AptaSure™ MRSA – Clinical / Laboratory Update
December 2022

As you may recall, Invenio Medical Inc. developed successful prototypes of the AptaSure™ MRSA device in Quarter 1, 2018, with further enhancements of tubing opacity modifications in subsequent months to follow. Once the configuration appeared optimal, the device underwent prospective studies, with a total of 2126 nasal swab specimens collected from subjects at risk for MRSA nasal colonization.

Of these 2126 specimens, 469 were excluded from the study based on inclusion/exclusion criteria leaving a total of 1628 unique specimens that met the predetermined eligibility criteria for inclusion in the study. In total, 1628 specimens were enrolled in the study and tested for methicillin-resistant *Staphylococcus aureus* by both the reference method and the AptaSure™ MRSA. There were16 specimens that when tested with AptaSure™ MRSA yielded an invalid result due to run failure or instrument error, giving an invalid rate of 0.98% (16/1628). None of these specimens were re-tested due to insufficient specimen volume.

The initial device performance calculations, clinical sensitivity of the AptaSure™ MRSA against direct and enriched bacterial culture was 92.9%, with a lower bound 95% confidence interval of 85%. Clinical specificity of the AptaSure™ MRSA was 92.6%, with a lower bound 95% confidence interval of 89%. Of the specimens that were MRSA-negative by culture but MRSA-positive by the AptaSure™ MRSA, culture showed that 67 specimens were S. *aureus* and 45 were negative (no growth). When AptaSure™ MRSA was compared to Direct Culture only, the positive percent agreement of the AptaSure™ MRSA was 92.5%, with a lower bound 95% confidence interval of 86%. Negative percent agreement of the AptaSure™ MRSA was 91.29%, with a lower bound 95% confidence interval of 89%. Overall, performance was determined to require additional testing.

In conclusion, our last update indicated for the need for additional substantial equivalence and device performance testing, including the need to test additional devices for performance. This also included the need for additional device stability testing of control reagents and solutions used in the assay.

Update Clinical Equivalency Testing – June – November, 2022

In order to obtain additional data points for our substantial equivalence (SE) determination for the AptaSure™ MRSA, Invenio Medical, Inc., procured an additional 1150 functional prototypes to be used in additional testing and run in parallel to bacteriophage amplification specific to *S. aureus,* and to assess the phenotypic response of the target organism to cefoxitin as an analog to methicillin.

A. Type of Test:

Qualitative lateral flow identification and AST test using bacteriophage amplification growth-based detection

B. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OUS	I	866.2050 - Staphylococcal typing bacteriophage	Microbiology (83)

C. Intended Use:

1. Intended use(s):
   The AptaSure™ MRSA is a qualitative *in vitro* diagnostic test for the timely identification of *Staphylococcus aureus* (*S. aureus*) and determination of methicillin susceptibility (MSSA) or methicillin resistance (MRSA) directly from positive nasopharygeal/nares cultures.

   The test uses bacteriophage lysing to identify the presence of *S. aureus* and to assess the phenotypic response of the target organism to cefoxitin, an indicator of oxacillin (a methicillin analog) resistance.

   The assay is performed directly on positive culture specimens that are determined as Gram Positive Cocci in singles (GPC) or as Gram Positive Cocci in Clusters (GPCC) by Gram stain.

   The AptaSure™ MRSA is performed directly on positive nasopharyngeal/ nares specimens from BD BACTEC™ culture bottles (Plus Aerobic/F and Plus Anaerobic/F).

   The Test is indicated for use in conjunction with other laboratory and clinical data available to the physician as an aid in the detection of MRSA/MSSA from positive cultures.

   Subculturing of positive cultures is necessary for additional susceptibility test determinations, differentiation of mixed growth and for epidemiological typing.

2. Indication(s) for use:
   The AptaSure™ MRSA is a qualitative *in vitro* diagnostic test for the timely identification of *Staphylococcus aureus* (*S. aureus*) and determination of methicillin susceptibility (MSSA) or methicillin resistance (MRSA) directly from positive nasopharyngeal/nares cultures.

   The test uses bacteriophage lysing to identify the presence of *S. aureus* and to assess the phenotypic response of the target organism to cefoxitin, an indicator of oxacillin (a methicillin analog) resistance.

   The assay is performed directly on positive culture specimens that are determined as Gram Positive Cocci in singles (GPC) or as Gram Positive Cocci in Clusters (GPCC) by Gram stain.

   The AptaSure™ MRSA is performed directly on positive nasopharyngeal/ nares specimens from BD BACTEC™ culture bottles (Plus Aerobic/F and Plus Anaerobic/F).

   The Test is indicated for use in conjunction with other laboratory and clinical data available to the physician as an aid in the detection of MRSA/MSSA from positive cultures.

   Subculturing of positive cultures is necessary for additional susceptibility test determinations, differentiation of mixed growth and for epidemiological typing.

3. Special conditions for use statement(s):
   For prescription use

4. Special instrument requirements:
   Manual readings only

D. Device Description:

The AptaSure™ MRSA test uses lytic bacteriophage, specific for *Staphylococcus aureus (S. aureus,* SA*),* as an amplification technology for detection of *S. aureus* and determination of methicillin resistance or susceptibility in positive cultures. To detect *S. aureus* (ID Reaction Tube), the bacteriophage infect the *S. aureus* (if present), replicate within the host (culminating in bacterial lysis) and over the incubation period, of 15 minutes, produce several cycles of bacteriophage amplification.

In order to further extract analyte for sample preparation, a separate Reaction Tube (RS), uses cefoxitin (an oxacillin and methicillin analog) which inhibits bacteriophage amplification for susceptible organisms (MSSA) and fails to inhibit bacteriophage amplification when the organism is resistant to methicillin (MRSA).

The Test then uses a self- performing immunoassay (Detector) to detect the increase in concentration of bacteriophage using antibodies specific to the Test bacteriophage, and optimized such that at above a threshold concentration, it produces a visible signal.

E. Substantial Equivalence Information:

1. Predicate device name(s):
   K071026 BD GeneOhm™ StaphSR Assay K851949
   Wellcome (Remel) Staphaurex® ZL30 K011710 Oxoid
   PBP2' Latex Agglutination
   (Preamendment) BBL (BD) cefoxitin 30 μg Sensi-Disc
   (Preamendment) BBL (BD) oxacillin 1 μg Sensi-Disc (Preamendment)
   Coagulase Test (multiple manufacturers) (Preamendment) Catalase
   Test (multiple manufacturers)

2. Predicate 510(k) number(s):
   K071026 BD GeneOhm™ StaphSR Assay
   K851949 Wellcome (Remel) Staphaurex® ZL30
   K011710 Oxoid PBP2' Latex Agglutination

3. Comparison with predicate:

SIMILARITIES		
Items	**AptaSure™ MRSA**	**BD GeneOhm™ StaphSR Assay**
Intended Use –	The AptaSure™ MRSA is a qualitative *in vitro* diagnostic test for the timely	The BD GeneOhm™ StaphSR Assay is a qualitative *in vitro* diagnostic test for the rapid detection of

	identification of *Staphylococcus aureus* (*S. aureus*) and determination of methicillin susceptibility (MSSA) or methicillin resistance (MRSA) directly from positive cultures. The test uses bacteriophage amplification to identify the presence of *S. aureus* and assess the phenotypic response of the target organism to cefoxitin, an indicator of oxacillin (a methicillin analog) resistance. The assay is performed directly on positive blood culture specimens that are determined as Gram Positive Cocci in singles (GPC) or as Gram Positive Cocci in Clusters (GPCC) by Gram stain. The AptaSure™ MRSA is performed directly on positive culture specimens from BD BACTEC™ culture bottles (Plus Aerobic/F and Plus Anaerobic/F). The Test is indicated for use in conjunction with other laboratory and clinical data available to the physician as an aid in the detection of MRSA/MSSA from positive cultures. Subculturing of positive cultures is necessary for additional susceptibility test determinations, differentiation of mixed growth and for epidemiological typing	*Staphylococcus aureus* (SA) and methicillin- resistant *Staphylococcus aureus* (MRSA) directly from positive blood culture. The assay utilizes polymerase chain reaction (PCR) for the amplification of specific targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed on gram positive cocci, identified by Gram stain, from positive blood cultures. The BD GeneOhm™ StaphSR Assay is not intended to monitor treatment for MRSA/SA infections. Subculturing of positive blood cultures is necessary for further susceptibility testing.
Single Use	Yes	Yes
Indications for Use	Professional Use	Professional Use
Interpretation of results	Visual	Visual
Patient population	Clinical patients	Clinical patients
Specimen type	Positive cultures	Positive blood/body fluid culture

Assay controls	Pos Control 1: MRSA Pos Control 2: MSSA Neg Control: NSA	Pos Control: MRSA Pos Control: SA Neg Control: NSA

DISSIMILARITIES		
Items	**AptaSure™ MRSA**	**BD GeneOhm™ StaphSR Assay**
Time to result	15 minutes	60-75 minutes
Mode of action	The test uses bacteriophage amplification with cefoxitin to rapidly determine the presence of MRSA in SA populations.	The assay utilizes polymerase chain reaction (PCR) for the amplification of specific targets and fluorogenic target specific hybridization probes for the real-time detection of the amplified DNA.
Assay format	Detection: Lateral flow immunoassay with colloidal gold particles with monoclonal antibodies specific to assay bacteriophage.	Amplification: polymerase chain reaction (PCR) Detection: Fluorogenic target-specific hybridization probes of the amplified DNA.

SIMILARITIES		
Items	**AptaSure™ MRSA**	**Coagulase Tube, Catalase Slide Tests (pre-amendment)**
Intended Use	See above	The Coagulase Tube and Catalase Slide Tests (multiple manufacturers) are qualitative *in vitro* diagnostic tests for the identification SA directly from isolated colonies from a positive blood culture. "Positive" results for both catalase and coagulase are indicative of *SA*. The assays contain rabbit serum with EDTA for Coagulase Test and peroxide (H_2O_2) for Catalase Test substrates which will react with coagulase and catalase enzymes expressed by SA. A clumping of rabbit serum confirms the presence of

		coagulase and production of O2 bubbles confirms the presence of catalase (i.e. both results indicating presence of SA). The assay is performed on gram positive cocci which have been isolated by streaking on culture plates, identified by Gram stain, from positive blood cultures. Further subculturing of positive blood cultures are necessary for susceptibility testing.
Single Use	Yes	Yes
Indications for Use	Professional Use	Professional Use
Interpretation of results	Visual	Visual
Patient population	Clinical patients	Clinical patients
Specimen type	Positive culture	Positive blood / body fluid culture
Assay controls	Pos Control 1: MRSA Pos Control 2: MSSA Neg Control: NSA	

DISSIMILARITIES		
Items	**AptaSure™ MRSA**	**Coagulase Tube, Catalase Slide Tests (pre-amendment)**
Specimen Type	Positive culture	Overnight purified plate culture (i.e. isolated colonies) originating from a positive blood culture.
Time to result	15 minutes	Catalase: 5 minutes Coagulase: 4-24 hours

Mode of action	The test uses bacteriophage amplification to rapidly identify the presence of SA.	The assays contain rabbit serum with EDTA for Coagulase Test and peroxide (H_2O_2) for Catalase Test substrates which will react with coagulase and catalase enzymes expressed by SA. A clumping of rabbit serum confirms the presence of coagulase and production of O2 bubbles confirms the presence of catalase (i.e. both results indicating presence of SA.)
Assay format	Detection: Lateral flow immunoassay with colloidal gold particles with monoclonal antibodies specific to assay bacteriophage.	Amplification: none Detection: Clumping of coagulase substrate and O2 gas production (i.e. bubbles) of catalase substrate when metabolized by the respective enzymes.

SIMILARITIES		
Items	**AptaSure™ MRSA**	**Oxoid PBP2' Latex Agglutination Test**
Intended Use	See above	This test is a rapid latex agglutination assay, detecting PBP2' (also called PBP2a), in isolates of *Staphylococcus*, as an aid in identifying MRSA and methicillin-resistant coagulase-negative staphylococci.
Single Use	Yes	Yes
Indications for Use	Professional Use	Professional Use
Interpretation of results	Visual	Visual
Patient population	Clinical patients	Clinical patients
Assay controls	Pos Control 1: MRSA Pos Control 2: MSSA Neg Control: NSA	Pos Control: MRSA Neg Control: NSA

DISSIMILARITIES		
Items	**AptaSure^{TM} MRSA**	**Oxoid PBP2' Latex Agglutination Test**
Specimen Type	Positive culture	Overnight Purified Culture (16 –24 hrs)
Time to result	15 minutes	45 minutes
Mode of action	The test uses bacteriophage amplification to rapidly identify the presence of MRSA in *S. aureus* populations.	Latex particles sensitized with a monoclonal antibody against PBP2′ will specifically react with methicillin-resistant staphylococci to cause agglutination visible to the unaided eye.
Assay format	Detection: Lateral flow immunoassay with colloidal gold particles with monoclonal antibodies specific to assay bacteriophage.	Amplification: none Detection: Agglutination of latex particles.

SIMILARITIES			
Items	**AptaSure™ MRSA**	**BD BBL cefoxitin 30ug Sensi-disc**	**BD BBL cefoxitin 1 ug Sensi-disc**
Intended Use	See above	These discs are used for semi-quantitative *in vitro* susceptibility testing by the agar disc diffusion test procedure of common, rapidly growing and certain fastidious bacterial pathogens.	These discs are used for semi-quantitative *in vitro* susceptibility testing by the agar disc diffusion test procedure of common, rapidly growing and certain fastidious bacterial pathogens.
Single Use	Yes	Yes	Yes
Indications for Use	Professional Use	Professional Use	Professional Use
Interpretation of results	Visual	Visual	Visual
Patient population	Clinical patients	Clinical patients	Clinical patients
Assay controls	Pos Control 1: MRSA Pos Control 2: MSSA Neg Control: NSA	Pos Control 1: MRSA Neg Control: MSSA	Pos Control: MRSA Neg Control: MSSA

DISSIMILARITIES			
Items	**AptaSure™ MRSA**	**BD BBL cefoxitin 30ug Sensi-disc**	**BD BBL cefoxitin 1 ug Sensi-disc**
Specimen Type	Positive culture	Overnight culture	Overnight culture
Time to result	15 minutes	18-24 hours	18-24 hours
Mode of action	The test uses bacteriophage amplification to rapidly identify the presence of MRSA in *S. aureus* populations.	Diffusion of antibiotic into lawn of SA.	Diffusion of antibiotic into lawn of SA.

Assay format	Detection: Lateral flow immunoassay with colloidal gold particles with monoclonal antibodies specific to assay bacteriophage.	Amplification: none Detection: Visual interpretation of zone of inhibition.	Amplification: none Detection: Visual interpretation of zone of inhibition.

Summary of Gold Standard and Predicate Use:
After Gram stain, samples were tested with: a) the AptaSure™ MRSA per MP2009-B and b) a cohort of Gold Standard and predicate tests for identification including: coagulase tube test, catalase slide test, and Staphaurex® Test. The identification Gold Standard is defined as concordant results between the catalase, coagulase and Staphaurex® tests.

For SA positive samples, PBP2' test, Cefoxitin Sensi-disc (30 ug) test and Oxacillin Sensi-disc (1 ug) test were performed to determine antibiotic susceptibility. The Cefoxitin Sensi-disc test was defined as the Gold Standard for MRSA/MSSA determination on *S. aureus*-positive samples.

Except for MRSA/MSSA Blood Culture Test – BT, all other Gold Standard/predicates used purified colonies from overnight culture of positive blood culture samples on 5% sheep blood-tryptic soy agar (TSA) plates.
Sensi-disc testing (i.e. Kirby-Bauer disc diffusion) included secondary plating on Mueller-Hinton plates.

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

Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) Systems

CLSI M100-S19, *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement* CLSI EP12-A2, User Protocol for Evaluation of Qualitative Test Performance, 9/9/2008

CLSI M100-S20 *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement*, M100-S20, 2010

CLSI EP25-A, *Evaluation of Stability of In Vitro Diagnostic Reagents, Approved Guideline*, Sept. 2009

CLSI M23-A2, *Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters*, 09/08/2009

CLSI EP7-A2, Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition, November 2005

G. Test Principle:

The AptaSure™ MRSA uses lytic bacteriophage, specific for *Staphylococcus aureus,*

as an amplification technology for detection of *S. aureus* and determination of methicillin resistance or susceptibility in positive nasopharyngeal / nares cultures to detect *S. aureus* (ID Reaction Tube), the bacteriophage infect the *S. aureus* (if present), replicate within the host (culminating in bacterial lysis) and over the incubation period, produce several cycles of bacteriophage amplification. In a separate Reaction Tube (RS), the test uses cefoxitin (methicillin analog) which inhibits bacteriophage amplification for susceptible organisms (MSSA) and fails to inhibit bacteriophage amplification when the organism is resistant to methicillin (MRSA).

The test then uses a self-performing immunoassay (Detector) to detect the increase in concentration of bacteriophage using antibodies specific to the Test bacteriophage, and calibrated such that at a known concentration, it will produce a visible signal.

The test formulation consists of 4 components: Identification (ID) liquid reagents (Reaction Media), ID dried reagents (Reaction Tubes), Resistance/Susceptibility (RS) liquid reagents (Reaction Media) and RS dried reagents (Reaction Tubes). The dried reagents are formulated specifically for use in the Bactec™ (Becton-Dickinson) culture systems with PLUS Aerobic and PLUS Anaerobic bottles.

The test is also composed of a dual lane self-performing lateral flow immunoassay (LFI Detector). The Test includes two 150 µL transfer pipettes that facilitate transfer of the incubated sample from each of the Reaction Tubes to each of the corresponding Detector wells. The pipettes are color coded (blue and red) to ensure proper transfer.

H. Performance Characteristics (if/when applicable):

1. Analytical performance:
   In the analytical in-house performance testing, all protocols used a single technician to set up the Test but this technician does not interpret the results. Multiple, independent, blinded, readers interpret the results.

   a. *Precision/Reproducibility:*

      Of 240 test points, 96.3% (231/240) of samples the AptaSure™ MRSA (Test) were correctly called as

      S. aureus. For Resistance and Susceptibility (RS) determinations, none (0/120) of the MSSA samples were incorrectly determined to be MRSA. There was a 100% agreement. (111/111) for MRSA samples which had been correctly classified as *S. aureus* by the Identification component of the MRSA/MSSA culture test. The breakout of performance by site and strain for the initial reproducibility showed inconsistencies so additional reproducibility studies were conducted. Inconsistencies were due to sample preparation for testing.

      In the additional study, reproducibility of the AptaSure™ MRSA was assessed over 6 days at 3 study sites (2 external, 1 internal), using 6 operators (2 at each site), with the following strains – 2 MRSA (JMI-520,

JMI-912), 2 MSSA, (JMI-2226, JMI-7812) 1 NSA (ATCC-12228, *S. epidermidis*).

Additionally, 3 GMP kit lots and 2 replicates per sample per session were tested. The analyte levels included 2 for MRSA and MSSA, 1 for NSA. The MRSA and MSSA samples were tested neat, and at 10-fold dilution, a level shown to be at or near the limit of detection. Accuracy was 644/648 = 99.4%.

All 4 errors were false-resistance calls of MSSA runs. The 4 errors were distributed between the three sites and the 3 GMP lots. Accuracy among MRSAs was 288/288, 100%.

Accuracy among MSSAs was 284/288 = 98.6%. Accuracy among NSAs was 100%.

b. *Linearity/assay reportable range:*
Not applicable

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

Controls
Quality control was performed each day of testing on both the reference method and the test method at three testing sites.

Reproducibility of the controls exceeded 94.5% at all sites during the course of the clinical trials. Invenio Medical, Inc. supplied frozen bacterial stocks of three control bacteria (Methicillin-resistant *Staphylococcus aureus* - MRSA, Methicillin-susceptible *Staphylococcus aureus* - MSSA, and non-*Staphylococcus aureus* - NSA) which required an additional 15 minutes of incubation before testing for clinical design validation trial.

External Quality Control Procedure Verification:
A new protocol was external quality control was developed, using American Type Culture Collection (ATCC) stocks of MRSA, MSSA, and NSA as listed in the table below.

Bacterial stocks of the three control strains were streaked to isolate single colonies which were grown overnight on trypticase soy agar with 5% sheep blood (TSA II). A 0.5 McFarland dilution was prepared with each isolate and 30 uL samples were directly inoculated into each Reaction Tube of the AptaSure™ MRSA. Tests were run as per the Test procedure. There were no invalid runs and no tests were repeated

Indicated control strains.

Bacteria	ATCC Strain Number
Methicillin-resistant *Staphylococcus aureus*	43300
Methicillin-susceptible *Staphylococcus aureus*	14775
Staphylococcus epidermidis (Coagulase Negative)	12228

The External QC Control Protocol was executed by 3 operators over 3 days. No differences were seen between days and operators. The total number of valid runs and results for each control strain are shown below.

QC validation results

Strain	N valid results	N correct results	% (95% CI) correct results
MRSA	110	110	100% (96.7% - 100%)
MSSA	110	110	100% (96.7% - 100%)
NSA	110	110	100% (96.7% - 100%)

The proposed External QC Control procedure demonstrated acceptable reliability between operators and days of testing. This change was accepted and used for the package insert procedure.

Stability

Three AptaSure™ MRSA kit lots were configured using a combination of unique lots of each of five kit components. Shelf-life stability of the MRSA/MSSA Test was assessed, per applicable CLSI Standards (CLSI EP25-A: Evaluation of Stability of *In Vitro* Diagnostic Reagents (2009)), by real-time storage at labeled storage conditions (2-8°C). The claimed shelf life is 24 weeks.

Detector devices, contained in their sealed storage pouches with desiccant pack, were placed at -20, 42 or 60°C for 4 hours. After exposure, pouches were opened and Detectors were used under ambient laboratory conditions. A total of three lots of Detector were evaluated during this study.

A study was also conducted to determine the interaction of temperature and humidity while the detector was in use. A two- factorial designed experiment, covering the interactions of effects of temperature ranges of 4-35°C (40-95°F) and relative humidity (RH) ranges of 2-90%, was employed. Detectors were placed in sealed chambers in the presence of saturated salt solutions (used to maintain a specific relative humidity), then chambers were placed in incubators (used to maintain a specific temperature) and left to equilibrate for 60 minutes. Temperature and RH achieved under these conditions was confirmed with a calibrated temperature/RH probe. A total of three lots of Detector were evaluated during this study.

Under normal ambient temperature conditions (18-25°C or 65-78°F), relative humidity does not affect the ability of the Detector to accurately call negative and positive bacteriophage-containing samples. In addition, short-term exposure of the Detector to extremes in temperature (i.e. -20 to 42°C or -4 to 105°F) which may arise during shipment of the product does not adversely affect the accuracy of call rates for negative and positive bacteriophage-containing samples.

However, the trend in call rates was observed at the extremes of the temperature range:

- For high negative samples, a significant increase in positive call rate was seen as humidity increased when the Detector was incubated at 35°C (95°F).
- For high negative and low positive samples, a significant decrease in positive call rates were seen when the incubation temperature was lowered to 4°C (40°F).

Specimen stability:
Invenio Medical, Inc. tested whether the age of the blood would affect bacteriophage amplification. It was determined that the MRSA AptaSure™ test must be performed ≤ 24 hours after the Bactec instrument alarms for positive culture bottles confirmed to have GPCC or GPC by Gram stain.

Invenio Medical, Inc. tested the robustness of the operating parameters and Twenty-seven combinations of parameters were tested, in addition to controls. The 27 runs were performed on each of five strains of *S. aureus* and *S. epidermidis.* The operating range was set by the highest minimum and lowest maximum for each of the five strain types and summarized in the following table.

Operating Parameters

Test parameter	Minimal	Maximal
mL blood added to culture bottle	5 ml	12 ml
Time after alarm, hrs	0	24
µl sample added	5	13
Min lag to incubation	0	25
Incubation temp	34	38
Hours incubation	4.5	5.5

Min lag before detector start	0	60

Research and development studies determined that the bacteriophage lose titer if they undergo more than one freeze-thaw cycle.

d. Detection limit:

The analytical sensitivity of the AptaSure™ MRSA, for correct call rate for *S. aureus* ID, correct call rate for MSSA, and correct call rate for MRSA, was determined to be 6.0×10^5 CFU/mL from Bactec blood culture bottle samples.

The Test Detectors return a negative result with >95% probability at bacteriophage concentrations 1.8×10^7 PFU/mL, and returned a positive result with >95% probability at bacteriophage concentrations 1.4×10^8 PFU/mL. The cutoff level (50% call probability) is 4.9×10^7 PFU/mL.

The prozone study used a high challenge concentration of bacteriophage (1×10^{11} PFU/mL), approximately 1.5 logs greater than the median phage output post-amplification of clinical samples, which failed to produce false negative results on the Detector indicating the Detector is visually insensitive to hook effect between 1×10^9 and 1×10^{11} PFU/mL.

e. Analytical specificity:

Co-Infection (or Cross-Contamination) Study
The following permutations of Target and Contaminant strains were tested in the Co-Infection Study: MRSA x MS-CoNS, MRSA x MSSA, hMRSA (Heteroresistant MRSA) x MSSA, and MSSA x MR- CoNS. Individual strains were spiked into charged Bactec™ blood culture bottles, per the Spiking Model, and grown to alarm on a Bactec™ 9050 blood culture incubator.

Target strains were mixed with contaminant strains at volumes of 10:1, 1:1, and 1:10, and then the MRSA/MSSA Test was run. Visual calls of MRSA, MSSA or NSA (not *S. aureus*) were made by a panel of three readers who were blinded to strain identity. Tests were run in triplicate from 3 kit lots on three successive days.

The Aptasure™ MRSA test showed a high ability to detect MRSA, even in a sample containing >90% MSSA (2 MSSA calls were made out of 81 total calls). Heteroresistant MRSA showed more sensitivity to mixture with an MSSA strain

The Aptasure™ MRSA test can reliably ($P \geq 95\%$) detect hMRSA from samples containing up to 37% MSSA. MRSA could be reliably detected in a culture comprised of > 90% methicillin-sensitive Coagulase Negative Staphylococci (MS-CoNS). Heteroresistant MRSA (hMRSA) could be reliably detected from samples containing up to 37% MSSA. At mixtures of ≥79% MSSA the test becomes more likely ($P \geq 50\%$) to return a call of

MSSA than of MRSA. Mixture of methicillin-resistant CoNS with MSSA yielded two types of results: 1) if the MR-CoNS strains were of a type that is intrinsically unreactive with the Test bacteriophage, no effect was seen, or 2) if the strain was intrinsically highly reactive (a rare phenotype), the Test returned a call of MRSA at contamination levels > 25%.

Individual strains were spiked into BACTEC™ blood culture bottles (Aerobic Plus) containing 8-10 mL whole blood, per the Spiking Model, and grown to alarm on a BACTEC™ 9050 blood culture incubator. MRSA target strains were mixed with contaminant strains at volumes of 9:1, 1:1, and 1:9, and then the MRSA/MSSA Blood Culture Test was run.

Visual calls of MRSA, MSSA or NSA (not *S. aureus*) were made by a panel of three technicians who were blinded to strain/sample identity. Bacterial input concentrations from blood bottle samples were determined by dilutional plate count techniques on tryptic soy agar, and colony forming unit (CFU) data was used to analyze percentage of MRSA call failures by cell percentage of non- MRSA co-infecting strain.

Tests were run as one test on a single MRSA/MSSA kit lot on three successive days. Pure cultures of MRSA or NSA strains were run as controls each day. Correct calls of 100% were observed for pure cultures of MRSA and NSA (54/54 and 81/81 for MRSA and NSA, respectively). Mixed cultures of MRSA and NSA generated 486 total calls that were classified as either True (T) or False (F). Using CFU data, the cell input fraction comprising MRSA (as % of NSA) for each run were calculated.

Assay outcome (T or F) was plotted against log (fractional MRSA) and a logistic regression fit was obtained. Based on the logistic fit, failure probabilities (10%, 50% and 90%) of NSA co-infection were calculated. In the case of Gram negative rods (e.g. *E. coli)* and Gram positive cocci in clusters and pairs (e.g. *S. anginosis*) the Test is sensitive enough to return an accurate call 90% of the time with as little as <1% of bacterial input being MRSA. In presence of Gram positive rods (e.g. *B. cereus)*, the Test was able to detect MRSA 90% of the time with ~10% of the bacteria input being MRSA.

When yeast was used as the co-infectant, 90% MRSA call accuracy required ~ 54% of the input CFU to be MRSA. This observation resulted from disparities in the cell densities of each organism at blood culture alarm. Yeast cultures alarm at ~1.6E+06 CFU/mL compared to average concentration of 5.4E+07 CFU/mL for MRSA cultures. As a consequence, the cell input of MRSA was reduced significantly relative to the other NSA strains evaluated in this study. As yeast reach cell density at a log lower than MRSA at alarm and grow slower than *S. aureus*, under true mixed infection of yeast and *S. aureus*, yeast CFU would reach only as high as 10% of total CFU at alarm. Additionally, the assay correctly returned a NSA call in all cases of yeast pure cultures run as control (18/18 calls) and indicates no risk of false response in the Test for this organism.

Study strains used and their characteristics.

Strain*	Type	Cefoxitin ZONE OF INHIBITION mm	Description
JMI 520	MRSA	7.9	*mecA⁺ Staphylococcal aureus*, used as Invenio Medical, Inc. QC and control strain for MRSA
NARSA 192	MRSA	12.2	*mecA⁺ Staphylococcal aureus*, well characterized MRSA strain
ATCC 43300	MRSA	12.2	*mecA⁺ Staphylococcal aureus*, used as Invenio Medical, Inc. QC and control strain for MRSA
ATCC 3555	NSA	NA	*Klebsiella pneumoniae*
JMI 017	NSA	NA	*Pseudomonas fluorescens*
JMI 27-10560	NSA	NA	*Streptococcus anginosis*
ATCC 51188	NSA	NA	*Enterococcus faecalis*
ATCC 13061	NSA	NA	*Bacillus cereus*
JMI 4220	NSA	NA	*Escherichia coli*
ATCC 14053	NSA	NA	*Candida albicans*

Cross Reactivity Studies:

The Test was challenged against 33 Gram-negatives, 7 yeasts and 123 Gram-positives organisms (including 65 *Staphylococcus* species not SA, 14 *Enterococcus* spp. (table below), 16 *Streptococcus* spp., 12 Gram positive rod species, and 16 additional miscellaneous gram positive organisms). No evidence of cross-reaction was seen with the seven yeasts (*C. albicans, C. glabrata, C. parapsilosis, C. krusei, C.tropicalis, Cryptococcus neoformans,* and *Rhodotorula mucilaginosa*), while there were 2 false-positive calls among the Gram-negatives and 12 false-positive calls among the Gram-positives.

The Gram-negative false-positive was observed in a *Proteus vulgaris* strain. The strain was retested on two subsequent occasions, but no phage amplification and no LFI signal was observed on any replicate. The initial false-positive result may have been an operator error. The Gram-positive false positives are accounted for by two strains, JMI-7302 (9 MRSA/0 NSA calls) and JMI-3716 (3 MSSA/6 NSA calls), both *S. epidermidis*.

These strains are known to be capable of cross-reacting with the bacteriophage cocktail, and thus likely represent true Test cross- reactions. The Gram negative false positives could not be repeated on secondary testing. Based on the strains tested, the overall analytical specificity of the Test is 98.8%. This value is consistent with the results of clinical validation, which showed a value of 98.3% specificity in *S. aureus* identification.

The following *Enterococcus* species were evaluated in the analytical specificity studies with no misidentifications.

Enterococcus species	Strain
Enterococcus faecalis	ATCC51188
Enterococcus faecalis	JMI 13133
Enterococcus faecalis	JMI 5515
Enterococcus faecalis	JMI 5991
Enterococcus faecium	JMI 3122
Enterococcus faecium	JMI 5430
Enterococcus faecium	VRE JMI 14275
Enterococcus faecium	VRE JMI 1698
Enterococcus avium	ATCC 14025
Enterococcus casseliflavus	ATCC 700327
Enterococcus durans	JMI 12-581
Enterococcus gallinarum	ATCC 49573
Enterococcus hirae	ATCC 10541
Enterococcus mundtii	ATCC 43178
Enterococcus raffiniosus	ATCC 49464

Thirty two different viruses, including Influenza Virus A H1, Influenza Virus A H2, Influenza Virus B, Respiratory Syncytial Virus (RSV) A, Respiratory Syncytial Virus (RSV) B , Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Adenovirus 7A, Enterovirus – Coxsackie, Rhinovirus, Metapneumovirus, Coronavirus 229E, Coronavirus SARS, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), Hepatitis D Virus (HDV), Human Immunodeficiency Virus (HIV) 1, Rubella Virus, Cytomegalovirus (CMV) , Mumps Virus ,Varicella Zoster Virus (VZV) , Coxsackie virus , Epstein-Barr Virus , Herpes Virus 1 , Herpes Virus 2, Human T-Lymphotrophic Virus (HTLV) I, Human T-Lymphotrophic Virus (HTLV) II, Human Immunodeficiency Virus (HIV), 2 strains West Nile Virus (WNV), and Echovirus were tested. None (0/33) of the Reaction

Media samples containing viral antigens or PBS-control resulted in false-reactive results when tested with the AptaSure™ MRSA test. In addition, 100% (33/33) of the bacteriophage-spiked Reaction Media samples containing viral antigens or PBS-control were detected (i.e. no false-negative results) when tested with the AptaSure™ MRSA test.

<u>Analytical Reactivity (Inclusivity) Studies:</u>

Multi-Locus Strain Type (MLST) Reactivity
Inclusivity studies for the AptaSure™ MRSA was evaluated on a total of 114 MRSA and MSSA strains from North America and Western Europe and represented geographic and phylogenetic diversity including 46 MLS (multilocus sequence) types representing 17 clonal complexes. The MRSA or MSSA category of each strain was confirmed by cefoxitin disk diffusion testing following the guidance of CLSI M100-S20. All disk diffusion results were in agreement with source categorization of the strain.

The 114 strains were tested in replicate for a total of 228 test runs. Of the 22 incorrect calls, 20 were ID false-negatives (NSA) and 2 were false resistant (a single strain, NRS 274, OXA MIC = 2 μg/mL). The overall sensitivity of detection for *S. aureus* was 208/228 = 91.8%.
Among *S. aureus* true positives, accuracy of methicillin resistance and susceptibility determination is 204/206 = 99.0%.

The only MLS Type with more than one false call was ST239, a member of CC8, although it has also been described as a chimera of CC8 and CC30. These strains grew extremely slowly on initial testing, requiring up to 36 hours before alarm in the BACTEC™ 9050. (Normal time to alarm in the spike model is 12-16 hours for *S. aureus*.) Upon repeat testing, the strains alarmed in the normal time window, and returned true positive results. The initial result was thought to be an artifact.

Pulsed-Field Gel Electrophoresis (PFGE) USA Type Strain Reactivity Ten PFGE USA Type strains (representing 20 NARSA strains) were tested for reactivity with the MP Test. These strains included USA100 (382,741), USA200 (383, 71, 722), USA 300 (643, 739),USA 300-0114 (384), USA 400 (123, 192, 193), USA500 (385, 708), USA 600 (648, 715), USA700(386), USA800 (387), USA 1000 (NRS 483) and USA1100 (NRS 484).

All of the major clinical types (100, 300, 500) were represented by multiple isolates, and all yield correct results (MRSA) upon initial testing with the exception of one of three USA 300 strains which tested as false negative initially, but was correctly identified at repeat testing.

Types 700 and 1000 were represented by single isolates and gave false results (NSA) on initial testing. NRS 386 (USA 700) gave correct results on retesting, but NRS 483 (USA 1000) repeated the NSA result on retesting.

Panton-Valentine Leukocidin (PVL) Strain Reactivity
Fourteen NARSA strains (8 positive for PVL (123, 192, 193, 384, 483, 484, 643, 739) and 6 negative for PVL (22, 648, 708, 715, 722, 741) strains were evaluated for this study. The AptaSure™ MRSA detected 6/6 PVL-negative strains and 6/7 (plus one mixed result (NRS384)) PVL-positive strains. The false negative PVL positive strain was NRS 483.

SCCmec Strain Reactivity
Strains with known SCC*mec* types from NARSA were obtained, and all isolates were MRSA by cefoxitin disk diffusion (≤ 21mm) and had a vancomycin MIC of ≤ 2 μg/mL. Seventeen strains representing two SCC

mec types were tested. These included SCC mec II type (71, 192, 382, 383, 648, 715, 722, and 741) and SCC mec IV / Iva type
(123, 193, 384, 385, and 386). Initial testing of the 17 strains (68 runs) demonstrated that the MP Test identified 56 of the runs as MRSA for an initial sensitivity of 85%. The three discordant strains were repeated and 67/68 runs (98.5% strain sensitivity) were correctly identified as MRSA by the AptaSure™ MRSA test.

Borderline Oxacillin Resistant (BORSA) S. aureus Reactivity
Fourteen Borderline Oxacillin Resistant (BORSA) *S. aureus* strains were obtained the Centers for Disease Control, Atlanta GA (CDC, n=6 isolates) and a private collection (n=10 isolates). Both institutions published on these and similar strains with the same definition for BORSA (Swenson et al. Diag. Microbiol- Infect. Dis. 58 (2007) 33-39, Louie et al. - J. Clin. Microbiol. 38 (6) (2000) 2170-2173).

Fourteen isolates were received by Invenio Medical, Inc. with oxacillin sensitivity classifications, which were determined by the source laboratory using Broth Micro-Dilution (BMD) techniques and were known by the source laboratory as *mecA* negative. ß-lactam resistance in these strains was also characterized using 1 µg oxacillin or 30 µg cefoxitin disk diffusion testing (DD) at Invenio Medical, Inc.. Criteria for antibiotic categorization followed CLSI M100-S19 as: ≥22 mm cefoxitin-sensitive; ≥13mm oxacillin-sensitive and 11-12 mm oxacillin-intermediate. As controls, ATCC 43300 (known MRSA) and ATCC 25923 (known MSSA) strains were tested. All strains were *mecA*-negative but had MIC values of 4-8µg/ml by oxacillin broth microdilution testing by OXA-BMD methods at the source laboratories.

Oxacillin disk diffusion testing performed at Invenio Medical, Inc., 3 strains tested R, 8 strains tested I, and 5 strains tested S. In cefoxitin disk diffusion testing, all 16 strains tested S. In the AptaSure™ MRSA test, 1 strain tested R, 2 strains tested R/S (meaning the replicates were equally divided between R and S) and 13 strains tested S. There were no differences in performance between kit lots or blood culture formulations.

Modified intrinsic PBP-bearing Staphylococcus aureus strains (MOD- SA)

The performance of the AptaSure™ MRSA on rare modified intrinsic PBP-bearing *Staphylococcus aureus* strains (MOD-SA) was not evaluated.

SCC mec drop outs
A panel of 28 empty cassette variant *S. aureus* strains was evaluated that harbor elements of the SCC*mec* cassette, but were phenotypically MSSA. These strains were obtained from two laboratories with private collections. These strains were extensively characterized with regard to susceptibility testing and molecular structure, the details of which have been published in peer-reviewed journals (Donnio et al.

JCM (2005) vol. 43 (8) pp. 4191-3). The strains were multiple independent isolates, with various mutations in the SCC*mec* cassette, and included a variety of MLSTs. The phenotype of these strains was confirmed by cefoxitin disk diffusion. The AptaSure™ MRSA called all 28 strains as MSSA.

Interferent Studies

Three different abnormal sera types (hemolytic, icteric and lipemic) for a total of 180 tests were performed (4 serum types x 3 strain types x 15 serum samples) and high concentrations of four different serum components (hemoglobin, bilirubin, triglycerides and Intralipids) were evaluated at for a total of 130 tests using 'high levels' of hemoglobin, bilirubin, triglycerides and Intralipids at 5 mg/mL, 200 µg/mL, 30 mg/mL and 3 mmol/L (834 ug/mL of Intralipid) respectively. It was determined that detector visual calls were not affected by any of four probable interfering agents or the 3 abnormal sera types tested with the three test strains (MRSA, MSSA and NSA). Test accuracy remained 100% for all circumstances.

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A 20-member Heterophilic Assessment Panel, comprised of 18 samples of human plasma samples containing heterophile antibodies, one sample of human anti-mouse antibodies (HAMA) and one purchased sample of Rheumatoid factor (Rf) and spiked into high negative (HN) and low positive (LP) bacteriophage samples. None of the HAMA, Rf, or heterophilic plasma samples used in this study interfered with interpretation of Detectors in the AptaSure™ MRSA when challenged at higher than normal sample volumes. Under normal clinical concentrations, these antibodies are not expected to interfere with interpretation of the Test. There was no effect of the component antibiotics on the functionality of bacteriophage in the AptaSure™ MRSA test.

Potential Drug Interferents and Culture Media Interferents

Five antibiotics: 1) cephalexin, 2) ciprofloxacin, 3) gentamicin, 4) sulfisoxazole and 5) tetracycline, and three analgesics/anti- inflammatories class 1) acetaminophen, 2) acetylsalicylic acid and 3) ibuprofen were tested. Acyclovir (antiviral), SPS (anticoagulant), and antibiotic-absorbing resin from blood culture bottles were also tested. Each test compound was spiked into the assay at the level corresponding to the CLSI recommended "test high dose" in blood. Where CLSI guidance was not available, the compound was tested above the highest likely or possible level. Two lots of the AptaSure™ MRSA test were used in the study. The control assay did not contain any additive. Three control strains were tested in presence of each additive and under the control condition.

Control strains included were methicillin resistant *Staphylococcus aureus* (MRSA), a methicillin sensitive *S. aureus* (MSSA) and two coagulase negative *Staphylococcus epidermidis* (NSA - not-*S. aureus*). A total of 330 tests were performed. Results show detection/call accuracy for MRSA, MSSA and NSA bacteria was 100% and indicated the MRSA/MSSA Blood Culture Test - BT was not adversely affected by any of the potential interfering antibiotics, analgesics/anti-inflammatories, antiviral, SPS or blood culture bottle resin compounds.

f. Assay cut-off:

Not Applicable

2. Comparison studies:

   a. Method comparison with predicate device:

   Not Applicable

   b. Matrix comparison:

   Not Applicable

3. Clinical studies:

   a. Prospective Clinical studies

Subjects included individuals 18 years of age or older with positive blood cultures on a Bactec™ System (9000 series or F/X), and the sample was tested on the AptaSure™ MRSA within 24 hours of positive determination on Bactec™ System (i.e. alarm).

Aliquots of the blood culture were used to perform standard culture identification for *S. aureus.* The AptaSure™ MRSA was compared to: 1) the traditional enzymatic methods of coagulase tube and catalase slide tests and the latex agglutination method of Staphaurex® (Remel) for detection of *Staphylococcus aureus* and 2) the latex agglutination PBP2' Test (Oxoid) and antibiotic susceptibility methods Cefoxitin and Oxacillin Sensi-disc (Becton-Dickinson) for determination of methicillin-resistant (MRSA) or methicillin–susceptible (MSSA) *S. aureus* in accordance with CLSI M100-S19. All clinical validation was performed and interpreted by single users, consistent with the intended use.

A total of 1116 (366 *S. aureus*, of which 191 were MRSA, 173 were MSSA, and 2 not evaluated) paired samples were tested for MRSA/MSSA by the AptaSure™ MRSA and the culture gold standard across all study sites. Sensitivity and specificity of the MRSA/MSSA Blood Culture Test vs. Gold Standard/predicate methods was 91.8% (88.5, 94.4; n=156) and 98.3% (97.1, 99.1; n = 180), respectively.

Invenio Medical, Inc. compared the results of the AptaSure™ MRSA to 30 µg cefoxitin disks and 1 µg oxacillin disks in accordance with CLSI M100-S19 in 336 paired tests. Additional MRSA characterization was performed using the PBP2' latex agglutination test.

The Very Major Errors (vmj) and Major Errors (maj) were calculated as described in the AST guidance. The MRSA detection was acceptable using the cefoxitin disk testing, but not with the oxacillin disk testing. For cefoxitin, the vmj rate 1.11 % with a 95% confidence interval (CI) of 0.1348, 3.956 and the maj rate was 0.6% with a 95% CI of 0.01644, 3.565. The oxacillin vmj rate was 8.76% with a 95% CI of 5.19, 13.66
and maj errors were 0.82% with a 95% CI 0.02058, 4.446.

The category NSA (not *Staphylococcus aureus*) indicates isolates which were not identified as *Staphylococcus aureus* and do not progress further to susceptibility testing. In these studies the percent of reference MRSA positive results which were AptaSure™ MRSA test NSA was 5.76% (11/191) and the percent of reference MSSA positive results which were NSA was 10.98% (19/173) as compared to cefoxitin.

Within *S. aureus* positives determined to be either MRSA or MSSA by AptaSure™ MRSA test, the MRSA call was 98.9% (178/180) accurate and the MSSA determination was 99.4% (153/154) accurate compared to the cefoxitin disk diffusion result. These results are summarized in the following tables:

AptaSure™ MRSA test VS. TUBE COAGULASE/STAPHAUREX

+ 30 UG CEFOXITIN DISK DIFFUSION STANDARD:

MicroPhage	SA/TCT + FOX DD std MRSA	MSSA	NSA
MRSA	178	1	4
MSSA	2	153	9
NSA	11	19	737

N = 1114

- Very Major Errors (False-susceptible) 2/180 = 1.11**%** CI: 0.13% – 4.0%
- Major Errors (False-resistant) 1/154 = 0.6**%** CI: 0.02% – 3.6%

Not *Staphylococcus aureus* (NSA) Categories with Cefoxitin Disk Testing

NSA categories	Calculation	95% Confidence interval
Percent of reference MRSA+ results that are Invenio Medical, Inc. NSA	11/191 = 5.759%	CI: 2.91, 10.07
Percent of reference MSSA+ results that are Invenio Medical, Inc. NSA	19/173 = 10.98%	CI: 6.743, 16.62
Percent of reference SA+ (MRSA+ or MSSA+) results that are Invenio Medical, Inc. NSA	30/364 = 8.242%	CI: 5.63, 11.56
Percent of reference NSA results that are Invenio Medical, Inc. SA+ (MRSA+ or MSSA+)	13/750 = 1.733 %	CI: 0.9261, 2.946

There were two isolates which were identified as MSSA by Invenio Medical, Inc. BT but identified as MRSA by the cefoxitin gold standard. The two strains, 52217 and 54011, were found to be mecA-positive based on PBP2a results, and neither appeared to be borderline resistant based on zone of inhibition results. Sample 54011 was the only sample tested from this patient, while sample 52217 was one of 9 positive blood cultures tested from its respective patient.

The additional 8 samples were all called MRSA by both the MP BT Test and the reference methods. Strain 52217 was not further characterized as it gave correct results on retesting (4/4 trials) and that clinical testing error of 52217 was attributed to either manufacturer defect or operator error. Strain 54011 was determined to be USA100/800, mecA-Type 2, PVL and TSST negative. It was noted that the MP BT Test discrepancy appeared to be due to inadequate bacteriophage amplification due to strain variance as molecular characterization. In this evaluation it was noted that of the 60 strains typed by the reference lab, 12 had the 100/800 PFGE type, and 11 were called correctly. Of 10 strains with mecA type 2, 9 were called correctly.

AptaSureTM MRSA test VS. TUBE COAGULASE/STAPHAUREX + 1 UG OXACILLIN DISK DIFFUSION STANDARD*:

MicroPhage	SA/TCT + OXA DD std MRSA	MSSA	NSA
MRSA	177	1	4
MSSA	17	121	9
NSA	11	14	737

N = 1091

Very Major Errors (False-susceptible) 17/194 = 8.8% CI: 5.2% - 13.7%

Major Errors (False-resistant) 1/122= 0.82% CI: 0.02% - 4.4%

*In the studies performed, the AptaSureTM MRSA test significantly under called MRSA by the oxacillin disk diffusion gold standard as compared to the cefoxitin disk diffusion gold standard. Of the 17 Very Major Errors, 15 disagreed with both the cefoxitin disk diffusion results and the Invenio Medical, Inc. Test results. Of 16 samples that were called MRSA by oxacillin and MSSA by cefoxitin, 15 were from one site. The difference in reading method at this site was thought to account for most or all of the discrepancies between oxacillin and cefoxitin diffusion test results. Further investigation revealed a systematic downshift of 3mm in zone of inhibition diameter.

Not *Staphylococcus aureus* (NSA) Categories with Oxacillin Disk Testing

NSA categories	Calculation	95% Confidence interval
Percent of reference MRSA+ results that are Invenio Medical, Inc. NSA	11/205 = 5.366 %	CI: 2.709, 9.398
Percent of reference MSSA+ results that are Invenio Medical, Inc. NSA	14/136 = 10.29 %	CI: 5.743, 16.67

Percent of reference SA+ (MRSA+ or MSSA+) results that are Invenio Medical, Inc. NSA	25/341 = 7.331 %	CI: 4.80, 10.63
Percent of reference NSA results that are Invenio Medical, Inc. SA+ (MRSA+ or MSSA+)	13/750= 1.733 %	CI: 0.9261, 2.946

One strain each of *Enterococcus faecalis*, *Staphylococcus warneri, Staphylococcus gallinarum, Staphylococcus lugdunensis* and 2 strains of *Staphylococcus epidermidis* cross reacted with the Test. At all sites 72 *Enterococcus* samples and 19 "Gram-positive, non-Staph" samples (comprised of *Enterococcus* and *Streptococcus*) were identified. Of these ~80 *Enterococcus* samples, only one gave a false-positive result. Fatty acid GC analysis and repeat sequencing data of this strain show that the best (not perfect) match to E. faecalis. This isolate was thought to be an atypical strain of *E. faecalis*.

b. *Retrospective Clinical studies*

Not applicable.

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

Not applicable.

a. *Clinical Sensitivity and b. Clinical Specificity*
Four clinical sites were included in the study. Per protocol, each site was instructed to collect samples from positive blood cultures, 0 – 24 hours after detection (i.e. alarm), from the Bactec™ Culture System. Acceptable blood culture bottle types included Bactec™ plus Aerobic and Plus Anaerobic bottles. Only patients >= 18 years of age were included in the study. A Gram stain was performed, bacteria were examined and only samples tested as Gram positive cocci in clusters (GPCC) were carried forward for additional testing.

After Gram stain, samples were tested with a) the AptaSure™ MRSA test per MP2009-B and b) a cohort of Gold Standard and predicate tests including: coagulase tube test, catalase slide test, and Staphaurex® Test. The ID Gold Standard is defined as concordant results between the catalase, coagulase and Staphaurex® tests. In order to preserve blinding of sample information/results different technologists performed the Gold Standard tests and another group of technologists performed the predicate and MRSA/MSSA Blood Culture Tests. For *S. aureus* positive samples, PBP2' Test, Cefoxitin Sensi-disc Test and Oxacillin Sensi-disc Test were performed to determine antibiotic susceptibility. The Cefoxitin Sensi-disc test was defined as the Gold Standard for MRSA/MSSA determination on *S. aureus*-positive samples.

The following chart summarizes all the clinical studies performed.

Summary of Clinical Studies (Lower –Confidence Interval % Sensitivity)	% Sensitivity (95% Confidence Interval)	% Specificity (95% Confidence Interval)
MRSA determination, Cefoxitin, Overall Population	93.2 (88.6, 96.3)	99.5 (98.7, 99.8)
MRSA determination, Oxacillin, Overall Population	86 (80.5, 90.4)	99.4 (98.7, 99.8)
MSSA determination, Cefoxitin, Overall Population	88.4 (82.7, 92.8)	98.7 (97.8, 99.3)
MSSA determination, oxacillin, Overall Population	80 (82.5, 93.7)	97.2 (95.9, 98.1)
MRSA determination, Cefoxitin, by lot	78.6, 81.8, 87.8	99.7, 99.7, 99.0
MSSA determination, Cefoxitin, by lot	88.7, 73.8, 74.6	98.2, 99.3, 98.9

Staph. aureus Detection, Overall Population	88.51-94.40 (91.80)	97.05-99.07 (98.27)
MRSA/MSSA Blood Culture Test Performance by Kit Lot: Detection of *Staph. aureus*	91-99.7 (97.4) 81.9 – 92. 7 (88.1) 87.2-96.8 (93)	98.0 99.6 97.4
Staph. aureus Detection, No antibiotic exposure	87.9 – 94.5 (91.6)	98.1 (96.6 – 99.1)
Staph. aureus Detection, Antibiotic exposure	83.4 – 97.5 (92.5)	98.6 (96-99.7)
Staph. aureus Detection, No antiviral exposure	88.4 -94.3 (91.7)	100 (89.7-100)
Staph. aureus Detection, Antiviral exposure	39.8- 100 (100) too few data points	89.7-100 (100)
Predicate % agreement cat/coag S. aureus population	Pos agreement 88.4-94.4 (91.8)	Neg agreement 94.4 -98.2 (96.7)
Predicate % agreement Staphurex S. aureus population	Pos agreement 87.7 - 93.8 (91.1)	Neg Agreement 95.1-98.5 (97.1)
Predicate % agreement PBP2' S. aureus population	Pos agreement 96.0-100 (98.9)	Neg Agreement 94.5-100 (98.1)
Staph aureus by site (Invenio Medical, Inc. vs ID Gold Standard) – except 1 site >= 90%	85.41 – 98.35 (94) 86.62 – 95.85 (92) 80.06 – 95.28 (89.33) 82.74 – 96.88 (94)	94.83 – 99.11 (97.59) 97.27-99.99 (99.50) 92.44-98.92 (96.69) 96.29-99.98 (99.32)
Staph aureus by bottle type Plus Aerobic Plus Anaerobic	86.77 - 94.20 (93) 86.98 – 97.33 (95)	96.49-99.01 (98.03) 98.07-100 (100)

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

There were no subjects less than 18 years of age, seven subjects were between 18 and 21 years of age. All other subjects, 1109 total, were greater than 21 years of age at the time their blood was drawn**.**

The table below lists the organism identifications reported by site microbiology. 2 sites employed a Microscan, 1 used a Vitek 2, and 1 used a variety of manual culture methods.

Organism 1	**Organism 2**	**N**
Not reported	None	3
Acinetobacter sp.	None	14
Acinetobacter sp.	CoNS	2
Anaerobe	None	2
B. cepacia	None	4
Bacillus sp.	None	8
Bacillus sp.	CoNS	1
Bacteroides sp.	None	2
Candida sp.	None	22
Candida sp.	CoNS	1
Candida sp.	Corynebacterium sp.	1
Capnocytophaga sp.	None	1
Clostridium sp.	None	3
CoNS	None	222
CoNS	CoNS	5
CoNS	Corynebacterium sp.	1
CoNS	E. coli	2
CoNS	GPNS	1
CoNS	S. epidermidis	4
Corynebacterium sp.	None	7
Corynebacterium sp.	CoNS	1
E. aerogenes	None	5
E. cloacae	None	17
E. cloacae	CoNS	1
E. cloacae	E. coli	1
E. cloacae	E. faecalis	3
E. coli	None	57
E. coli	E. cloacae	1
E. coli	S. epidermidis	2
E. faecalis	None	37
E. faecalis	GN	2
E. faecalis	S. haemolyticus	1
E. faecium	None	30
Enterococcus sp	None	2
Gemella sp.	None	1

GN	None	9
GPNS	None	18
GPNS	Corynebacterium sp.	1
K. pneumoniae	None	45
K. pneumoniae	E. cloacae	2
K. pneumoniae	S. aureus	3
Micrococcus sp.	None	4
P. aeruginosa	None	15
P. aeruginosa	E. faecalis	1
P. aeruginosa	S. aureus	1
P. aeruginosa	Viridans Strep	1
P. mirabilis	None	7
P. mirabilis	E. faecalis	1
S. agalactiae	None	15
S. agalactiae	CoNS	1
S. aureus	None	365
S. aureus	Acinetobacter sp.	1
S. aureus	CoNS	1
S. aureus	E. cloacae	1
S. capitis	None	6
S. epidermidis	None	67
S. epidermidis	Corynebacterium sp.	1
S. haemolyticus	None	7
S. hominis	None	13
S. lugdunensis	None	2
S. maltophilia	None	4
S. marcescens	None	10
S. marcescens	Citrobacter sp.	6
S. marcescens	E. faecalis	2
S. mutans	None	3

S. pneumoniae	None	17
S. warneri	None	1
Viridans Strep	None	16
Viridans Strep	GN	1
Y	None	1
Total		1116

Abbreviations: CoNS, coagulase-negative *Staphylococci*; GPNS, Gram-positive, not *Staphylococcus*; GN, Gram-negative; Y, yeast.

4. Clinical cut-off**:**

Not Applicable

5. Expected values/Reference range:

In the AptaSure™ MRSA clinical study, a total 1114 clinical specimens were tested and determined to be MRSA, MSSA or not-*S. aureus* by reference culture methods. The number and percentage of MRSA and MSSA positive cases by age group are shown in the table below. For comparison, 16.5% of Gram-positive blood cultures in the US in 2002 were determined to be *S. aureus* (Ann Clin Microbiology Antimicrobial (2004) vol. 3 pp. 7). Of these, 8.1% were methicillin-resistant (MRSA) and 8.4% were methicillin-susceptible (MSSA). The frequency of MRSA and MSSA isolated at a given location should be confirmed by the user.

Age Group	**Total specimens**	**n MRSA**	**% MRSA**	**n MSSA**	**% MSSA**
0-17	0	0	0	0	0%
18-20	8*	5	62.5%	0	0%
21-30	58	23	39.7%	0	0%
31-40	111	11	9.9%	28	25.2%
41-50	148*	31	20.9%	19	12.8%
51-60	218	30	13.8%	41	18.8%
61-70	251	45	17.9%	35	13.9%
>70	322	48	14.9%	50	15.5%
Total	**1116**	**193**	**17.3%**	**173**	**15.5%**

* One sample in this group was identified as S. aureus by the gold standard ID method, but did not get a gold standard disk diffusion test. Sample was PBP2' positive and thus is classified as MRSA.

Reference Range

Positive for *Staphylococcus aureus*
Negative for *Staphylococcus aureus*
Methicillin-resistant *Staphylococcus aureus* (MRSA)
Methicillin-susceptible *Staphylococcus aureus* (MSSA)

I. Conclusion:

The submitted equivalency information in this report supports a substantial equivalence.

Based on additional equivalency tests performed, Invenio Medical, Inc. plans on submission of the latest data in efforts to satisfy requirements of sensitivity and specificity for approval to proceed with SOP transmission to manufacturers for updated bidding/timeline proposals.