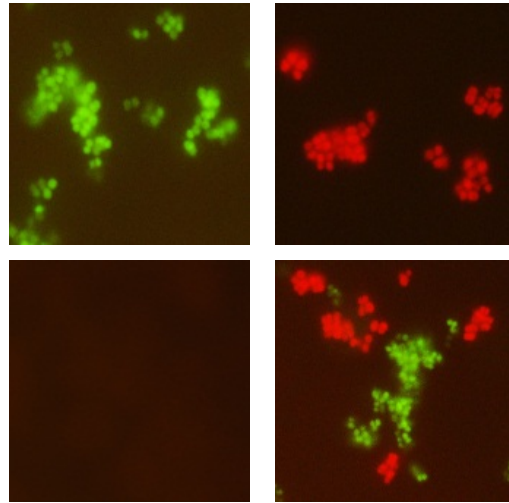
Temperature Control:

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. *Staphylococcus aureus* is identified as multiple bright green fluorescent cocci in multiple fields of view, whereas CoNS is identified as multiple bright red fluorescent cocci in multiple fields of view. Non-staphylococci appear non-fluorescent. Floating organisms or debris should not be interpreted or confused with positive organisms.



Representative examples (clockwise from upper left) of green-positive *S. aureus*, red-positive CoNS, mixture of green-positive *S. aureus* and red-positive CoNS, and negative test results.

Troubleshooting

False positive and/or negative Control and Sample test results may occur if the AdvanDx Microscope Filter (AC007) is not used, or by contamination of the specimens.

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

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 *Staphylococcus* PNA Blue and *Staphylococcus* PNA Yellow. If foam is expressed from bottles, DO NOT USE, discard the coverslip and prepare a new one using fresh hybridization reagents.

Limitations

- The following *Staphylococcus* species are negative by *Staphylococcus* QuickFISH BC: *Staphylococcus simulans* and *Staphylococcus felis*.
- The analytical specificity studies demonstrated that *Macrocooccus caseolyticus* (formerly *Staphylococcus cohnii* subsp. *cohnii*), and *Macrocooccus equipericus* (formerly *Staphylococcus equipericus*) tested negative with the *Staphylococcus* QuickFISH BC assay.
- In clinical studies, one *Micrococcus* spp. tested false green-positive and one *S. aureus* tested false red-positive.
- Coagulase-Negative *Staphylococcus* species other than those listed in the analytical and clinical studies have not been evaluated; therefore, the performance is unknown.
- *Staphylococcus* QuickFISH BC is not compatible with blood culture media containing charcoal or VersaTREK REDOX 2 blood culture bottles.
- Clinical studies were conducted using the BACTEC Plus aerobic, BACTEC Lytic/10 anaerobic, BACTEC Peds Plus and BacT/ALERT SA and SN blood culture bottles. The performance of *Staphylococcus* QuickFISH BC with other blood culture bottle types has not been evaluated.
- The performance of VersaTREK REDOX 1, BACTEC (Anaerobic Plus, Standard 10 Aerobic, Standard 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 result.
- The product has not been validated with specimens other than blood cultures.

<i>S. epidermidis</i>	96
<i>S. haemolyticus</i>	5
<i>S. hominis</i>	18
<i>S. hyicus</i> ¹	1
<i>S. intermedius</i> ¹	1
<i>S. lugdunensis</i>	1
<i>S. saccharolyticus</i>	1
<i>S. schleiferi</i>	1
<i>S. simulans</i>	4
<i>S. warneri</i>	1
<i>S. xylosus</i>	1

¹ *S. intermedius* and *S. hyicus* are coagulase positive

Expected Results

The *S. aureus* and CoNS positive result rates from the clinical studies ranged from 23-38% and 59-74%, respectively. Non-staphylococcus species (*Micrococcus*, *Streptococcus*, *Kocuria* and *Enterococcus* species) were identified in 2-3% of the samples. The study population of Gram-positive cocci in clusters-positive blood culture bottles was derived from 5 health care centers in the United States and included 516 blood cultures from 431 patients. Rates presented are a percentage of the number of each target species identified in each blood culture by routine methods as a percent of the total number of all species identified in the studies (refer to the Performance Characteristics section). Rates of positive and negative species results obtained with *Staphylococcus* QuickFISH BC may vary depending on institution and patient population (3).

Performance Characteristics

The performance of *Staphylococcus* QuickFISH BC versus routine laboratory methods has been assessed in five clinical laboratory studies. A total of 516 routine GPCC positive blood culture bottles (from 431 patients) and 31 spiked samples were included in the studies. The studies showed 99.3% (150/151) positive percent agreement for *S. aureus* and 98.3% (351/357) for CoNS. The negative percent agreement was 95.6% (43/45) from positive blood culture bottles containing GPCC.

Clinical Studies

	<i>S. aureus</i>	CoNS*	Other
Green (<i>S. aureus</i>)	150	0	1 ⁴
Red (CoNS)	1 ¹	351	1 ⁵
Negative (Non- <i>Staphylococcus</i> spp.)	0	6 ^{2,3}	43

Total	Positive Percent Agreement	Positive Percent Agreement	Negative Percent Agreement
	99.3% (150/151) ⁶	98.3% (351/357) ⁶	95.6% (43/45)
	95% CI (96.4-100)	95% CI (96.4-99.4)	95% CI (84.9-99.5)

¹False positive red result, culture ID was *S. aureus*. Result of retest was green fluorescence.

²Result of retesting of 2 false negatives was red fluorescence for each.

³Includes 4 samples identified as *S. simulans*, a known limitation of the assay.

⁴Repeat testing of one false positive (green) was negative. Culture identification was *Micrococcus* spp.

⁵Results of one test (*S. aureus* by culture ID) were both green and red. Technically a false positive red result; however, the test was correctly positive (green) for *S. aureus*. Specimen was not available for retesting.

⁶Includes five mixed cultures (*S. aureus* and CoNS) correctly identified as green and red.

*The following CoNS were identified in the clinical studies:

Organism	Number
Coagulase negative staphylococci (not further speciated)	212
<i>S. auricularis</i>	2
<i>S. capitis</i>	9
<i>S. caprae</i>	4

In the clinical studies, the time between routine Gram stain and *Staphylococcus* QuickFISH BC testing varied for each of the laboratories. Bottles were stored at room temperature after Gram stain and before QuickFISH testing. Bottles were tested within 2 hours 13% (67/516) of the time, 31% (159/516) within 4 hours and 48% (248/516) within 8 hours. Fifty percent (256/516) of the samples were tested between 8 and 48 hours from Gram stain and 2% (12/516) were greater than 48 hours when tested with QuickFISH. No discrepancies were reported within the first 6 hour time frame and only one in less than 8 hours (at 6 ½ hours). The four other discrepancies (not counting *S. simulans*, a known limitation) occurred at greater than 8 hours.

Limit of Detection

The detection limit for *S. aureus* and *S. epidermidis* were both determined to be approximately 10⁵ 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

Staphylococcus QuickFISH BC has been tested on 142 clinical laboratory and reference strains including 29 *Staphylococcus aureus* strains and 40 strains of other *Staphylococcus*. All 29 *S. aureus* strains tested green-positive and 38 out of 40* other staphylococci were red-positive. The 2 negative results (*S. felis* and *S. simulans*) were expected because these organisms have unique rRNA sequences which are not complimentary to the assay probes. Additionally, 10 GPCC (including 2 *Micrococcus* spp.) tested negative by the *Staphylococcus* QuickFISH BC assay. Testing of 51 strains of other bacteria and 12 yeasts all resulted in negative results.

*The following CoNS were tested in the analytic studies:

Organism	Strain
<i>Staphylococcus arlettae</i>	ATCC-43957
<i>Staphylococcus auricularis</i>	ATCC-33753
<i>Staphylococcus capitis</i>	ATCC-27840
<i>Staphylococcus caprae</i>	ATCC-51548
<i>Staphylococcus chromogenes</i>	ATCC-43764
<i>Staphylococcus cohnii</i>	ATCC-29974
<i>Staphylococcus cohnii</i> subsp. <i>cohnii</i>	ATCC 29972
<i>Staphylococcus cohnii</i> subsp. <i>cohnii</i>	ATCC 29973
<i>Staphylococcus cohnii</i> subsp. <i>urealyticus</i>	ATCC 49328
<i>Staphylococcus cohnii</i> subsp. <i>urealyticus</i>	ATCC 49329
<i>Staphylococcus cohnii</i> subsp. <i>urealyticus</i>	ATCC 49330
<i>Staphylococcus cohnii</i> subsp. <i>urealyticus</i>	ATCC 49331
<i>Staphylococcus delphini</i>	ATCC-49171
<i>Staphylococcus epidermidis</i>	ATCC-14990
<i>Staphylococcus epidermidis</i>	ATCC-49461
<i>Staphylococcus epidermidis</i>	ATCC-51625
<i>Staphylococcus equorum</i>	ATCC-43958
<i>Staphylococcus felis</i>	ATCC-49168
<i>Staphylococcus fleurettii</i>	BAA-274
<i>Staphylococcus haemolyticus</i>	ATCC-29970
<i>Staphylococcus hominis</i>	ATCC-27844
<i>Staphylococcus intermedius</i>	ATCC-49052
<i>Staphylococcus kloosii</i>	ATCC-43959
<i>Staphylococcus lentus</i>	ATCC-29070
<i>Staphylococcus lugdunensis</i>	ATCC-49576
<i>Staphylococcus lutrae</i>	ATCC-700373
<i>Staphylococcus muscae</i>	ATCC-49910
<i>Staphylococcus pasteurii</i>	ATCC-51128

Organism	Strain
<i>Staphylococcus piscifermentans</i>	ATCC-51136
<i>Staphylococcus pseudintermedius</i>	ATCC 49444
<i>Staphylococcus pulvereri</i>	ATCC-51699
<i>Staphylococcus saccharolyticus</i>	ATCC-14953
<i>Staphylococcus saprophyticus</i>	ATCC-15305
<i>Staphylococcus schleiferi</i>	ATCC-43808
<i>Staphylococcus schleiferi</i>	ATCC-49545
<i>Staphylococcus sciuri</i>	ATCC-29061
<i>Staphylococcus simulans</i>	ATCC-27851
<i>Staphylococcus succinus</i>	ATCC-700337
<i>Staphylococcus warneri</i>	ATCC-49454
<i>Staphylococcus xylosus</i>	ATCC-29971

Reproducibility

A reproducibility study was performed with *Staphylococcus* QuickFISH BC and the results are presented below by site across 3 days and by day across 3 sites, with 2 operators at each site.

Summary of Reproducibility Results by Site Across 3 Days

	Site 1	Site 2	Site 3	Total
Positive Agreement Green	45/45	45/45	45/45	100% (135/135)
Positive Agreement Red	45/45	45/45	45/45	100% (135/135)
Negative Agreement	36/36	36/36	36/36	100% (108/108)
Total Agreement	100% (126/126)	100% (126/126)	100% (126/126)	100% (378/378)

Summary of Reproducibility Results by Day Across 3 Sites

	Day 1	Day 2	Day 3	Total
Positive Agreement Green	45/45	45/45	45/45	100% (135/135)
Positive Agreement Red	45/45	45/45	45/45	100% (135/135)
Negative Agreement	36/36	36/36	36/36	100% (108/108)
Total Agreement	100% (126/126)	100% (126/126)	100% (126/126)	100% (378/378)

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.
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3. **Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahn 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 Antibiol. 3(7).
4. **Ly, T., Gulia, J., Pyrgos, V., Waga, M., Shoham, S.** 2008. Impact Upon Clinical Outcomes of Translation of PNA FISH

Definitions



Product code/catalog number



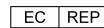
Consult the instructions for use



Contains sufficient for <n> tests



Manufacturer



Authorized representative



Use by



Batch code



Storage temperature limitations

Technical Advice and Customer Service

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



AdvanDx, Inc.
400 TradeCenter Suite 6990
Woburn, MA 01801
USA

Tel: +1 781 376 0009
Fax: +1 781 376 0111

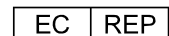
techsupport@advandx.com

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The product must not be used for Slide-Based human Cytochemistry, ISH-based Cancer Cytogenetics and Flow Cytometry.

31 July 2012

PN1878. Rev. D

Purchase of this kit licenses its use under patent numbers: US 5,985,563; US 5,888,733; US 6,664,045; US 6,395,474; US 6,357,163; US 5,539,082; US 7,223,833; US 6,361,942; US 7,816,50; EP 862,650; EP 804,456



AdvanDx A/S
Bygstubben 11
2950 Vedbæk
Denmark

Tel: +45 45 16 07 99
Fax: +45 45 16 07 98

www.PNA-FISH.com