**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION**
**DECISION SUMMARY**

**A. 510(k) Number:**

K082140

**B. Purpose for Submission:**

To obtain a 510(k) SE determination for a qualitative automated real-time polymerase chain reaction (PCR) assay.

**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:**

Automated real-time polymerase chain reaction (PCR) for unique gene specific sequence amplification of *staphylococcus aureus* (SA) and methicillin-resistant *staphylococcus aureus* (MRSA) DNA from positive blood cultures and fluorogenic target-specific hybridization for the detection of the amplified DNA.

**E. Applicant:**

Cepheid

**F. Proprietary and Established Names:**

Cepheid Xpert™ MRSA/SA Blood Culture Assay

**G. Regulatory Information:**

1. Regulation section:

   21 CFR section 866.1640, Antimicrobial susceptibility test powder

2. Classification:

Class II

3. Product code:

NQX

4. Panel:

Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

The Cepheid Xpert™ MRSA/SA Blood Culture Assay performed on 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) DNA directly from patient positive blood cultures. The assay utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA/SA specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed directly on positive blood culture specimens using BD BACTEC™ Plus Aerobic/F blood culture bottles that are determined as Gram Positive Cocci in Clusters (GPCC) or as Gram Positive Cocci in singles (GPC) by Gram stain. The Cepheid Xpert™ MRSA/SA Blood Culture Assay is not intended to monitor treatment for MRSA/SA infections. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing or for epidemiological typing.

2. Indication(s) for use:

The Cepheid Xpert™ MRSA/SA Blood Culture Assay performed on 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) DNA directly from patient positive blood cultures. The assay utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA/SA specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed directly on positive blood culture specimens using BD BACTEC™ Plus Aerobic/F blood culture bottles that are determined as Gram Positive Cocci in Clusters (GPCC) or as Gram Positive Cocci in singles (GPC) by Gram stain. The Cepheid Xpert™ MRSA/SA Blood Culture Assay is not intended to monitor treatment for MRSA/SA infections. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing or for epidemiological typing.

3. Special conditions for use statement(s):

For 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 Cepheid Xpert™ MRSA/SA Blood Culture Assay system performs real-time, multiplex polymerase chain reaction (PCR) for the amplification and detection of specific DNA targets after an initial sample processing and reagent addition step. 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 DNA targets in about 50 minutes. Each instrument contains 2 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 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 cells/spores, (5) dry real-time PCR reagents, and (6) an integrated PCR reaction tube that can be automatically filled by the instrument. All fluids including amplicons are contained within the disposable cartridge to minimize test-to test contamination. The instrument never comes into contact with any fluids within the cartridge. Each disposable cartridge tests one sample, and is not re-usable.

The Cepheid Xpert™ MRSA/SA Blood Culture Assay includes reagents for the detection of *staphylococcus aureus* and methicillin-resistant *staphylococcus aureus* specific DNA sequences. The primers and probes in the Xpert™ MRSA/SA Blood Culture Assay specifically detect DNA sequences of the staphylococcal protein A (*spa*) (a pair of primers and one FAM labeled TaqMan probe), the gene for methicillin/oxacillin resistance (*mecA*) (a pair of primers and one CF4-3 labeled TaqMan probe), and staphylococcal chromosomal cassette (*SCCmec*) inserted into the *staphylococcus aureus* chromosomal *attB* site (one forward primer and seven reverse primers, and one CF5-6 labeled TaqMan probe). The test also 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 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.

An aliquot of the positive blood culture that is determined as Gram Positive Cocci in Clusters (GPCC) or as Gram Positive Cocci in singles (GPC) by Gram stain is collected and transported to the GeneXpert® area for testing. Using the small transfer pipettes provided with the kit, one drop of the patient positive blood culture specimen is transferred to the elution reagent vial. Following a brief vortex step, the entire contents of the mixed material and two other reagents are transferred to uniquely identified chambers of the cartridge. The cartridge is then loaded into the instrument. The GeneXpert® performs sample preparation by mixing the sample with the SPC (*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.

## J. Substantial Equivalence Information:

1. Predicate device name(s):

   BD Diagnostics' GeneOhm™ Staph SR Assay
   Wellcome Diagnostics' Staphaurex ZL30
   Gibco Laboratories Life Technologies Inc.' Mueller Hinton Agar w/4% NaCl and Oxacillin
   Becton Dickinson & CO' BBL CHROMagar MRSA
   Becton Dickinson & CO' BD Phoenix Automated Microbiology Systems

2. Predicate K number(s):

   (K071026), (K851949), (K863821), (K042812), (K020322) and (K023301)

3. Comparison with predicates:

**Similarities and Differences between the Cepheid Xpert MRSA/SA Blood Culture Assay and the molecular-based predicate device**

| Similarities | | |
|---|---|---|
| **Item** | **Device** | **Predicate** |
| | **Cepheid Xpert™ MRSA/SA Blood Culture Assay** | **BD GeneOhm™ Staph SR Assay (510(k) #k071026)** |
| **Features/Technical Information** | | |
| Intended Use | The Cepheid Xpert™ MRSA/SA Blood Culture Assay performed on 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) DNA directly from patient positive blood cultures. The assay utilizes automated real-time polymerase chain reaction (PCR) for the amplification of MRSA/SA specific DNA targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed directly on positive patient blood culture specimens using BD BACTEC™ Plus Aerobic/F blood culture bottles that are determined as Gram Positive Cocci in Clusters (GPCC) or as Gram Positive Cocci in singles (GPC) by Gram stain. The Cepheid Xpert™ MRSA/SA Blood Culture Assay is not intended to monitor treatment for MRSA/SA infections. Subculturing of positive blood cultures is necessary to recover organisms for susceptibility testing or for epidemiological typing. | The BD GeneOhm™ StaphSR Assay is a qualitative *in vitro* diagnostic test for the rapid detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) directly from positive blood culture. The assay utilizes polymerase chain reaction (PCR) for the amplification of specific targets and fluorogenic target-specific hybridization probes for the real-time detection of the amplified DNA. The assay is performed on Gram positive cocci, identified by Gram stain, from positive blood cultures. The BD GeneOhm™ StaphSR Assay is not intended to monitor treatment for MRSA/SA infections. Subculturing of positive blood cultures is necessary for further susceptibility testing. |
| Qualitative/Quantitative | Qualitative | Qualitative |
| Test Principle | Real-time PCR | Real-time PCR |
| DNA Target Sequence | Sequence incorporating the insertion site (*attB*) of staphylococcal Cassette Chromosome *mec* (SCC*mec*) for detection of MRSA | Sequence incorporating the insertion site (*attB*) of staphylococcal Cassette Chromosome *mec* (SCC*mec*) for detection of MRSA |
| Specimen Type | Positive Blood Culture specimens that contain either Gram Positive Cocci in Clusters (GPCC) or Gram Positive Cocci in singles (GPC), as identified by Gram stain | Positive Blood Culture specimens that contain Gram positive cocci, as identified by Gram stain |

| Differences | | |
|---|---|---|
| **Item** | **Device** | **Predicate** |
| | **Cepheid Xpert™ MRSA/SA Blood Culture Assay** | **BD GeneOhm™ Staph SR Assay (510(k) #k071026)** |
| **Features/Technical Information** | | |
| Test Cartridge | Disposable single-use, multi-chambered fluidic cartridge. | Disposable single-use PCR tube |
| Instrument System | Cepheid GeneXpert Dx System | Cepheid SmartCycler |
| Sample Preparation | Self-contained and automated after mixed specimen and two single-dose reagents are added to cartridge. | Manual |
| Probes | TaqMan Probes | Molecular Beacons |
| Internal Controls | Sample Processing Control (SPC) and Probe Check Control (PCC) | One internal reagent control and external assay positive and negative controls required per PCR run |
| DNA Target Sequence | Sequence specific to methicillin/oxacillin resistance (*mecA*) gene<br><br>Sequence specific to *staphylococcus aureus* species (*spa* gene) | N/A<br><br>Sequence specific to *staphylococcus aureus* species (*nuc* gene) |
| End Users | Operators with no clinical lab experience to experienced clinical laboratory technologists | CLIA High Complexity Laboratory Users |
| Ability to identify correctly "Empty Cassette Variants" | Yes, sequence specific to methicillin/oxacillin resistance (*mecA*) gene | No |
| Rapid test results | Approximately 50 minutes to result. | Approximately 60-75 minutes to results. |

**Similarities and Differences between the Cepheid Xpert MRSA/SA Blood Culture Assay and the Conventional Microbiology-based predicate devices for *staphylococcus aureus* (SA) only**

| **Similarities** | | | |
|---|---|---|---|
| **Item** | **Device** | **Predicates (SA only)** | |
| | **Cepheid Xpert™ MRSA/SA Blood Culture Assay** | **Staphaurex Latex Agglutination Test for SA (510(k) #k851949)** | **BD Phoenix Automated Microbiology System for SA (510(k) #k020322)** |
| **Features/Technical Information** | | | |
| Intended Use | Detection of SA | Same | Same |
| Single Use | Yes | Same | Same |
| Assay Controls | External Positive Control: SA<br>External Negative Control: *S. epidermidis* | Same | Same |

| **Differences** | | | |
|---|---|---|---|
| **Item** | **Device** | **Predicates (SA only)** | |
| | **Cepheid Xpert™ MRSA/SA Blood Culture Assay** | **Staphaurex Latex Agglutination Test for SA (510(k) #k851949)** | **BD Phoenix Automated Microbiology System for SA (510(k) #k020322)** |
| **Features/Technical Information** | | | |
| Mode of Detection | Sequence specific to *staphylococcus aureus* species (*spa* gene) | Clumping factor and Protein A | Microbial utilization and degradation of specific substrates |
| Specimen Type | Direct positive blood culture specimens that contain either Gram Positive Cocci in Clusters (GPCC), Gram Positive Cocci in singles (GPC), or no organisms were seen (NOS), as identified by Gram stain | Cultured isolates of Staphylococcus species | Gram positive organisms |
| Assay Format | Amplification: PCR<br>Detection: Fluorogenic target-specific hybridization | Agglutination with latex particles | Conventional chromogenic and fluorogenic |

| | | | |
|---|---|---|---|
| | | sensitized with fibrinogen and IgG | biochemical tests for identification (ID) and antimicrobial resistance test (AST) |
| Interpretation of Test Results | Diagnostic software of the GeneXpert Dx System | Visual interpretation | Automated |

**Similarities and Differences between the Cepheid Xpert MRSA/SA Blood Culture Assay and the Conventional Microbiology-based predicate devices for methicillin-resistant *staphylococcus aureus* (MRSA)**

| **Similarities** | | | | |
|---|---|---|---|---|
| **Item** | **Device** | **Predicates (MRSA only)** | | |
| | **Cepheid Xpert™ MRSA/SA Blood Culture Assay** | **Mueller Hinton Agar w/4% NaCl and Oxacillin (Oxacillin Screen Agar Test) (510(k) #k863861)** | **BBL CHROMagar MRSA (510(k) #k042812)** | **BD Phoenix Automated Microbiology System (510(k) #k023301)** |
| **Features/Technical Information** | | | | |
| Intended Use | Detection of SA | Same | Same | Same |
| Single Use | Yes | Same | Same | Same |
| Assay Controls | External Positive Control: MRSA<br>External Negative Control: SA | Same | | |

| **Differences** | | | | |
|---|---|---|---|---|
| **Item** | **Device** | **Predicates (MRSA only)** | | |
| | **Cepheid Xpert™ MRSA/SA Blood Culture Assay** | **Mueller Hinton Agar w/4% NaCl and Oxacillin (Oxacillin Screen Agar Test) (510(k) #k863861)** | **BBL CHROMagar MRSA (510(k) #k042812)** | **BD Phoenix Automated Microbiology System (510(k) #k023301)** |

| **Features/Technical Information** | | | | |
|---|---|---|---|---|
| Mode of Detection for methicillin resistance | SCC*mec* gene specific for MRSA<br><br>mecA gene specific for methicillin/oxacillin resistance | Growth on Mueller Hinton Agar with 4% NaCl and 6ug/ml oxacillin | Use of specific Chromogenic substrates and cefoxitin to differentiate MRSA from other organisms | Utilizes a redox indicator for detection of organism growth in the presence of an antimicrobial agent |
| Specimen Type | Direct positive blood culture specimens that contain either Gram Positive Cocci in Clusters (GPCC), Gram Positive Cocci in singles (GPC), or no organisms were seen (NOS), as identified by Gram stain | Pure culture isolate of *staphylococcus aureus* | Swab from Anterior nares | Pure culture isolate of *staphylococcus aureus* |
| Assay Format | Amplification: PCR Detection: Fluorogenic target-specific hybridization | Phenotypic detection based on a 24 hour growth of SA inoculated on media | Phenotypic detection based on a 24-48 hour growth of SA (mauve colonies) inoculated on media | AST panels containing MIC tests for several antimicrobial agents |
| Interpretation of test results | Diagnostic software of the GeneXpert Dx System | Manual: Visual interpretation | Manual: Visual interpretation | Automated |

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

Not applicable.

**L. Test Principle:**

The Cepheid Xpert™ MRSA/SA Blood Culture Assay system performs real-time, multiplex polymerase chain reaction (PCR) for the amplification and detection of specific DNA targets after an initial sample processing and reagent addition step. 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 DNA targets in about 50 minutes. Each instrument contains 2 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.

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## M. Performance Characteristics (if/when applicable):

1. Analytical performance:

*a. Precision/Reproducibility:*

The reproducibility of the Xpert™ MRSA/SA Blood Culture Assay was evaluated using a panel of 10 simulated samples (cultured MRSA and SA bacteria spiked at varying levels to a mixture of human blood and blood culture media which were mixed at the dilution of 10 ml *staphylococcus aureus*- free whole blood per BD BACTEC™ Plus Aerobic/F blood culture bottle) that included high negative (0.1 X the assay LoD), low positive (the assay LoD) and moderate positive (2-3 X the assay LoD) MRSA or SA samples, and a negative sample contained a methicillin-sensitive *staphylococcus epidermidis* strain. The MRSA strains used to prepare the simulated samples were N315 (MRSA1, *SCCmec* type II, USA 100, representing a prevalent healthcare-associated MRSA strain) and MW2 (MRSA2, *SCCmec* type IVa, USA 400, representing a prevalent community-associated MRSA strain). The SA strain used to prepare the simulated samples was N7129. Bacterial dilutions were quantified prior to testing. Panel samples were tested at each of the 3 testing sites twice per day for 10 days using one Xpert™ MRSA/SA Blood Culture Assay lot (10 samples X 2 runs/day X 10 days X 3 sites = 600). The total percent agreement for the Xpert™ MRSA/SA Blood Culture Assay was 100%. The Xpert™ MRSA/SA Blood Culture Assay is a qualitative assay based partially on numerical Cycle Threshold (Ct) values. The overall Ct value %CV across all sites for all samples ranged from 1.3% to 3.6% depending upon analyte type, target type, and concentration tested. This data is acceptable for this device. Detailed reproducibility study results are presented in the following table:

| | Panel Member ID | SA High Negative | SA Low Positive | SA Moderate Positive | MRSA1 High Negative | MRSA1 Low Positive | MRSA1 Moderate Positive | MRSA2 High Negative | MRSA2 Low Positive | MRSA2 Moderate Positive | Neg (MSSE) | Total % Agreement |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Bacterial Concentration | 0.1 X LoD | 1 X LoD | 2-3 X LoD | 0.1 X LoD | 1 X LoD | 2-3 X LoD | 0.1 X LoD | 1 X LoD | 2-3 X LoD | N/A | |
| Site 1 | Agreement with Expected result | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **200/200 (100%)** |
| | Mean *Spa* Ct Value | N/A | 32.93 | 32.25 | N/A | 31.14 | 29.23 | N/A | 30.94 | 29.56 | N/A | |
| | *Spa* Ct % CV | N/A | 2.3% | 2.2% | N/A | 1.9% | 1.2% | N/A | 1.5% | 1.8% | N/A | |
| | Mean *mecA* Ct Value | N/A | N/A | N/A | N/A | 31.37 | 29.48 | N/A | 31.22 | 30.01 | N/A | |
| | *mecA* Ct % CV | N/A | N/A | N/A | N/A | 1.6% | 1.2% | N/A | 1.6% | 1.9% | N/A | |
| | Mean SCC*mec* Ct Value | N/A | N/A | N/A | N/A | 33.13 | 31.15 | N/A | 33.01 | 31.74 | N/A | |
| | SCC*mec* Ct % CV | N/A | N/A | N/A | N/A | 2.0% | 1.3% | N/A | 1.4% | 1.7% | N/A | |
| | Mean SPC (BG) Ct Value | 34.09 | N/A | N/A | 34.58 | N/A | N/A | 34.44 | N/A | N/A | 34.37 | |
| | SPC (BG) Ct % CV | 2.3% | N/A | N/A | 4.8% | N/A | N/A | 2.3% | N/A | N/A | 3.0% | |
| Site 2 | Agreement with Expected result | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **200/200 (100%)** |
| | Mean *Spa* Ct Value | N/A | 32.85 | 31.95 | N/A | 30.62 | 29.03 | N/A | 30.54 | 29.28 | N/A | |
| | *Spa* Ct % CV | N/A | 2.2% | 1.8% | N/A | 1.2% | 1.8% | N/A | 1.5% | 1.4% | N/A | |
| | Mean *mecA* Ct Value | N/A | N/A | N/A | N/A | 30.92 | 29.27 | N/A | 31.02 | 29.62 | N/A | |
| | *mecA* Ct % CV | N/A | N/A | N/A | N/A | 1.5% | 1.4% | N/A | 2.1% | 1.2% | N/A | |
| | Mean SCC*mec* Ct Value | N/A | N/A | N/A | N/A | 32.65 | 30.99 | N/A | 32.65 | 31.37 | N/A | |
| | SCC*mec* Ct % CV | N/A | N/A | N/A | N/A | 1.7% | 1.4% | N/A | 2.2% | 1.4% | N/A | |
| | Mean SPC (BG) Ct Value | 34.39 | N/A | N/A | 34.27 | N/A | N/A | 34.38 | N/A | N/A | 34.35 | |
| | SPC (BG) Ct % CV | 1.5% | N/A | N/A | 2.5% | N/A | N/A | 1.9% | N/A | N/A | 1.6% | |
| Site 3 | Agreement with Expected result | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | 20/20 (100%) | **200/200 (100%)** |
| | Mean *Spa* Ct Value | N/A | 32.57 | 31.81 | N/A | 30.53 | 28.88 | N/A | 30.77 | 29.49 | N/A | |
| | *Spa* Ct % CV | N/A | 1.2% | 1.4% | N/A | 1.0% | 1.2% | N/A | 1.3% | 1.5% | N/A | |
| | Mean *mecA* Ct Value | N/A | N/A | N/A | N/A | 30.78 | 29.23 | N/A | 31.26 | 29.79 | N/A | |
| | *mecA* Ct % CV | N/A | N/A | N/A | N/A | 0.9% | 1.2% | N/A | 1.9% | 1.5% | N/A | |
| | Mean SCC*mec* Ct Value | N/A | N/A | N/A | N/A | 32.47 | 31.04 | N/A | 33.03 | 31.62 | N/A | |
| | SCC*mec* Ct % CV | N/A | N/A | N/A | N/A | 0.9% | 1.7% | N/A | 1.7% | 1.3% | N/A | |
| | Mean SPC (BG) Ct Value | 34.23 | N/A | N/A | 34.57 | N/A | N/A | 34.60 | N/A | N/A | 34.27 | |
| | SPC (BG) Ct % CV | 2.6% | N/A | N/A | 3.3% | N/A | N/A | 2.9% | N/A | N/A | 2.4% | |
| | Total Agreement with Expected result | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | 60/60 (100%) | **600/600 (100%)** |
| | 95% CI | 94.0% - 100% | 94.0% - 100% | 94.0% - 100% | 94.0% - 100% | 94.0% - 100% | 94.0% - 100% | 94.0% - 100% | 94.0% - 100% | 94.0% - 100% | 94.0% - 100% | **99.4% - 100%** |
| | Overall Mean *Spa* Ct Value | N/A | 32.78 | 32.00 | N/A | 30.76 | 29.05 | N/A | 30.75 | 29.44 | N/A | |
| | Overall *Spa* Ct % CV | N/A | **2.0%** | **1.9%** | N/A | **1.7%** | **1.5%** | N/A | **1.5%** | **1.6%** | N/A | |
| | Overall Mean *mecA* Ct Value | N/A | N/A | N/A | N/A | 31.02 | 29.33 | N/A | 31.17 | 29.80 | N/A | |
| | Overall *mecA* Ct % CV | N/A | N/A | N/A | N/A | **1.6%** | **1.3%** | N/A | **1.9%** | **1.6%** | N/A | |
| | Overall Mean SCC*mec* Ct Value | N/A | N/A | N/A | N/A | 32.75 | 31.06 | N/A | 32.90 | 31.57 | N/A | |
| | Overall SCC*mec* Ct % CV | N/A | N/A | N/A | N/A | **1.8%** | **1.5%** | N/A | **1.8%** | **1.5%** | N/A | |
| | Overall Mean SPC (BG ) Ct Value | 34.23 | N/A | N/A | 34.47 | N/A | N/A | 34.47 | N/A | N/A | 34.33 | |
| | Overall SPC (BG ) Ct % CV | **2.2%** | N/A | N/A | **3.6%** | N/A | N/A | **2.4%** | N/A | N/A | **2.4%** | |

*b. Linearity/assay reportable range:*

Not applicable.

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

**Controls**

Internal Controls

The Xpert™ MRSA/SA Blood Culture Assay includes internal controls, including 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 pivotal clinical study data validated the pre-determined SPC Ct cut-offs of 3 to 45.

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 using the Lot Specific Parameters (LSP) determination process.

External Controls

The external controls utilized in the clinical trial consisted of 1 negative and 5 different MRSA positive controls in human blood/simulated wound matrix (concentrated white blood cells resuspended in human plasma, mixed 1:2 with PBS buffer containing 15% glycerol). The negative control is just matrix basepool without bacteria. The MRSA positive controls are 9924 (*SCCmec* type I, 8,000 CFU/tube), 2926 (*SCCmec* type II, 4,300 CFU/tube), 11515 (*SCCmec* type III, 13,300 CFU/tube), 9897 (*SCCmec* type IV, 9,100 CFU/tube), and ST45-MRSA-V (*SCCmec* type V, 13,600 CFU/tube). One negative and one positive external control were run on each day that GeneXpert testing was performed, prior to testing any patient specimens, during the clinical trial. The external positive controls representing the five different *SCCmec* cassette types were rotated daily. Of the 273 negative and positive external controls tested, 99.3% (217/273) of these controls gave correct results. Correct results were obtained after retesting for the remaining 2 external controls.

Performance of ready-to-use external control materials manufactured by MicroBioLogics (St. Cloud, Minnesota) was also evaluated. MicroBioLogics KWIK-STIK™ is a self-contained unit including a single microorganism strain in a lyophilized pellet, a reservoir of hydrating fluid, and inoculating swab. The selected KWIK-STIK™ controls include a negative control containing methicillin-sensitive *staphylococcus epidermidis* cells (MicroBioLogics catalog number 0371; ATCC number 12228), methicillin-sensitive *staphylococcus aureus* cells (MicroBioLogics catalog number 0360; ATCC number 25923), and methicillin-resistant *staphylococcus aureus* cells (MicroBioLogics catalog number 0158;

ATCC number 700699; SCC*mec* type II). Replicates of 20 each of the external controls were run per the manufacturer's instructions using the Xpert™ MRSA/SA Blood Culture Assay. Reproducible performance was observed when using the ready to use KWIK-STIK™ lyophilized reference stock cultures. The sponsor is recommending these external controls to the end users in the package insert.

**Stability**

Assay Reagent Stability

The Xpert™ MRSA/SA Blood Culture Assay reagent stability 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 2-8ºC. The real-time stability testing is ongoing with 3 lots when stored under 5±3ºC, 25±3ºC, 35±3ºC and 45±3ºC at predefined time intervals up to 24 months. 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.

An open package study was conducted to evaluate functional performance after leaving the pouch open for 24 hours and 48 hours at 2-8ºC and 25ºC. It was determined that there was no significant performance difference after leaving the package open for up to 48 hours at 2-8ºC and 24 hours at 25ºC.

Shipping condition studies were conducted to demonstrate that the Xpert™ MRSA/SA Blood Culture Assay package systems were suitable for shipment of assay kits, and the assay performance was not affected after being subjected to specified worst case shipping conditions.

Specimen stability

The recommended maximum time of 4 hours between sample aliquot and Xpert start was verified using the clinical study data stratified in the following 4 categories: 0-1 hour; 1-2 hours; 2-3 hours; and 3-4 hours. Relative to sensitivity and specificity, the Fisher's Exact test showed no statistical difference for either MRSA or SA among any of the four time groups.

Sample stability as a function of time the sample sits in the sample chamber of the GeneXpert cartridge in the GeneXpert instrument for up to 3 hours was established by testing the unprocessed sample remaining in the sample chamber of the cartridge at 0 hour, 0.5 hour, 1 hour, 2 hour and 4 hour time points. One-way ANOVA indicated that there was no

statistically significant performance differences observed when positive blood culture samples were left in the sample chamber of the cartridge in the GeneXpert instrument.

**GeneXpert Dx System Calibrations**

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 1,000 runs per module (current labeling recommendation) by Cepheid.

The data from the thermal calibration study demonstrated that a 2,000 run thermal stability claim can be supported with a predicted failure rate of 0.7%. The data from the optical calibration variation study reveled that the Xpert assays can continue to function effectively even when the instrument optical calibrations are off by as much as +/- 50%. The data from the decay of optical calibration study demonstrated that the average optical calibration change after 2,000 runs ranged from -3.96% to -8.82% for four different optical channels. With a 99% confidence interval, the worst case change ranged from -9.66% to -13.8% after 2,000 runs.

*d. Detection limit:*

The analytical sensitivity (limit of detection or LoD) of the Xpert™ MRSA/SA Blood Culture Assay was determined using quantified (CFU/mL) cultures of 6 isolates and 3 MSSA isolates, serially diluted in a simulated sample matrix consists of a mixture of human blood and blood culture media which were mixed at the dilution of 10 ml *Staphylococcus aureus*- free whole blood per 25 ml BD BACTEC™ Plus Aerobic/F blood culture bottle media. Methicillin-sensitive *Staphylococcus epidermidis* (MSSE) at $10^6$ CFU/ml was also added to the simulated sample matrix to simulate the most common skin contaminant organism. The MRSA isolates used in this study were 64/6146 (*SCCmec* type I, USA 500), N315 (*SCCmec* type II, USA 100, representing a prevalent healthcare-associated MRSA strain), 11373 (*SCCmec* type III, unknown PFGE type), MW2 (*SCCmec* type IVa, USA 400, representing a prevalent community-associated MRSA strain), ST59-MRSA-V (*SCCmec* type V, USA 1000), and HDE288 (*SCCmec* type VI, USA 800). The SA isolates used in this study were N7129 (USA900), 102-04 (USA1200), and 29213 (unknown PFGE type). These well characterized bacterial isolates were obtained from various bacterial strain collections and investigators worldwide, and most of them were typed by pulsed-field gel electrophoresis (PFGE). Simulated specimens were quantified by plate counts prior to testing. Each bacterial isolate was tested in replicates of 20 per concentration of

simulated specimens.

The LoD point estimates were determined using Logistic Regression Analysis, and the 95% upper and lower confidence intervals were determined using maximum likelihood estimates on the logistic model parameters using the large sample variance-covariance matrix. The data are presented in the following tables:

| MRSA Strain ID | *SCCmec* type | PFGE | LoD Point Estimate (CFU/test) | Lower 95% CI (CFU/test) | Upper 95% CI (CFU?test) |
|---|---|---|---|---|---|
| 64/4176 | I | USA500 | 193 | 168 | 235 |
| N315 | II | USA100 | 54 | 47 | 67 |
| 11373 | III | unknown | 88 | 77 | 108 |
| MW2 | IVa | USA400 | 24 | 19 | 33 |
| ST59-MRSA-V | V | USA1000 | 178 | 158 | 209 |
| HDE288 | VI | USA800 | 194 | 175 | 260 |

| MSSA Strain ID | PFGE | LoD Point Estimate (CFU/test) | Lower 95% CI(CFU/test) | Upper 95% CI(CFU/test) |
|---|---|---|---|---|
| N7129 | USA900 | 79 | 69 | 99 |
| 102-04 | USA1200 | 69 | 60 | 88 |
| 29213 | unknown | 75 | 63 | 103 |

The LoD 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. The sponsor has chosen to claim LoDs of 250 CFU/test for MRSA and 100 CFU/test for MSSA.

Due to the enrichment process involved in blood cultures, all positive blood culture specimens contain very high bacterial loads resulting in CFUs per test well above the estimated LoDs of the assay. Therefore, it is not necessary to confirm the estimated LoDs of the assay for this specific intended use.

*e. 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 MRSA isolates. The analytical study was conducted to test MRSA specimens at the claimed 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, $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$ and $1:10^6$). Bacterial cells used in this study were diluted into a simulated sample matrix consists of a mixture of human blood and blood culture media which were mixed at the dilution of 10 ml *Staphylococcus aureus*- free whole blood per 25 ml BD BACTEC™ Plus Aerobic/F blood culture bottle media. Methicillin-

sensitive *Staphylococcus epidermidis* (MSSE) at $10^6$ CFU/ml was also added to the simulated sample matrix. No significant competitive inhibitory effects were observed on the analytical LoD of MRSA *SCCmec* types I, II, III, Iva, V or VI in the presence of competing SA cells at MRSA to SA ratios of less than $1:10^5$. At MRSA to SA ratios of greater than $1:10^5$, the competitive inhibitory effect of SA at the claimed LoD concentrations for all *SCCmec* types is quite evident.

The following warning language was added to the "Interpretation of Results" section of the package insert in light of the results:

> "A False Negative for MRSA (a result of "MRSA NEGATIVE; SA POSITIVE" instead of "MRSA POSITIVE; SA POSITIVE") could be obtained if both MRSA and SA are present in the sample at an MRSA:SA ratio of $1:1x10^6$ or greater."

*f.* ***Analytical inclusivity:***

The analytical inclusivity of the Xpert™ MRSA/SA Blood Culture Assay was evaluated in two studies. The first study evaluated 25 SA and MRSA specimens supplied by CDC as representative of strains currently found in the healthcare community. The second study was performed to evaluate a larger and geographically broader number of SA and MRSA strains selected to broadly represent the range of genetic diversity found in the species *staphylococcus aureus* based on its phylogenetic structure. Selections were made to represent the primary lineages with emphasis placed on the 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.

**Analytical Inclusivity Study on CDC *Staphylococcus aureus* Specimens**

Twenty-five *Staphylococcus aureus* strains from multiple sources provided by the CDC were tested using the Xpert™ MRSA/SA Blood Culture 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 Xpert™ MRSA/SA Blood Culture Assay, except one specimen. Further investigation reveled that the particular specimen was actually mislabeled by the CDC.

**Analytical Inclusivity Study on Expanded Panel of *Staphylococcus aureus* Specimens**

One hundred twenty-one *Staphylococcus aureus* strains (78 MRSA strains and 43 SA strains from 11 countries) were tested using the Xpert™

MRSA/SA Blood Culture Assay. Strains broadly represent Cooper and Feil Groups 1A, 1B, and 2, *SCCmec* types I, II, III, IV, Iva, IVb, IVc, V and VI, 24 sequence types (STs), 83 *spa*-types and 18 clonal complexes (CC). Overnight cultures were grown in Brian Heart Infusion (BHI) and adjusted to 0.5 Mcfarland units (~ 3 x $10^8$ CFU/ml). All strains were tested in triplicate using cultures further diluted one hundred thousand-fold or one million-fold, suggesting theoretically estimated bacterial concentrations of 300 or 3,000 CFU/ml. Bacterial strain identification, PFGE type, *SCCmec* type, *spa*-type, CC and STs were determined by the source supplier.

The Xpert™ MRSA/SA Blood Culture Assay correctly identified 116 of 121 strains. The 5 discordant samples were further characterized by Gram stain (GS), catalase (Cat), tube coagulase (Coag) tests. Methicillin susceptibility was also further assessed by disk diffusion (DD) test using a 30 ug cefoxitin disk and a diameter cut-off of 21/22 mm (disk diffusion results less than 21 mm indicate resistance). Further investigation results indicated that the Xpert™ MRSA/SA Blood Culture Assay correctly identified all 5 initially discordant strains. 5 of 7 suspected "empty cassette variants" defined by the source supplier were correctly reported. Further investigation reveled that the remaining 2 were actually methicillin-resistant. Each of the 12 known USA300 isolates was correctly identified by the assay.

*g. Evaluation of Empty Cassette Variants:*

Twenty-two *Staphylococcus aureus* isolates identified as "empty cassette variants" by the supplier, Rhode Island Hospital, Providence, RI, were tested using the Xpert™ MRSA/SA Blood Culture Assay. Overnight cultures were grown in Brian Heart Infusion (BHI) and adjusted to 0.5 Mcfarland units (~ 3 x $10^8$ CFU/ml). All isolates were tested using cultures further diluted one hundred-fold (high) and one hundred thousand-fold (low), suggesting theoretically estimated bacterial concentrations of 3 x $10^8$ CFU/ml (high) and 3,000 CFU/ml (low) respectively.

All 22 isolates were reported MRSA negative: SA positive at both testing concentrations using the Xpert™ MRSA/SA Blood Culture Assay. These results demonstrated that the Xpert™ MRSA/SA Blood Culture Assay will not likely to report a false positive MRSA result for an "empty cassette variants".

*h. Evaluation of BORSA Strains:*

Seven well characterized borderline oxacillin-resistant *Staphylococcus aureus* (BORSA) isolates (BORSA strains are *mec*A negative, but exhibit an oxacillin minimum inhibitory concentration (MIC) >= 2 and <= 8 ug/ml by a mechanism not completely understood, however infections by these strains can be treated with *B*-lactams.) were received from University of Toronto. These isolates were tested as triplicates at two concentrations using the Xpert™ MRSA/SA Blood Culture Assay. Each of the 7 BORSA strains was streaked for isolation on blood agar. A single colony was chosen from each to inoculate overnight cultures. At 18 hours these tryptic soy broth (TSB) cultures were diluted one hundred-fold (high) and one hundred thousand-fold (low). Dilutions of all cultures were plated in triplicate and were found to range from 255,000 to 1,288,333 CFU/test (high) and 255 to 1,288 CFU/test (low). The BORSA strains were also characterized by Gram stain (GS), catalase (Cat), tube coagulase (Coag) tests. Methicillin susceptibility was further assessed by disk diffusion (DD) test using a 30 ug cefoxitin disk and a diameter cut-off of 21/22 mm (disk diffusion results less than 21 mm indicate resistance). All 7 BORSA isolates were reported MRSA negative: SA positive at both testing concentrations using the Xpert™ MRSA/SA Blood Culture Assay.

These results demonstrated that the Xpert™ MRSA/SA Blood Culture Assay will not report a false positive MRSA result for a BORSA strain. The detailed results of this study are presented in the following table:

| Strain ID | MIC (ug/ml) | Xpert Test Results | Conc. | Mean *spa* Ct | Mean *mecA* Ct | Mean *SCCmec* Ct |
|---|---|---|---|---|---|---|
| MA4 | 4 | MRSA Neg; SA Pos. | High | 20.4 | 0 | 0 |
| MA6 | 4 | MRSA Neg; SA Pos. | High | 20.8 | 0 | 22.7* |
| MA8 | 4 | MRSA Neg; SA Pos. | High | 20.9 | 0 | 0 |
| MA14 | 8 | MRSA Neg; SA Pos. | High | 20.0 | 0 | 0^ |
| MA15 | 4 | MRSA Neg; SA Pos. | High | 20.9 | 0 | 0 |
| MSH7 | 2 | MRSA Neg; SA Pos. | High | 20.2 | 0 | 0^ |
| MSH12 | 8 | MRSA Neg; SA Pos. | High | 20.8 | 0 | 0^ |
| | | | | | | |
| MA4 | 4 | MRSA Neg; SA Pos. | Low | 30.5 | 0 | 0 |
| MA6 | 4 | MRSA Neg; SA Pos. | Low | 31.2 | 0 | 33.3* |
| MA8 | 4 | MRSA Neg; SA Pos. | Low | 30.8 | 0 | 0 |
| MA14 | 8 | MRSA Neg; SA Pos. | Low | 30.4 | 0 | 0 |
| MA15 | 4 | MRSA Neg; SA Pos. | Low | 31.1 | 0 | 0 |
| MSH7 | 2 | MRSA Neg; SA Pos. | Low | 30.8 | 0 | 0 |
| MSH12 | 8 | MRSA Neg; SA Pos. | Low | 31.0 | 0 | 0 |
| | | | | | | |

* Strain MA6 exhibits a *Staphylococcus aureus* "empty cassette" profile (MSSA)
^ 1 of 3 replicates reported a *SCCmec* Ct between 43.6 and 44.1, well above the

cut-off Ct of 36.0.

*i. Analytical specificity:*

The analytical specificity of the Xpert™ MRSA/SA Blood Culture Assay was evaluated by testing a panel consisting of 105 microorganism strains, 98 strains were from the American Type Culture Collection (ATCC) and 7 strains were from the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA), which are phylogenetically related to *Staphylococcus aureus* or potentially encountered in patients with positive blood cultures or in a hospital environment. Of these, 29 strains of methicillin-sensitive coagulase negative staphylococci and 9 strains of methicillin-resistant coagulase negative staphylococci were included. The organisms 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). Stock cultures were prepared by suspending the organism growth from an agar plate in PBS buffer containing 15% glycerol. Each strain was tested using 100 ul of culture adjusted to 1.7 – 3.2 McFarland units. Positive and negative controls (approximately $10^3$ to $10^4$ CFU/test) were also included in the study. At least 2 replicates were run for each strain.

None of the isolates tested were detected by the Xpert™ MRSA/SA Blood Culture Assay. The analytical specificity of the Xpert™ MRSA/SA Blood Culture Assay is 100%.

***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 Xpert™ MRSA/SA Blood Culture Assay.
During clinical testing, 2.3% (7/298) eligible samples generated initial error test results due to PCC setting violations or reported valve motion failures. Upon repeat testing, 6 of the 7 provided valid results and 1 could not be retested due to insufficient reagents available at the site to perform the retest.

**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 (*spa, mecA, SCCmec*) | Manual setting = **36** |
| Maximum Pressure (Max PSI) | Default setting = **120** |

The valid cycle range for the three MRSA targets (*spa, mecA, SCCmec*) was set to 3 to 36 cycles based upon pre-clinical positive blood culture data (n=406) and pre-clinical SA negative blood culture data (n=228) collected during development to maximize percent sensitivity and percent specificity. These cut-offs were subsequently validated in the pivotal clinical study.

The valid cycle range (3 to 45) for the SPC was derived from analytical data generated from MRSA/SA LSP testing of 3 development reagent lots, simulated inhibitory studies with potentially interfering substances including anticoagulated whole blood and blood culture media components containing the anticoagulant sodium polyanetholesulfonate (SPS) or ion exchange and nonionic adsorbent resins (to remove antimicrobials), and pre-clinical SA negative blood culture data (n=228). The cut-off was also subsequently validated in the pivotal clinical study. During clinical testing, 3.7% (11/298) eligible samples generated initial invalid test results due to SPC failure. Upon repeat testing, 10 of the 11 provided valid SA negative results and 1 was 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, no tests (out of a total of 298 eligible samples) exceeded the maximum pressure limit.

***k. Interfering Substances:***

Substances that may be present in blood cultures with potential to interfere with the Xpert™ MRSA/SA Blood Culture Assay were tested in the interfering substance study. Potentially interfering substances include, but are not limited to, anticoagulated whole blood and blood culture media components containing the anticoagulant sodium polyanetholesulfonate (SPS) or ion exchange and nonionic adsorbent resins (to remove antimicrobials). Negative samples (n=8) were tested in each substance to determine the effect on the performance of the sample processing control (SPC). Positive samples (n=8) with MRSA cells spiked near the LoD at 500 CFU/test were also tested in each substance. All results were compared to positive and negative buffer controls.

All negative specimens were correctly reported MRSA negative: SA negative using the Xpert™ MRSA/SA Blood Culture Assay. None of the potentially interfering substances had a statistically significant inhibitory effect on SPC performance in negative samples (p-value = 0.260). All positive specimens were correctly reported MRSA positive; SA positive using the assay. Fisher's exact tests conducted on the data generated with and without these potentially interfering substances demonstrated that their presence did not affect assay performance.

***l. Carry-Over Contamination:***

A study was conducted to demonstrate that single-use, self-contained GeneXpert cartridges prevent carry-over contamination in negative samples run following very high 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 MRSA positive sample (roughly $10^7$ CFU/test). This was repeated 20 times between 2 GeneXpert modules for a total of 42 runs.

There was no evidence of any carry-over contamination. All 21 positive samples were correctly reported MRSA positive: SA positive (mean *spa* Ct 15.0, mean *mecA* Ct 15.0 and mean *SCCmec* Ct 16.8). All 21 negative samples were correctly reported MRSA negative; SA negative (mean SPC Bg Ct 34,2, and mean Cts for all three MRSA targets 0).

2. Comparison studies:

*a. Method comparison with predicate device:*

Not applicable.

*b.* *Matrix comparison:*

Not applicable.

3. Clinical studies:

***a. Prospective Clinical studies***

Performance characteristics of the Xpert MRSA/SA Blood Culture Assay were determined in a multi-site prospective investigational study. Three U.S. clinical sites having Methicillin-resistant *Staphylococcus aureus* (MRSA) culture-based methods already in place participated in the study. Patients were included in the study only if the doctor requested blood culture testing to be performed for routine patient care. All specimens in the study meeting the inclusion and exclusion criteria represented excess, left over material from normal standard of care laboratory testing. All clinical sites were granted waivers of informed consent by their IRB for this study. Inclusion criteria included, but were not limited to: positive blood culture specimen was taken from a BD BACTEC™ Plus Aerobic/F bottle, and Gram Stain contained either Gram Positive Cocci in Clusters (GPCC) or Gram Positive Cocci in singles (GPC). Patients previously enrolled in the study were denied repeat entry.

As a secondary goal of gathering information to potentially identify patients with early MRSA or SA infections that as yet to show any organisms on Gram stain, positive blood culture specimens taken from BD BACTEC™ Plus Aerobic/F bottles that were determined as no organisms were seen (NOS) by Gram stain were also included in the clinical study initially.

Each clinical site's standard-of-care culture that conformed to CLSI M47-A was used as comparative method for assessing the performance of the Xpert MRSA/SA Blood Culture Assay, rather than sending samples to a central lab for culture, since the sub-culturing of positive blood culture bottles after transport and delay had not been validated. The culture methods all consisted of initial analysis on blood agar plate (and chocolate agar, MacConkey and CNA plates) after 24 and 48 hours of incubation using Gram Stain. Presumptive colonies of *Staphylococcus aureus* were confirmed with either tube or slide coagulation testing, latex agglutination assay, or automated identification system. Susceptibility testing was performed on all confirmed *Staphylococcus aureus* colonies at all study sites in accordance with the CLSI documents M2-A9 and M100-S17, using the Cefoxitin disc testing to detect methicillin/oxacillin resistance. (<= 21 mm = resistant; >= 22 mm = susceptible). Fisher's Exact Test was used to determine whether pooling the sensitivity and specificity data from three sites is appropriate. It was determined that the data can be pooled across sites.

Of the Xpert MRSA/SA Blood Culture Assays run on eligible specimens, 92.8% (233/251) of these specimens were successful on the first attempt. The remaining 18 gave indeterminate results on the first attempt (10 "INVALID", 7 "ERROR" and 1 "NO RESULT"). One of the indeterminate specimens could not be retested due to insufficient reagents available at the site to perform the retest (not eligible for inclusion). Of the 17 indeterminate on the first attempt with sufficient sample for retest, 94.1% (16/17) gave a result on the second attempt; one was indeterminate on the second attempt (not eligible for inclusion).

The performance data from all study sites are presented in the following table:

| | | Culture | | | |
|---|---|---|---|---|---|
| | | MRSA+ | SA+/MRSA- | Neg/No Growth | Total |
| Xpert | MRSA+ | 53 | 0 | 0 | 53 |
| | SA+/MRSA- | 0 | 24 | 1 | 25 |
| | SA- | 0 | 0 | 171 | 171 |
| | Total | 53 | 24 | 172 | 249 |
| | | | | | |
| Xpert Performance | MRSA: | | | | |
| | Positive Percent Agreement: 53/53 100% 95% CI: 93.3% - 100%<br>Negative Percent Agreement: 196/196 100% 95% CI: 98.1% - 100% | | | | |
| | SA: | | | | |
| | Positive Percent Agreement: 77/77 100% 95% CI: 95.3% - 100%<br>Negative Percent Agreement: 171/172 99.4% 95% CI: 96.8% - 100% | | | | |

There was a total of 30 positive patient blood culture specimens that were determined as no organisms seen (NOS) by Gram stain included in the clinical study. All of them tested negative (MRSA Neg; SA Neg) by the Xpert MRSA/SA Blood Culture Assay. The culture results of these specimens were all "no growth" except 3 samples {1 Coagulase negative *Staphylococcus* (CNS), 1 yeast and 1 *Candida albicans*}. Therefore, the sponsor agreed to modify language to the labeling to limit the use of the test to patients with Gram positive cocci (alone or in clusters).

Eleven positive blood culture bottles showed mixed cultures by Gram stain. Only 1 positive blood culture bottle containing MRSA or SA showed confirmed mixed cultures. Final culture results and the Xpert MRSA/SA Blood Culture Assay Results for these specimens are presented in the following table:

| Sample ID Number | Gram Stain Result | Culture Result | Cepheid Xpert MRSA/SA Blood Culture Assay Result |
|---|---|---|---|
| BL14099 | GPCC, YST | Staphylococcus coagulase negative; Candida tropicalis | Negative |
| BL14100 | GPC, GPCCH | Alpha hemolytic streptococcus | Negative |
| BL14104 | GPC, GPCCH | Streptococcus pneumoniae | Negative |
| BL14125 | GPC, GPCCH | Alpha hemolytic streptococcus | Negative |
| BL14129 | GPC, GPCCH | Streptococcus agalactiae | Negative |
| BL21116 | GPCC, YST | Staphylococcus coagulase negative; Yeast | Negative |
| BL21118 | GPC, GNB | Alpha streptococcus not Group D; Bacteriodes fragilis | Negative |
| BL21127 | GPC, GNB | Streptococcus bovis; Klebsiella pneumoniae; Staphylococcus coagulase negative | Negative |
| BL21150 | GPC, GPCCH, GNB | Enterococcus species; Gram negative bacilli | Negative |
| BL21161 | GPC, GNB | Enterococcus faecalis; Escherichia coli | Negative |
| BL21162 | GPC, GNB | Enterococcus faecalis, vancomycin resistant; Klebsiella pneumoniae | Negative |
| | | | |
| BL21119 | GPCC | MRSA; Corynebacterium species | MRSA |

The performance of the Xpert MRSA/SA Blood Culture Assay in testing positive blood culture specimens that are mixed culture specimens containing MRSA or SA could not be thoroughly evaluated based on test result from only 1 such specimen in the clinical study. The following standard warning language in the package insert was included to address this issue:

"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."

*b.* *Retrospective Clinical studies*

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 Blood Culture Assay clinical study, a total of 279 blood culture specimens were tested from three 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 following table:

| Age Group | Total N | MRSA BY Culture | | SA (non MRSA) By Culture | |
|---|---|---|---|---|---|
| | | Number Positive | Observed Prevalence | Number Positive | Observed Prevalence |
| 0-20 years | 2 | 0 | 0.0% | 0 | 0.0% |
| 21-30 years | 19 | 1 | 5.3% | 2 | 10.5% |
| 31-40 years | 18 | 2 | 11.1% | 0 | 0.0% |
| 41-50 years | 58 | 12 | 20.7% | 7 | 12.1% |
| 51-60 years | 67 | 16 | 23.9% | 8 | 11.9% |
| 61-70 years | 48 | 13 | 27.1% | 5 | 10.4% |
| >70 years | 37 | 9 | 24.3% | 2 | 5.4% |
| Total | 249 | 53 | 21.3% | 24 | 9.6% |

**N. Instrument Name:**

GeneXpert® Dx System

**O. System Descriptions:**

1. Modes of Operation:

The GeneXpert DX System operates in random access mode. The instrument is available with a four cartridge base, and a 16 cartridge base. The multiple

cartridge bases allow up to 4 and 16 single use cartridge, respectively, to be run simultaneously. Up to four of the four-cartridge 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 _____

3. Specimen Identification:

   User enters Patient ID/Sample ID using hand-held barcode scanner or by typing it in.

4. Specimen Sampling and Handling:

   Not applicable

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 1,000 runs per module (current labeling recommendation) by Cepheid.

   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 Xpert™ MRSA/SA Blood Culture Assay.

6. Quality Control:

   The Xpert™ MRSA/SA Blood Culture Assay includes internal controls, including 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 pivotal clinical study data validated the pre-determined SPC Ct cut-offs of 3 to 45.

   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 using the Lot Specific Parameters (LSP) determination process.

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:**

Not applicable

**Q. Proposed Labeling:**

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

**R. Conclusion:**

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