510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number

k113371

B. Purpose for Submission:

To determine substantial equivalence of the device for the identification of *Staphylococcus aureus* and other *Staphylococci* on smears from positive blood cultures containing Gram positive cocci in clusters.

C. Measurand:

Staphylococcus aureus, and other *Staphylococci* species (CoNS) specific ribosomal RNA sequences

D. Type of Test:

Fluorescence In Situ Hybridization (FISH) using protein nucleic acid (PNA) probes

E. Applicant:

AdvanDx, Inc

F. Proprietary and Established Names:

*Staphylococcus Quick*FISH™ BC

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3700

2. Classification:

   Class I

3. Product code:

   NXX
   FISH (Fluorescent *in situ* hybridization) kit, Protein Nuclei Acid, RNA, *Staphylococcus aureus*

4. Panel:

   83 Microbiology

H. Intended Use:

1. Intended use(s):

   The *Staphylococcus Quick*FISH 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.

2. Indication(s) for use:

   The *Staphylococcus Quick*FISH 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.

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   AdvanDx Microscope Dual Band Filter (Cat. No. AC007)
   AdvanDx *Quick*FISH Slides (Cat. No. CS012)

I. Device Description:

*Staphylococcus Quick*FISH BC is a is a fluorescence *in situ* hybridization (FISH) assay which uses PNA probes hybridizing to *S. aureus*-specific ribosomal RNA sequences and PNA probes hybridizing to ribosomal RNA of other *Staphylococcus* species (CoNS). The test provides rapid (20 minutes assay time) identification of *S. aureus* and CoNS on smears made from positive blood cultures.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   S. aureus/CNS PNA FISH

2. Predicate 510(k) number(s):

   k092166

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Technology	Fluorescence In Situ Hybridization (FISH) using protein nucleic acid (PNA) probe	Same
Function	Identification of *S. aureus* and other *Staphylococci*	Same
Sample	Positive blood culture	Same
Interpretation of results	Qualitative Fluorescence microscope	Same

Differences		
Item	Device	Predicate
Reagents	Two fixation solutions (QuickFix 1,2), at 55ºC	One fixation solution, at room temperature
	Two PNA probe solutions (PNA Blue, and PNA Yellow)	One single solution
	Wash solution, and mounting fluid not required	Require wash solution and mounting media
Control Organisms	Pre-fixed on the same slide as sample	Positive/negative control organisms prepared separately
Sample preparation	Secondary vessel required to hold the sample for pipetting 10µL onto a slide	One drop of sample from positive blood culture bottle
Hybridization	15 minutes	30 minutes

Differences		
Item	Device	Predicate
Assay time	20 minutes	1.5 hrs

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

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

The fluorophore-labeled PNA probes and quencher-labeled PNA probes are added to a smear prepared from a culture. Hybridization is performed at 55°C for 15-20 minutes then the smear is ready for examination by fluorescence microscopy. While maintaining the morphology of the cells, *S. aureus* and other *Staphylococcus* species (CoNS) cells become fluorescent by specific binding of the fluorophore-labeled PNA probes. With the Staphylococcus *Quick*FISH BC, *Staphylococcus aureus* cells yield green fluorescence whereas CoNS cells yield bright red fluorescence.

The fluorescence microscopy is performed using a dual band microscope filter (AdvanDx, Cat. No. AC007).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The assay was performed on 14 slides consisting of five *S. aureus* (pos-green), five CoNS (pos- red), and four non-*Staphylococci* species (negative), in triplicate on three separate days at three separate sites. Each batch was run independently by at least two different operators at each site. The reproducibility was >95%.

b. Linearity/assay reportable range:

Not applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

The *Quick*FISH slides are provided in individually sealed pouches with nitrogen and a desiccant. Slides must be used immediately after breaking pouch seal and within expiration. Slides are store s at 2-8°C.

The *Quick*FISH microscope slides with controls (i.e. CS012) consist of the following for the *Staphylococcus Quick*FISH BC:

Positive Control:

Staphylococcus aureus ATCC 29213	Green
Staphylococcus lugdunensis ATCC 700328	Red
Staphylococcus epidermidis ATCC 12228	Red

Negative Control:

Micrococcus luteus ATCC 10240	No fluorescence
Cryptococcus neoformans ATCC 204092	No fluorescence
Klebsiella oxytoca ATCC 43086	No fluorescence
Streptococcus pyogenes ATCC 12384	No fluorescence

All results were as expected.

Compatibility Study

A compatibility study was performed with BacT/Alert (SA, SN), BACTEC (Lytic 10 anaerobic, aerobic plus, anaerobic plus, PEDS Plus, Standard 10 aerobic, Standard anaerobic) and VERSA TREK (REDOX 1 aerobic) on a panel of six *S. aureus,* seven CoNS, and four negatives. The study demonstrated that compatibility among these bottle types.

The *Staphylococcus Quick*FISH BC is not compatible with bottles supplemented with charcoal and VERSA TREK Redox-2 anaerobic bottles.

Fixed Smear Stability (BacT/ALERT SA)

An in-house study was conducted to determine the stability of fixed slides. There were six *S. aureus* (green pos), six CoNS (red pos), and four negatives (no fluorescence). Positive and negative controls were also included in the slides.

The data demonstrated that fixed QuickFISH smears were stable on the slide warmer at 55°C for up to 5 minutes, at room temperature for 1 hour, and at 2-8 °C for up to 24 hrs before testing.

d. Detection limit:

The detection limit was determined to be approximately 10^5 CFU/mL by serial dilutions of *S. aureus* and *S. epidermidis* cultures from BacT/ALERT blood culture bottles. The average number of colonies per mL (CFU/mL) was calculated from three plates.

The data sets support the LoD of 10^5 CFU/mL for positive results (green- *S. aureus,* red- *S. epidermidis*) with the *Staphylococcus Quick*FISH BC assay.

Co-infection Study

Co- infection studies were performed using BacT/ALERT SA blood culture bottle with sterile human blood. The target organism was held at LoD while the competing organisms (i.e. *Enterococcus faecalis, E. coli, Candida albicans*) in increased concentrations, which varied and were up to 10^8, 10^9, 10^{10}, 10^{11} CFU/mL. Results demonstrated that the target organisms at LoD were detected in the presence of other competing organisms; both *S. aureus* and *S. epidermidis* were detected at and above LoD in co-infection studies.

Analytical sensitivity

The *Staphylococcus Quick*FISH BC was tested on 29 *S. aureus* and 40 *Staphylococcus* spp that were not *S. aureus*, including three *S. cohnii* subspp. *cohnii* strains. All 29 *S. aureus* tested were Green-positive and 38 out of the 40 *Staphylococcus* spp, not *S. aureus* were Red-positive. The two negative results were *S. felis* and *S. simulans.*

The following CoNS were tested in the analytic studies:

Organism	**Strain**
Staphylococcus arlettae	ATCC-43957
Staphylococcus auricularis	ATCC-33753
Staphylococcus capitis	ATCC-27840
Staphylococcus caprae	ATCC-51548
Staphylococcus chromogenes	ATCC-43764
Staphylococcus cohnii	ATCC-29974
Staphylococcus cohnii subsp. *cohnii*	ATCC 29972
Staphylococcus cohnii subsp. *cohnii*	ATCC 29973
Staphylococcus cohnii subsp. *urealyticus*	ATCC 49328
Staphylococcus cohnii subsp. *urealyticus*	ATCC 49329
Staphylococcus cohnii subsp. *urealyticus*	ATCC 49330
Staphylococcus cohnii subsp. *urealyticus*	ATCC 49331
Staphylococcus delphini	ATCC-49171
Staphylococcus epidermidis	ATCC-14990
Staphylococcus epidermidis	ATCC-49461
Staphylococcus epidermidis	ATCC-51625
Staphylococcus equorum	ATCC-43958
Staphylococcus felis	ATCC-49168
Staphylococcus fleurettii	BAA-274
Staphylococcus haemolyticus	ATCC-29970
Staphylococcus hominis	ATCC-27844
Staphylococcus intermedius	ATCC-49052
Staphylococcus kloosii	ATCC-43959
Staphylococcus lentus	ATCC-29070

Organism	Strain
Staphylococcus lugdunensis	ATCC-49576
Staphylococcus lutrae	ATCC-700373
Staphylococcus muscae	ATCC-49910
Staphylococcus pasteuri	ATCC-51128
Staphylococcus piscifermentans	ATCC-51136
Staphylococcus pseudintermedius	ATCC 49444
Staphylococcus pulvereri	ATCC-51699
Staphylococcus saccharolyticus	ATCC-14953
Staphylococcus saprophyticus	ATCC-15305
Staphylococcus schleiferi	ATCC-43808
Staphylococcus schleiferi	ATCC-49545
Staphylococcus sciuri	ATCC-29061
Staphylococcus simulans	ATCC-27851
Staphylococcus succinus	ATCC-700337
Staphylococcus warneri	ATCC-49454
Staphylococcus xylosus	ATCC-29971

e. Analytical specificity:

The study included ten Gram positive cocci in clusters including *Rothia (Stomatococcus) mucilaginosa*, twelve yeasts with six strains of *C. krusei* and 51 strains of bacteria. They all tested negative with the *Staphylococcus Quick*FISH BC assay. However, two *Macrococcus* spp, namely *M. caseolyticus* (formerly *Staphylococcus cohnii* subspp. *cohnii*), and *M. equipericus* (formerly *Staphylococcus equipericus*) were all formerly *Staphylococcus* spp.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison of device to conventional methods, as the reference method:

The performance of the *Staphylococcus Quick*FISH BC assay was compared to the routine blood culture identification methods.

b. Matrix comparison:

Not applicable

3. Clinical studies:

The performance of *Staphylococcus* QuickFISH BC versus routine laboratory methods has been assessed in five clinical laboratory studies. A total of 516 routine Gram Positive Cocci in clusters (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% (45/47) from positive blood culture bottles containing GPCC.

	S. aureus	**CoNS***	**Other**
Green (*S. aureus*)	150	0	1[4]
Red (CoNS)	1[1]	351	1[5]
Negative (Non-*Staphylococcus* spp.)	0	6[2,3]	43
Total	**Positive Percent Agreement** 99.3% (150/151)[6] 95% CI (96.4-100)	**Positive Percent Agreement** 98.3% (351/357)[6] 95% CI (96.4-99.4)	**Negative Percent Agreement** 95.6% (43/45) 95% CI (84.9-99.5)

[1]False positive red result, culture ID was *S. aureus*. Result of retest was green fluorescence.
[2]Result of retesting of 2 false negatives was red fluorescence for each.
[3]Includes 4 samples identified as *S. simulans*, a known limitation of the assay.
[4]Repeat testing of one false positive (green) was negative. Culture identification was *Micrococcus* spp.
[5]Results 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.
[6]Includes 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
S. auricularis	2
S. capitis	9
S. caprae	4
S. epidermidis	96
S. haemolyticus	5
S. hominis	18
S. hyicus[1]	1
S. intermedius[1]	1
S. lugdunensis	1
S. saccharolyticus	1
S. schleiferi	1
S. simulans	4
S. warneri	1
S. xylosus	1

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

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: 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.

a. Clinical Sensitivity:

See table above

b. Clinical specificity:

See table above

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

S. aureus: multiple bright green fluorescent cocci in multiple fields
CoNS: multiple bright red fluorescent cocci in multiple fields
Non-staphylococci are non-fluorescent

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The information submitted in this premarket notification is complete and supports a substantial equivalence decision.