

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

A. 510(k) Number:

k100822

B. Purpose for Submission:

To obtain a substantial equivalence determination for this 510k for MRSA/SA from nasal specimens.

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™ Xpert SA Nasal Complete Assay

G. Regulatory Information:

1. Regulation section:

21 CFR 866. 1640 Antimicrobial susceptibility test powder

2. Classification:

Class II

3. Product code:

NQX- nucleic acid amplification test, DNA, methicillin resistant *Staph aureus*, direct specimen

- OOI- Nucleic acid amplification systems, real time
4. Panel:

83 Microbiology

H. Intended Use:

1. Intended use(s):

The Cepheid® Xpert® SA Nasal Complete Assay performed in the GeneXpert® Dx System is a qualitative *in vitro* diagnostic test designed for rapid detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal swabs in patients at risk for nasal colonization. The test utilizes automated real-time polymerase chain reaction (PCR) to detect MRSA/SA DNA. The Xpert SA Nasal Complete Assay is intended to aid in the prevention and control of MRSA/SA infections in healthcare settings. The Xpert SA Nasal Complete Assay is not intended to diagnose, guide or monitor treatment for MRSA/SA infections, or provide results of susceptibility to methicillin. A negative result does not preclude MRSA/SA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

2. Indication(s) for use:

The Cepheid® Xpert® SA Nasal Complete Assay performed in the GeneXpert® Dx System is a qualitative *in vitro* diagnostic test designed for rapid detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal swabs in patients at risk for nasal colonization. The test utilizes automated real-time polymerase chain reaction (PCR) to detect MRSA/SA DNA. The Xpert SA Nasal Complete Assay is intended to aid in the prevention and control of MRSA/SA infections in healthcare settings. The Xpert SA Nasal Complete Assay is not intended to diagnose, guide or monitor treatment for MRSA/SA infections, or provide results of susceptibility to methicillin. A negative result does not preclude MRSA/SA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

3. Special conditions for use statement(s):

Prescription Use

4. Special instrument requirements:

GeneXpert® Dx System, Software version 2.1

I. Device Description:

The Cepheid Xpert SA Nasal Complete Assay is a rapid, automated *in vitro* diagnostic test designed for rapid and simultaneous detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) from nasal swabs in patients at risk for nasal colonization. The Xpert SA Nasal Complete 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 realtime PCR for detection of MRSA and SA in approximately 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 I-CORE® 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 SA Nasal Complete Assay includes reagents for the simultaneous detection of the target organisms, MRSA and SA. The primers and probes in the Xpert SA Nasal Complete Assay detect nucleic acid sequences of the staphylococcal protein A (*spa*), the gene for methicillin/oxacillin resistance (*mecA*), and the staphylococcal cassette chromosome (*SCCmec*) inserted in the SA chromosomal *attB* site.

The primers and probes in the Xpert SA Nasal Complete Assay detect nucleic acid sequences of the staphylococcal protein A (*spa*), the gene for methicillin/oxacillin resistance (*mecA*), and staphylococcal cassette chromosome (*SCCmec*) inserted into the SA chromosomal *attB* site. Nasal swab specimens from the anterior nares are collected and transported to the GeneXpert® area. The swab specimen is placed in a tube containing elution reagent. Following a brief vortex, 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 processing 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*.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Xpert™ MRSA/SA SSTI Assay
Xpert MRSA Assay

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

K080837
K070462

3. Comparison with predicate:

Similarities

Item	Device		Predicate
	Xpert SA Nasal Complete Assay	Xpert MRSA/SA SSTI Assay (K080837)	Xpert MRSA Assay (K070462)
Intended Use	Rapid detection of MRSA and SA	Same	MRSA only
Indication for Use	Identification of MRSA and SA	Same	MRSA only
Technological Principles	Fully-automated nucleic acid amplification (DNA); real-time PCR	Same	Same
Test Cartridge	Disposable single-use, multi-chambered, fluidic cartridge.	Same	Same
Instrument System	Cepheid GeneXpert Dx System	Same	Same
Fluidics	Self-contained and automated after specimen preparation	Same	Same
Probes	TaqMan® Probes	Same	Same
Internal Controls	Sample processing control (SPC) and probe check control (PCC).	Same	Same
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)	Same	N/A, assay does not detect SA
Rapid test results	Approximately 50 minutes to results.	Same	Approximately 75 minutes.

Differences

Intended Use	Simultaneous rapid detection of SA and MRSA.	Same	Only detects MRSA
Specimen Type	Direct from nasal swabs.	Direct from skin and soft tissue infection swabs.	Direct from nasal swabs.
DNA Target Sequence	Sequence specific to <i>Staphylococcus aureus</i> species (<i>spa</i> gene)	Same	NA
Software version	2.1	1.6b	1.6

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

Not applicable

L. Test Principle:

The primers and probes in the Xpert SA Nasal Complete 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. Nasal swabs from the anterior nares are collected and transported to the GeneXpert® area. The swab is placed in a tube containing elution reagent. Following a brief vortex, the eluted material and two other liquid reagents are transferred to different chambers of the 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

A Reproducibility study was performed using a panel of 10 specimens with varying concentrations of SA, MRSA added to a mixture of human blood and porcine mucin in a background of methicillin-susceptible *Staphylococcus epidermidis* (MSSE) 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 SA Nasal Complete kit was used at each of the 3 testing sites.

Summary of Reproducibility Results ^a

Specimen ID	Site 1	Site 2	Site 3	Total Agreement
Negative (MSSE)	100.0% (20/20)	100.0% (20/20)	100.0% (20/20)	100.0% (60/60)
MSSA – High Neg	95.0% (19/20)	95.0% (19/20)	95.0% (19/20)	95.0% (57/60)
MSSA – Low Pos	85.0% (17/20)	95.0% (19/20)	100.0% (20/20)	93.3% (56/60)
MSSA – Mod Pos	100.0% (20/20)	100.0% (20/20)	100.0% (20/20)	100.0% (60/60)
MRSA1 – High Neg	100.0% (20/20)	95.0% (19/20)	85.0% (17/20)	93.3% (56/60)
MRSA1 – Low Pos	95.0% (19/20)	95.0% (19/20)	100.0% (20/20)	96.7% (58/60)
MRSA1 – Mod Pos	95.0% (19/20)	100.0% (20/20)	100.0% (20/20)	98.3% (59/60)
MRSA2 – High Neg	60.0% (12/20)	60.0% (12/20)	50.0% (10/20)	56.7% (34/60)
MRSA2 – Low Pos	95.0% (19/20)	95.0% (19/20)	95.0% (19/20)	95.0% (57/60)
MRSA2 – Mod Pos	100.0% (20/20)	100.0% (20/20)	100.0% (20/20)	100.0% (60/60)
% Total Agreement by Site	92.5% (185/200)	93.5% (187/200)	92.5% (185/200)	92.8% (557/600)

^a For negative and high negative samples, %Agreement = (# negative results/total samples run); for low and moderate positive samples, %Agreement = (# positive results/total samples run).

Summary of Ct value results by sample level and probe

SPC			
Level	Mean	Std Dev	%CV
Neg (MSSE)	34.3	0.72	2.1%
MSSA High Neg	34.3	0.75	2.2%
MRSA1 High Neg	34.6	0.86	2.5%
MRSA2 High Neg	34.6	0.75	2.2%

<i>spa</i>			
Level	Mean	Std Dev	%CV
MSSA Low Pos	33.7	0.91	2.7%
MSSA Moderate Pos	31.6	0.71	2.2%
MRSA1 Low Pos	32.6	1.53	4.7%
MRSA1 Moderate Pos	31.7	0.79	2.5%
MRSA2 Low Pos	32.7	0.97	3.0%
MRSA2 Moderate Pos	30.6	0.85	2.8%

<i>mecA</i>			
Level	Mean	Std Dev	%CV
MRSA1 Low Pos	33.3	0.88	2.6%
MRSA1 Moderate Pos	32.2	0.82	2.5%
MRSA2 Low Pos	33.4	1.02	3.1%
MRSA2 Moderate Pos	31.1	0.75	2.4%

SCC <i>mec</i>			
Level	Mean	Std Dev	%CV
MRSA1 Low Pos	34.1	0.86	2.5%
MRSA1 Moderate Pos	32.9	0.79	2.4%
MRSA2 Low Pos	34.6	1.19	3.4%
MRSA2 Moderate Pos	32.5	0.80	2.5%

b. Linearity/assay reportable range:

Not applicable

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

The Xpert SA Nasal Complete Assay includes a Sample Processing Control (SPC) and a Probe Check Control (PCC). The success rate was >95% on the first attempt and was acceptable.

Recommended external quality control organisms were also tested at the sites. External Controls may be used in accordance with accrediting institutions and government regulations. External Controls are not provided in the test kit; however, the outside source and the catalog numbers are provided in the “Materials Available but Not Provided” section of the Xpert SA Nasal Complete 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 simulated nasal matrix. The nasal matrix consisted of mucin and 1% of blood in PBS with 15% glycerol. The limit of detection is defined as the lowest number of colony forming units (CFU) per sample that can be reproducibly distinguished from negative samples with 95% confidence or the lowest concentration at which 19 of 20 replicates were positive.

For SA, replicates of 20 were evaluated at various concentrations for three (3) individual isolates. USA types USA900 and USA1200 were represented.

For MRSA, replicates of 20 were evaluated at various concentrations for ten (10) individual isolates representing SCC mec types I, II, III, IVa, IVd, V, VI, VII, and VIII. When characterized by pulsed-field gel electrophoresis (PFGE), USA100 and USA400 are represented. Isolates of heterogeneous oxacillin resistant subpopulations were included.

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. The LoD point estimates and 95% upper and lower confidence intervals for each SA and each MRSA SCC mec type tested are summarized in tables below:

95% Confidence Intervals for Analytical LoD – SA

SA Strain ID	PFGE	LoD (CFU/swab)	Lower 95% CI (CFU/swab)	Upper 95% CI (CFU/swab)
N7129	USA900	154	132	197
102-04	USA1200	128	109	177
29213	unknown	94	76	138

95% Confidence Intervals for Analytical LoD – MRSA

MRSA Strain ID	SCC mec Type	PFGE	LoD (CFU/swab)	Lower 95% CI (CFU/swab)	Upper 95% CI (CFU/swab)
64/4176	I	USA500	79	64	119
N315	II	USA100	94	76	131
BK2464	II	USA100	143	116	212
11373	III	unknown	52	42	77
MW2	IVa	USA400	85	69	130
BK2529*	IVd	USA500	256	216	334
ST59-MRSA-V	V	USA1000	127	105	170
HDE288*	VI	USA800	97	78	141
JCSC6082	VII	unknown	214	182	276
WA MRSA16	VIII	unknown	292	259	384

(*) Heterogeneous oxacillin-resistant isolates

The results of this study indicate that the Xpert SA Nasal Complete Assay will

produce a positive SA result 95% of the time with 95% confidence for a nasal swab containing 175 CFU and a positive MRSA result 95% of the time with 95% confidence for a nasal swab containing 300 CFU.

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

An additional study was conducted to determine the effect of increasing amounts of methicillin-susceptible *Staphylococcus aureus* (MSSA) cells on the Limit of Detection (LoD) of methicillin-resistant *Staphylococcus aureus* (MRSA) cells established for the Xpert SA Nasal Complete Assay.

The competitive inhibitory effect of increasing amounts of SA relative to MRSA at LoD was evaluated for each SCC*mec* type I, II, III, IVa, V, VI, VII, and VIII.

For MRSA SCC*mec* types I, II, III, IVa, V, VI, and VII, the effect of competing MSSA is inhibitory at the claimed LoD at MRSA: MSSA ratios of 1:1x10⁶.

For MRSA SCC*mec* type VIII, the effect of competing MSSA is inhibitory at the claimed LoD at a MRSA: MSSA ratio of 1:1x10³.

Analytical Inclusivity

A total of 248 isolates (199 MRSA strains and 49 methicillin sensitive *S. aureus* strains) were tested using the Xpert SA Nasal Complete Assay. All strains were tested in triplicate using bacterial cell stocks diluted to concentrations at or near the assay cut-off. Known USA300 isolates (39 in total), including USA300-0114, are represented. Other PFGE types included in the study are USA100 (54), USA200 (5), USA400 (1), USA500 (7), USA600 (2), USA700 (2), USA800 (9), USA1000 (8), USA1100 (2), IBERIAN isolates (2), and strains designated PGFE types A, B, and F. All results are 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 SA Nasal Complete Assay. All strains were tested at cell concentrations near the analytical LoD, at ~300 CFU/test and at ~3X10⁵ CFU/test.

All 22 isolates were identified as MRSA negative and SA positive. No *mecA* signals were reported.

e. Analytical specificity:

Cross Reactivity

A total of 114 strains (83 Gram positive, 28 Gram negative, and three yeasts) phylogenetically related to *Staphylococcus aureus* or those potentially present in nasopharyngeal flora were tested. There were 103 strains from the American Type Culture Collection (ATCC), seven strains from the Network

on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA); the remaining four isolates were from Sweden, Germany and Japan. The isolates included 34 methicillin-sensitive coagulase negative staphylococci (MScoNS), and 12 methicillin-resistant coagulase negative staphylococci (MRCoNS).

Each strain was tested using 100 µL of culture adjusted to 4.5 to 9.5x10⁸ CFU/mL. Three replicates of each strain were tested. All isolates were reported MRSA Negative; SA Negative by the Xpert SA Nasal Complete Assay.

Evaluation of BORSA Strains:

All seven BORSA isolates (including the apparent "empty cassette" isolate) were reported MRSA negative; SA positive at both high and low cell concentrations using the Xpert SA Nasal Complete Assay. No *mecA* signals were reported.

Interference Study

In another analytical study, potential interfering substances that may be present in clinical nasal specimen were tested with the following results:

Summary of Test Results per Substance and Concentration Tested

Substance	Conc Tested	Accurate Tests per # of Replicates Tested				
		Negs*	MSSA		MRSA [^]	
			N7129	102-04	type II	type IVa
Buffer (Control)	100% (v/v)	24/24	16/16	8/8	16/16	8/8
Anefrin Nasal Spray	100% (v/v)	8/8	8/8	8/8	8/8	8/8
Blood	100% (v/v)	8/8	8/8	8/8	8/8	8/8
Flonase	100% (v/v)	2/8	8/8	-	-	-
	50% (v/v)	3/8	8/8	8/8	-	-
	25% (v/v)	4/8	8/8	8/8	-	-
	10% (v/v)	7/8	-	-	6/8	7/8
	5% (v/v)	8/8	-	-	7/8	8/8
	1% (v/v)	8/8	-	-	8/8	-
Mucin	5% (w/v)	8/8	8/8	8/8	8/8	8/8
NasalCrom	100% (v/v)	8/8	8/8	8/8	8/8	8/8
Nasonex	100% (v/v)	2/8	8/8	-	-	-
	50% (v/v)	4/8	8/8	8/8	-	-
	25% (v/v)	6/8	-	8/8	-	-
	10% (v/v)	8/8	-	-	8/8	7/8
	5% (v/v)	8/8	-	-	-	8/8
Neo-Syneprhine	100% (v/v)	8/8	8/8	8/8	8/8	8/8
Rhinolast (Astelin)	100% (v/v)	8/8	8/8	8/8	8/8	8/8
Saline Nasal Spray	100% (v/v)	8/8	8/8	8/8	8/8	8/8
Zicam Nasal Gel	100% (v/v)	8/8	8/8	8/8	8/8	8/8

(*) In negatives per substance (excluding buffer control), if not 8/8, then Invalid or Error test result(s) reported

(^) In MRSA positives per substance (excluding buffer control), if not 8/8, then false negative test result(s) reported

(-) Level of substance not tested

Inhibitory effects resulting in invalid test results were observed in the presence of Flonase or Nasonex in negative samples at concentrations greater than 5% (v/v) and 10% (v/v), respectively.

Inhibitory effects resulting in MRSA false negative test results were observed in the presence of Flonase at greater than 1% (v/v) and in the presence of Nasonex greater than 5% (v/v).

Carry-Over Contamination study consisted of a negative sample (no MRSA cells spiked into the elution buffer) processed within the same GeneXpert module immediately following a very high positive sample (1×10^7 MRSA cells spiked into the elution buffer). This was repeated 20 times between two GeneXpert modules for a total of 42 runs (10 positive samples per module and 11 negative samples per module). The study demonstrated no evidence of carry-over contamination.

f. Assay cut-off:

The valid minimum cycle threshold setting for *spa*, *mecA* and *SCCmec* is 10 because in practice, a Ct cannot be calculated before the end of the minimum background subtraction range, which is 10 cycles. The earliest *spa* Ct reported in true SA and MRSA Positive results during pre-clinical testing was 16.5.

The valid maximum cycle threshold (Ct) setting for *spa*, *mecA*, and *SCCmec* is 35.0, 36.0, and 38.0 respectively.

To obtain a valid SA Positive test result, the *spa* Ct must be reported within the valid cycle range. To obtain a valid MRSA Positive test result, all *spa*, *mecA*, and *SCCmec* Cts must be reported within the valid cycle range. To obtain a valid MRSA or SA Negative test result, the *spa*, *mecA*, and *SCCmec* Cts must not be reported within the valid cycle range and the SPC Ct must be reported within its valid cycle range. If the SPC falls outside the valid cycle range, the test result is Invalid and must be repeated.

The valid maximum cycle settings (assay cut-offs) for the Xpert SA Nasal Complete targets (*spa*, *mecA*, and *SCCmec*) were derived from pre-clinical data (n=736) collected using clinical specimens to maximize percent sensitivity and percent specificity relative to enriched culture. The SA specificity was poor if the *spa* Ct cut-off was moved from 35 (91.5%) to 36 (88.6%) and poor SA sensitivity was calculated if the *spa* Ct cut-off was moved from 35 (92.7%) to 34 (90.0%). These assay cut-offs were subsequently validated in the pivotal clinical study, Protocol 128. The clinical study supports and validates the Ct cut-offs chosen for *spa*, *mecA*, and *SCCmec* targets. From the analysis it is demonstrated that the established assay settings maximize sensitivity (highest priority) and specificity.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

Clinical Sensitivity/Specificity:

Performance characteristics were determined in a multi-site prospective investigation study at eight US institutions by comparing the Xpert SA Nasal Complete Assay with Reference Culture. Double swabs were collected from each subject. One swab was tested by the Xpert SA Nasal Complete Assay at the enrolling center and the other swab 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 a sheep 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 30µg cefoxitin disk and cutoff of ≤21 mm (R), ≥22 mm (S).

Performance of the Xpert SA Nasal Complete Assay was calculated relative to the Reference Culture (enriched blood agar culture) results. The overall performance of the Xpert SA Nasal Complete Assay relative to direct CHROMagar, and enriched CHROMagar methods was for information only.

Overall Results

A total of 2487 specimens were tested for MRSA and SA by Xpert the SA Nasal Complete Assay and enriched blood agar culture. Among the eligible 2487 cases, there were 141 subjects who had antibiotic use within 7 to 21 days prior to sample collection, 2323 subjects with confirmed no antibiotic use, and 23 cases of unknown antibiotic status. There was no statistically significant difference in the culture positivity rate or the Xpert SA Nasal Complete Assay performance based on antibiotic status.

The performance of the Xpert SA Nasal Complete Assay is summarized in the tables below:

		Reference Culture			Total
		MRSA+	SA+/MRSA-	Neg/No Growth	
Xpert	MRSA+	159	24	25	208
	SA+/MRSA-	9	393	152	554
	SA-	5	37	1683	1725
	Total	173	454	1860	2487
MRSA: Sensitivity:		91.9% (159/173) (95% CI: 86.8-95.5%)			

Specificity:	97.9% (2265/2314) (95% CI: 97.2-98.4%)
PPV:	76.4% (159/208) (95% CI: 70.1-82.0%)
NPV:	99.4% (2265/2279) (95% CI: 99.0-99.7%)
SA:	
Sensitivity:	93.3% (585/627) (95% CI: 91.1-95.1%)
Specificity:	90.5% (1683/1860) (95% CI: 89.1-91.8%)
PPV:	76.8% (585/762) (95% CI: 73.6-79.7%)
NPV:	97.6% (1683/1725) (95% CI: 96.7-98.2%)

Of the Xpert Assays run on eligible specimens, 96.5% (2487/2578) of these specimens were successful on the first attempt. The remaining 91 gave indeterminate results on the first attempt (31 “INVALID”, 51 “ERROR” and 9 “NO RESULT”). The study design did not allow for repeat testing.

Empty Cassette Variants

There were 14 isolates that fit the empty cassette profile (i.e. *spa* positive, *SCCmec* positive, *mecA* negative) in the clinical studies. All of the 14 specimens were verified true negative MRSA isolates, and true positive SA isolates relative to Reference Culture.

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:

A total of 2487 nasal specimens were included from eight institutions 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 Reference Culture

Age Group	Total N	MRSA By Culture		SA By Culture	
		Number Positive	Observed Prevalence	Number Positive	Observed Prevalence
Ages 22 to 30	325	10	3.1%	71	21.8%
Ages 31 to 40	359	17	4.7%	84	23.4%
Ages 41 to 50	459	28	6.1%	118	25.7%
Ages 51 to 60	487	36	7.4%	141	29.0%
Ages 61 to 70	315	25	7.9%	75	23.8%
Ages >70	542	57	10.5%	138	25.5%

N. Instrument Name: The GeneXpert® Dx Instrument

O. System Descriptions:

1. Modes of Operation:

The GeneXpert Dx System operates in random access mode. The instrument is available in configurations with one module base, four module bases, and 16 module bases. The multiple module bases allow one (1), up to four (4) and up to 16 single use cartridges, respectively, to be run simultaneously. Up to four of the four module base GeneXpert Dx Systems may be linked together.

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 2.1 software.

3. Specimen Identification:

Barcode

3. 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 SA Nasal Complete Assay test includes a Sample Processing Control (SPC) and Probe Check Control (PCC) pre-loaded in the cartridge and provided with the assay.

The SPC verifies that conditions adequate for the processing of the target SA and MRSA bacteria have occurred. It consists of *Bacillus globigii* spores formulated into a dry reagent bead included in each Xpert SA Nasal Complete cartridge. Furthermore the SPC verifies (1) the effectiveness of each sample preparation step, (2) reaction tube filling, (3) that all reaction components are present and functioning, and monitors the presence of potential inhibitor(s) in the PCR assay.

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

if the fluorescence generated meets the validated acceptance criteria. If the PCC fails for any SA or MRSA target or SPC, a probe check error is reported and the test will not continue. For these conditions, the test will need to be repeated using a new specimen, a new cartridge and new reagents.

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 at 2-28°C; open package for up to 24 hours at 2-28°C. Currently there are three lots that have 9 months of real time data, and one lot has 12 months of real time data.

The stability of the product is being evaluated at four temperatures ($5^{\circ} \pm 3^{\circ}\text{C}$, $25^{\circ} \pm 3^{\circ}\text{C}$, $35^{\circ} \pm 3^{\circ}\text{C}$ and $45^{\circ} \pm 3^{\circ}\text{C}$) at predefined time point intervals up to 36 months following the study plan described in the 510k submission. One lot has an extra time point for both 4°C and 25°C storage conditions.

A **Specimen Stability study** was conducted to establish specimen transport and storage claims for the Xpert SA Nasal Complete Assay. The following collection device types were tested:

LSD: Liquid Stuart-Dry (Cepheid Sample Collection Device)

LSW: Liquid Stuart-Wet (swab pre-moistened with sterile 0.9% NaCl solution)

LAW: Liquid Amies-Wet (swab pre-moistened with the provided transport medium)

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. The study supported this claim.

Failure Modes Testing was performed to determine the effect of failure modes that might occur with the Xpert SA Nasal Complete Assay. Failures may be due to operator errors, manufacturing errors or instrument malfunction.

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 manufacturing error might result in beads being loaded incorrectly into the cartridge before packaging (missing beads or double beads), or a cartridge being assembled incorrectly (missing the filter required for cell capture).

An instrument malfunction examples include an ultrasonic horn failure, motion of the syringe drive not detected, syringe pressure reading exceeds protocol limit, the system failed to find the plunger home position, a valve positioning error was detected, digital temperature reading of thermistor(s) not within acceptable range, and the optical signal from the detector(s) did not reach the expected value. Because the software performs self-check procedures prior to the start of each

test, if any of the instrument malfunctions described above occur the test is aborted; no assay results are reported.

Replicates of eight (8) MRSA negative and eight (8) MRSA positive samples were tested per condition. Positive samples contained MRSA cells at ~600 CFU/test. Results are compared to positive and negative controls in which all cartridges are assembled correctly (1 EZR- enzyme bead, 1 TSR-primer/probe bead, 1 SPC- sample processing bead, and a capture filter) and all liquid reagents (reagent 1, reagent 2, and elution buffer) are dispensed correctly and completely. Of the 354 runs, 3 provided uninformative GeneXpert results (Error). Errors consisted of 1 pressure abort, 1 probe check control error, and 1 valve positioning error.

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.