

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION MEMORANDUM  
ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

K140619

**B. Purpose for Submission:**

To obtain a Substantial Equivalence determination for the AdvanDx *mecA XpressFISH*<sup>®</sup> assay.

**C. Measurand:**

mRNA expressed from the *mecA* gene for methicillin resistance in smears from blood cultures that are positive for *Staphylococcus aureus*.

**D. Type of Test:**

Qualitative fluorescence *in situ* hybridization (FISH) assay

**E. Applicant:**

AdvanDx, Inc.

**F. Proprietary and Established Names:**

*mecA XpressFISH*<sup>®</sup>

**G. Regulatory Information:**

1. Regulation section:

21 CFR 866.1640: Antimicrobial susceptibility powder

2. Classification:

Class II

3. Product code:

MYI

4. Panel:

83 - Microbiology

**H. Intended Use:**

1. Intended use(s):

*mecA XpressFISH*<sup>®</sup> is a qualitative nucleic acid fluorescence *in situ* hybridization assay intended for the detection of *mecA* mRNA on smears from blood cultures that are positive for *Staphylococcus aureus* by the *Staphylococcus QuickFISH*<sup>™</sup> BC assay.

The *mecA XpressFISH*<sup>®</sup> assay is indicated for use in conjunction with other laboratory tests and clinical data available to the clinician as an aid in the detection of *mecA* mRNA from methicillin-resistant *S. aureus* (MRSA) from patient positive blood cultures. The *mecA XpressFISH*<sup>®</sup> assay is not intended to monitor treatment for MRSA infections or for use with mixed cultures including those containing both *S. aureus* and coagulase negative staphylococci.

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

2. Indication(s) for use:

Same as Intended Use.

3. Special conditions for use statement(s):

For prescription use only.

For use with *Staphylococcus QuickFISH*<sup>™</sup> BC assay.

4. Special instrument requirements:

AdvanDx Microscope Filter

AdvanDx SlideStation-10

AdvanDx 10 $\mu$ L Pipette

Water Bath

Incubator (33-35°C)

Fluorescence microscope equipped with 60X or 100X oil objective

**I. Device Description:**

*mecA XpressFISH*<sup>®</sup> is a qualitative Fluorescence *in situ* Hybridization (FISH) assay that utilizes Peptide Nucleic Acid (PNA) probes which hybridize to *mecA* messenger RNA (mRNA) sequences of *Staphylococcus aureus* cells. The test is performed on smears made from blood cultures that are positive for *Staphylococcus aureus* by the *Staphylococcus QuickFISH*<sup>™</sup> BC assay.

The device consists of a means to induce *S. aureus* to produce *mecA* mRNA, reagents, laboratory ware, and controls sufficient to perform the assay and detect the fluorescence output. The device kit includes a cefoxitin coated swab, tryptic soy broth (TSB), a fixation reagent, a PNA hybridization reagent, a wash solution concentrate, and a microscope slide, which has a sample well as well as positive and negative control wells.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

BinaxNOW<sup>®</sup> PBP2a

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

K090301

3. Comparison with predicate:

Similarities		
Item	<i>mecA XpressFISH</i> <sup>®</sup> K140619	BinaxNOW <sup>®</sup> PBP2a K090301
Intended Use	<p><i>mecA XpressFISH</i><sup>®</sup> is a qualitative nucleic acid fluorescence <i>in situ</i> hybridization assay intended for the detection of <i>mecA</i> mRNA on smears from blood cultures that are positive for <i>Staphylococcus aureus</i> by the <i>Staphylococcus QuickFISH</i><sup>™</sup> BC assay.</p> <p>The <i>mecA XpressFISH</i><sup>®</sup> assay is indicated for use in conjunction with other laboratory tests and clinical data available to the</p>	<p>The BinaxNOW<sup>®</sup> PBP2a Test is a qualitative, in vitro immuno-chromatographic assay for the rapid detection of penicillin-binding protein 2a (PBP2a) present in methicillin-resistant <i>Staphylococcus aureus</i> (MRSA). The test is performed directly on blood culture samples positive for <i>S. aureus</i>.</p> <p>The BinaxNOW<sup>®</sup> PBP2a Test is not intended to diagnose MRSA nor to</p>

<b>Similarities</b>		
<b>Item</b>	<b><i>mecA XpressFISH</i><sup>®</sup> K140619</b>	<b>BinaxNOW<sup>®</sup> PBP2a K090301</b>
	<p>clinician as an aid in the detection of <i>mecA</i> mRNA from methicillin-resistant <i>S. aureus</i> (MRSA) from patient positive blood cultures. The <i>mecA XpressFISH</i><sup>®</sup> assay is not intended to monitor treatment for MRSA infections or for use with mixed cultures including those containing both <i>S. aureus</i> and coagulase negative staphylococci.</p> <p>Sub-culturing of positive blood cultures is necessary to recover organisms for susceptibility testing, epidemiological typing, and/or differentiation of mixed growth.</p>	<p>guide or monitor treatment for MRSA infections.</p> <p>Sub-culturing positive blood cultures is necessary to recover organisms for susceptibility testing or epidemiological typing.</p>
Sample Type	<i>S. aureus</i> positive blood cultures	Same
Test Principle	Detection of <i>mecA</i> gene expression	Same
Method of Result Interpretation	Visual	Same
Mode of Operation	Manual	Same

<b>Differences</b>		
<b>Item</b>	<b><i>mecA XpressFISH</i><sup>®</sup> K140619</b>	<b>BinaxNOW<sup>®</sup> PBP2a K090301</b>
Target Analyte	<i>mecA</i> mRNA	PBP2a protein
Test Method	Molecular detection of gene expression	Phenotypic detection of gene expression
Technology	<i>In situ</i> hybridization of fluorescently labeled peptide nucleic acid (PNA) probe	Immunochromatography with monoclonal antibodies that bind to the penicillin binding protein PBP2a
Sample Preparation	Cefoxitin induced expression of <i>mecA</i>	Recovery and washing of cells by centrifugation,

Differences		
Item	<i>mecA XpressFISH</i> <sup>®</sup> K140619	BinaxNOW <sup>®</sup> PBP2a K090301
	expression	followed by lysis
Controls	Integrated Positive and Negative Controls to monitor hybridization, washing and result interpretation	Separate preparation of test strip(s) for control organisms

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

Not applicable

**L. Test Principle:**

Methicillin resistance in *S. aureus* is most commonly associated with the expression of the *mecA* gene that encodes a unique penicillin-binding protein (PBP2a) with low affinity for methicillin. The *mecA XpressFISH*<sup>®</sup> assay uses fluorescein-labeled Peptide Nucleic Acid (PNA) probes to hybridize to *mecA* mRNA sequences on smears from blood cultures containing *S. aureus*. Routine clinical blood cultures that have been signaled as positive on an automated blood culture instrument are initially characterized by Gram stain. Those exhibiting Gram-positive cocci in clusters are tested using the *Staphylococcus QuickFISH*<sup>™</sup> BC assay (K113371) to confirm the presence of a pure culture of *S. aureus*. Once this has been established, an aliquot of the blood culture is incubated in Trypticase Soy Broth containing cefoxitin to induce the expression of *mecA* mRNA.

Cefoxitin resistance has been established by the Clinical and Laboratory Standards Institute as the preferred method for detection of methicillin resistance conferred by the *mecA* gene in *S. aureus*. In the *mecA XpressFISH*<sup>®</sup> assay, the cefoxitin does not function to establish or measure the methicillin susceptibility of the *S. aureus*; rather, the induction step activates the expression of the *mecA* gene thereby increasing the amount of available mRNA targets for detection in the assay. An aliquot of the induced sample is mixed with a fixation solution and heat fixed onto a glass slide. Fixation is followed by hybridization with a cocktail of fluorescent PNA probes, then a stringent wash to remove unbound probes. Finally, the slide is mounted with mounting medium and a glass coverslip for examination by fluorescence microscopy. *mecA*-positive cells exhibit green fluorescence through the specific binding of the fluorophore-labeled PNAs to *mecA* mRNA. *mecA*-negative cells appear as non-fluorescent cocci in clusters.

Each sample slide contains separate positive and negative controls in small wells adjacent to the sample well. The controls are provided pre-fixed to the slide and monitor the procedural steps of the assay following cefoxitin induction and slide fixation. The control wells are oriented such that they can be processed together under a second coverslip. The

hybridization, wash and mounting steps are performed on the control wells in the same manner as for the sample well.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

**Overall Reproducibility**

Two Reproducibility Studies were performed to evaluate overall site-to-site and operator-to-operator and lot-to-lot reproducibility of the *mecA XpressFISH*<sup>®</sup> assay. For the first study, contrived blood cultures of two strains of MRSA and one strain of MSSA were prepared using the BacT/ALERT 3D automated blood culture system. Aliquots of each culture were coded and distributed to each of the 3 study sites and tested over 5 days by two operators at each site. There was 100% concordance with expected results except for Day 2 at one site on which one operator had 6 false-positive results (Positive Percent Agreement: 100% [360/360]; Negative Percent Agreement: 96.6% [171/177]). A definitive root cause for these failures could not be established and therefore a second Reproducibility Study was conducted with additional control measures in place to safeguard the integrity of study results that included examination of the Internal Positive and Negative Controls on each slide as well as daily testing of Positive and Negative External Controls by each operator.

For the second Reproducibility Study contrived blood cultures were also prepared using the BacT/ALERT 3D automated blood culture device. Two strains of MRSA and one of MSSA were each used to inoculate aerobic bottles containing sterile whole human blood. In order to simulate testing of clinical samples, the bottles were incubated in the BacT/ALERT device for different durations. One set was harvested after about 12 hours of incubation (“bottle ring”) and the other set was harvested after about 20 hours of incubation (bottle ring plus 8 hours). At inoculation, and again upon harvest, quantitative plating was used to determine the titer of each of the cultures. Aliquots of each culture were coded A-F, randomized for each day of testing, and distributed to the study sites. Details of the Reproducibility Panel are presented below in **Table 1**.

**Table 1. Sample Matrix for *mecA XpressFISH*<sup>®</sup> Reproducibility**

Organism	Strain ID	CFU/mL	
		Set 1: Bottle Ring (12 hours)	Set 2: Bottle Ring + 8 Hours (20 hours)
MRSA-1	NRS 674	4.5 x 10 <sup>7</sup>	9.6 x 10 <sup>8</sup>
MRSA-2	ATCC 43300	3.4 x 10 <sup>7</sup>	1.1 x 10 <sup>8</sup>
MSSA	ATCC 29213	7.7 x 10 <sup>7</sup>	2.8 x 10 <sup>7</sup>

Testing was performed over five days at two external sites and internally at AdvanDx using two different lots of *mecA XpressFISH*<sup>®</sup> slides and cefoxitin swabs, three lots of External Controls, and blinded Reproducibility Panel samples. At each site, two operators each performed duplicate tests with each panel member on each of the five days of the study (3 sites X 2 operators X 5 days). Ten test results were obtained by each operator at each study site for a cumulative total of 30 replicates per panel member.

Because the *mecA XpressFISH*<sup>®</sup> assay requires visual observation and interpretation of the test results, operators with different levels of experience were included in the study. Testing was conducted by two operators at each site. At the external sites, one operator was experienced (Operator 1) and the other operator was trained, but inexperienced with the *mecA XpressFISH*<sup>®</sup> assay (Operator 2). Both operators at AdvanDx were experienced.

On Day 4, Operator 2 at Site 3 found the Internal Positive Control on the slide for the External Positive Control to be negative. This observation was confirmed by Operator 1. The root cause of this error could not be confirmed and but was likely due to either a manufacturing defect or operator error. The External Control and associated panel members were retested and the expected results were obtained.

In total, 248 *mecA XpressFISH*<sup>®</sup> tests were performed, including the repeat testing of the 8 samples that was required at Site 3 due to failure of the Internal Positive Control with an External Positive Control sample. As shown in **Table 2**, 247/248 (99.6%) Internal Positive Controls produced the expected result as did all the Internal Negative Controls (248/248; 100%). All the External Controls (60/60; 100%) and all the reproducibility panel members (180/180; 100%) also produced the expected results. There were no differences in performance by site, operator or reagent lot.

**Table 2. *mecA* XpressFISH® Reproducibility Study Results by Site**

Sample	Agreement (%; 95% CI)				
	Site 1	Site 2	Site 3	Total	Overall
<b>Positive Internal Control</b>	80/80	80/80	87/88 *	247/248 (99.6; 97.8 - 99.9)	495/496 (99.8; 98.9 - 100)
<b>Negative Internal Control</b>	80/80	80/80	88/88 *	248/248 (100; 98.5 - 100)	
<b>Positive External Control</b>	10/10	10/10	10/10	30/30 (100; 88.7 - 100)	60/60 (100; 94.0 - 100)
<b>Negative External Control</b>	10/10	10/10	10/10	30/30 (100; 88.7 - 100)	
<b>MRSA-1 12 hours</b>	10/10	10/10	10/10	30/30 (100; 88.7 - 100)	180/180 (100; 97.9 - 100)
<b>MRSA-1 20 hours</b>	10/10	10/10	10/10	30/30 (100; 88.7 - 100)	
<b>MRSA-2 12 hours</b>	10/10	10/10	10/10	30/30 (100; 88.7 - 100)	
<b>MRSA-2 20 hours</b>	10/10	10/10	10/10	30/30 (100; 88.7 - 100)	
<b>MSSA 12 hours</b>	10/10	10/10	10/10	30/30 (100; 88.7 - 100)	
<b>MSSA 20 hours</b>	10/10	10/10	10/10	30/30 (100; 88.7 - 100)	

CI: Confidence Interval

\* One set of 8 samples was re-tested due to failure of the Internal Positive Control with an External Positive Control Sample; because the External Positive Control result could not be reported, all results associated with the Reproducibility Panel members in the run were excluded from the analysis

### **Cefoxitin Induction Reproducibility Study**

An additional study was conducted to evaluate the reproducibility of the cefoxitin induction step. Testing was performed on 25 strains of MRSA (**Table 3**). Contrived blood cultures were prepared at AdvanDx using the BacT/ALERT 3D automated blood culture instrument by inoculating each strain into culture medium containing blood. The bottles were allowed to incubate until bottle ring (growth periods ranged from 11h 46 min to 22h 15min), after which each blood culture was divided into aliquots that were stored at 2-8°C until shipment to the test sites.

Prior to being sent to the testing laboratories, the morphology of each organism was confirmed as Gram Positive Cocci in Clusters (GPCC) and all 25 strains were also confirmed as *S. aureus* by *Staphylococcus QuickFISH*<sup>™</sup> BC.

The study was performed with two different lots of *mecA XpressFISH*<sup>®</sup> slides, two lots of cefoxitin swabs on Day 1 and one lot of swabs with no cefoxitin (denoted as “MOCK”) on Day 2, and one lot of External Controls. All operators were blinded as to the identity of the panel members which were tested over two days at two external sites and internally at AdvanDx.

There were two operators at each site. At the two external sites, one operator was experienced (Operator 1) and the other operator was trained, but inexperienced with the *mecA XpressFISH*<sup>®</sup> assay (Operator 2). Both operators at AdvanDx were experienced.

The results of the study are summarized in **Table 4**. All the operators at each of the three sites obtained 100% (25/25) agreement with expected results on Day 1 when testing was performed with swabs containing cefoxitin. On Day 2 when testing was performed with “MOCK” swabs without cefoxitin, there was variation in percent agreement between operators and sites. Of the 25 samples tested without cefoxitin induction, a total of seven (7) samples were scored as negative. For both operators combined at each site the overall agreement with expected results was 48/50 (96.0%; 95% CI 86.5-98.9%) at Site 1, 50/50 (100%; 95% CI: 92.9-100%) at Site 2 and 45/50 (90.0%; 95% CI 78.6-95.7%) at Site 3.

All Internal and External Positive and Negative Controls that were tested during the course of the study produced the expected results.

The results of the study confirm that the cefoxitin induction step, performed as instructed in the *mecA XpressFISH*<sup>®</sup> Package Insert, is necessary to ensure the reproducibility of assay performance.

**Table 3. MRSA Strains used in the Cefoxitin Induction Reproducibility Study**

#	Strain	PFGE	SCC <i>mec</i>	spa type	Clonal Complex	Origin	Level Tested (CFU/mL)
1	ATCC 33591	-	III	t037	-	-	8.42 x 10 <sup>6</sup>
2	ATCC 43300	-	II	t002	-	-	1.90 x 10 <sup>8</sup>
3	ATCC 700699	-	II	t002	5	Japan	1.61 x 10 <sup>7</sup>
4	ATCC BAA-1556	USA300	IV	t008	-	-	4.80 x 10 <sup>7</sup>
5	ATCC BAA-1681	USA100	II	-	-	MI	9.40 x 10 <sup>7</sup>
6	ATCC BAA-1683	USA400	IVa	-	-	MI	4.30 x 10 <sup>7</sup>
7	ATCC BAA-1685	USA600	II	-	-	MI	3.23 x 10 <sup>7</sup>
8	NRS100	-	I	t008	8	US	9.10 x 10 <sup>8</sup>
9	NRS123	USA400	IVa	t128	1	ND	1.91 x 10 <sup>8</sup>
10	NRS172	-	IV	t008	8	France	2.25 x 10 <sup>8</sup>
11	NRS382	USA100	II	t002	5	OH	1.85 x 10 <sup>8</sup>
12	NRS383	USA200	II	t018	30 (36)	NC	1.92 x 10 <sup>8</sup>
13	NRS386	USA700	IVa	t126	8	LA	1.85 x 10 <sup>8</sup>
14	NRS387	USA800	IV	t088	5	WA	7.43 x 10 <sup>7</sup>
15	NRS483	USA1000	IV	-	-	VT	3.20 x 10 <sup>8</sup>
16	NRS484	USA1100	IV	-	-	AK	3.90 x 10 <sup>8</sup>
17	NRS643	USA300	IV	-	-	CA	3.33 x 10 <sup>8</sup>
18	NRS651	USA200	II	-	-	CA	4.20 x 10 <sup>8</sup>
19	NRS657	USA300	IV	-	-	CA	4.47 x 10 <sup>7</sup>
20	NRS686	Iberian	IV	-	-	GA	1.11 x 10 <sup>8</sup>
21	NRS691	USA500	IV	-	-	GA	2.25 x 10 <sup>8</sup>
22	NRS694	USA300	IV	-	-	GA	4.32 x 10 <sup>7</sup>
23	NRS739	USA300	IV	-	-	TN	6.38 x 10 <sup>7</sup>
24	NRS745	USA1000	V	-	-	GA	5.87 x 10 <sup>7</sup>
25	NRS752	USA100	IV	-	-	CA	2.08 x 10 <sup>8</sup>

**Table 4. Summary of Results from the Cefoxitin Induction Reproducibility Study**

		Site 1		Site 2		Site 3	
		Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2
<b>Day 1 (With Induction)</b>	<b>Swab Lot</b>	1	2	2	1	1	1
	<b>Samples</b>	1-25	1-25	1-25	1-25	15-25	1-13
	<b>Swab Lot</b>					2	2
	<b>Samples</b>					1-14	14-25
	<b>Positive Agreement</b>	25/25	25/25	25/25	25/25	25/25	25/25
	<b>Percent</b>	100	100	100	100	100	100
	<b>95% CI</b>	87.7-100	87.7-100	87.7-100	87.7-100	87.7-100	87.7-100
<b>Day 2 (Without Induction)</b>	<b>Swab Lot</b>	MOCK	MOCK	MOCK	MOCK	MOCK	MOCK
	<b>Samples</b>	1-25	1-25	1-25	1-25	1-25	1-25
	<b>Positive Agreement</b>	24/25	24/25	25/25	25/25	22/25	23/25
	<b>Percent</b>	96.0	96.0	100	100	88.0	92.0
	<b>95% CI</b>	80.5-99.3	80.5-99.3	87.7-100	87.7-100	70.0-95.8	75.0-97.8

CI: Confidence Interval

*b. Linearity/assay reportable range:*

Not applicable

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

**Internal Controls**

Each *mecA* Control Slide for use with the *mecA* XpressFISH<sup>®</sup> assay contains separate Internal Positive and Negative Controls in small wells adjacent to the sample well. The controls comprise well-characterized organisms that are grown, killed, deposited and fixed within the control wells to aid in result interpretation. The positive well contains methicillin resistant *Staphylococcus aureus* (MRSA) and the negative control well contains methicillin susceptible *Staphylococcus aureus* (MSSA). The hybridization, wash and mounting steps are performed on the control wells in the same manner as for the sample well.

Quality control with the *mecA* Control Slides must be performed each time testing is performed. After processing, the Positive Control well should display multiple fluorescent green cocci in clusters. The Negative Control well should not contain fluorescent green cells. If the Positive and Negative Controls do not perform accordingly, results for the test sample are considered invalid and the patient result should not be reported.

Because the Positive and Negative Internal Controls are not exposed to the cefoxitin induction process they do not monitor this step of the assay procedure. This is indicated as a Limitation in the Package insert. The cefoxitin induction process can only be monitored through testing of appropriate Positive and Negative External Controls.

### External Controls

A recommended procedure for preparation of External Positive and Negative Controls based on culture of commercially available strains of MRSA and MSSA is provided in the Package Insert. The ability of External Controls that were prepared according to the recommended procedure to monitor the performance of the *mecA* XpressFISH<sup>®</sup> assay was evaluated under four different failure modes (**Table 5**).

**Table 5. Potential Failure Modes Evaluated with External Controls**

Process Step	Nominal Condition	Failure Mode
mRNA induction	Induction with cefoxitin	Omission of cefoxitin induction
Hybridization	With <i>mecA</i> PNA probe	No <i>mecA</i> PNA probe
Wash Temperature	57±1°C	25°C
Wash Duration	10-20 min	1 min

Under each of the potential failure modes tested either the Positive or the Negative External Control was shown to fail, confirming that External Controls prepared in the manner described in the Package Insert are appropriate to monitor for substantial deviations from the prescribed *mecA* XpressFISH<sup>®</sup> test procedure. As noted in the Package Insert, the Positive and Negative Internal Controls are designed to monitor steps in the assay workflow downstream of the cefoxitin induction process.

#### *d. Detection limit:*

The limit of detection (LOD) of the *mecA* XpressFISH<sup>®</sup> assay was determined using 3 strains of MRSA that were grown under simulated conditions of blood culture and serially diluted in half-log increments in a blood culture matrix. Four replicates of each dilution were initially tested to obtain a preliminary estimate of the LOD (*i.e.*, the lowest level detected reproducibly with both the *Staphylococcus QuickFISH*<sup>™</sup> BC and *mecA* XpressFISH<sup>®</sup> assays). Colony counts were performed to determine the target levels tested.

The LOD for each strain was confirmed by testing an additional 20 replicates at the estimated LOD target level (**Table 6**). Across the three strains of MRSA tested, the LOD of the *mecA* XpressFISH<sup>®</sup> assay was estimated to be approximately 10<sup>5</sup> CFU/mL, which is also consistent with the claimed analytical sensitivity of the *Staphylococcus QuickFISH*<sup>™</sup> BC assay.

**Table 6. Limits of Detection of *mecA* XpressFISH®**

<b>MRSA Strain</b>	<b>LOD (CFU/mL)</b>
<b>NRS 383</b>	2.79 x 10 <sup>5</sup>
<b>ATCC 33591</b>	1.47 x 10 <sup>5</sup>
<b>ATCC 43300</b>	8.13 x 10 <sup>4</sup>

*e. Analytical reactivity:*

The analytical reactivity of the *mecA* XpressFISH® assay was assessed using a panel of 64 strains of MRSA representing different genotypes, clonal complexes and both geographic and temporal diversity. Testing was performed on cultures grown under simulated conditions of blood culture. Details of the strains and levels tested are shown in **Table 7**. All the strains produced positive results with *Staphylococcus QuickFISH*™ BC and, as expected, the *S. aureus mecC* variant LGA251 was the only strain not to yield a positive result with the *mecA* XpressFISH® assay. The inability of the *mecA* XpressFISH® assay to detect methicillin resistance conferred by mechanisms other than the presence of the *mecA* gene is reflected as a Limitation in the Package Insert.

Minimum Inhibitory Concentrations for cefoxitin were determined for 20 strains of MRSA, 6 strains of MSSA, 5 strains of BORSA (Borderline Oxacillin Resistant *S. aureus*) and 2 strains of *S. epidermidis*. MICs were determined by the Macrodilution (Tube) Broth Method and breakpoints were interpreted according to Clinical and Laboratory Standards Institute (CLSI) M100-S24 (24<sup>th</sup> edition, 2014) (*i.e.*, cefoxitin MIC ≤4µg/mL: susceptible; ≥8µg/mL: resistant). The results from testing these strains with the *mecA* XpressFISH® assay are summarized in **Table 8**. These data demonstrate the ability of the *mecA* XpressFISH® assay to detect strains of MRSA with a range of different MIC values. As expected the *mecC* variant MRSA strain tested negative, as did all 5 strains of BORSA. One strain of MSSA that is *mecA* positive produced a *mecA* XpressFISH® positive result.

**Table 7. Strains of MRSA Tested in the Analytical Reactivity Study**

#	Strain ID	CFU/mL	PFGE (USA#)	SCC <i>mec</i>	Spa Type	Clonal Complex	Location	Patient Age	Culture Source	Year of Isolation	Notes
1	ATCC 33591	2.3 x 10 <sup>8</sup>	-	III	t037	-	-	-	-	-	-
2	ATCC 700699	2.0 x 10 <sup>8</sup>	-	II	t002	5	Japan	0.3	Wound	1996	-
3	ATCC 43300	1.7 x 10 <sup>8</sup>	-	II	t002	-	-	-	-	-	-
4	ATCC 49476	6.0 x 10 <sup>7</sup>	-	-	-	-	-	-	-	-	-
5	Clinical DH2-72	2.7 x 10 <sup>8</sup>	-	-	-	-	CA	-	Clinical	-	VISA
6	Clinical DH2-80	3.4 x 10 <sup>8</sup>	-	-	-	-	CA	-	Clinical	-	VISA
7	Clinical DH2-90	3.3 x 10 <sup>8</sup>	-	-	-	-	CA	-	Clinical	-	VISA
8	ATCC BAA-1556	2.9 x 10 <sup>8</sup>	300	IV	t008	-	-	-	-	-	-
9	ATCC BAA-1764	4.5 x 10 <sup>8</sup>	1100	-	-	-	-	-	-	-	-
10	ATCC BAA-1683	1.4 x 10 <sup>8</sup>	400	IVa	-	-	MI	-	Abscess	2004	-
11	ATCC BAA-1755	3.7 x 10 <sup>8</sup>	700	-	-	-	-	-	-	-	-
12	ATCC BAA-1758	4.8 x 10 <sup>8</sup>	800	-	-	-	-	-	-	-	-
13	ATCC BAA-1685	1.5 x 10 <sup>8</sup>	600	II	-	-	MI	-	Skin	2004	-
14	ATCC BAA-1747	1.2 x 10 <sup>8</sup>	1000	-	-	-	-	-	-	-	-
15	Clinical 320	2.9 x 10 <sup>7</sup>	-	IVh	t022	22	Denmark	-	Clinical	-	-
16	Clinical 321	8.9 x 10 <sup>7</sup>	-	V	t084	15	Denmark	-	Clinical	-	-
17	Clinical M142	5.9 x 10 <sup>8</sup>	-	IVc	t005	22	Denmark	-	Clinical	-	-
18	Clinical M357	1.9 x 10 <sup>7</sup>	-	IVh	t032	22	Denmark	-	Clinical	-	-
19	LGA251	2.5 x 10 <sup>8</sup>	-	XI	t6300	425	England	-	Cow	2007	<i>mecC</i> variant <sup>1</sup>
20	Clinical 26a3	3.2 x 10 <sup>8</sup>	-	IVc	t019	30	DK	-	Clinical	-	-
21	NRS022	1.3 x 10 <sup>6</sup>	600	II	t266	45	NY	77	Blood	1999	-
22	NRS123	2.1 x 10 <sup>8</sup>	400	IVa	t128	1	ND	1.3	Blood	1998	-
23	NRS382	7.2 x 10 <sup>8</sup>	100	II	t002	5	OH	-	-	-	-
24	NRS385	6.1 x 10 <sup>8</sup>	500	IVa	t064	8	CT	-	Blood	-	-
25	NRS386	2.4 x 10 <sup>8</sup>	700	IVa	t126	8 (72)	LA	-	Blood	-	-
26	NRS387	2.4 x 10 <sup>8</sup>	800	IV	t088	5	WA	-	Wound	-	-

#	Strain ID	CFU/mL	PFGE (USA#)	SCC <i>mec</i>	Spa Type	Clonal Complex	Location	Patient Age	Culture Source	Year of Isolation	Notes
27	NRS657	3.5 x 10 <sup>8</sup>	300	IV	-	-	CA	42	Blood	2007	-
28	NRS658	3.5 x 10 <sup>8</sup>	100	II	-	-	CA	60	Bone	2007	-
29	NRS694	3.4 x 10 <sup>8</sup>	300	IV	-	-	GA	55	Blood	2005	-
30	NRS697	2.5 x 10 <sup>8</sup>	100	II	-	-	MN	70	Blood	2005	-
31	NRS739	4.3 x 10 <sup>8</sup>	300	IV	-	-	TN	0.9	Pleural Fluid	2005	-
32	Clinical 126 <sup>2</sup>	2.1 x 10 <sup>8</sup>	-	II	t002	-	Germany	-	Clinical	-	-
33	Clinical 35 <sup>2</sup>	2.6 x 10 <sup>8</sup>	-	II	t002	-	Germany	-	Clinical	-	-
34	NRS483	3.0 x 10 <sup>8</sup>	1000	IV	-	-	VT	-	Wound	-	-
35	NRS484	4.1 x 10 <sup>8</sup>	1100	IV	-	-	AK	-	Wound	-	-
36	NRS745	2.7 x 10 <sup>8</sup>	1000	V	-	-	GA	58	CSF	2006	-
37	Clinical 03-16918	3.4 x 10 <sup>7</sup>	-	V	-	1	Australia	-	Wound	-	-
38	Clinical 04-17021	3.6 x 10 <sup>8</sup>	-	V	-	59	Australia	-	Skin	-	-
39	Clinical 04-17116	2.1 x 10 <sup>8</sup>	-	V	-	5	Australia	-	Nose	-	-
40	Clinical WBG8318	4.1 x 10 <sup>8</sup>	-	V	-	45	Australia	-	Skin	-	-
41	NRS070	7.1 x 10 <sup>7</sup>	-	II	t002	5	Japan	-	Respiratory	1982	-
42	NRS100	1.1 x 10 <sup>8</sup>	-	I	t008	8	US	-	-	-	-
43	NRS648	6.5 x 10 <sup>7</sup>	600	II	-	-	CA	57	Blood	2005	-
44	NRS651	1.4 x 10 <sup>8</sup>	200	II	-	-	CA	61	Peritoneal Fluid	2006	-
45	NRS686	2.6 x 10 <sup>8</sup>	Iberian	IV	-	-	GA	74	Blood	2006	-
46	NRS691	1.9 x 10 <sup>8</sup>	500	IV	-	-	GA	72	Blood	2005	-
47	NRS692	5.2 x 10 <sup>8</sup>	800	IV	-	-	GA	51	Blood	2006	-
48	NRS708	1.2 x 10 <sup>8</sup>	500	IV	-	-	NY	90	BURSA	2005	-
49	NRS722	3.1 x 10 <sup>7</sup>	200	II	-	-	OR	-	-	-	-
50	NRS740	7.9 x 10 <sup>7</sup>	200	II	-	-	TN	70	Peritoneal Fluid	2005	-
51	NRS752	3.4 x 10 <sup>8</sup>	100	IV	-	-	CA	-	Synovial Fluid	2007	-
52	NRS805	2.8 x 10 <sup>8</sup>	100	IV	-	-	MN	-	Blood	2008	-
53	NRS036	1.3 x 10 <sup>8</sup>	-	I	t051	8	France	-	Blood	1995	-
54	NRS056	4.0 x 10 <sup>8</sup>	-	III	t037	239	Brazil	11	Wound	1999	-

#	Strain ID	CFU/mL	PFGE (USA#)	SCC <i>mec</i>	Spa Type	Clonal Complex	Location	Patient Age	Culture Source	Year of Isolation	Notes
55	NRS064	1.9 x 10 <sup>6</sup>	-	III	t037	239	Oman	50	Blood	1998	-
56	Clinical PHRI22949	1.8 x 10 <sup>8</sup>	400	IV	t125	1	-	-	-	-	-
57	Clinical PHRI22960	3.3 x 10 <sup>8</sup>	1100	IV	t019	30	-	-	-	-	-
58	NRS108	3.5 x 10 <sup>7</sup>	-	I	t008	8	France	-	-	-	-
59	NRS248	1.1 x 10 <sup>8</sup>	400	IV	t128	1	-	-	-	-	-
60	PHRI22951	3.3 x 10 <sup>7</sup>	700	IV	t126	72	-	-	-	-	-
61	ATCC BAA-1681	1.9 x 10 <sup>8</sup>	100	II	-	-	MI	-	Nose	2003	-
62	NRS383	1.7 x 10 <sup>8</sup>	200	II	t018	30 (36)	NC	-	-	-	-
63	NRS643	3.5 x 10 <sup>8</sup>	300	IV	-	-	CA	26	Bone	2005	-
64	Clinical JHH-8	2.5 x 10 <sup>8</sup>	-	-	-	-	-	-	Clinical	-	-

<sup>1</sup> Garcia-Alvarez L., *et al.* Lancet Infect Dis. 2011 11(8): 595-603

<sup>2</sup> Grobner S., *et al.* J Clin Microbiol. 2009 47(6): 1689-94

VISA: Vancomycin Intermediate *S. aureus*

**Table 8. Characteristics and Cefoxitin MIC Values for Strains Tested with the *mecA* XpressFISH® Assay**

Strain		USA clone#	SCC <i>mec</i> Type	spa Type	Clonal Complex	Region	Patient Age	Clinical Source	Year Isolated	MIC (µg/mL)	<i>mecA</i> XpressFISH® Fluorescence	Notes
MRSA	DH2-72					CA				<b>32</b>	G	VISA
MRSA	ATCC BAA-1556	300	IV	t008						<b>32</b>	G	
MRSA	ATCC BAA-1683	400	IVa			MI		abscess	2004	<b>32</b>	G	
MRSA	LGA251		XI	t6300	425	England		Cow	2007	<b>8</b>	N	<i>mecC</i> variant
MRSA	NRS386	700	IVa	t126	8 (72)	LA		blood		<b>32</b>	G	
MRSA	NRS657	300	IV			CA	42	blood	2007	<b>32</b>	G	
MRSA	NRS694	300	IV			GA	55	blood	2005	<b>32</b>	G	
MRSA	NRS739	300	IV			TN	0.9	pleural fluid	2005	<b>32</b>	G	
MRSA	NRS745	1000	V			CA	58	CSF	2006	<b>16</b>	G	
MRSA	04-17021		V		59	Australia		skin		<b>16</b>	G	
MRSA	NRS070		II	t002	5	Japan		respiratory	1982	<b>&gt;32</b>	G	
MRSA	NRS648	600	II			CA	57	blood	2005	<b>&gt;32</b>	G	
MRSA	NRS651	200	II			CA	61	peritoneal fluid	2006	<b>&gt;32</b>	G	
MRSA	NRS692	800	IV			GA	51	blood	2006	<b>32</b>	G	
MRSA	NRS708	500	IV			NY	90	bursa	2005	<b>&gt;32</b>	G	
MRSA	NRS805	100	IV			MN		blood	2008	<b>32</b>	G	
MRSA	NRS036		I	t051	8	France		blood	1995	<b>&gt;32</b>	G	
MRSA	NRS064		III	t037	239	Oman	50	blood	1998	<b>&gt;32</b>	G	

Strain		USA clone#	SCC <i>mec</i> Type	spa Type	Clonal Complex	Region	Patient Age	Clinical Source	Year Isolated	MIC (µg/mL)	<i>mecA</i> XpressFISH® Fluorescence	Notes
MRSA	PHRI22960	1100	IV	t019	30					32	G	
MRSA	NRS 643	300	IV			CA	26	bone	2005	32	G	
MSSA	ATCC 29213			t021				wound	pre-1977	4	N	
MSSA	ATCC 25923			t002		WA			1945	2	N	
MSSA	ATCC BAA-1718	300				TX	12	abscess	pre-2007	4	N	
MSSA	114			t004		Germany				4	N	MRSA revertant
MSSA	NRS052			t242	5	CA	27	bile	2000	4	N	
MSSA	ATCC BAA-2421		II			MA		blood	2010	8	G	<i>mecA</i> + MSSA <sup>1</sup>
BORSA	PHRI23735			t227	25					4	N	
BORSA	PHRI23736			t273	1					2	N	
BORSA	PHRI23737			t081	25					4	N	
BORSA	PHRI23739			t026	45					4	N	
BORSA	PHRI23740			t167	25					4	N	
<i>S. epidermidis</i>	ATCC-51625									8	G	ATCC: resistant
<i>S. epidermidis</i>	ATCC-14990									4	N	

G: Green fluorescence (positive); N: No fluorescence (negative); BORSA: Borderline Oxacillin Resistant *S. aureus*

<sup>1</sup> This strain is characterized by ATCC as *mecA*<sup>+</sup>, methicillin susceptible. However, in this study, the cefoxitin MIC was above the breakpoint for susceptibility and the organism would be considered phenotypically resistant.

f. Analytical specificity:

**Cross-Reactivity Study**

The analytical specificity of the *mecA XpressFISH*<sup>®</sup> assay was evaluated in a Cross-Reactivity Study by testing 30 strains of MSSA (including 5 strains of BORSA), 40 coagulase negative strains of staphylococci, 36 other isolates of Gram positive species and 20 Gram negative organisms and yeasts. Testing was performed after growing each organism under simulated conditions of blood culture. Colony counts were performed to determine target levels (CFU/mL). Testing was performed with both the *Staphylococcus QuickFISH*<sup>™</sup> BC and *mecA XpressFISH*<sup>®</sup> assays. The results from both assays are shown in **Tables 9 to 12**. One strain of *S. aureus* (BAA-2421) that is characterized by the American Type Culture Collection (ATCC) as methicillin susceptible but which is known to carry the *mecA* gene tested positive with the *mecA XpressFISH*<sup>®</sup> assay (refer also to **Table 8**). Six strains of coagulase negative staphylococci also tested positive with *mecA XpressFISH*<sup>®</sup>, although the risk of obtaining false-positive results from such strains is mitigated by the requirement that a positive result must first be obtained with the *Staphylococcus QuickFISH*<sup>™</sup> BC prior to testing with *mecA XpressFISH*<sup>®</sup>. All six coagulase negative staphylococci that produced positive *mecA XpressFISH*<sup>®</sup> results were negative for *S. aureus* by *Staphylococcus QuickFISH*<sup>™</sup> BC. Similarly, both *Lactococcus lactis* and *Candida parapsilosis* produced positive results with *mecA XpressFISH*<sup>®</sup> but tested negative by *Staphylococcus QuickFISH*<sup>™</sup> BC.

The following species that have potential to cross-react in the *mecA XpressFISH*<sup>®</sup> assay are listed in the limitations section of the Package Insert: *Lactococcus lactis*, *Candida parapsilosis* and *Micrococcus luteus*.

**Table 9. Strains of MSSA Tested with *mecA XpressFISH*<sup>®</sup>**

Strain	CFU/mL	<i>Staphylococcus QuickFISH</i> <sup>™</sup> BC Result	<i>mecA XpressFISH</i> <sup>®</sup> Fluorescence
ATCC 29213	2.3 x 10 <sup>8</sup>	<i>S. aureus</i>	N
ATCC 25923	1.1 x 10 <sup>8</sup>	<i>S. aureus</i>	N
ATCC 11632	3.9 x 10 <sup>7</sup>	<i>S. aureus</i>	N
ATCC 6538	1.3 x 10 <sup>8</sup>	<i>S. aureus</i>	N
JHH-7	1.3 x 10 <sup>8</sup>	<i>S. aureus</i>	N
AIS2006-033	1.6 x 10 <sup>8</sup>	<i>S. aureus</i>	N
OTT-004	3.6 x 10 <sup>8</sup>	<i>S. aureus</i>	N
ATCC 12600	1.3 x 10 <sup>8</sup>	<i>S. aureus</i>	N
ATCC BAA-1749	2.5 x 10 <sup>8</sup>	<i>S. aureus</i>	N
ATCC BAA-1718	3.7 x 10 <sup>8</sup>	<i>S. aureus</i>	N
pr.nr.115724	1.6 x 10 <sup>8</sup>	<i>S. aureus</i>	N
114 <sup>1</sup>	2.8 x 10 <sup>8</sup>	<i>S. aureus</i>	N
30 <sup>1</sup>	1.9 x 10 <sup>8</sup>	<i>S. aureus</i>	N
13 <sup>1</sup>	2.2 x 10 <sup>7</sup>	<i>S. aureus</i>	N

Strain	CFU/mL	<i>Staphylococcus</i> <i>QuickFISH</i> <sup>™</sup> BC Result	<i>mecA</i> <i>XpressFISH</i> <sup>®</sup> Fluorescence
NRS102	1.0 x 10 <sup>8</sup>	<i>S. aureus</i>	N
NRS103	1.1 x 10 <sup>8</sup>	<i>S. aureus</i>	N
ATCC 35844	5.8 x 10 <sup>6</sup>	<i>S. aureus</i>	N
NRS052	7.8 x 10 <sup>7</sup>	<i>S. aureus</i>	N
NRS072	3.6 x 10 <sup>7</sup>	<i>S. aureus</i>	N
NRS161	1.1 x 10 <sup>8</sup>	<i>S. aureus</i>	N
NRS180	1.4 x 10 <sup>8</sup>	<i>S. aureus</i>	N
ATCC BAA-2421 <sup>2</sup>	1.8 x 10 <sup>8</sup>	<i>S. aureus</i>	G
PHRI22946	2.1 x 10 <sup>8</sup>	<i>S. aureus</i>	N
PHRI22955	3.5 x 10 <sup>8</sup>	<i>S. aureus</i>	N
PHRI23735 <sup>3</sup>	5.1 x 10 <sup>8</sup>	<i>S. aureus</i>	N
PHRI23736 <sup>3</sup>	4.1 x 10 <sup>6</sup>	<i>S. aureus</i>	N
PHRI23737 <sup>3</sup>	2.5 x 10 <sup>8</sup>	<i>S. aureus</i>	N
PHRI23739 <sup>3</sup>	7.1 x 10 <sup>6</sup>	<i>S. aureus</i>	N
PHRI23740 <sup>3</sup>	1.5 x 10 <sup>8</sup>	<i>S. aureus</i>	N
S68024	1.8 x 10 <sup>8</sup>	<i>S. aureus</i>	N

<sup>1</sup> MRSA revertant lacking *mecA*

<sup>2</sup> Reported by ATCC as a *mecA* positive strain of MSSA, although when tested the MIC was above the breakpoint for susceptibility (refer to **Table 9**)

<sup>3</sup> BORSA: Borderline Oxacillin Resistant *S. aureus*

G: Green fluorescence (positive); N: No fluorescence (negative)

**Table 10. Other *Staphylococcus* Species Tested with *mecA XpressFISH*<sup>®</sup>**

Species	Strain ID	CFU/mL	<i>Staphylococcus</i> <i>QuickFISH</i> <sup>™</sup> BC Result	<i>mecA</i> <i>XpressFISH</i> <sup>®</sup> Fluorescence	Methicillin Susceptibility Status <sup>1</sup>
<i>S. arlettae</i>	ATCC-43957	2.0x10 <sup>6</sup>	CoNS	N	S
<i>S. auricularis</i>	ATCC-33753	3.2x10 <sup>8</sup>	CoNS	N	S
<i>S. capitis subsp. capitis</i>	ATCC-27840	7.4x10 <sup>7</sup>	CoNS	N	S
<i>S. capitis</i>	ATCC-35661	2.3x10 <sup>6</sup>	CoNS	N	S
<i>S. caprae</i>	ATCC-35538	5.3x10 <sup>7</sup>	CoNS	N	Not tested
<i>S. caprae</i>	ATCC-51548 <sup>2</sup>	3.3x10 <sup>7</sup>	CoNS	N	S
<i>S. lentus</i>	ATCC-29070	8.5x10 <sup>7</sup>	CoNS	N	Not tested
<i>S. carnosus</i>	ATCC-51365	7.0x10 <sup>8</sup>	CoNS	N	S
<i>S. chromogenes</i>	ATCC-43764	3.2x10 <sup>8</sup>	CoNS	N	S
<i>S. cohnii subsp. urealyticum</i>	ATCC-29974	1.2x10 <sup>6</sup>	CoNS	N	S
<i>S. epidermidis</i>	ATCC-51625	3.4x10 <sup>7</sup>	CoNS	G	R
<i>S. epidermidis</i>	ATCC-14990	1.8x10 <sup>6</sup>	CoNS	N	S
<i>S. epidermidis</i>	ATCC-12228	2.1x10 <sup>7</sup>	CoNS	N	S
<i>S. epidermidis</i>	ATCC-49461	5.2x10 <sup>7</sup>	CoNS	G <sup>1</sup>	R

Species	Strain ID	CFU/mL	<i>Staphylococcus QuickFISH™ BC</i> Result	<i>mecA XpressFISH®</i> Fluorescence	Methicillin Susceptibility Status <sup>1</sup>
<i>S. equorum</i>	ATCC-43958	2.0x10 <sup>7</sup>	CoNS	N	S
<i>S. felis</i>	ATCC-49168	3.4x10 <sup>8</sup>	N <sup>3</sup>	N	S
<i>S. felis</i>	Adx# 2029 Clinical	1.3x10 <sup>7</sup>	CoNS	N	S
<i>S. gallinarum</i>	ATCC-35539	1.1x10 <sup>6</sup>	CoNS	N	S
<i>S. haemolyticus</i>	ATCC-29970	2.2x10 <sup>7</sup>	CoNS	N	S
<i>S. haemolyticus</i>	Adx# 1960 Clinical	5.5x10 <sup>7</sup>	CoNS	N	R
<i>S. haemolyticus</i>	Adx# 1961 Clinical	2.1x10 <sup>7</sup>	CoNS	N	R
<i>S. hominis</i>	ATCC-27844	5.5x10 <sup>7</sup>	CoNS	N	S
<i>S. hominis</i>	Adx# 1962 Clinical	1.7x10 <sup>7</sup>	CoNS	G	R
<i>S. hominis</i>	Adx# 1981 Clinical	2.1x10 <sup>7</sup>	CoNS	N	R
<i>S. intermedius</i>	ATCC-49052	3.3x10 <sup>7</sup>	CoNS	N	S
<i>S. kloosii</i>	ATCC-43959	1.1x10 <sup>8</sup>	CoNS	N	S
<i>S. lentus</i>	ATCC-29070	8.5x10 <sup>7</sup>	CoNS	N	S
<i>S. lugdunensis</i>	ATCC-49576	1.2x10 <sup>8</sup>	CoNS	N	S <sup>4</sup>
<i>S. pettenkoferi</i>	Adx# 2078 Clinical	7.0x10 <sup>7</sup>	CoNS	N	R
<i>S. vitulinus</i>	ATCC-51698	1.2x10 <sup>8</sup>	CoNS	N	S
<i>S. pseudintermedius</i>	ATCC 49444	8.7x10 <sup>7</sup>	CoNS	G	S
<i>S. saprophyticus</i>	ATCC-15305	6.4x10 <sup>7</sup>	CoNS	N	S
<i>S. schleiferi subsp. schleiferi</i>	ATCC-43808	1.1x10 <sup>7</sup>	CoNS	N	S
<i>S. schleiferi subsp. coagulans</i>	ATCC-49545	8.2x10 <sup>7</sup>	CoNS	N	S
<i>S. sciuri</i>	ATCC-29061	5.4x10 <sup>6</sup>	CoNS	G	R
<i>S. simulans</i>	ATCC-27851	2.3x10 <sup>6</sup>	CoNS	N	S
<i>S. simulans</i>	Adx# 2030 Clinical	2.3x10 <sup>8</sup>	CoNS	G	R
<i>S. warneri</i>	ATCC-49454	6.1x10 <sup>7</sup>	CoNS	N	S
<i>S. warneri</i>	Adx# 2037 Clinical	4.7x10 <sup>6</sup>	CoNS	N	S
<i>S. xylosus</i>	ATCC-29971	2.8x10 <sup>7</sup>	CoNS	N	S

CoNS: Coagulase-negative staphylococci; G: Green fluorescence (positive); N: No fluorescence (negative)

<sup>1</sup> Based on cefoxitin disk diffusion performed at AdvanDx: R ≤24mm; S ≥25mm

<sup>2</sup> Initially *Staphylococcus QuickFISH™ BC* negative on testing at 5.0x10<sup>6</sup> CFU/mL

<sup>3</sup> *S. felis* is known to produce false-negative results with *Staphylococcus QuickFISH™ BC* as indicated in the Package Insert

<sup>4</sup> Based on cefoxitin disk diffusion performed at AdvanDx: R ≤21mm; S ≥22mm

**Table 11. Gram Positive Species Tested with *mecA* XpressFISH®**

Species	Strain ID	Testing CFU/mL	<i>Staphylococcus QuickFISH™</i> BC Result	<i>mecA</i> XpressFISH® Fluorescence
<i>Abiotrophia defectiva</i>	ATCC 49176	9.3x10 <sup>8</sup>	N	N
<i>Aerococcus viridans</i>	ATCC 11563	3.7x10 <sup>7</sup>	N	N
<i>Bacillus cereus</i>	ATCC 10876	1.1x10 <sup>7</sup>	N	N
<i>Corynebacterium jeikeium</i> MDR	BAA-949	2.3x10 <sup>8</sup>	N	N
<i>Corynebacterium renale</i>	ATCC 19412	3.9x10 <sup>8</sup>	N	N
<i>Enterococcus faecalis</i>	NCTC 775	4.2x10 <sup>8</sup>	N	N
<i>Enterococcus faecium</i>	ATCC 700221	5.7x10 <sup>8</sup>	N	N
<i>Granulicatella adiacens</i>	ATCC 43205	5.2x10 <sup>8</sup>	N	N
<i>Kocuria kristinae</i>	ATCC BAA-752	1.5x10 <sup>8</sup>	N	N
<i>Kytococcus schroeteri</i>	ATCC BAA-2410	8.0x10 <sup>7</sup>	N	N
<i>Lactobacillus fermentum</i>	ATCC 9338	7.3x10 <sup>7</sup>	N	N
<i>Lactococcus lactis</i> subsp. <i>lactis</i>	ATCC 11454	7.6x10 <sup>8</sup>	N	G
<i>Leuconostoc mesenteroides</i>	ATCC 19255	3.3x10 <sup>8</sup>	N	N
<i>Macrococcus equipercicus</i>	ATCC 51833	4.1x10 <sup>6</sup>	N	N
<i>Micrococcus luteus</i>	ATCC 49732	4.2x10 <sup>6</sup>	N	N
<i>Micrococcus luteus</i>	ATCC 4698	2.8x10 <sup>6</sup>	N	N
<i>Micrococcus luteus</i>	NCIMB 8166	1.5x10 <sup>8</sup>	N	N
<i>Micrococcus luteus</i>	ATCC 7498	2.1x10 <sup>7</sup>	N	N
<i>Micrococcus luteus</i>	Clinical 35673	1.2x10 <sup>7</sup>	N	N
<i>Micrococcus luteus</i>	Clinical M3992181-W	3.1x10 <sup>7</sup>	N	N
<i>Micrococcus luteus</i>	Clinical M3992181-Y	6.6x10 <sup>7</sup>	N	N
<i>Micrococcus luteus</i>	Clinical T24413-B	6.0x10 <sup>7</sup>	N	N
<i>Micrococcus luteus</i>	Clinical RMA-04-13	1.7x10 <sup>8</sup>	N	N
<i>Micrococcus luteus</i>	Clinical M42320003	1.7x10 <sup>8</sup>	N	N
<i>Micrococcus lylae</i>	ATCC 27566	5.4x10 <sup>6</sup>	N	N
<i>Pediococcus damnosus</i>	ATCC 11308	8.8x10 <sup>7</sup>	N	N
<i>Peptostreptococcus anaerobius</i>	ATCC 27337	1.6x10 <sup>6</sup>	N	N
<i>Planococcus citreus</i>	ATCC 14404	1.1x10 <sup>7</sup>	N	N
<i>Rothia dentocariosa</i>	ATCC 31918	6.7x10 <sup>7</sup>	N	N
<i>Rothia mucilaginosa</i>	ATCC 25296	7.8x10 <sup>7</sup>	N	N
<i>Streptococcus agalactiae</i>	ATCC 13813	4.6x10 <sup>8</sup>	N	N
<i>Streptococcus mitis</i>	ATCC 6249	9.1x10 <sup>8</sup>	N	N
<i>Streptococcus mutans</i>	ATCC 25175	5.6x10 <sup>7</sup>	N	N
<i>Streptococcus pneumoniae</i>	ATCC 10015	2.2x10 <sup>7</sup>	N	N
<i>Streptococcus pyogenes</i>	ATCC 12384	1.2x10 <sup>9</sup>	N	N
<i>Vagococcus fluvialis</i>	ATCC 49515	2.5x10 <sup>8</sup>	N	N

G: Green fluorescence (positive); N: No fluorescence (negative)

**Table 12. Additional Species Tested with *mecA* XpressFISH®**

Species	Strain ID	Testing CFU/mL	Gram Stain	<i>Staphylococcus QuickFISH™</i> BC Result	<i>mecA</i> XpressFISH® Fluorescence
<i>Pseudomonas aeruginosa</i>	ATCC 10145	6.6x10 <sup>7</sup>	GNR	N	N
<i>Enterobacter aerogenes</i>	ATCC 13048	1.4x10 <sup>8</sup>	GNR	N	N
<i>Enterobacter cloacae</i>	ATCC 23355	4.5x10 <sup>7</sup>	GNR	N	N
<i>Acinetobacter baumannii</i>	ATCC 19606	6.5x10 <sup>8</sup>	GNR	N	N
<i>Escherichia coli</i> 0157:H7	ATCC 43888	1.8x10 <sup>9</sup>	GNR	N	N
<i>Escherichia coli</i> ESBL	ATCC BAA-197	2.3x10 <sup>7</sup>	GNR	N	N
<i>Escherichia coli</i> ESBL	Clinical 790	2.5x10 <sup>7</sup>	GNR	N	N
<i>Klebsiella pneumoniae</i>	ATCC 700603	7.8x10 <sup>7</sup>	GNR	N	N
<i>Klebsiella pneumoniae</i> KPC	ATCC BAA-1705	1.1x10 <sup>8</sup>	GNR	N	N
<i>Klebsiella pneumoniae</i> KPC	Clinical KPC-15	4.0x10 <sup>7</sup>	GNR	N	N
<i>Serratia marcescens</i>	ATCC 14756	9.1x10 <sup>6</sup>	GNR	N	N
<i>Proteus mirabilis</i>	ATCC 7002	1.1x10 <sup>8</sup>	GNR	N	N
<i>Moraxella catarrhalis</i>	ATCC 25240	6.1x10 <sup>7</sup>	GNR	N	N
<i>Klebsiella oxytoca</i>	ATCC 43086	9.2x10 <sup>6</sup>	GNR	N	N
<i>Stenotrophomonas maltophilia</i>	ATCC 49130	1.7x10 <sup>9</sup>	GNR	N	N
<i>Citrobacter freundii</i>	ATCC 8090	7.0x10 <sup>6</sup>	GNR	N	N
<i>Candida glabrata</i>	ATCC 15126	8.0x10 <sup>6</sup>	YEAST	N	N
<i>Candida parapsilosis</i>	ATCC 22019	2.1x10 <sup>6</sup>	YEAST	N	G
<i>Candida albicans</i>	ATCC 18804	1.5x10 <sup>7</sup>	YEAST	N	N
<i>Candida krusei</i>	ATCC 44507	1.2x10 <sup>7</sup>	YEAST	N	N

GNR: Gram Negative Rod

N: No fluorescence (negative); G: Green fluorescence (positive)

### Cross-Contamination Study

According to the instructions for use, *mecA* XpressFISH® slides can be washed in batches of up to five slides following the hybridization step. This is the only step in the *mecA* XpressFISH® procedure where slides prepared from different samples are present and exposed together to a common reagent and where they might become cross-contaminated. AdvanDx conducted an in-house study with the *mecA* XpressFISH® assay to assess the potential for cross-contamination during the wash step to lead to False Positive results.

A well characterized *mecA* positive MRSA strain (NRS 383) and a strain of MSSA (ATCC BAA-1718) were used for testing. For each run, a set of four MRSA slides and one MSSA slide was prepared from simulated blood cultures at >10<sup>8</sup> CFU/mL. Three sets of five slides were processed through the hybridization step according to the *mecA* XpressFISH® procedure and each slide set was then placed into a slide holder for the wash step. The placement of the MSSA slide relative to the four MRSA slides within the holder was varied between sets (*i.e.*, the MSSA slide within a set was placed at a different

middle or outside position relative to the four MRSA slides within the holder). The slide sets were each then washed according to the *mecA XpressFISH*<sup>®</sup> procedure, and the slides were mounted and examined by fluorescence microscopy. All MRSA samples were Green Positive (12/12; 100%) and all MSSA samples were Negative (3/3; 100%) with no Green Positive organisms found in the MSSA sample wells (or Internal Negative Control wells). The results of this study verify that cross-contamination of slides is unlikely to occur when up to five slides are washed together.

*g. Assay cut-off:*

The *mecA XpressFISH*<sup>®</sup> assay Package Insert stipulates that, in order to report a negative test result, a minimum of 50 microscopic fields of view should be examined with either a 60X or a 100X objective lens. To report a sample as positive by *mecA XpressFISH*<sup>®</sup>, multiple fields of view that contain multiple fluorescent cells should be observed.

The requirement to examine 50 fields of view was determined by examining mixtures of MSSA and MRSA in different proportions. The levels of MRSA that were tested are close to the limit of detection of the assay. Five slides were viewed with both 60X and 100X objective lenses and under each test condition. The results are summarized in **Table 13**.

With MRSA at a concentration of  $4.73 \times 10^5$  CFU/mL, an average of 15.4 fluorescent cells was observed in 50 fields of view when visualized with a 60X objective. On the same slides, an average of 7.6 fluorescent cells was found in 50 fields of view when visualized with a 100X objective. MSSA at  $2.30 \times 10^8$  CFU/mL did not show any positive fluorescent cells in 50 fields of view.

With MRSA at  $5.15 \times 10^5$  CFU/mL in the presence of MSSA at  $1.15 \times 10^8$  CFU/mL, an average of 53.4 fluorescent cells was observed in 50 fields of view. Under a 60X objective lens when the same MRSA culture was mixed with MSSA at  $1.15 \times 10^4$  CFU/mL, an average of 64.4 fluorescent cells was observed in 50 fields. With a 100X objective under these conditions, the average numbers of fluorescent cells in 50 fields were 27.6 and 30.4, respectively.

The data from this study support the Package Insert instructions regarding the number of fields of view to examine in order to report results for the *mecA XpressFISH*<sup>®</sup> assay.

**Table 13. Quantitative Microscopic Analysis of MRSA Cultures**

MRSA	MSSA	<i>mecA</i> <i>XpressFISH</i> <sup>®</sup>		Cells Observed in 50 fields of view					
		QC	Result	Slide 1	Slide 2	Slide 3	Slide 4	Slide 5	Mean
				<b>60x Objective</b>					
$4.73 \times 10^6$	none	Pass	G+	138	111	95	112	167	124.6
$4.73 \times 10^5$	none	Pass	G+	17	15	15	18	12	15.4
				<b>100x Objective</b>					
$4.73 \times 10^6$	none	Pass	G+	59	53	57	60	74	60.6
$4.73 \times 10^5$	none	Pass	G+	8	8	9	7	6	7.6
				<b>60x Objective</b>					
$5.15 \times 10^5$	$1.15 \times 10^8$	Pass	G+	87	58	67	30	25	53.4
$5.15 \times 10^5$	$1.15 \times 10^4$	Pass	G+	71	58	36	89	68	64.4
				<b>100x Objective</b>					
$5.15 \times 10^5$	$1.15 \times 10^8$	Pass	G+	40	14	31	28	25	27.6
$5.15 \times 10^5$	$1.15 \times 10^4$	Pass	G+	35	34	11	27	45	30.4
				<b>60x Objective</b>					
$5.15 \times 10^7$	$1.15 \times 10^6$	Pass	G+	>50	>50	>50	>50	>50	>50
$5.15 \times 10^7$	$1.15 \times 10^5$	Pass	G+	>50	>50	>50	>50	>50	>50
$5.15 \times 10^7$	$1.15 \times 10^4$	Pass	G+	>50	>50	>50	>50	>50	>50
				<b>100x Objective</b>					
$5.15 \times 10^7$	$1.15 \times 10^6$	Pass	G+	>25	>25	>25	>25	>25	>25
$5.15 \times 10^7$	$1.15 \times 10^5$	Pass	G+	>25	>25	>25	>25	>25	>25
$5.15 \times 10^7$	$1.15 \times 10^4$	Pass	G+	>25	>25	>25	>25	>25	>25

G+ = Green Positive

*h. Assay Interference:*

**Interfering Substances Study**

Testing was performed with simulated blood cultures to assess the performance of *mecA XpressFISH*<sup>®</sup> in the presence of various drugs and other compounds that may be present in human blood samples as well as components of different culture media. The substances evaluated and their concentrations are listed in **Table 14**. Testing was performed with two strains each of MRSA and MSSA.

**Table 14. Substances Tested for Potential Interference with *mecA XpressFISH*<sup>®</sup>**

<b>Substance</b>	<b>Concentration Tested *</b>
Amoxicillin	12 µg/mL
Clavulanate	3 µg/mL
Sulbactam	45 µg/mL
Ampicillin	120 µg/mL
Clindamycin	10 µg/mL
Daptomycin	130 µg/mL
Ibuprofen	50 µg/mL
Linezolid	38 µg/mL
Oxacillin	230 µg/mL
Vancomycin	50 µg/mL
Amoxicillin and Clavulanate	12 µg/mL 3 µg/mL
Sulbactam and Ampicillin	45 µg/mL 120 µg/mL
Bilirubin	150 µg/mL
Hemoglobin	~30 mg/mL
Triglycerides	3500 µg/mL

\* Per milliliter of blood

No interference was observed with any of the compounds that were tested except linezolid. Both strains of MRSA that were tested in the presence of linezolid produced weak positive green fluorescence. There was no impact from linezolid on correct reporting of results for MSSA. There is therefore a potential to obtain false-negative results with *mecA XpressFISH*<sup>®</sup> with samples from patients who are being treated with linezolid. This is noted as a limitation within the *mecA XpressFISH*<sup>®</sup> Package Insert.

For 0.04% sodium polyanethol sulfonate (SPS) and ~0.2% saponin, a lack of interference with *mecA XpressFISH*<sup>®</sup> was inferred from testing of culture media that contain these substances.

#### **Mixed Infection Study**

*mecA XpressFISH*<sup>®</sup> is intended to be used with blood cultures of *S. aureus* as determined by *Staphylococcus QuickFISH*<sup>™</sup> BC and is not intended to be used with mixed cultures identified either by Gram stain or *Staphylococcus QuickFISH*<sup>™</sup> BC. Because of the inherent analytic sensitivity of slide based microbial tests and the variable rate of growth of microbes, there exists a possibility that a blood culture which appears to be positive solely for *S. aureus* by Gram stain and *Staphylococcus QuickFISH*<sup>™</sup> BC is in fact a mixed culture (*i.e.*, *S. aureus* and another species). In addition, should MRSA and MSSA

occur together in the same blood culture, the culture would not be distinguishable as a mixed culture by either Gram stain or *Staphylococcus QuickFISH™* BC. A microbial interference study was therefore conducted in order to evaluate whether the presence of a non-MRSA organism would interfere with the detection of MRSA by *mecA XpressFISH®*.

Testing was performed with simulated blood cultures to determine the impact of mixed populations of MRSA and MSSA or MRSA and coagulase negative staphylococci on the *mecA XpressFISH®* assay. Two strains of MRSA were tested in the presence of different co-infecting species of *Staphylococcus* (**Table 15**). No interference was observed in detection of either strain of MRSA at levels close to the limits of detection of the assay when the co-infecting species were present at  $>10^8$  CFU/mL.

**Table 15. Mixed Infection Study**

MRSA		Co-infecting Organism		<i>Staphylococcus QuickFISH™</i> BC		<i>mecA XpressFISH®</i>	
Strain	CFU/mL	Strain	CFU/mL	Controls	Result	Controls	<i>mecA</i> Result
NRS 674	$2.57 \times 10^5$	Methicillin-susceptible <i>S. aureus</i> ATCC 29213	$1.15 \times 10^8$	Pass	Green Positive	Pass	Green Positive
ATCC 43300	$5.15 \times 10^5$			Pass	Green Positive	Pass	Green Positive
None	n/a			Pass	Green Positive	Pass	Negative
NRS 674	$2.57 \times 10^5$	<i>Staphylococcus simulans</i> ATCC 27851	$1.70 \times 10^8$	Pass	Green Positive	Pass	Green Positive
ATCC 43300	$5.15 \times 10^5$			Pass	Green Positive	Pass	Green Positive
None	n/a			Pass	Negative	Pass	Negative
NRS674	$1.07 \times 10^5$	Methicillin-susceptible <i>Staphylococcus epidermidis</i> ATCC 14990	$1.23 \times 10^8$	Pass	Green/Red Positive	Pass	Green Positive
ATCC 43300	$2.37 \times 10^5$			Pass	Green/Red Positive	Pass	Green Positive
None	n/a			Pass	Red Positive	Pass	Negative

**Culture Media Compatibility**

In order to evaluate the compatibility of alternative culture media with *mecA XpressFISH®*, testing was performed with 20 strains of MRSA and 3 strains of MSSA, representing a range of geographic and genotypic diversity. Samples were grown under simulated conditions of blood culture and colony counts were determined prior to testing to estimate target levels. Each sample was tested by Gram stain and *Staphylococcus QuickFISH™* BC prior to analysis with *mecA XpressFISH®*. The expected results for both *Staphylococcus QuickFISH™* BC and *mecA XpressFISH®* were obtained for each of the media shown in **Table 16**.

BacT/ALERT FA, FN and PF (containing charcoal) and VersaTREK REDOX 2 bottle/media types should not be used with *mecA XpressFISH* because they are not

compatible with *Staphylococcus QuickFISH*<sup>™</sup> BC. This is included as a Limitation in the *mecA XpressFISH*<sup>®</sup> Package Insert.

**Table 16. Culture Media Shown to be Compatible with *mecA XpressFISH*<sup>®</sup>**

Manufacturer	System	Culture Medium
BD	BACTEC	Plus Aerobic/F
		Plus Anaerobic/F *
		Standard/10 Aerobic/F *
		Standard Anaerobic/F *
		Peds Plus/F *
		Lytic/10 Anaerobic/F
bioMérieux	BacT/ALERT	SA Standard Aerobic
		SN Standard Anaerobic *
Thermofisher	VersaTREK	REDOX-1 Aerobic *

\* Clinical performance with *mecA XpressFISH*<sup>®</sup> not established. Refer to Clinical Studies (Section 3) and Package Insert Limitations.

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable.

b. *Matrix comparison:*

Refer to section 1h above.

3. Clinical studies:

a. *Clinical Sensitivity:*

A multicenter clinical study was conducted at laboratories in the United States and one in the United Kingdom. The study sites utilized two commercially available, continuously monitoring blood culture systems (bioMérieux BacT/ALERT and BD BACTEC). Blood culture bottles that were positive for Gram-Positive Cocci in Clusters (GPCC) by Gram stain were tested with *Staphylococcus QuickFISH*<sup>™</sup> BC. Methicillin susceptibility status of *S. aureus* isolates (*i.e.*, resistant or susceptible) was determined at each site by cefoxitin disk in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. Quality control was performed for each *mecA XpressFISH*<sup>®</sup> sample that was tested in the study using the in-built Positive and Negative Controls.

During the course of the study the sites identified 1,101 blood culture bottles as positive for GPCC. One hundred and twenty five bottles were identified as paired or duplicates and these were removed such that performance would be determined using only one bottle per patient/episode. Ten samples were excluded because >60h transpired since the bottle was called positive and before *mecA XpressFISH*<sup>®</sup> testing could occur.

*Staphylococcus QuickFISH*<sup>™</sup> BC results for 602 of the remaining samples showed the presence organisms other than *S. aureus* and therefore these samples were not tested with *mecA XpressFISH*<sup>®</sup>. Five samples were identified as mixed cultures of *S. aureus* and coagulase negative staphylococci (CoNS) for which *mecA XpressFISH*<sup>®</sup> is not indicated, and these were therefore also not tested. This left a total of 359 blood cultures which met the inclusion criteria for *mecA XpressFISH*<sup>®</sup> testing during which protocol violations (e.g., incorrect temperature, or incubation time) were recorded for 20 samples which were also excluded from the analysis. Thus, the remaining 339 test results were used to determine performance of *mecA XpressFISH*<sup>®</sup> in comparison to cefoxitin disk diffusion as the reference method. The interval between a culture turning positive and *mecA XpressFISH*<sup>®</sup> testing ranged from <2h up to 57h (**Table 17**).

**Table 17. Length of Time from Culture Positivity to *mecA XpressFISH*<sup>®</sup> Testing**

<b>Time (hours)</b>	<b>&lt;2</b>	<b>2-8</b>	<b>8-24</b>	<b>24-48</b>	<b>48-60</b>
<b>Number</b>	2	55	132	137	13
<b>Percent</b>	0.6	16.2	39.9	40.4	3.8

The results of the study are summarized by site and blood culture medium in **Table 18**. Overall results of the study are shown in **Table 19**. There were no Positive or Negative Control failures during the course of the study (339/339 paired positive and negative results, as expected; 100%).

**Table 18. Summary of Clinical Study Results for *mecA* XpressFISH® vs. Cefoxitin Disk Diffusion Reference Method Stratified by Site, Blood Culture Medium and Methicillin Susceptibility**

Bottle Type	Site	<i>mecA</i> XpressFISH/Cefoxitin Disk Diffusion		
		Methicillin Resistant ( <i>mecA</i> +)	Methicillin Susceptible ( <i>mecA</i> -)	Total (%; 95% CI)
BACTEC Plus Aerobic/F	A	2/2	12/12	14/14 (100)
	B	18/19	17/17	35/36 (97.2)
	C	7/7	18/19	25/26 (96.2)
	D	7/7	14/14	21/21 (100)
	E	19/19	19/19	38/38 (100)
	<b>Total (%; 95% CI)</b>	<b>53/54 (98.1; 90.2-99.7)</b>	<b>80/81 (98.8; 93.3-99.8)</b>	<b>133/135 (98.5; 94.8-99.6)</b>
BACTEC Lytic/10 Anaerobic/F	A	7/7	10/10	17/17 (100)
	B	10/10	17/17	27/27 (100)
	C	10/10	14/14	24/24 (100)
	D	19/19	4/4	23/23 (100)
	E	13/14	13/13	26/27 (96.3)
	<b>Total (%; 95% CI)</b>	<b>59/60 (98.3; 91.1-99.7)</b>	<b>58/58 (100; 93.8-100)</b>	<b>117/118 (99.2; 95.4-99.9)</b>
BACTEC Peds Plus	B	1/1	1/1	2/2 (100)
	C	2/2	5/5	7/7 (100)
	D	0	1/1	1/1 (100)
	<b>Total (%; 95% CI)</b>	<b>3/3 (100; 43.9-100)</b>	<b>7/7 (100; 64.6-100)</b>	<b>10/10 (100; 72.3-100)</b>
BACTEC Plus Anaerobic/F	<b>B [Total] (%; 95% CI)</b>	<b>7/7 (100; 64.6-100)</b>	<b>5/5 (100; 56.6-100)</b>	<b>12/12 (100; 75.8-100)</b>
BACTEC Standard/10 Aerobic/F	<b>D [Total] (%; 95% CI)</b>	<b>0</b>	<b>1/1 (100; 20.7-100)</b>	<b>1/1 (100; 20.7-100)</b>
<b>BACTEC All Media</b>	<b>Total (%; 95% CI)</b>	<b>122/124 (98.4; 94.3-99.6)</b>	<b>151/152 (99.3; 96.4-99.9)</b>	<b>273/276 (98.9; 96.9-99.6)</b>

Bottle Type	Site	<i>mecA</i> XpressFISH/Cefoxitin Disk Diffusion		
		Methicillin Resistant ( <i>mecA</i> +)	Methicillin Susceptible ( <i>mecA</i> -)	Total (%; 95% CI)
BacT/ALERT SA Standard Aerobic	F	24/24	18/18	42/42 (100)
	G	1/1	8/8	9/9 (100)
	<b>Total (%; 95% CI)</b>	<b>25/25 (100; 86.7-100)</b>	<b>26/26 (100; 87.1-100)</b>	<b>51/51 (100; 93.0-100)</b>
BacT/ALERT SN Standard Anaerobic	F	4/4	2/2	6/6 (100)
	G	0	6/6	6/6 (100)
	<b>Total (%; 95% CI)</b>	<b>4/4 (100; 51.0-100)</b>	<b>8/8 (100; 67.6-100)</b>	<b>12/12 (100; 75.8-100)</b>
BacT/ALERT All Media	<b>Total (%; 95% CI)</b>	<b>29/29 (100; 88.3-100)</b>	<b>34/34 (100; 89.9-100)</b>	<b>63/63 (100; 94.2-100)</b>

**Table 19. Overall *mecA* XpressFISH® Performance vs. Cefoxitin Disk Diffusion**

		Cefoxitin Disk Diffusion		
		Methicillin Resistant (≤ 21mm)	Methicillin Susceptible (≥ 22mm)	Total
<i>mecA</i> XpressFISH®	Positive	151	1 <sup>1</sup>	152
	Negative	2 <sup>2</sup>	185	187
	<b>Total</b>	<b>153</b>	<b>186</b>	<b>339</b>
<b>Sensitivity</b>		151/153 = 98.7% (95% CI: 95.4-99.6%)		
<b>Specificity</b>		185/186 = 99.5% (95% CI: 97.0-99.9%)		
<b>PPV</b>		151/152 = 99.3% (95% CI: 96.4-99.9%)		
<b>NPV</b>		185/187 = 98.9% (95% CI: 96.2-99.7%)		

<sup>1</sup>False positive *mecA* (weak green); negative upon repeat testing. cefoxitin disk = 28mm.

<sup>2</sup>Two false negative results for *mecA*:

a) repeat test negative for *mecA* XpressFISH®; cefoxitin disk = 19mm, resistant;

b) repeat test positive for *mecA* XpressFISH® (weak green); cefoxitin disk = 11mm, resistant.

b. *Clinical specificity:*

Refer to Section 3a, above.

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

The Clinical Study was performed with blood cultures from six health care centers in the United States and one in the United Kingdom. *S. aureus* positive blood culture bottles were identified by testing with *Staphylococcus QuickFISH™* BC. In total, 339 blood cultures were included in study, with one culture per subject. The rate of MRSA in *S. aureus* positive cultures varied by geographic location and ranged from 6.7% at the site in the United Kingdom to 29.0% - 58.3% at the sites in the US (**Table 20**). The incidence of MRSA infection will vary depending on institution, patient population as well as geographic location and other factors.

**Table 20. Rate of MRSA Positive Blood Cultures and Percent Agreement of *mecA* XpressFISH® with Cefoxitin Disk Diffusion**

Study Site	Rate of MRSA/ <i>S. aureus</i>
<b>A</b>	9/31 29.0%
<b>B</b>	37/77 48.1%
<b>C</b>	19/57 33.3%
<b>D</b>	26/46 56.5%
<b>E</b>	33/65 50.8%
<b>F</b>	28/48 58.3%
<b>G</b>	1/15 6.7%

**N. Proposed Labeling:**

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

**O. Conclusion:**

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