

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

A. 510(k) Number:

K133851

B. Purpose for Submission:

To obtain substantial equivalence for the Alere™ PBP2a SA Culture Colony Test

C. Measurand:

Penicillin Binding Protein 2a (PBP2a) in isolates identified as *Staphylococcus aureus*

D. Type of Test:

The Alere PBP2a SA culture colony test is a rapid immunochromatographic membrane assay that uses monoclonal antibodies to detect the PBP2a protein directly from isolated colonies identified as *Staphylococcus aureus*

E. Applicant:

Alere Scarborough, Inc.

F. Proprietary and Established Names:

Alere™ PBP2a SA Culture Colony Test.

G. Regulatory Information:

1. Regulation section:

CFR 866.1640 – Antimicrobial susceptibility test powder

2. Classification:

II

3. Product code:

MYI - System, Test, Genotypic Detection, Resistant Markers, *Staphylococcus* Colonies

4. Panel:

83, Microbiology

H. Intended Use:

1. Intended use(s):

The Alere™ PBP2a SA Culture Colony Test is a qualitative, *in vitro* immunochromatographic assay for the rapid detection of penicillin-binding protein 2a (PBP2a) in isolates identified as *Staphylococcus aureus*, as an aid in identifying methicillin-resistant *Staphylococcus aureus* (MRSA).

2. Indication(s) for use:

The Alere™ PBP2a SA Culture Colony Test is a qualitative, *in vitro* immunochromatographic assay for the rapid detection of penicillin-binding protein 2a (PBP2a) in isolates identified as *Staphylococcus aureus*, as an aid in identifying methicillin-resistant *Staphylococcus aureus* (MRSA).

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Not applicable

I. Device Description:

The Alere™ PBP2a SA Culture Colony Test is a rapid immunochromatographic membrane assay intended for the detection of penicillin-binding protein 2a (PBP2a) in isolates identified as *Staphylococcus aureus* as an aid in the identification of MRSA. The test uses recombinant monoclonal antibody fragments (rFabs) to detect the PBP2a protein directly from bacterial isolates. The rFab and a control antibody are immobilized onto a nitrocellulose membrane as two distinct lines and combined with a sample pad, a pink/purple conjugate pad, and an absorption pad to form a test strip. Isolates are sampled directly from the culture plate and eluted into an assay tube containing Reagent 1. Reagent 2 is then added and the test strip is placed in the assay tube. Results are read visually at 5 minutes.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Oxoid Penicillin-Binding Protein (PBP2a) Latex Agglutination Test

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

K011710

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The Alere™ PBP2a SA Culture Colony Test is a qualitative, <i>in vitro</i> immunochromatographic assay for the rapid detection of penicillin-binding protein 2a (PBP2a) in isolates identified as <i>Staphylococcus aureus</i> , as an aid in identifying methicillin-resistant <i>Staphylococcus aureus</i> (MRSA).	The test is a rapid latex agglutination assay, detecting PBP2' (also called PBP2a) in isolates of <i>Staphylococcus</i> , as an aid in identifying methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) and methicillin-resistant coagulase-negative staphylococci.
Analyte	PBP2a	Same
Specimen Type	Isolates identified as <i>S. aureus</i>	Same

Differences		
Item	Device	Predicate
Technology	A qualitative, <i>in vitro</i> immunochromatographic assay.	A rapid latex agglutination assay
Time to Results	5 minute after sample preparation	Within 3 minutes after sample/test preparation
Performance	Compared to Cefoxitin (30 µg) Disk Diffusion (95% confidence intervals included in parenthesis)	Compared to NCCLS methods (95% confidence intervals included in parenthesis and estimated per performance values and known sample size in product insert)
	Tryptic Soy Agar with 5% Sheep Blood Plate:	
	<u><i>S. aureus</i> Isolates:</u> Sensitivity: 99.1% (96.7%, 99.8%) Specificity: 99.2% (97.0%, 99.8%)	<u><i>S. aureus</i> Isolates:</u> Sensitivity: 100% (94.7%, 100%) Specificity: 99.0% (95.8%, 99.9%) <u>CoNS Isolates:</u> Sensitivity: 96.5% (91.4%, 98.6%) Specificity: 100% (92.1%, 100%)
	Columbia Agar with 5% Sheep Blood Plate:	
<u><i>S. aureus</i> Isolates:</u> Sensitivity: 98.6% (96.0%, 99.5%) Specificity: 99.2% (97.0%, 99.8%)	<u><i>S. aureus</i> Isolates:</u> Sensitivity: 100.0% (94.7%, 100.0%) Specificity: 100.0% (97.1%, 100%)	

Differences		
Item	Device	Predicate
		<u>CoNS Isolates:</u> Sensitivity: 99.5% (97.4%, 99.9%) Specificity: 99.5% (97.3%, 99.9%)
		<p style="text-align: center;">Mueller Hinton Plate:</p> <u>S. aureus Isolates:</u> Sensitivity: 99.1% (96.7%, 99.8%) Specificity: 99.6% (97.7%, 99.9%)
		<u>S. aureus Isolates:</u> Sensitivity: 100.0% (94.7%, 100.0%) Specificity: 100% (97.1%, 100%) <u>CoNS Isolates:</u> Sensitivity: 95.6% (90.2%, 98.1%) Specificity: 98.0% (88.4%, 99.6%)

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

CLSI M100-S24, *Performance Standards for Antimicrobial Susceptibility Testing; Twenty Fourth Informational Supplement*, 2014.

Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems; Guidance for Industry and FDA, 2009.

Draft Guidance for Industry and FDA: *Establishing the Performance Characteristics of Nucleic Acid-Based In vitro Diagnostic Devices for the Detection and Differentiation of Methicillin-Resistant Staphylococcus aureus (MRSA) and Staphylococcus aureus (SA)*, 2011.

L. Test Principle:

Alere™ PBP2a SA Culture Colony Test is a rapid immunochromatographic membrane assay that uses recombinant monoclonal antibody fragments (rFabs) to detect the PBP2a protein directly from bacterial isolates. The rFab and a control antibody are immobilized onto a nitrocellulose membrane as two distinct lines and combined with a sample pad, a pink/purple conjugate pad, and an absorption pad to form a test strip.

Isolates are sampled directly from the culture plate and eluted into an assay tube containing Reagent 1. Reagent 2 is then added and the dipstick is placed in the assay tube. Results are read visually at 5 minutes based on the presence or absence of a pink/purple Sample Line. A pink/purple Control Line appears in the top half of the test strip in a valid assay.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

A reproducibility study was performed at three sites. Testing was done at three sites, using 18-24 hrs isolates that have been sub-cultured, by two operators in duplicates for five days. The reproducibility panel consists of six MRSA, and four MSSA; 10 isolates x 2 tests (duplicates) x 2 operators x 5 days x 3 sites = 600

Testing was done from each of the following media: Tryptic Soy Agar with 5% sheep blood, and Mueller-Hinton Agar induced with a 30 µg Cefoxitin disk. There was 100% (600/600) agreement with expected test results.

b. Linearity/assay reportable range:

Not applicable

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

Traceability:

Not applicable.

Stability:

The following Product Stability Studies were performed to generate real time and accelerated stability data to support the expiration date of each lot under stated conditions.

The materials used in this study were as follows:

- Alere PBP2a SA Culture Colony Test Devices
- Alere PBP2a Culture Colony Test Reagent 1
- Alere PBP2a Culture Colony Test Reagent 2

Control Panel:

- *S. aureus* ATCC 43300 Positive Plate Control
- *S. aureus* ATCC 25923 Negative Plate Control
- *S. epidermidis* ATCC 35547 Positive Plate Control
- *S. epidermidis* ATCC 14990 Negative Plate Control
- Alere PBP2a SA Culture Colony Test QC Negative Control
- Alere PBP2a SA Culture Colony Test estimated Low Control
- Alere PBP2a SA Culture Colony Test estimated LOD Control

Stability conditions were set up as follows:

1. Accelerated –at 45°C and at 55°C
2. Real-Time at room temperature and at 30°C

Results are outlined in Table 1 below:

Transfer Lot 1 devices, Reagent 1 and Reagent 2 remained stable at 52 weeks of real time at 2-8°C and continued to hold after 40 weeks at 30°C, 20 weeks at 45°C and 10 weeks at 55°C. Transfer Lot 2 devices, Reagent 1, and Reagent 2 remained stable at 36 weeks at 2-8°C, 30°C, and continued to hold at 20 weeks at 45°C and 10 weeks at 55°C. See below Table 1 for Stability Summary.

Table 1: Stability Testing Summary

Weeks of Stability at Various Temperatures				
Lot	2-8°C	30°C	45°C	55°C
Transfer lot 1	52 weeks	40 weeks	20 weeks	10 weeks
Transfer lot 2	36 weeks*	36 weeks*	20 weeks	10 weeks
Transfer lot 3	28 weeks*	28 weeks*	20 weeks	10 weeks

*Stability testing continues on these lots at the stated temperatures.

It is anticipated that the Alere PBP2a SA Culture Colony Test will have at least 12 months of stability.

Quality Control:

The Alere PBP2a SA culture colony test has built-in positive and negative procedural controls. The manufacturer recommends that these controls be included in each test run.

1. Procedural Controls
 - a. The appearance of a blue line at the “control line” position can be considered an internal positive procedural control. If capillary flow has occurred, this line will always appear.
 - b. In comparison to the color of the control line, the background color on the dipstick should be white within 5 minutes.

2. External Positive and Negative Controls:

The external controls tested during clinical and analytical testing are described in Table 2 below.

Table 2: Controls

QC Organism	Expected Result	Result With Device
<i>S. aureus</i> ATCC 43300	Positive	Positive
<i>S. aureus</i> ATCC 25923	Negative	Negative

During the clinical study, external positive and negative controls were used for each new lot and each day of testing.

A total of 130 positive and negative external controls were tested in the clinical study and demonstrated 100% concordance with the expected results.

In addition to the above QC organisms, the analytical study included additional supplemental positive and negative controls that were tested in triplicate on each day of testing with results interpreted by two operators. All positive and negative daily controls generated the expected results on each day of testing.

d. Detection limit:

A limit of detection study was conducted using *Staphylococcus aureus* (ATCC BAA44) and the detection limit was determined to be 7.3×10^8 CFU/ml.

Table 3: Alere PBP2a SA Limit of Detection Results

<i>Staphylococcus</i> species	Concentration (CFU/ml)	# Detected per Total Tests	% Detected
<i>S. aureus</i> (ATCC BAA44)	7.30×10^8	19/20	95%

e. Analytical reactivity and specificity:

A total of 162 strains of methicillin resistant *Staphylococcus aureus* (MRSA), 112 strains of methicillin-sensitive *Staphylococcus aureus* (MSSA) were tested with the Alere PBP2a SA Culture Colony Test from each of the following media: Tryptic Soy Agar with 5% sheep blood, and Mueller-Hinton Agar induced with a 30 µg Cefoxitin disk and all tests showed the expected results.

f. Assay cut-off:

Not applicable

2. Comparison studies:

A clinical study was conducted at three geographically diverse sites throughout the United States including Alere.

A total of 454 *S. aureus* fresh isolates were tested from each of the following media: Tryptic Soy Agar with 5% sheep blood, Columbia agar with 5% sheep blood and Mueller-Hinton Agar induced with a 30 µg Cefoxitin disk. Results were compared to those of 30 µg Cefoxitin disk diffusion, according to Clinical and laboratory Standards Institute (CLSI) interpretation. Alere PBP2a SA Culture Colony Test performance, including 95% confidence intervals, versus Cefoxitin disk diffusion, stratified by plate type is provided in Table 4 below.

Table 4: Summary of Performance Characteristics by Plate Type:

Plate Type	Sensitivity (# positive/total)	95% C. I.	Specificity (# negative/total)	95% C.I.
Primary Plate ¹	100% (129/123)	(97.1, 100.0)	98.5% (134/136)	(94.8, 99.6)
Tryptic Soy Agar with 5% sheep blood	99.1% (213/215)	(96.7%, 99.8%)	99.2% (237/239)	(97.0%, 99.8%)
Columbia Agar with 5% sheep blood	98.6% (212/215)	(96.0%, 99.5%)	99.2% (237/239)	(97.0%, 99.8%)
Mueller Hinton with 30 µg Cefoxitin Induction	99.1% (213/215)	(96.7, 99.8%)	99.6% (238/239)	(97.7%, 99.9%)

¹Alere test was performed from primary plates at 2 out of 3 clinical sites. Primary plates were either Tryptic Soy Agar or Columbia Agar, with the exception of two samples of unknown plate type.

a. Method comparison with predicate device:

The sponsor conducted the Cefoxitin disk screen test as the reference method for detecting resistant strains of Staphylococci. Cefoxitin disk at concentration of 30 µg is a surrogate for oxacillin resistance and is used in the clinical studies. The CLSI recommendations are:

Table 5: Interpretive Criteria for 30µg Cefoxitin Disk Diffusion Test

Interpretive Criteria (zone diameter in mm) for 30 µg Cefoxitin Disk Diffusion Test		
	Oxacillin Susceptible	Oxacillin Resistant
<i>S. aureus</i> and <i>S. lugdunensis</i>	≥22 mm	≤21 mm

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Refer to Table 4 (above).

b. Clinical specificity:

Refer to Table 4 (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:

Not applicable

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.