

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number:

k080837

B. Purpose for Submission:

New 510k

C. Measurand:

Target DNA sequences for the *staphylococcal* protein A (*spa*), for methicillin/oxacillin resistance (*mecA*), and for the *staphylococcal* chromosomal cassette (*SCCmec*) insertion event into the *staphylococcus aureus* chromosomal *attB* site.

D. Type of Test:

Nucleic Acid Amplification Test, DNA, Methicillin-resistant *Staphylococcus aureus* (MRSA) / *Staphylococcus aureus* (SA), qualitative

E. Applicant:

Cepheid

F. Proprietary and Established Names:

Xpert™ MRSA/SA SSTI Assay

G. Regulatory Information:

1. Regulation section:
21 CFR 866. 1640 Antimicrobial susceptibility test powder
2. Classification:
Class II
3. Product code:
NQX
4. Panel:
83 Microbiology

H. Intended Use:

1. Intended use(s):

The Cepheid Xpert MRSA/SA Skin and Soft Tissues Infection Assay (Xpert MRSA/SA SSTI Assay) performed in the GeneXpert® Dx System is a qualitative in vitro diagnostic test intended for the detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) from skin and soft tissue infection swabs. The test utilizes automated real-time polymerase chain reaction (PCR) to detect MRSA/SA DNA.

2. Indication(s) for use:

The Xpert MRSA/SA SSTI Assay is indicated for use in conjunction with other laboratory tests such as microbiology culture, and clinical data available to the clinician as an aid in the detection of MRSA/SA from skin and soft tissue infections. The Xpert MRSA/SA SSTI Assay is not intended to monitor treatment for MRSA/SA infections. Concomitant cultures for SA and MRSA are necessary to recover organisms for susceptibility testing or epidemiological typing.

In a mixed culture containing MRSA/SA and other organisms (e.g. Gram negative bacilli, yeast), results can be false negative or variable depending on the concentration of MRSA/SA present, particularly if the concentration of MRSA/SA is close to the LoD of the assay.

3. Special conditions for use statement(s):

Prescription Use

4. Special instrument requirements:

To be used with the GeneXpert® Dx System (GX-4 or GX-16 instruments, and the GeneXpert® Dx System Software 1.6b)

I. Device Description:

The Xpert MRSA/SA SSTI Assay system performs real-time, multiplex polymerase chain reaction (PCR) for detection of DNA after an initial sample processing step. In this platform, additional sample preparation, amplification, and real-time detection are all fully-automated and completely integrated. The assay is performed on a GeneXpert® Dx System, which consists of the GeneXpert instrument, personal computer, hand-held barcode scanner, and disposable fluidic cartridges that are designed to complete sample preparation and real-time PCR for detection of MRSA and SA in about 50 minutes. Each instrument contains 1 to 16 randomly accessible modules that are each capable of performing separate sample preparation and real-time PCR tests. Each module contains a syringe drive for dispensing fluids, an ultrasonic horn for lysing cells or spores, and ICORE® thermocycler for performing real-time PCR and detection.

The patented single-use cartridges contain: (1) eleven chambers for holding sample, reagents, or other materials, (2) a valve body composed of a plunger and syringe barrel, (3) a rotary valve system for controlling the movement of fluids between chambers, (4) an area for capturing, concentrating, washing, and lysing spores/cells, (5) dry real-time PCR reagents, and (6) an integrated PCR reaction tube that can be automatically filled by the instrument. To eliminate test-to-test contamination, all fluids including amplicons, are contained within the disposable

cartridge. The instrument never comes into contact with any fluids within the cartridge. Each disposable cartridge is intended to test one sample. Cartridges are not re-usable.

The Xpert MRSA/SA SSTI Assay includes reagents for the detection *Staphylococcus aureus*, and also detection of the gene insertion that causes methicillin resistance. The test includes a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR assay to avoid false negative results. The SPC also ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

The primers and probes in the Xpert MRSA/SA SSTI Assay detect nucleic acid sequences of the *staphylococcal* protein A (*spa*), the gene for methicillin/oxacillin resistance (*mecA*), and *staphylococcal* cassette chromosome (SCC*mec*) inserted into the SA chromosomal *attB* site. Swab specimens from skin and soft tissue infections are collected and transported to the GeneXpert® area. The specimen swab is placed in a tube containing elution reagent. Following a brief vortexing, the eluted material and two other reagents are transferred to different chambers of the cartridge. The GeneXpert® performs sample preparation by mixing the eluted sample with the sample preparation control (*Bacillus globigii* in the form of a dry spore cake within the cartridge) and treatment reagents, capturing the bacterial cells on a filter, lysing the cells using glass beads and an ultrasonic horn, then eluting the released DNA. The DNA solution is then mixed with dry PCR reagents and transferred into the PCR tube for real-time PCR and detection.

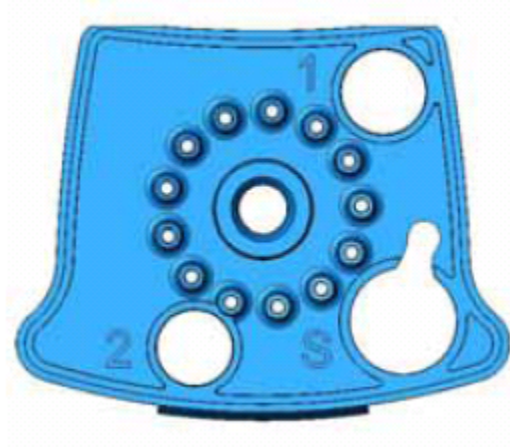
Note: *Bacillus globigii* spores are used as the sample preparation control because they are more difficult to lyse than *Staphylococcus aureus*.

Xpert MRSA/SA SSTI Assay



Xpert MRSA/SA SSTI Assay self-contained Cartridge

1 = Reagent 1, 2 = Reagent 2, S = Sample



GeneXpert Dx System



J. Substantial Equivalence Information:

1. Predicate device name(s):
Cepheid Xpert™ MRSA Assay
BD GeneOhm StaphSR Assay
2. Predicate 510(k) number(s):
K070462

K071026

3. Comparison with predicate:

Similarities			
	Device	Predicate	
Item	Xpert MRSA/SA SSTI Assay	Xpert MRSA Assay (K070462)	BD GeneOhm™ StaphSR Assay (K071026)
Intended Use	Rapid detection of MRSA and SA	MRSA only	Same
Indication for Use	Identification of MRSA and SA	MRSA only	Same
Technological Principles	Fully-automated nucleic acid amplification (DNA); real-time PCR	Same	Same
Test Cartridge	Disposable single-use, multichambered, fluidic cartridge.	Same	Disposable single-use PCR tube
Instrument System	Cepheid GeneXpert Dx System	Same	Cepheid SmartCycler
Fluidics	Self-contained and automated after swab elution and two single-dose reagent additions.	Same	Manual
Probes	TaqMan® Probes	Same	Molecular Beacons
Internal Controls	Sample processing control (SPC) and probe check control (PCC).	Same	One internal reagent control and external positive and negative controls required per run
DNA Target Sequence	Sequence incorporating the insertion site (<i>attB_{ssc}</i>) of <i>Staphylococcal</i> Cassette Chromosome <i>mec</i> (SCC <i>mec</i>) for detection of MRSA.	Same	Same
	Sequence specific to methicillin/oxacillin resistance (<i>mecA</i> gene)	N/A	N/A
Rapid test results	Approximately 50 minutes to results.	Approximately 75 minutes to results.	Approximately 60-75 minutes.

Differences			
	Device	Predicate	
Item	Xpert MRSA/SA SSTI Assay	Xpert MRSA Assay (K070462)	BD GeneOhm™ StaphSR Assay (K071026)
Intended Use	Simultaneous rapid detection of SA and MRSA.	Only detects MRSA.	Same
Specimen Type	Direct from skin and soft tissue infection swabs.	Direct from nasal swabs.	Direct from Positive Blood Cultures
DNA Target	Sequence specific to	N/A	Sequence specific to

Item	Xpert MRSA/SA SSTI Assay	Xpert MRSA Assay (K070462)	BD GeneOhm™ StaphSR Assay (K071026)
Sequence	<i>Staphylococcus aureus</i> species (<i>spa</i> gene)		<i>Staphylococcus aureus</i> species (<i>nuc</i> gene)

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

Not applicable

L. Test Principle:

The primers and probes in the Xpert MRSA/SA SSTI Assay detect the presence of proprietary sequences for the *staphylococcal* protein A (Spa), the gene for methicillin/oxacillin resistance (*mecA*), and the *staphylococcal* cassette chromosome (SCC*mec*) inserted into the SA chromosomal *attB* site. Swabs from skin and soft tissue infections are collected and transported to the GeneXpert® System area. The swab is placed in a tube containing 2.0 mL elution reagent. Following a brief vortex, the eluted material and two other liquid reagents are transferred to different chambers of the cartridge.

The user initiates a test from the system user interface, the instrument signals the user where to place the cartridge by flashing a green light, and the cartridge is placed into the indicated module in the GeneXpert Dx System instrument. The instrument moves the sample and reagents to and from different chambers within the Xpert MRSA/SA SSTI Assay cartridge. The GeneXpert® Dx System performs sample preparation by mixing the eluted sample with the sample preparation control (*Bacillus globigii* in the form of a dry spore cake within the cartridge) and treatment reagents, capturing the bacterial cells on a filter, lysing the cells using glass beads and an ultrasonic horn, then eluting the released DNA. The DNA solution is then mixed with dry PCR reagents and transferred into the PCR tube for real-time PCR and detection. The Xpert MRSA/SA SSTI Assay completes sample preparation and real-time PCR in approximately 50 minutes. Internal controls in Xpert MRSA/SA SSTI Assay check key automated steps.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility study was performed using a panel of 10 specimens with varying concentrations of SA, MRSA and *Staphylococcus epidermidis* (negative) were tested in duplicate on 10 different days at each of the three sites (10 specimens x 2 times/ day x 10 days x 3 sites). One lot of Xpert MRSA/SA kit was used at each of the 3 testing sites. Xpert MRSA/SA assays were performed according to the Xpert MRSA/SA procedure.

Summary of Reproducibility Results

Specimen ID	Site 1	Site 2	Site 3	Total Agreement
Neg (MSSE)	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
SA High Neg	100% (20/20)	100% (20/20)	90% (18/20)	96.7% (58/60)
SA Low Pos	100% (20/20)	100% (20/20)	95% (19/20)	98.3% (59/60)
MRSA1 High Neg	100% (20/20)	90% (18/20)	100% (20/20)	96.6% (58/60)
MRSA1 Low Pos	100% (20/20)	100% (20/20)	90% (18/20)	96.6% (58/60)
MRSA2 High Neg	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
MRSA2 Low Pos	100% (20/20)	95% (19/20)	95% (19/20)	96.6% (58/60)
% Total Agreement by Site	100% (140/140)	97.9% (137/140)	95.7% (134/140)	97.9% (411/420)

Summary of Ct Value Results by Sample Level and Probe

SPC			
Level	Mean	Std Dev	%CV
MRSA1 High Neg	34.52	0.82	2.36
MRSA2 High Neg	34.46	0.85	2.46
Neg (MSSE)	34.44	0.90	2.62
SA High Neg	34.38	0.92	2.66

<i>Spa</i>			
Level	Mean	Std Dev	%CV
MRSA1 Low Pos	32.96	0.8	2.44
MRSA2 Low Pos	31.05	0.69	2.21
SA Low Pos	33.91	0.8	2.35

<i>mecA</i>			
Level	Mean	Std Dev	%CV
MRSA1 Low Pos	33.25	0.80	2.40
MRSA2 Low Pos	31.50	0.68	2.16

SCC <i>mec</i>			
Level	Mean	Std Dev	%CV
MRSA1 Low Pos	34.19	0.90	2.63
MRSA2 Low Pos	33.13	0.68	2.05

A second reproducibility study was performed using a panel of four specimens of (SA: 10X LoD, MRSA1: 10X LoD, MRSA2: 10X LoD, and Negative Control: *Staphylococcus epidermidis*). The panels were tested in duplicate on 10 different days at each of the three sites (4 specimens x 2 times/ day x 10 days x 3 sites). One lot of Xpert MRSA/SA SSTI Assay was used at each of the 3 testing sites. The results of the second reproducibility study are demonstrated in the table below.

Summary of Second Reproducibility Results

Specimen ID	Site 1	Site 2	Site 3	Total Agreement
Neg (MSSE)	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
SA Moderate Pos ¹	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
MRSA1 Moderate Pos ¹	100% (20/20)	100% (20/20)	100% (20/20)	100% (60/60)
MRSA2 Moderate Pos ¹	100% (20/20)	100% (20/20)	95% (19/20)	98.3% (59/60)
% Total Agreement by Site	100% (80/80)	100% (80/80)	98.8% (79/80)	99.6% (239/240)

¹10X LoD

Summary of Ct Value Results by Sample Level and Probe

SPC			
Level	Mean	Std Dev	%CV
MRSA1 Moderate Pos	35.72	1.87	5.24
MRSA2 Moderate Pos	36.29	2.66	7.34
SA Moderate Pos	34.55	1.19	3.44
NEG	34.45	1.06	3.09

Spa			
Level	Mean	Std Dev	%CV
MRSA1 Moderate Pos	29.52	1.30	4.40
MRSA2 Moderate Pos	28.91	1.03	3.57
SA Moderate Pos	30.59	0.91	2.99

mecA			
Level	Mean	Std Dev	%CV
MRSA1 Moderate Pos	29.78	1.28	4.29
MRSA2 Moderate Pos	29.32	1.24	4.22

SCCmec			
Level	Mean	Std Dev	%CV
MRSA1 Moderate Pos	31.49	1.26	3.99
MRSA2 Moderate Pos	31.05	1.12	3.59

b. Linearity/assay reportable range:

Not applicable

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

The Xpert MRSA/SA SSTI Assay includes a system control, referred to as the Probe Check Control (PCC), and an internal control, referred to as the Sample Processing Control (SPC). These internal controls are contained in the Xpert MRSA/SA SSTI Assay cartridge. An additional control is the System Control Check for Temperature. This check ensures that the GeneXpert Dx Instrument is operating within validated heating and cooling specifications.

Recommended external quality control organisms were tested at the sites with acceptable results. External Controls may be used in accordance with accrediting institutions and government regulations. External Controls are not provided in the test kit; however they are available for purchase from an outside source (Microbiologics). The outside source and the catalog numbers are provided to the customer in the 'Materials Available but Not Provided' section of the Xpert MRSA/SA SSTI Assay Package Insert.

d. Detection limit:

Limit of Detection Study

The Limit of Detection study was determined using *Staphylococcus aureus* (SA) cells and methicillin-resistant *Staphylococcus aureus* (MRSA) cells diluted into a surrogate wound matrix of human origin. The surrogate wound matrix consisted of a white blood cell (WBC) concentrate prepared from whole blood by centrifugation. The matrix also contained red blood cells (RBC) and plasma, and a negligible amount of anticoagulant (CPD or CPDA-1).

For MRSA, replicates of 20 were evaluated at each MRSA concentration tested (CFU/swab) for 6 individual isolates representing SCCmec types I, II, III, IVa, V, and VI. Well characterized MRSA isolates by pulsed-field gel electrophoresis (PFGE) such as the USA100 and USA400 were represented.

For SA, replicates of 20 were evaluated at each SA concentration (CFU/swab) for 3 individual SA isolates. USA types USA900 and USA1200 were represented. The estimate and confidence intervals were determined using logistic regression with data (number of positive results per number of replicates at each level) over the range of CFU/swab tested. The confidence intervals were determined using maximum likelihood estimates on the logistic model parameters using the large sample variance-covariance matrix.

Effect of Competing Amounts of SA on the Limit of Detection of MRSA

The competitive inhibitory effect of increasing amounts of SA relative to MRSA at LoD was evaluated for each SCCmec type I, II, III, IVa, V, and VI. This analytical study was conducted to

test MRSA specimens at the claimed Xpert MRSA/SA SSTI LoD concentration for each SCCmec type in the presence of SA at ten-fold increasing concentrations (i.e. MRSA to SA ratios of 1:1, 1:10, 1:1e2, 1:1e3, 1:1e4, 1:1e5, and 1:1e6). Cells used in this study were diluted into the same background surrogate wound matrix of human origin. Replicates of 20 each were evaluated over a span of five days at the LoD established for 6 individual MRSA isolates representing SCCmec types I, II, III, IVa, V, and VI in the presence of increasing concentrations of SA (ATCC strain 29213) each for the various MRSA/SA combinations should be tested for a span of three to four days.

At a MRSA:SA ratio of 1:1x10⁶, greater than 50% of MRSA samples were detected at the LoD for SCCmec types II, III, IVa, V, and VI. Approximately 25% of MRSA samples were detected at the LoD for SCCmec type I. Under these conditions (MRSA:SA ratio of 1:1 x10⁶), it can be concluded that the effect of competing SA is inhibitory at the claimed LoD concentrations for all 6 SCCmec types tested.

Analytical Inclusivity

Analytical Inclusivity Study on CDC Staphylococcus aureus Specimens

Twenty five *Staphylococcus aureus* strains from multiple sources provided by the CDC were tested using the XpertTM MRSA/SA SSTI Assay. All strains were tested in triplicate using 100 ul of stationary phase cell suspension diluted 10 million-fold. Colony forming units per assay (CFU/test) were determined by plate counts in triplicate. Bacterial strain identification, PFGE type and SCCmec type were determined by the CDC.

All results were reported correctly by the XpertTM MRSA/SA SSTI Assay, except one specimen. Further investigation revealed that the particular specimen was actually mislabeled by the CDC.

Analytical Inclusivity Study on Expanded Panel of Staphylococcus aureus Specimens

One hundred twenty one additional *Staphylococcus aureus* strains were tested using the Xpert MRSA/SA Assay. Overnight cultures were grown in Brain Heart Infusion (BHI) media and adjusted to 0.5 McFarland units. All strains were tested in triplicate using 100 µL of cultures further diluted 100 thousand to one million-fold. MRSA (78) and SA (43) strains were selected to broadly represent the range of genetic diversity found in the species *Staphylococcus aureus* based on phylogenetic structure. Selections represent primary lineages with emphasis on specific clonal complexes within which MRSA is predominantly observed. Lineages that contain MRSA and SA, as well as those that contain SA exclusively were included.

The Xpert MRSA/SA Assay correctly identified 116 of 121 strains. The 5 discordants were characterized by catalase, tube coagulase, and Gram stain. *MecA*- Mediated Oxacillin resistance was assessed by disk diffusion using a 30 µg cefoxitin disk and a diameter cut-off of 21/22 mm.

Three of 78 MRSA strains were reported MRSA negative; SA positive using the Xpert assay. Further characterization indicates these strains are not resistant and were correctly reported MRSA negative; SA positive.

Two of 43 SA strains were reported MRSA positive; SA positive using the Xpert assay. Further characterization indicates these strains are resistant and were correctly reported MRSA positive; SA positive. Each of the 12 known USA300 isolates were correctly reported MRSA positive and SA positive as expected.

Evaluation of Empty Cassette Variants:

In another study, 22 *Staphylococcus aureus* isolates identified as “empty cassette variants” received from a hospital were tested using the Xpert MRSA/SA Assay. Overnight cultures were adjusted to 0.5 McFarland units. All strains were tested from cultures further diluted 100-fold (high) and 100 thousand-fold (low).

The Xpert MRSA/SA Assay correctly identified all 22 isolates as MRSA negative and SA positive. At both cell concentrations tested, only Cts for the *spa* and *SCCmec* targets were reported. No *mecA* Cts were reported.

e. Analytical specificity:

Cross Reactivity

One hundred five strains were tested using the Xpert MRSA/SA Assay. There were 98 strains from the American Type Culture Collection (ATCC) and 7 strains from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) representing species phylogenetically related to *Staphylococcus aureus* or those potentially encountered in a hospital environment.

Of these, 29 methicillin-sensitive coagulase negative staphylococci and 9 methicillin-resistant coagulase negative staphylococci were included. The organisms tested were identified as either Gram positive (74), Gram negative (28), or yeast (3). The organisms were further classified as either aerobic (95) or anaerobic (10).

Evaluation of BORSA Strains:

All 7 BORSA isolates (including an “empty cassette” isolate) were reported MRSA negative; SA positive at both high and low cell concentrations using the Xpert MRSA/SA Assay. No *mecA* signals were reported. These results demonstrate that a BORSA strain will be correctly identified as MRSA negative; SA positive and will not report a false positive MRSA test result using the Xpert MRSA/SA Assay.

Interference Study

In a non-clinical study, potential interfering substances in skin and soft tissue infections were tested with the following results. Inhibition of the MRSA/SA assay has been observed with StaphA + Septic (5% w/v), Hydrocortisone (5% w/v), and antibacterial hand sanitizer (5% w/v).

Samples containing Mercurochrome may not be used due to its fluorescent nature.

Potential Interfering SSTI Substances Tested

Substance	Active Ingredient	% Tested
TET Buffer (control)	Control	Control
Buffy Coat (wound stimulant)	WBC (1.5e9/mL)	50% (v/v)
Whole Blood (MRSA/SA free)	N/A	50% (v/v)
Plasma		50% (v/v)
Neosporin	400 units Bacitracin 5,000 units Polymyxin B 3.5 mg Neomycin	1% and 5% (w/v)
StaphA ⁺ Septic	0.2% Benzethonium Chloride, 2.5% Lidocaine HCl	1% and 5% (w/v)
Hyrdocortisone	1% Hyrdocortisone	1% and 5% (w/v)
Biol-Ease	20% Benzocaine	1% and 5% (w/v)
Iodine Tincture	2% Iodine	50% (v/v)

Potential Interfering SSTI Substances Tested

Substance	Active Ingredient	% Tested
TET Buffer (control)	Control	Control
Mupirocin	0.2% Benzethonium Chloride 2.5% Lidocaine HCl	5% (w/v)
Saline	0.65% Sodium Chloride	50% (v/v)
Antibacterial hand sanitizer	62% Ethyl alcohol	1% (w/v)
Antibacterial hand sanitizer	62% Ethyl alcohol	5% (w/v)
70% Isopropyl alcohol	70% Isopropyl alcohol	50% (v/v)

Carry-Over Contamination study consisted of a negative sample (no MRSA cells spiked onto swabs) processed in the same Gene Xpert module immediately following a very high positive sample (1×10^7 MRSA cells spiked into the elution buffer). This was repeated 20 times between 2 Gene Xpert modules for a total of 42 runs. The study demonstrated no evidence of carry-over contamination.

f. Assay cut-off:

Lot Specific Parameters and Assay Settings

Lot specific assay settings are generated for every lot manufactured to account for slight variations in reagent production. The lot specific assay settings (LSP file) (Normalization Factor and Probe Check Limits) are incorporated into the 2-D barcode on each cartridge label and are transferred to the GeneXpert Dx system via a hand-held barcode scanner prior to initiating the XpertTM MRSA/SA SSTI Assay.

General Assay Settings

These parameters are general assay settings that are used for all reagent lots. They are fixed and not part of the LSP process. The following table lists general assay settings:

Attribute	Setting
Background Subtraction	Always ON
Background Minimum Cycle	Default setting = 5
Background Maximum Cycle	Manual setting = 30
Manual Threshold (all targets and SPC)	Manual setting = 20
Curve Analysis	Primary
Boxcar Average Cycles	Zero (Off)
Valid Minimum Ct (all targets and SPC)	Default setting = 3
Valid Maximum Ct (SPC)	Manual setting = 45
Valid Maximum Ct (<i>spa</i> , <i>mecA</i> , <i>SCCmec</i>)	Manual setting = 36
Maximum Pressure (Max PSI)	Default setting = 120

The valid cycle range for the three MRSA targets (*spa*, *mecA*, and *SCCmec*) was set to 3 to 36 cycles based upon pre-clinical SSTI data (n=114) collected during development and verified using clinical specimens. These cut-offs were subsequently validated in the pivotal clinical study.

The valid cycle range (3 to 45 cycles) for the SPC were derived from analytical data (MRSA/SA LSP testing of 3 development reagent lots), simulated inhibitory studies with potentially interfering substances (IS) including blood, wound pus surrogate of human origin, and common over the counter topical ointments and tinctures, and pre-clinical negative SSTI data (n=70) collected during the development phase and subsequently validated in the pivotal clinical study. During clinical testing, 1.8% (16/886) of eligible subjects yield initial INVALID test results. Upon repeat testing, 11 of the 16 were TN, 1 was SA TP, 1 was SA FN, and 3 were invalid a second time.

The maximum pressure setting of 120 psi ensures the integrity of the cartridge and main valve body filter, preventing the potential for fluidic leaks either internal or external to the cartridge. If the Max PSI setting is exceeded during any fluidic movements within the cartridge, the GeneXpert run is aborted. During clinical testing, four tests (out of a total of 886 eligible samples) exceeded the maximum pressure limit. Valid test results were reported upon repeat testing for 2 of the 4 pressure aborted runs. One was invalid (failed SPC) and one was a probe check error upon repeat testing.

2. Comparison studies:

a. *Method comparison with predicate device:*

Performance characteristics of the Xpert MRSA/SA SSTI Assay were determined in a multi-site prospective investigation study at four US institutions by comparing the Xpert MRSA/SA SSTI Assay with reference culture. Subjects included individuals whose routine care called for collection of a swab from the patient's skin and soft tissue infection for culture.

Double swabs were collected from each subject. One swab was tested by the Xpert MRSA/SA SSTI Assay at the enrolling center and the other swab was tested by the site's standard method, and the remaining specimen was sent to the central laboratory for reference culture testing.

At the centralized laboratory, the specimen was enriched overnight in trypticase soy broth with 6.5% NaCl. The trypticase soy broth was then streaked onto plates with cefoxitin (for MRSA) and without cefoxitin (for SA). If the cultures for both the SA and MRSA plates were determined to be negative, the overall sample result was interpreted as "Negative". If either or both the SA or MRSA plates showed *S. aureus* presumptive colonies, the colonies were sub-cultured onto a blood agar plate. Confirmation of presumptive positive colonies was performed with catalase, tube coagulase, and Gram stain. *MecA*-Mediated Oxacillin resistance was tested by disk diffusion test using a 30 µg cefoxitin disk and cutoff of 21/22 mm.

The clinical testing consisted of 848 specimens which were tested for MRSA and SA by Xpert MRSA/SA SSTI Assay and culture.

Overall Results

A total of 848 specimens were tested for MRSA and SA by Xpert MRSA/SA SSTI Assay and culture.

Among the 848 cases in the eligible dataset, antibiotic use within the 3 weeks prior to sample collection was reported for 207 subjects, and no antibiotic use was confirmed for 441 subjects; for 200 cases, antibiotic status was unknown. There was a significant decrease in the positivity rate of SA with respect to culture results when antibiotics were used. This phenomenon has also been reported in the literature. The MRSA positivity rate for culture was also decreased. The decrease in positivity was not observed with the Xpert MRSA/SA Assay when antibiotics were used nor was any inhibition observed in the assay in the presence of topical antibiotics (see Interfering Substances). The decreased culture positivity rates for MRSA and SA in the presence of antibiotics caused the higher than expected false positive rates observed with the Xpert MRSA/SA SSTI Assay.

Five (5) of the 246 MRSA positive cultures had mixed infections of MRSA and SA. Xpert MRSA/SA SSTI identified 3 of the 5 mixed infections as MRSA positive and 2 of the five as SA positive/MRSA negative.

The performance of the Xpert MRSA/SA SSTI Assay is summarized in the tables below.

MRSA/SA Performance in Subjects with No Antibiotic Use (within 3 weeks of sample collection) vs. Reference Culture

		Culture		
		MRSA+	SA+/MRSA-	Neg/No Growth
Xpert	MRSA+	137 ^a	2	6
	Total	145		

SA+/MRSA-	3 ^b	79	16	98
SA-	6	4	188	198
Total	146	85	210	441

^a1 of the 137 were mixed infections of MRSA and SA.

^b2 of the 3 were mixed infections of MRSA and SA.

Positive Percent Agreement (MRSA+) = 93.8%; 95%Confidence Interval = 88.6-97.1

Negative Percent Agreement (MRSA+) = 97.3%; 95%Confidence Interval = 94.7-98.8

Positive Percent Agreement (SA+/MRSA+) = 95.7%; 95%Confidence Interval = 92.2-97.9

Negative Percent Agreement (SA+/MRSA+) = 89.5%; 95%Confidence Interval = 84.6-93.3

Among subjects with no antibiotic use within the 3 weeks prior to sample collection, the Xpert MRSA/SA SSTI Assay identified 93.8% of the specimens positive for MRSA and 97.3% of the specimens negative for MRSA relative to the reference culture method, and 95.7% of the specimens positive for SA and 89.5% of the specimens negative for SA relative to the reference culture method.

Among these subjects with no antibiotic use, 96.8% (427/441) were successful on the first attempt with the Xpert MRSA/SA Assay. The remaining 14 gave indeterminate results on the first attempt (6 “INVALID”, 7 “ERROR” and 1 “NO RESULT”). Of the 14 indeterminate on the first attempt, all gave a result on the second attempt.

MRSA/SA Performance in Subjects with Unknown Antibiotic Use (within 3 weeks of sample collection) vs. Reference Culture

		Culture			
		MRSA+	SA+/MRSA-	Neg/No Growth	Total
Xpert	MRSA+	47 ^c	0	4	51
	SA+/MRSA-	2	45	8	55
	SA-	1	2	91	94
	Total	50	47	103	200

^c2 of the 47 were mixed infections of MRSA and SA.

Positive Percent Agreement (MRSA+) = 94.0%; 95%Confidence Interval = 83.5-98.7

Negative Percent Agreement (MRSA+) = 97.3%; 95%Confidence Interval = 93.3-99.3

Positive Percent Agreement (SA+/MRSA+) = 96.9%; 95%Confidence Interval = 91.2-99.4

Negative Percent Agreement (SA+/MRSA+) = 88.3%; 95%Confidence Interval = 80.5-93.8

When it was unknown if subjects took antibiotics within the 3 weeks prior to sample collection, the Xpert MRSA/SA SSTI Assay identified 94.0% of the specimens positive for MRSA and 97.3% of the specimens negative for MRSA relative to the reference culture method, and 96.9% of the specimens positive for SA and 88.3% of the specimens negative for SA relative to the reference culture method.

Among these subjects with unknown antibiotic use, 97.0% (194/200) were successful on the first attempt with the Xpert MRSA/SA Assay. The remaining 6 gave indeterminate results on the

first attempt (2 “INVALID”, 3 “ERROR” and 1 “NO RESULT”). Of the 6 indeterminate on the first attempt, all gave a result on the second attempt.

MRSA/SA Performance in Subjects with Known Antibiotic Use (within 3 weeks of sample collection) vs. Reference Culture

		Culture			
Xpert		MRSA+	SA+/MRSA-	Neg/No Growth	Total
	MRSA+	44	2	10	56
	SA+/MRSA-	3	31	19	53
	SA-	3	1	94	98
	Total	50	34	123	207

Positive Percent Agreement (MRSA+) = 88.0%; 95%Confidence Interval = 75.7-95.5

Negative Percent Agreement (MRSA+) = 92.4%; 95%Confidence Interval = 87.0-96.0

Positive Percent Agreement (SA+/MRSA+) = 95.2%; 95%Confidence Interval = 88.3-98.7

Negative Percent Agreement (SA+/MRSA+) = 76.4%; 95%Confidence Interval = 67.9-83.6

Among subjects with known antibiotic use within the 3 weeks prior to sample collection, the Xpert MRSA/SA SSTI Assay identified 88.0% of the specimens positive for MRSA and 92.4% of the specimens negative for MRSA relative to the reference culture method, and 95.2% of the specimens positive for SA and 76.4% of the specimens negative for SA relative to the reference culture method.

Among these subjects with antibiotic use, 96.1% (199/207) of these eligible specimens were successful on the first attempt with the Xpert MRSA/SA Assay. The remaining 8 gave indeterminate results on the first attempt (5 “INVALID” and 3 “ERROR”). Of the 8 indeterminate on the first attempt, all gave a result on the second attempt.

Empty Cassette Variants

A total of 16 isolates that fit the empty cassette profile resulting in positive *spa* and *SCCmec* test results, but no *mecA* detection (Ct = 0) were tested on the Xpert MRSA/SA Assay. Fifteen of the 16 were verified MRSA true negative isolates relative to culture, and 14 of 16 were verified true positive SA isolates relative to culture. One isolate was identified as MRSA by culture and 2 isolates were both MRSA and SA negative by culture. Results are presented in the table below.

MRSA/SA SSTI Performance vs. Reference Culture - Empty Cassette Variants

Subject #	Xpert Result	<i>spa</i> (Ct)	<i>mecA</i> (Ct)	SCC <i>mec</i> (Ct)	Culture	Xpert v. Culture	
						MRSA	SA
1	SA	23.6	0	26.0	SA	TN	TP
2	SA	14.7	0	16.5	SA	TN	TP
3	SA	20.5	0	34.0	SA	TN	TP
4	SA	18.4	0	21.0	SA	TN	TP
5	SA	15.6	0	28.4	MRSA	FN	TP

Subject #	Xpert Result	spa (Ct)	mecA (Ct)	SCCmec (Ct)	Culture	Xpert v. Culture	
						MRSA	SA
6	SA	17.2	0	31.6	SA	TN	TP
7	SA	34.1	0	35.6	Neg	TN	FP
8	SA	29.1	0	33.0	SA	TN	TP
9	SA	12.7	0	23.5	SA	TN	TP
10	SA	18.2	0	27.6	SA	TN	TP
11	SA	18.4	0	22.0	SA	TN	TP
12	SA	25.5	0	27.7	SA	TN	TP
13	SA	20.0	0	22.1	Neg	TN	FP
14	SA	26.0	0	28.3	SA	TN	TP
15	SA	23.9	0	25.7	SA	TN	TP
16	SA	19.9	0	34.0	SA	TN	TP

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

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:

In the Xpert MRSA/SA clinical study, a total of 848 SSTI specimens were included from four large hospitals across the United States. The number and percentage of positive cases by the reference culture method, calculated by age group, are presented in the tables below.

Observed Prevalence of MRSA and SA by Culture

Age Group	Total N	MRSA By Culture		SA By Culture	
		Number Positive	Observed Prevalence	Number Positive	Observed Prevalence
Ages Less Than 3	34	11	32.4%	21	61.8%
Ages 3 to 18	100	25	25.0%	55	55.0%
Ages 19 to 65	614	188	30.6%	300	48.9%
Ages 66 and over	100	22	22.0%	35	35.0%

N. Instrument Name: The GeneXpert® Dx instrument, GX IV (contains one to four modules) and GX XVI (contains four to sixteen modules).

O. System Descriptions:

1. Modes of Operation:

The GeneXpert Dx System automates and integrates random access sample preparation, and amplification and real-time detection of DNA from swab specimens of skin and soft tissue infections. Each instrument module contains the hardware components that enable automated sample processing in the cartridge and filling of the reaction tube with the sample-reagent mixture for PCR.

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes X or No

The software that controls the operation of the sample processing and the I-CORE module, and collects, analyzes and interprets the acquired optical data is the GeneXpert Dx Version 1.6b software.

3. Specimen Identification: Barcode

4. Specimen Sampling and Handling: Automated

GeneXpert Dx System Hardware Components for Automated Sample Processing

Module Hardware Components	Function
Valve Drive	Rotates the cartridge valve body to address the different cartridge chambers.
Syringe Pump drive	Dispenses fluids to and from the different cartridge chambers.

Ultrasonic horn	Lyses the bacterial cells and sample prep control.
I-CORE® module	Performs PCR amplification and detection. As the user inserts the cartridge into the system, the reaction tube component of the cartridge is inserted into the I-CORE module. After sample preparation within the cartridge, the sample and reagent mixture is transferred from the cartridge chamber into the reaction tube. During the amplification process, the I-CORE heater heats up and the fan cools down the reaction tube contents. Two optical blocks positioned within the ICORE excite the dye molecules that make up the probes and detect the fluorescence emitted. The system uses calibration and data analysis algorithms to determine a relative fluorescence value for each reporter dye after each thermal cycle.
Hand-held Barcode Scanner	Scans cartridge barcode and optional Patient or Sample ID barcode into the GeneXpert Dx System.

5. Calibration:

Optical and thermal calibration of the GeneXpert Dx System is performed by Cepheid at the time of manufacture prior to installation and once yearly or after 1000 runs per module. The user does not calibrate or perform any serviceable functions on the instrument. The normalization function compensates for any optical degradation between calibrations.

The thermal reaction chamber thermistors are calibrated to $\pm 0.50^{\circ}\text{C}$ using National Institute of Standards and Technology (NIST)-traceable standards. During the manufacturing process, the temperature of the heating system is measured at two temperatures: 60°C and 95°C . Calibration coefficients that correct for small errors in the raw thermistor readings of the heaters are stored in the memory of each I-CORE module.

The optical system is calibrated using standard concentrations of individual unquenched fluorescent dye-oligos. For each optical channel, the signal produced by a tube alone (the blank signal) is subtracted from the raw signal produced by the dye-oligo standard to determine the spectral characteristics. Using the individual spectral characteristics of the pure dye-oligos, signals from an unknown mixture of dye-oligos can be resolved into corrected signals for the individual dye-oligos in the mixture.

6. Quality Control:

The Xpert MRSA/SA SSTI test includes a Sample Processing Control (SPC) and Probe Check Control (PCC) pre-loaded in the cartridge and provided with the assay.

The sample processing control (SPC) consist of *Bacillus globigii* spores to control for adequate lysing and processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR assay to avoid false-negative results. *Bacillus globigii* spores are used as the sample preparation control because they are more difficult to lyse than the

Staphylococcal target bacteria. The SPC also ensures the PCR reaction conditions (temperature and time) are appropriate for the amplification reaction and that the PCR reagents are functional.

The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

A cartridge loading and unloading mechanism assures the proper positioning of the cartridge in the instrument. In addition, internal quality controls perform a self-test before each test starts to verify that the system is functioning properly. These tests consist of verification of heaters, fan, and optics. There are also continuous checks for syringe drive and valve stalling. The software also verifies ultrasonic actuation by monitoring horn current during operation.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

Shelf-life

The Xpert MRSA/SA Assay shelf-life was demonstrated in stability studies using real-time stability results and linear regression analysis to support a **shelf-life of 12 months** when the reagents and cartridge are stored refrigerated at 2-8°C. Stability testing is ongoing with three lots and the actual shelf-life dating will be determined by the results of real time stability studies and approved by the stability committee in compliance with approved procedures at the time of product clearance.

The stability of the product is being evaluated at four temperatures (5° + 3°C, 25° + 3°C, 35° + 3°C and 45°+ 3°C) at predefined timepoint intervals up to 24 months following the study plan described in the 510k submission.

A **Specimen Stability study** was conducted to establish specimen transport and storage claims for the Xpert MRSA/SA SSTI Assay. The recommended storage conditions are room temperature (15 - 28°C) of up to 24 hours or refrigerated 2-8°C for up to five days until testing is performed. The study supported this claim.

Elution Efficiency study was performed to determine the efficiency of eluting MRSA cells from a clinical swab using the Xpert MRSA Assay. Four concentrations of MRSA type II cells (1×10^8 CFU/swab, 1×10^6 CFU/swab, 1×10^4 CFU/swab, and 1×10^2 CFU/swab) spiked onto swabs were used to determine the elution efficiency. As a control, each concentration was also spiked directly into the elution reagent and processed identically as the tests with the swab. Cells added directly to elution reagent represent an elution efficiency of 100%. Replicates of 4 were run at each cell concentration. There was no significant difference between cells added directly to the elution buffer (representing 100% elution efficiency) and cells spiked onto a swab and then eluted in the elution buffer by a 10 second vortex step.

Failure Modes Testing was performed to determine the effect of failure modes that might occur with the Xpert MRSA/SA Assay. Failures may be due to instrument malfunction, manufacturing errors or operator errors. An instrument malfunction might result in an ultrasonic horn failure. A manufacturing error might result in beads being omitted from the cartridge before packaging. Operator error might include adding an insufficient amount of reagent to the cartridge or omitting a liquid reagent or adding a liquid reagent to the wrong chamber. A gross instrument malfunction such as ultrasonic horn failure resulted in an “Invalid” test result. All reagent bead filling errors resulted in “Invalid” or “Error” test results, except for the double SPC bead. In the event of a double SPC bead, impact is minimal and a believable output is expected. Conditions where both liquid reagents 1 and 2 were omitted, the elution buffer was omitted, the addition location of reagents 1 and 2 was switched, and only reagent 1 was omitted resulted in “Error” test results due to probe check failures. Conditions where only liquid reagent 2 was omitted resulted in a valid test result. Probe checks pass under this condition because reagent 1 is used to rehydrate the TSR and EZR beads, not reagent 2. Reagent 2 is used to reduce potential sample inhibition. If reagent 2 is omitted, a sample containing potentially inhibitory substances will likely result in an “Invalid” test due to an SPC failure. Valid test results are expected if at least 90% of all liquid reagents are added.

Q. Proposed Labeling:

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

R. Conclusion:

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