DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
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Silver Spring, MD 20993-0002

November 23, 2016

CEPHEID
JIM KELLY, Ph.D.
EXECUTIVE DIRECTOR, REGULATORY AFFAIRS
904 CARRIBEAN DRIVE
SUNNYVALE
CA 94089

Re: K162444
Trade/Device Name: Xpert MRSA NxG
Regulation Number: 21 CFR 866.1640
Regulation Name: Antimicrobial test powder
Regulatory Class: II
Product Code: NQX, OOI
Dated: August 30, 2016
Received: August 31, 2016

Dear Dr. Kelly:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Ribhi Shawar -S

For Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K162444

Device Name
Xpert MRSA NxG

Indications for Use *(Describe)*
The Xpert® MRSA NxG Assay, performed on the GeneXpert® Instrument Systems, is a qualitative in vitro diagnostic test intended for the detection of methicillin-resistant Staphylococcus aureus (MRSA) DNA directly from nasal swabs in patients at risk for nasal colonization. The test utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA-specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The Xpert MRSA NxG Assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. The Xpert MRSA NxG Assay is not intended to diagnose, guide, or monitor treatment for MRSA infections, or provide results of susceptibility to methicillin. A negative result does not preclude MRSA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

As required by 21 CFR Section 807.92(c).

Submitted by:	Cepheid 904 Caribbean Drive Sunnyvale, CA 90489 Phone number: (847) 228-3299 Fax number: (847) 890-6589
Contact:	Scott A. Campbell, PhD, MBA
Date of Preparation:	November 8, 2016
Device:	
Trade name:	Xpert® MRSA NxG
Common name:	Xpert MRSA NxG Assay
Type of Test:	Qualitative real-time polymerase chain reaction (PCR) assay for the amplification and detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA.
Regulation number/ Classification name/ Product code:	866.1640 /System, Nucleic acid amplification test, DNA, methicillin resistant *Staphylococcus aureus*, Direct specimen/NQX 862.2570/Instrumentation for clinical multiplex test systems/OOI
Classification Advisory Panel	Microbiology (83)
Prescription Use	Yes
Predicate Device Assay:	BD MAX™ MRSA *XT* [510(k) # K133605]

Device Description:

The Xpert MRSA NxG Assay is an automated real-time polymerase chain reaction (PCR) *in vitro* diagnostic test for qualitative detection of methicillin-resistant *Staphylococcus aureus* (MRSA) directly from nasal swab specimens of patients at risk for colonization with MRSA in a healthcare setting.

The Xpert MRSA NxG Assay is performed on the Cepheid GeneXpert® Instrument Systems (GeneXpert Dx, GeneXpert Infinity-48, GeneXpert Infinity-48s, and GeneXpert Infinity-80 systems). The GeneXpert Instrument System platform automates sample preparation, amplification and real-time detection.

The GeneXpert Instrument Systems require the use of single-use, disposable cartridges (the Xpert MRSA NxG cartridges) that hold the PCR reagents and host the PCR process. Because the cartridges are self-contained and specimens never come into contact with working parts of the instrument modules, cross-contamination between samples is minimized.

The Xpert MRSA NxG Assay includes reagents for the detection of target MRSA DNA. The primers and probes in the Xpert MRSA NxG Assay detect proprietary sequences for the gene for methicillin/oxacillin resistance (*mecA* and *mecC*), and staphylococcal cassette chromosome *mec* (SCC*mec*), which is inserted into the SA chromosomal *attB* site. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are internal controls utilized by the GeneXpert Instrument System platform. The SPC is present to control for adequate processing of the target bacteria and to monitor for the presence of inhibitor(s) in the PCR assay to avoid false-negative results. The Probe Check Control verifies reagent rehydration, real-time PCR tube filling in the cartridge, probe integrity, and dye stability.

The single-use, multi-chambered fluidic cartridges are designed to complete sample preparation and real-time PCR for the detection of genomic DNA from methicillin-resistant *Staphylococcus aureus* (MRSA*)* in 70 minutes or less. The GeneXpert Instrument Systems, comprised of the GeneXpert Dx Systems and the GeneXpert Infinity Systems, have 1 to 80 randomly accessible modules, depending upon the instrument, that are each capable of performing separate sample preparation and real-time PCR and RT-PCR tests. Each module contains a syringe drive for dispensing fluids (i.e., the syringe drive activates the plunger that works in concert with the rotary valve in the cartridge to move fluids between chambers), an ultrasonic horn for lysing cells or spores, and a proprietary I-CORE® thermocycler for performing real-time PCR and RT-PCR and detection.

Nasal swab specimens are collected using the Cepheid Sample Collection Device and are transported to the laboratory or designated GeneXpert testing area. The nasal swab specimen is placed in a tube containing 2 mL of Elution Reagent. Following a brief vortexing of the sample, the entire contents of the Elution Reagent tube are transferred to the sample chamber of the Xpert MRSA NxG cartridge using a transfer pipette. For nasal

swab specimens that are collected using Copan Liquid Amies Elution Swab (ESwab) collection and transport system, 300 µL of liquid sample is transferred to a tube containing 2 mL of Elution Reagent. Following a brief vortexing of the sample, the entire contents of the Elution Reagent tube are transferred to the sample chamber of the Xpert MRSA NxG cartridge using a transfer pipette. The user initiates a test from the system user interface and places the cartridge into the GeneXpert instrument platform, which performs hands-off real-time, multiplex PCR for detection of DNA. The results are automatically generated at the end of the process in a report that can be viewed and printed.

Device Intended Use:

The Xpert® MRSA NxG Assay, performed on the GeneXpert® Instrument Systems, is a qualitative *in vitro* diagnostic test intended for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA directly from nasal swabs in patients at risk for nasal colonization. The test utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA-specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The Xpert MRSA NxG Assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. The Xpert MRSA NxG Assay is not intended to diagnose, guide, or monitor treatment for MRSA infections, or provide results of susceptibility to methicillin. A negative result does not preclude MRSA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

Substantial Equivalence:

The Xpert MRSA NxG Assay is substantially equivalent to the BD MAX™ MRSA *XT* assay [510(k) #K133605]. The performance of the Xpert MRSA NxG Assay was evaluated in two multi-site prospective clinical studies in which the performance of the Xpert MRSA NxG Assay was determined relative to culture and susceptibility testing. The results of the studies demonstrated that the performance of the Xpert MRSA NxG Assay is substantially equivalent to the predicate device.

Table 8-1 shows the similarities and differences between the Xpert MRSA NxG Assay and the predicate device.

Table 8-1: Comparison of Similarities and Differences of the Xpert MRSA NxG Assay with the Predicate Device

Similarities		
Item	**Device**	**Predicate Device**
	Cepheid Xpert MRSA NxG Assay	**BD MAX™ MRSA *XT* Assay**
510(k) Number	To be assigned	K133605
Regulation	Same	866.1640
Product Code	Same	NQX
Device Class	Same	II
Intended Use	The Xpert® MRSA NxG Assay, performed on the GeneXpert® Instrument Systems, is a qualitative in vitro diagnostic test intended for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA directly from nasal swabs in patients at risk for nasal colonization. The test utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA-specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The Xpert MRSA NxG Assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. The Xpert MRSA NxG Assay is not intended to diagnose, guide, or monitor treatment for MRSA infections, or provide results of susceptibility to methicillin. A negative result does not preclude MRSA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.	The BD MAX™ MRSA *XT* assay performed on the BD MAX™ System is an automated qualitative *in vitro* diagnostic test for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA from nasal swabs in patients at risk for nasal colonization. The test utilizes real-time polymerase chain reaction (PCR) for the amplification of MRSA DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD MAX™ MRSA *XT* assay is intended to aid in the prevention and control of MRSA infections in healthcare settings. It is not intended to diagnose MRSA infections nor guide or monitor treatment for MRSA infections. A negative result does not preclude nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

Similarities		
Item	**Device**	**Predicate Device**
	Cepheid Xpert MRSA NxG Assay	**BD MAX™ MRSA *XT* Assay**
Mode of Detection of Methicillin Resistance in *S. aureus*	Same	Presence of SCC*mec* cassette at *orfX* junction (specific to *S. aureus*) and *mecA* or *mecC* genes
Specimen Type	Same	Nasal swabs
Technological Principles	Same	Nucleic acid amplication (DNA); real-time PCR; fluorogenic target-specific hybridization probes
Assay Controls	Same	Specimen Processing Control (SPC)
Assay Results	Same	Qualitative
Laboratory Users	Same	CLIA Moderate Complexity and High Complexity labs

Primary Differences		
	New Device	**Predicate Device**
Item	**Cepheid Xpert MRSA NxG Assay**	**BD MAX™ MRSA *XT* Assay**
Instrument System	Cepheid GeneXpert Instrument System	BD MAX System
Early assay termination function	Yes (for positive samples)	No

The Xpert MRSA NxG Assay has the same general intended use as the predicate device and has the same technological characteristics as the predicate device. The differences between the Xpert MRSA NxG Assay and the predicate device do not raise different questions of safety and effectiveness. The clinical study demonstrates that the Xpert MRSA NxG Assay is acceptable for its intended use with inexperienced lab users and is substantially equivalent to the predicate device described above.

Non-Clinical Studies:

Analytical Sensitivity (Limit of Detection)

Studies were performed to determine the analytical sensitivity or Limit of Detection (LoD) of the Xpert MRSA NxG Assay using two different collection kits (the Cepheid Sample Collection Device P/N 900-0370 or Copan P/N 139CFA, referred to as the "rayon swab" and the ESwab collection kit, Copan P/N 480C or Becton Dickinson P/N 220245 referred to as the "ESwab" refer to Section 6.3). The LoD is the lowest concentration of sample (reported as CFU/swab or CFU/mL in Elution Reagent) that can be reproducibly distinguished from negative samples 95% of the time with 95% confidence. This study determined the lowest concentration of methicillin-resistant *Staphylococcus aureus* (MRSA) cells diluted into simulated nasal matrix that can be detected using the Xpert MRSA NxG Assay The simulated nasal matrix consisted of 5% (w/v) porcine mucin and 1% (v/v) human whole blood in a 1X Phosphate Buffered Saline (PBS) solution with 15% (v/v) glycerol.

The analytical sensitivity of the Xpert MRSA NxG Assay was assessed following the guidance in Clinical and Laboratory Standards Institute (CLSI) document EP17-A2 using two lots of reagents tested across three testing days with thirteen (13) individual MRSA strains and the two types of swabs (rayon swab and ESwab). The 13 individual strains represent SCC*mec* types I, II, III, IV, IVa, V, VI, VII, VIII, IX, X and XI. These strains in the LoD study represent the most common healthcare-acquired (USA100) and most common community-acquired (USA400) MRSA strains that are characterized by pulsed-field gel electrophoresis (PFGE). Strains that contained heterogeneous subpopulations with respect to their oxacillin resistance phenotype were also included in the study.

The LoD was established by testing five concentration levels with two reagent lots. The LoD and 95% confidence interval (CI) were then estimated for each lot using logistic regression analysis. The logistic regression analysis does not rely on a single concentration but utilizes the logit function to incorporate the information from all levels tested in the model. The point estimates were calculated using a method of maximum likelihood estimates (MLE) of the logistic regression model parameters. The maximum estimated LoD observed per strain from the logistic regression analysis was used to establish the LoD claim. The LoD point estimates and 95% upper and lower confidence intervals for each MRSA SCC*mec* type tested are summarized in Table 8-2 and Table 8-3.

The results of this study indicate that the Xpert MRSA NxG Assay will produce a positive MRSA result 95% of the time with 95% confidence for a nasal swab (Rayon) containing 302 CFU (Table 8-2).

Table 8-2: 95% Confidence Intervals for Analytical LoD — MRSA (Rayon swab)

MRSA Strain	PFGE ID[a]	LOD Estimate (Logistic Regression) (CFU/Swab)			LoD Estimate In Elution Reagent (CFU/mL)
		Lower 95% CI	LoD Point Estimate	Upper 95% CI	
Type I	USA500	72	**91**	136	**46**
Type II	USA100	127	**161**	236	**81**
Type III	unknown	50	**64**	96	**32**
Type IVa	USA400	46	**58**	84	**29**
Type IV (Fin 7)	unknown	256	**302**	392	**151**
Type IVa	USA300	143	**182**	282	**91**
Type V	USA1000	85	**102**	138	**51**
Type VI	USA800	32	**42**	64	**21**
Type VII	unknown	95	**128**	235	**64**
Type VIII	unknown	139	**163**	233	**82**
Type IX	unknown	142	**169**	227	**85**
Type X	unknown	86	**97**	119	**49**
Type XI (*mecC*)	unknown	219	**266**	358	**133**

a. PFGE = Pulsed-field gel electrophoresis

The results of this study indicate that the Xpert MRSA NxG Assay will produce a positive MRSA result 95% of the time with 95% confidence for a nasal swab (ESwab) containing 812 CFU (Table 8-3).

Table 8-3: 95% Confidence Intervals for Analytical LoD — MRSA (ESwab)

MRSA Strain	PFGE ID[a]	LoD Estimate (Logistic Regression) (CFU/swab)			LoD Estimate In Elution Reagent (CFU/mL)
		Lower 95% CI	LoD Point Estimate	Upper 95% CI	
Type I	USA500	285	**343**	469	**45**
Type II	USA100	184	**218**	293	**28**
Type III	unknown	215	**254**	338	**33**
Type IVa	USA400	134	**167**	245	**22**
Type IV (Fin 7)	unknown	656	**812**	1145	**106**
Type IVa	USA300	470	**563**	733	**73**
Type V	USA1000	378	**465**	671	**61**
Type VI	USA800	71	**89**	128	**12**
Type VII	unknown	201	**245**	338	**32**
Type VIII	unknown	520	**631**	851	**82**
Type IX	unknown	311	**377**	533	**49**
Type X	unknown	149	**166**	215	**22**
Type XI (*mecC*)	unknown	597	**734**	998	**96**

a. PFGE = Pulsed-field gel electrophoresis

Analytical Specificity (Cross-reactivity)

The analytical specificity of the Xpert MRSA NxG Assay was evaluated by testing a panel of one hundred and fifty-two potentially cross-reactive microorganisms that are methicillin-susceptible *Staphylococcus aureus* (MSSA), organisms phylogenetically related to *Staphylococcus aureus* (SA), and members of the nasal commensal microflora (e.g., other bacteria, viruses, and yeast) with the potential to cross-react with the Xpert MRSA NxG Assay. The one hundred and fifty two organisms tested were identified as either gram-positive (104), gram-negative (25), yeast (3), viruses (17), or Gram reaction indeterminate (3). Of these organisms, eighty-four were characterized as follows: twenty-three (23) were methicillin-susceptible, coagulase-negative *Staphylococcus* (MSCoNS), five (5) were methicillin-resistant, coagulase-negative *Staphylococcus* (MRCoNS), forty-seven (47) were methicillin-susceptible *Staphylococcus aureus* (MSSA), including two (2) empty cassette MSSA, and seven (7) borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) strains. Human cells were also tested in the study.

Evaluation of BORSA Strains: The seven well-characterized borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) strains that were tested included one "empty cassette" MSSA strain. Methicillin-resistant *Staphylococcus aureus* is resistant to all ß-lactam drugs (with the exception of ceftaroline) through the alternative penicillin-binding protein PBP2a encoded by *mecA* or *mecC*. BORSA strains do not carry *mecA/mecC* gene, but exhibit an oxacillin minimum inhibitory concentration (MIC) ≥ 2 and ≤ 8 µg/mL. It is especially valuable to distinguish MRSA from BORSA to aid in implementing appropriate management and isolation precaution options for patients infected with methicillin-susceptible strains of *S. aureus*. BORSA strains tested with Xpert MRSA NxG assay were reported as MRSA NOT DETECTED.

All potentially cross-reactive microorganisms were tested in triplicate in Elution Reagent containing simulated nasal matrix at $>10^6$ CFU/mL for bacteria and $>10^5$ $TCID_{50}$/mL for viruses. Human cells were tested at 10^5 cells/mL.

All microorganisms and the human cells were reported as **MRSA NOT DETECTED** by the Xpert MRSA NxG Assay. For the panel of one hundred and fifty-two potentially cross-reactive microorganisms and human cells evaluated in the study, the analytical specificity of the Xpert MRSA NxG Assay was 100%.

In silico analysis indicates that the Xpert MRSA NxG Assay may produce positive results with strains of *Staphylococcus argenteus*, a recently described species of *Staphylococcus* that is closely related to *S. aureus*, that carry an SCC*mec* cassette and *mecA* or *mecC*.

Microbial Interference

A study was conducted to assess the inhibitory effects of commensal microorganisms in nasal swab specimens on the performance of the Xpert MRSA NxG Assay. A panel of nine (9) bacterial strains, reported to be present in 10% or more of nasal cavities of healthy subjects, were evaluated using the Xpert MRSA NxG Assay (Table 8-4).

The nine commensal bacteria were spiked into the simulated nasal matrix at

approximately 1.0 x 10^6 CFU/ mL in Elution Reagent and tested in the presence of MRSA (cross-reactivity) or in the absence of MRSA (interference). Two MRSA strains (Table 8-5) were used in this study and these strains prepared at approximately 3X LoD and tested in replicates of four. None of the potentially interfering microorganisms evaluated in the study were found to cross-react or interfere with the detection of any of the MRSA strains using the Xpert MRSA NxG Assay.

Table 8-4: Commensal Bacterial Strains Tested in Microbial Interference

Strain	Strain ID
Staphylococcus aureus (MSSA)	15280
Staphylococcus epidermidis (MSSE)	ATCC 35984
Corynebacterium bovis	ATCC 7715
Streptococcus mutans	ATCC 25175
Proteus vulgaris	ATCC 29905
Haemophilus influenzae	ATCC 9007
Neisseria meningitidis	ATCC 700111
Moraxella catarrhalis	ATCC 43628
Streptococcus pneumoniae	ATCC 6303

Table 8-5: MRSA Strains

Target	Strain ID
MRSA (*mecA*)	MRSA Type II (NRSA70,N315)
MRSA (*mecC*)	MRSA Type XI LGA251

Analytical Reactivity (Inclusivity)

One hundred and ninety-six methicillin-resistant *Staphylococcus aureus* strains were tested in this study. The strains tested represented Cooper and Feil Groups 1A, 1B, and 2, SCC*mec* types and subtypes (I, IA, II, III, IIIA, III-Hg, IV, IVa, IVb, IVc, IVd, V, VI, VII, VIII, IX, X and XI), - sequence types (STs), *spa*-types, PFGE types, and clonal complexes (CC). Known USA100, USA200, USA300, USA400, USA500, USA600, USA700, USA800, USA1000, USA1100, IBERIAN strains, heteroresistant strains, and novel *mecC* strain $MRSA_{LGA251}$ were also included in this study. A "challenge panel" of 59 well characterized MRSA strains that have Minimum Inhibitory Concentrations (MIC) for cefoxitin/oxacillin that span the measurable dynamic range were also included in this study. Oxacillin MIC values for these 59 strains ranged from 0.5 to >32 µg/mL.

All 196 MRSA strains were correctly reported as **MRSA DETECTED** using the Xpert MRSA NxG Assay.

Interfering Substances Study

Nineteen substances that may be present in nasal swab specimens with the potential to interfere with the performance of the Xpert MRSA NxG Assay were evaluated. The

potentially interfering substances included mucous, human blood, nasal sprays or drops, nasal gels, nasal corticosteroids, FluMist, oral nasal anesthetics or analgesics, nasal antibiotics, antibacterials and antivirals. The substances, active ingredients, and concentrations tested are listed in Table 8-6. All interfering substances, with the exception of mucin, were initially tested at 50% (v/v) in a simulated nasal matrix for Negative (simulated matrix only) and MRSA-positive samples. Mucin was tested at 7% (w/v) in simulated nasal matrix for Negative (simulated matrix only) and MRSA-positive samples.

Buffer Controls (negative and positive) without interfering substances were included.

Positive samples were tested per interfering substance with two clinical MRSA strains, SCC*mec* Type II (*mecA*) and SCC*mec* Type XI ($mecC_{LGA251}$), spiked at approximately 3X analytical LoD in simulated nasal matrix.

Replicates of eight positive and negative samples with each interfering substance were evaluated in this study. Negative samples in the presence of potentially interfering substance were tested to determine the impact on the performance of the sample processing control (SPC).

The effect of each potentially interfering substance on positive and negative samples was assessed by comparing the target cycle threshold (Ct) values generated in the presence of the potentially interfering substance to the Ct values of the buffer controls in the absence of the potentially interfering substance.

The positive and negative samples for 16 potentially interfering substances were correctly identified. Potentially inhibitory effects were observed in positive samples tested with Nasonex 50% (v/v), Flonase 50% (v/v), and Beconase at 40% (v/v) and 50% (v/v) due to delay in Ct values; however, none of the substances reported false negative test results. No interference was observed in positive samples tested with Nasonex 40% (v/v), Flonase 40% (v/v), and Beconase at 30% (v/v). This is addressed in Section 16, Limitations, in the package insert.

Table 8-6. Potential Interfering Nasal Substances Tested

Substance	**Active Ingredient**	**Concentration Tested**
Mucous (Mucin)	Porcine mucin representing densely glycosylated proteins (mucous)	7% (w/v)
Blood	Blood (Human)	50% (v/v)
Aneferin Decongestant Spray	0.05% Oxymetazoline Hydrochloride	50% (v/v)
Azelastin Antihistamine Spray	0.1% Azelastine Hydrochloride	50% (v/v)
NasalCrom Allergy Symptom	5.2 mg Cromolyn Sodium	50% (v/v)
Neo-Synephrine Decongestant Spray	0.5% Phenylephrine Hydrochloride	50% (v/v)
Saline Nasal Moisturizing Spray	0.65% Sodium Chloride	50% (v/v)

Substance	Active Ingredient	Concentration Tested
Zicam Nasal Gel (Upper Respiratory Allergy Symptom Relief)	4x, 12x, 30x Luffa operculata 12x, 30x Galphimia glauca 12x, 30x, 200x Histaminum hydrochloricum 12x, 30x, 200x Sulphur	50% (v/v)
Nasonex (Nasal Allergy Symptom Medication, inhaled	0.05% Mometasone Furoate Monohydrate	40%(v/v), 50% (v/v) [a]
Flonase	0.05% Fluticasone Propionate	40% (v/v), 50% (v/v) [a]
FluMist	Live intranasal influenza virus vaccine	50% (v/v)
Finafta Multioral	7.5% Benzocaine	50% (v/v)
TobraDex	0.3% Tobramycin, 0.1% Dexamethasone	50% (v/v)
Bactroban	2% Mupirocin	50% (v/v)
Relenza	5 mg Zanamivir	50% (v/v)
Beconase® AQ	0.05% or $3.6x10^{-5}$ g Beclomethasone	30% (v/v), 40% (v/v) [a], 50% (v/v) [a]
Nasacort® AQ	0.06% or $4.4x10^{-5}$ g Triamcinolone acetonide	50% (v/v)
Rhinocort aqua®	0.06% or $4.4x10^{-5}$ g Budesonide	50% (v/v)
Flunisolide Nasal Solution USP, 0.025%	0.03% or $1.9x10^{-5}$ g Flunisolide	50% (v/v)

a. Potential inhibitory effect observed for the concentration tested due to delay in Ct values.

Carry-Over Contamination

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples tested following very high MRSA-positive samples in the same GeneXpert module. The study consisted of a negative sample processed in the same GeneXpert module immediately following a very high positive sample. The MRSA-negative samples were composed of MSSE prepared in a simulated nasal matrix at a concentration $\geq 1.0 \times 10^7$ CFU/mL in the Elution Reagent. The MRSA-positive samples were composed of MRSA in a simulated nasal matrix at a concentration $\geq 1 \times 10^7$ CFU/mL in the Elution Reagent. The testing scheme was repeated 40 times between 2 GeneXpert instruments (one module per instrument) for a total of 41 runs per instrument (20 high positive samples per instrument and 21 negative samples per instrument). All 40 positive samples were correctly reported as **MRSA DETECTED**. All 42 negative samples were correctly reported as **MRSA NOT DETECTED**.

Linearity

Not applicable, the Xpert MRSA NxG Assay is a qualitative assay.

Clinical Studies

Clinical Performance

Performance characteristics of the Xpert MRSA NxG Assay were determined in two separate prospective, multi-site investigational studies using nasal specimens collected from individuals at risk for nasal colonization of methicillin-resistant *S. aureus* (MRSA). In the first study, eight investigational sites within the US and outside of the US tested the Xpert MRSA NxG Assay with nasal swabs collected using the Cepheid Sample Collection Device (Rayon Swab). In the second study, six investigational sites within the US tested the Xpert MRSA NxG Assay with nasal swabs collected using the Liquid Amies Elution Swab (ESwab) Collection and Transport System. Not more than one specimen per subject was included in the studies and analyses.

The Xpert MRSA NxG Assay results were compared to reference culture and susceptibility results.

The comparative reference method consisted of both a direct culture on MRSA selective chromogenic medium and enriched culture. Enrichment of the specimen was performed in Trypticase Soy Broth (TSB) with 6.5% Sodium Chloride followed by subculture of the TSB 6.5% NaCl on to Blood Agar (BA) and MRSA selective chromogenic medium. Identification of presumptive *S. aureus* colonies from BA and MRSA colonies from the selective chromogenic medium plates was confirmed with Gram stain, and catalase and coagulase testing. MRSA was confirmed by susceptibility testing with a Cefoxitin disk (30µg). The reference method result was considered positive for MRSA if the presence of MRSA was confirmed in either direct culture or enriched culture.

Results Obtained with the Xpert MRSA NxG Assay in Comparison to the Reference Method using the Rayon Swab

A total of 1103 eligible Rayon swab specimens were tested by the Xpert MRSA NxG Assay and by the reference method. Relative to the reference method, the Xpert MRSA NxG Assay demonstrated a sensitivity and specificity of 91.0% and 96.9%, respectively (Table 8-7). For the population tested, the MRSA positive predictive value (PPV) was 78.7% and the negative predictive value (NPV) was 98.9%.

Table 8-7: Xpert MRSA NxG Assay with Rayon Swab vs. Reference Method

		Reference Method		
	MRSA	**Positive**	**Negative**	**Total**
Xpert MRSA NxG	Positive	111	30[a]	141
	Negative	11[b]	951	962
	Total	122	981	1103
		Sensitivity:	91.0% (95% CI: 84.6-94.9)	
		Specificity:	96.9% (95% CI: 95.7-97.8)	
		PPV:	78.7% (95% CI: 71.3-84.7)	
		NPV:	98.9% (95% CI: 98.0-99.4)	

a. 30/30 specimens with Xpert MRSA NxG false positive results were also MRSA culture negative upon repeat subculture of the enrichment broth.
b. 11/11 specimens with Xpert MRSA NxG false positive results were MRSA culture positive upon repeat subculture of the enrichment broth.

Results Obtained with the Xpert MRSA NxG Assay in Comparison to the Reference Method using the ESwab

A total of 846 eligible ESwab specimens were tested by the Xpert MRSA NxG Assay and by the reference method. Relative to the reference method, the Xpert MRSA NxG Assay demonstrated a sensitivity and specificity of 92.9% and 97.6%, respectively (Table 8-8). For the population tested, the MRSA positive predictive value (PPV) was 83.5% and the negative predictive value (NPV) was 99.1%.

Table 8-8: Xpert MRSA NxG Assay with ESwab vs. Reference Method

		Reference Method		
	MRSA	**Positive**	**Negative**	**Total**
Xpert MRSA NxG	Positive	91	18[a]	109
	Negative	7[b]	730	737
	Total	98	748	846
		Sensitivity:	92.9% (95% CI: 86.0-96.5)	
		Specificity:	97.6% (95% CI: 96.2-98.5)	
		PPV:	83.5% (95% CI: 75.4-89.3)	
		NPV:	99.1% (95% CI: 98.1-99.5)	

a. 17/18 specimens with Xpert MRSA NxG false positive results were also MRSA culture negative after repeat of subculture of the enrichment broth.
b. 6/7 specimens with Xpert MRSA NxG false negative results were MRSA culture positive after repeat subculture of the enrichment broth.

Results Obtained with the Xpert MRSA NxG Assay in Comparison to the Reference Method for the Rayon Swab and ESwab Combined

Table 8-9 shows the sensitivity and specificity analyses of the combined Xpert MRSA NxG Assay results with Rayon Swab and ESwab relative to the reference method.

Table 8-9: Xpert MRSA NxG Assay with Rayon Swab and ESwab Combined vs. Reference Method[a]

	Reference Method			
	MRSA	**Positive**	**Negative**	**Total**
Xpert MRSA NxG	Positive	202	48	250
	Negative	18	1681	1699
	Total	220	1729	1949
		Sensitivity:	91.8% (95% CI: 87.4-94.8)	
		Specificity:	97.2% (95% CI: 96.3-97.9)	
		PPV:	80.8% (95% CI: 75.5-85.2)	
		NPV:	98.9% (95% CI: 98.3-99.3)	

a. Using the data from Tables 8-7 and 8-8, the Fisher's Exact Test (*p-value* = 0.81 for sensitivity and *p-value* = 0.46 for specificity) demonstrated that the data are poolable across collection devices (Rayon Swab and ESwab).

Reproducibility Study

A panel of five samples with varying concentrations of MRSA was tested four times per day on six different days by two different operators, at three sites (5 samples x 4 times/day x 6 days x 2 operators x 3 sites). Three lots of Xpert MRSA NxG Assay cartridges were used, with each representing two days of testing. The Xpert MRSA NxG Assay was performed according to the Xpert MRSA NxG Assay procedure. Each of the 5 samples was prepared in simulated nasal matrix at the concentration levels in Table 8-10. Results are summarized in Table 8-11.

Table 8-10: Reproducibility Panel

Panel Sample	Concentration Level
Neg	True negative (no target)
ModPos1, MRSA Type XI (*mecC*)	Moderate positive (~2-3x LoD)
LowPos1, MRSA Type XI (*mecC*)	LOD (~1x LoD)
ModPos2, MRSA Type II (*mecA*)	Moderate positive (~2-3x LoD)
LowPos2, MRSA Type II (*mecA*)	LoD (~1x LoD)

Table 8-11: Summary of Reproducibility Results: % Agreement by Study Site/Operator

Sample	Site 1			Site 2			Site 3			% Total Agreement by Sample
	Op 1	Op 2	Site	Op 1	Op 2	Site	Op 1	Op 2	Site	
Neg	100% (24/24	100% (24/24	100% (48/48	100% (24/24	100% (24/24	100% (48/48	100% (24/24	100% (24/24	100% (48/48	100% (144/144
ModPos1	100% (24/24	100% (24/24	100% (48/48	100% (24/24	100% (24/24	100% (48/48	100% (24/24	100% (24/24	100% (48/48	100% (144/144
LowPos1	100% (24/24	100% (24/24	100% (48/48	100% (24/24	100% (24/24	100% (48/48	100% (24/24	100% (24/24	100% (48/48	100% (144/144
ModPos2	100% (24/24	100% (24/24	100% (48/48	100% (24/24	100% (24/24	100% (48/48	100% (24/24	100% (24/24	100% (48/48	100% (144/144
LowPos2	95.8% (23/24	100% (24/24	97.9% (47/48	100% (24/24	100% (24/24	100% (48/48	100% (24/24	95.8% (23/24	97.9 (47/48	98.6% (142/144

The reproducibility of the Xpert MRSA NxG Assay was also evaluated in terms of the fluorescence signal expressed in Ct values for each target detected. The mean, standard deviation (SD), and coefficient of variation (CV) between-sites, between-days, between-lots, between-operators and within-assay for each panel member are presented in Table 8-12.

Table 8-12: Summary of Reproducibility Data[a]

Sample	Assay Channel (Analyte)	N[b]	Mean Ct	Between-Site		Between-Day		Between-Lot		Between-Operator		Within-Assay		Total	
				SD	CV (%)[c]	SD	CV (%)[c]	SD	CV (%)[c]	SD	CV (%)[c]	SD	CV (%)[c]	SD	CV (%)[c]
Neg	SPC	144	32.3	0.0	0.0	0.0	0.0	0.3	0.9	0.3	0.8	0.8	2.3	0.8	2.6
ModPos1	*mec*	144	29.9	0.0	0.0	0.0	0.0	0.4	1.4	0.0	0.0	1.1	3.5	1.1	3.8
	SCC	144	32.6	0.0	0.0	0.0	0.0	0.5	1.5	0.0	0.0	1.0	3.0	1.1	3.3
LowPos1	*mec*	144	31.7	0.0	0.0	0.0	0.0	0.4	1.4	0.0	0.0	1.0	3.2	1.1	3.5
	SCC	144	34.3	0.0	0.0	0.0	0.0	0.5	1.5	0.0	0.0	0.9	2.7	1.1	3.1
ModPos2	*mec*	144	31.2	0.0	0.0	0.3	0.9	0.2	0.5	0.0	0.0	0.9	3.0	1.0	3.1
	SCC	144	32.8	0.0	0.0	0.3	0.8	0.3	1.0	0.0	0.0	0.9	2.7	1.0	3.0
LowPos2	*mec*	144	32.7	0.0	0.0	0.4	1.1	0.0	0.0	0.2	0.6	1.0	3.0	1.1	3.2
	SCC	144	34.4	0.0	0.0	0.4	1.1	0.0	0.0	0.1	0.3	1.0	3.0	1.1	3.3

a. There were a total of 12 indeterminate results over the course of the study (11 reported as "Error" and 1 as "Invalid"). All 12 produced valid test results upon repeat.

b. Results with non-zero Ct values out of 144.

c. (%) is contribution of variance of component to overall CV.

Conclusions

The results of the nonclinical analytical and clinical performance studies summarized above demonstrate that the Xpert MRSA NxG Assay is substantially equivalent to the predicate device.