EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
cobas vivoDx MRSA

DECISION SUMMARY

A. DEN Number:

DEN190016

B. Purpose for Submission:

De Novo request for evaluation of automatic class III designation for the cobas vivoDx MRSA test

C. Measurand:

Bioluminescence produced by viable bacterial cells under selective growth conditions

D. Type of Test:

Bioluminescent assay for the determination of antimicrobial susceptibility

E. Applicant:

Roche Molecular Systems, Inc.

F. Proprietary and Established Names:

cobas vivoDx MRSA

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.1655

2. Classification:

   Class II

3. Product code:

   QIV

4. Panel:

   83 - Microbiology

H. Indications for Use:

1. Indications for use:

   cobas vivoDx MRSA:
   The cobas vivoDx MRSA test performed on the cobas vivoDx System is an automated qualitative *in vitro* diagnostic test for the direct detection of live methicillin-resistant *Staphylococcus aureus* (MRSA) cells in nasal swab samples from patients who are at risk for nasal colonization by MRSA. The test utilizes selective agents and bioparticles (Smarticles technology) to introduce a luciferase gene into targeted bacteria to create an amplified luminescent signal in only viable (live) MRSA cells. The cobas vivoDx MRSA test is intended to aid in the prevention and control of MRSA infections in healthcare settings. It is not intended to diagnose MRSA infections, nor to guide, or monitor treatment. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

   cobas vivoDx MRSA Collection and Transport Kit:
   The cobas vivoDx MRSA Collection and Transport Kit is used to collect, transport and store human nasal swab specimens for use with the cobas vivoDx MRSA test.

2. Special conditions for use statement(s):

   For prescription use only.

   cobas vivoDx MRSA is only for use with nasal samples collected with cobas vivoDx MRSA Collection and Transport Kits.

   Nasal swab specimens for testing with cobas vivoDx MRSA should be processed as soon as possible after collection and should be stored in the cobas vivoDx MRSA Primary Tube for no more than 15 hours at 15-30°C. Analytical studies have shown that there is increased potential for false positive results with specimens containing high levels of MSSA when specimen processing is delayed.

   Valid results must be obtained with appropriately prepared Positive and Negative External Controls for results from the cobas vivoDx MRSA test to be displayed by the system. Follow the directions for preparation of External Controls using the recommended strains. Deviation from the recommended control procedures may lead to erroneous results.

   In analytical studies, high levels of *Staphylococcus equorum*, *Listeria monocytogenes* and the following species of *Enterococcus*: *E. faecium*, *E. flavescens* and *E. gallinarum*, were found to cross react in the cobas vivoDx MRSA test and produce positive results.

   In analytical studies, high levels of *Citrobacter koseri, Corynebacterium jeikeium,*

Enterobacter cloacae, and *Klebsiella pneumoniae* were found to interfere with detection of low levels of MRSA in the cobas vivoDx MRSA test.

Interfering substances that may cause false negative results include but are not limited to:

- Visible blood on the cobas vivoDx MRSA swab or in the primary tube
- Mupirocin

3. Special instrument requirements:

cobas vivoDx system

I. Device Description:

The cobas vivoDx MRSA test, performed on the cobas vivoDx Instrument, is an automated, phenotypic assay for the direct, qualitative detection of MRSA in nasal swab specimens that are collected and transported to the testing laboratory using the cobas vivoDx MRSA Collection and Transport Kit.

Upon receipt in the laboratory, the test operator vortexes the specimen to elute the target organisms (if present) and transfers an aliquot of the transport medium to an MRSA Test Tube containing a dried bead of a selective reagent (cefoxitin) that is solubilized with the sample. The operator then applies a MRSA Reagent Cap to the Test Tube, briefly vortexes the assembled cartridge and loads it onto the cobas vivoDx Instrument for automated processing.

The MRSA Reagent Cap comprises two reagent-filled blisters, one containing *Staphylococcus aureus*-specific bacteriophage-based Smarticles that encode the enzyme luciferase, and the other, a luminescent substrate. The cobas vivoDx Instrument automatically completes incubation of the cartridge, addition of the Smarticles and substrate to the test sample, as well as measurement and analysis of the luminescent signal. Results are reported as "Positive" (MRSA detected) or "Negative" (no MRSA detected).

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

Not applicable.

K. Test Principle:

The cobas vivoDx MRSA test is an automated, phenotypic assay for the qualitative detection of MRSA in nasal swab specimens from patients who are at risk of colonization. The device uses selective bacteriophage-based Smarticle technology to transduce cells of *Staphylococcus aureus* that may be present in nasal swab samples with a reporter plasmid that carries the genes for bacterial luciferase (*luxA/luxB*). The luciferase genes are constitutively expressed in viable *S. aureus* cells, resulting in continuous production of the luciferase enzyme. When substrate is added, the luciferase catalyzes a reaction that results in emission of light which can be detected by the cobas vivoDx system. In the absence of viable cells of *S. aureus*, no luciferase activity is observed.

To discriminate methicillin susceptible and resistant *S. aureus*, after Smarticle transduction,

the nasal swab sample is incubated in the presence of a fixed concentration of cefoxitin. Methicillin susceptible *S. aureus* (MSSA) does not grow under these conditions and therefore does not accumulate luciferase and does not emit light upon addition of substrate. In contrast, methicillin resistant *S. aureus* remains viable, resulting in accumulation of luciferase and emission of a detectable signal upon addition of the luciferase substrate. The results of the test are interpreted automatically by the cobas vivoDx system.

The major components of the cobas vivoDx MRSA test system are summarized in **Table 1**.

Table 1. Major components of the cobas vivoDx MRSA test system

Test Component	Description
cobas vivoDx System	Automated, continuous loading instrument for processing of cobas vivoDx MRSA test cartridges, comprised of the following sub-systems: Transfer Unit • Movement of test cartridges; barcode reading Temperature Control Station • Incubation and shaking of test cartridges Dispense Station • Dispense of Smarticle-containing liquid reagent Measurement Station • Dispense of luciferase substrate and kinetic measurement of luminescence Input/Output Racks • For insertion and removal of test cartridges LED Indicator Interface • To indicate operating status Embedded Computer • Responsible for running the Instrument Control software Tablet Computer • Provides the user interface, result display and interfaces with the LIS External Barcode Reader • Used for sample log-in
cobas vivoDx MRSA Collection and Transport Kit	Sterile collection swab (Puritan Hydraflock) and a primary transport tube containing proprietary medium that is designed to support the viability of MRSA and inhibit growth other organisms that may be present in the sample
cobas vivoDx MRSA	MRSA Reagent Cap • Comprises two separate blister packs containing Smarticles and luciferase substrate that are punctured by the cobas vivoDx system at specific points in the automated workflow • Fitted onto the MRSA Test Tube by the operator after addition of sample MRSA Test Tube • Contains a dried bead of cefoxitin that is rehydrated with the test sample
cobas vivoDx MRSA Control Media	The same proprietary medium as in the cobas vivoDx MRSA Collection and Transport Kit for use in preparation of Positive and Negative External Controls

LED: Light Emitting Diode; LIS: Laboratory Information System

External Positive and Negative Controls comprised of cultured cells of known methicillin susceptible and resistant strains of *S. aureus* must be tested with cobas vivoDx MRSA at a frequency specified by the user. Unless the expected results for both External Controls are obtained, test results from patient samples are not displayed by the cobas vivoDx instrument. Patient samples may be reported as "Positive" (MRSA detected), "Negative" (MRSA not detected), "Invalid" (due to repeated control failure), "Failed" (due to an instrument error) or "Results pending" (display pending receipt of valid External Control results).

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Site-to-Site Reproducibility Study
The between-laboratory reproducibility of the cobas vivoDx MRSA test was evaluated by testing a (b) (4) member panel of samples over a period of (b) (4) at each of (b) (4) sites, using a single lot of reagents and one cobas vivoDx system at each site. (b) (4) operators participated at each site.

Because the cobas vivoDx MRSA test is designed to detect viable organisms, panel members were prepared on each day of testing by serial dilution of bacterial suspensions (b) (4)

The MRSA-positive panel members comprised (b) (4)

(**Table 2**). (b) (4)

Table 2. Strains of MRSA used to evaluate the reproducibility of the cobas vivoDx MRSA test

Characteristic	MRSA Strain			
	NRS (b) (4)		NRS (b) (4)	
PFGE Type				
SCC*mec* Type				
Cefoxitin MIC (µg/mL)				

PFGE: Pulsed Field Gel Electrophoresis
SCC*mec*: Staphylococcal Cassette Chromosome *mec*
MIC: Minimal Inhibitory Concentration

(b) (4)

(Table 3). (b) (4)

(b) (4) These results are acceptable.

Table 3. Summary of results from the cobas vivoDx MRSA Site-to-Site Reproducibility Study

Factor	Agreement by Panel Member (observed range of CFU/mL) [1] (b) (4)
Site	(b) (4)
Day	
Operator	
Overall	(b) (4)

Refer to Table 2 for a detailed description of each panel member

(b) (4)

Lot-to-Lot Reproducibility

The reproducibility of the cobas vivoDx MRSA test between reagent lots was evaluated by testing (b) (4)

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Table 4. Summary of results from the cobas vivoDx MRSA Lot-to-Lot Reproducibility Study

Lot	Agreement by Panel Member (range of CFU/mL) [1]
(b) (4)	(b) (4)
Overall	

(b) (4)

Reproducibility with Borderline Cefoxitin Susceptible/Resistant S. aureus

To challenge the cobas vivoDx MRSA system, an additional Reproducibility Study was conducted using isolates of *S. aureus* that exhibited cefoxitin/oxacillin MICs close to the breakpoints for susceptibility/resistance. The study was conducted at a (b) (4) reagents. Panel members were prepared fresh daily in simulated nasal matrix and organism concentrations were verified by performing viable counts (**Table 5**). Each panel member was tested (b) (4) The results are summarized in **Table 6** and demonstrated acceptable reproducibility for detection of borderline cefoxitin susceptible/resistant *S. aureus* within and between instruments, reagent lots and days.

Table 5. Strains of borderline cefoxitin susceptible/resistant *S. aureus* used to evaluate the reproducibility of the cobas vivoDx MRSA test

Phenotype [1]	Panel Member	Strain [2]	Cefoxitin MIC (µg/mL)	CFU/mL [3]
MRSA [4]	(b) (4)			
MSSA [5]				

MRSA: Methicillin Resistant *S. aureus*; MSSA: Methicillin Susceptible *S. aureus*; MIC: Minimum Inhibitory Concentration

[1] Phenotype based on cefoxitin MIC and *mecA* status (positive/negative); (b) (4))

[2] Obtained from the CDC & FDA Antimicrobial Resistance Isolate Bank

[3] Observed viable counts ranged from (b) (4) CFU/mL (mean = 1,506 CFU/mL) for MRSA and (b) (4) CFU/mL (mean = 1.4 x 10^6 CFU/mL) for MSSA

Table 6. Reproducibility of cobas vivoDx MRSA test performance with borderline cefoxitin susceptible/resistant *S. aureus*

Factor		Agreement by Panel Member and Strain (observed range of CFU/mL) [1]	
		MRSA	MSSA
		(b) (4)	
Reagent Lot	(b) (4)		
Instrument			
Day			
Overall			

MRSA: Methicillin Resistant *S. aureus*; MSSA: Methicillin Susceptible *S. aureus*
Refer to **Table 5** for detailed description of each panel member
[1] To help ensure viability, panel members were prepared fresh each day from standardized bacterial suspensions and viable counts were performed to verify their concentration

b. Linearity/assay reportable range:

Not applicable.

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

External Controls
Instructions for preparation of External Positive and Negative Controls using commercially available strains of *S. aureus* (ATCC 33591 [MRSA] and ATCC 25923 [MSSA]) are provided in the device labeling. Valid results must be obtained with appropriately prepared Positive and Negative External Controls for results from the cobas vivoDx MRSA test to be displayed by the system, although the frequency of control testing is at the discretion of the laboratory based on applicable regulations and accreditation requirements. If either member of a control pair fails, patient test results are withheld until valid External Control results are obtained. If repeated control failure occurs, patient test results are reported as "Invalid."

During the Clinical Study described in **Section L(3)(a)**, (b) (4) pairs of External Quality Control pairs were tested, of which (b) (4) (99.7%) produc lid results.

The following additional studies were also performed to verify the reproducibility of the recommended Quality Control procedures:

Stability of Primary Cultures: Primary culture plates of the recommended External Control strains of *S. aureus* were (b) (4)

(b) (4) using two different lots of cobas vivoDx MRSA reagents. All results were as expected (b) (4) Positive and (b) (4) Negative Control results), demonstrating the stability of primary subcultures of the External Control strains when stored at room temperature for up to four days.

Verification of Positive Control Strain: Fresh secondary and tertiary subcultures of MRSA strain ATCC (b) (4) were used to prepare External Positive Controls according to the recommended procedure. The controls were tested usin (b) (4) All cobas vivoDx MRSA control and phenotypic test results were as expected (b) (4) in each case), demonstrating the reproducibility of suspensions prepared with ATCC (b) (4) External Positive Controls.

Maintenance of Quality Control Strains: Fresh secondary and tertiary subcultures of the recommended strains were used to prepare Positive and Negative External Controls on each day over a period of (b) (4) The controls were tested using two different lots of cobas MRSA reagents (b) (4) . All the Negative External Controls produced the expected results (b) (4) of the Positive External Controls, demonstrating the Reproducibility of External Control performance over the period of the study.

Based on the results of these studies, the instructions for use of the cobas vivoDx MRSA test indicate that only fresh secondary and tertiary cultures should be used to prepare external controls. Primary and secondary cultures may be stored at (b) (4) , respectively, prior to subculture.

The instructions for preparation and maintenance of the cobas vivoDx MRSA test External Positive and Negative Controls and associated performance are acceptable.

Specimen Stability

The stability of nasal swab specimens in cobas vivoDx MRSA Collection and Transport Tubes was evaluated by testing simulated samples that were seeded at clinically relevant concentrations with either MRSA, methicillin susceptible *S. aureus* (MSSA) or *Staphylococcus epidermidis* and which were then stored for different durations. The results of the study demonstrated that nasal swab specimens for use with the cobas vivoDx MRSA test may be transported and stored in cobas vivoDx MRSA Collection and Transport Tubes for up to 15 hours when held at 15-30°C.

The results of the study also showed that, when specimen processing is delayed and high levels of MSSA are present, there is increased potential for false positive results to occur. This is noted as a Limitation in the device labeling (refer to **Section H2**).

Reagent Stability

cobas vivoDx MRSA reagent kits comprised of cobas vivoDx MRSA Reagent Caps and Test Tubes, should be stored at 2-8°C. The cobas vivoDx MRSA Collection and Transport Kit and Control Media should be stored at 15-30°C. Stability testing of the

cobas vivoDx MRSA reagents to establish expiration dating under their respective storage conditions is on-going.

Open Kit Stability
cobas vivoDx MRSA kits containing cobas vivoDx MRSA Reagent Caps and Test Tubes were shown to produce the expected results with simulated samples and controls when stored at 2-8°C for up to 7 days after opening.

d. *Detection Limit:*

The Limit of Detection of the cobas vivoDx MRSA test was determined by testing serial dilutions of five different strains of MRSA in simulated nasal matrix (refer to **Section L(2)(b)**). For each strain, at least 20 replicates were tested at each target level with each of three lots of cobas vivoDx MRSA reagents. The concentration of organisms present in each dilution was confirmed by performing viable counts. The LoD for each strain and reagent lot combination, defined as the lowest target level at which 95% of replicates are expected to produce positive results, was estimated by Probit analysis (**Table 7**). The highest LoD point estimate was 315 CFU/mL for strain NRS 642 with Reagent Lot #2. This value was used as the basis for establishing appropriate target levels for subsequent Analytical Studies.

Table 7. Summary of results from the LoD Study for the cobas vivoDx MRSA test

MRSA Strain [1]	PFGE Type	SCC*mec* Type	Reagent Lot	LoD (CFU/mL) [2]
ATCC BAA-44	Iberian	(b) (4)	(b) (4)	17 (b) (4) (b) (4)
NRS 642	100			(b) (4) 315 (b) (4) (b) (4)
NRS 651	200			(b) (4) 108 (b) (4) (b) (4)
NRS 685	500			(b) (4) 78 (b) (4) (b) (4)
NRS 725	300			(b) (4) 69 ((b) (4) (b) (4)

PFGE: Pulsed Field Gel Electrophoresis; SCC*mec*: Staphylococcal Cassette Chromosome *mec*
Note: The highest predicted LoD for each strain is shaded
[1] The cefoxitin MIC of each strain included in the study was >16 µg/mL
[2] LoD point estimate with 95% confidence intervals
[3] The LoD point estimate for strain NRS 642 with Reagent Lot #2 was used to determine appropriate target levels for subsequent Analytical Studies
[4] Confidence intervals are broad due to a non-monotonic increase in hit rate with organism concentration

In addition to the five strains of MRSA shown in **Table 7**, the analytical sensitivity of the cobas vivoDx MRSA test for the heteroresistant ATCC 43300 strain was also

evaluated. The highest LoD point estimate for this strain was 320 (95% confidence interval: 190-772) CFU/mL with Reagent Lot #1.

e. *Analytical Reactivity:*

The inclusivity of the cobas vivoDx MRSA test was evaluated in two separate Analytical Reactivity Studies as described below:

Testing of Alternative PFGE and SCCmec Types

(b) (4)

A summary of the results of the study is provided in **Table 8**. On initial testing, the cobas vivoDx MRSA test produced (b) (4)

(b) (4)

Table 8. Results of the cobas vivoDx MRSA Inclusivity Study, stratified by SCC*mec* and PFGE type

SCC*mec* Type	PFGE Type	Number	Positive [1]	% Positive
I	Iberian	(b) (4)		
II	USA 100			
	USA 200			
	USA 600			
	N/A			
III	ST239			
	N/A			
IV	Iberian			
	USA 300			
	USA 300-114			
	USA 400			
	USA 500			
	USA 700			
	USA 800			
	USA 1000			
	USA 1100			
IVa	USA 400			
V	WA-MRSA			
	USA 700			
	USA 1000			
VI	USA 600			
IX (*mecC*)	N/A			
Total				

(b) (4)

Borderline Cefoxitin Susceptibility Panel

The second Analytical Reactivity Study included a panel of strains of *S. aureus* obtained from the CDC & FDA Antimicrobial Resistance Isolate Bank that exhibited cefoxitin MICs which were close to the respective breakpoints for susceptibility and resistance (≤4 and ≥8µg/mL). Each strain was initially tested in triplicate in simulated nasal matrix at approximately 1.2 x 10^3 CFU/mL (~4X the highest LoD point estimate for strain NRS 642 (**Table 7**)). Additional testing at higher concentrations was performed for any strain that did not yield 3/3 positive results. Results are summarized in **Table 9**. Thirteen of 22 isolates (59.1%) that were resistant to cefoxitin (MIC 8 to ≥16 µg/mL) were successfully detected at 1.2 x 10^3 CFU/mL and the remaining nine (40.9%) that were not detected (n = 8)

or gave variable results (n = 1) at this level were reported positive by cobas vivoDx MRSA when tested at 1.2 x 10^4 CFU/mL. Of 10 isolates with MICs that were borderline susceptible (cefoxitin MIC = 4 µg/mL), nine (90.0%) were reported as negative by cobas vivoDx MRSA even at the highest target level tested (1.2 x 10^6 CFU/mL). One cefoxitin susceptible isolate produced 2/3 positive results at 1.2 x 10^6 CFU/mL but was reported as negative at all other target levels (1.2 x 10^3 to 1.2 x 10^5 CFU/mL).

Table 9. Summary of results from testing strains of *S. aureus* with borderline cefoxitin susceptibility/resistance

Cefoxitin Phenotype	Cefoxitin MIC (µg/mL)	*mecA*	Number of Strains				
			Total Tested	Positive at Target Level (CFU/mL) [1]			
				1.2 x 10^3	1.2 x 10^4	1.2 x 10^5	1.2 x 10^6
Resistant	8	Positive	8	4	4 [2]	Not done	Not done
	16	Positive	10	6	4	Not done	Not done
	>16	Positive	4	3	1	Not done	Not done
Susceptible	4	Positive	1	0	0	0	0
	4	Negative	9	0	0	0	0 [3]

Cefoxitin breakpoints: ≤4 µg/mL, Susceptible; ≥8 µg/mL, Resistant

[1] Lowest level at which 3/3 replicates reported as positive by the cobas vivoDx MRSA test

[2] One strain that was positive at 1.2 x 10^4 CFU/mL also had 2/3 positive results at 1.2 x 10^3 CFU/mL

[3] One strain produced 2/3 positive results at 1.2 x 10^6 CFU/mL

f. Analytical Specificity:

Cross-reactivity

The analytical specificity of the cobas vivoDx MRSA test was evaluated by testing (b) (4) strains of bacteria in triplicate at a concentration of (b) (4) CFU/mL, depending on the strain. The list of species tested is shown in **Table 10** and included 30 strains of Methicillin Susceptible *S. aureus* (MSSA) with cefoxitin MICs (b) (4) as well as species that are phylogenetically related to *S. aureus* and other organisms that may be found as part of the nasal flora. One yeast species was also tested at a concentration of (b) (4). No false positive results were obtained with any of the strains of MSSA, although cross-reaction was observed with five other organisms, including *Listeria monocytogenes*, *Staphylococcus equorum* and three different species of *Enterococcus* (**Table 11**). In each case, the expected results were obtained when testing of the cross-reactive species was repeated at a lower concentration. The potential for false positive results with organisms other than MRSA is included as a Limitation in the device labeling (refer to **Section H2**).

(b) (4)

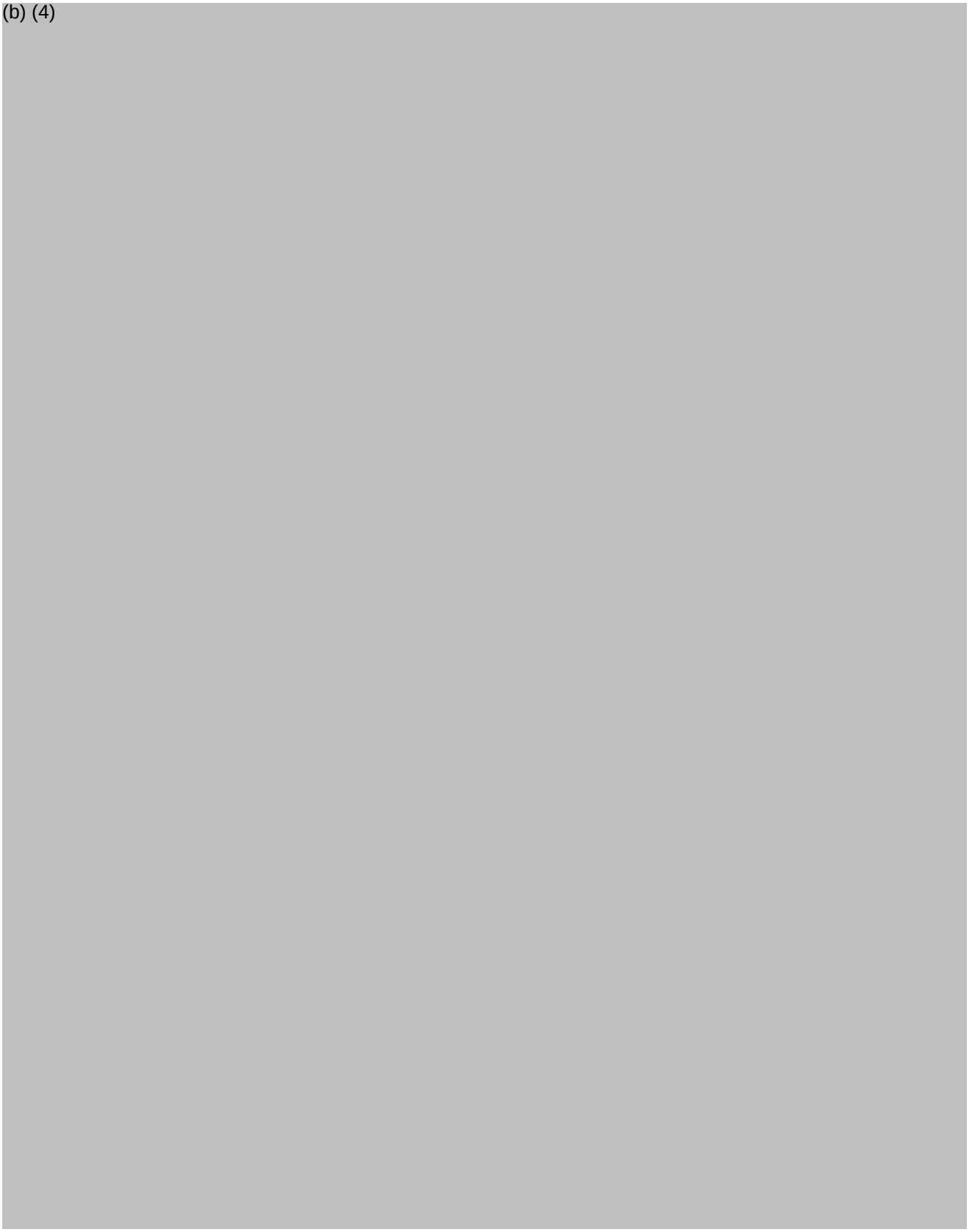

Table 11. Species found to cross-react in the cobas vivoDx MRSA test

Cross-reactive Species	CFU/mL		
	Cross-reactive	Not Cross-reactive [1]	No Interference [2]
Enterococcus faecium	(b) (4)	3.7×10^5	6.1×10^4
Enterococcus flavescens		3.6×10^5	3.4×10^4
Enterococcus gallinarum		1.1×10^5	1.1×10^5
Listeria monocytogenes		7.0×10^4	4.3×10^4
Staphylococcus equorum		4.6×10^3	3.8×10^3

[1] Highest level tested at which no cross-reaction was observed
[2] Highest level tested at which no interference was observed (i.e., no false negative results obtained with low levels of MRSA) [see below]

Microbial Interference

The potential for interference with cobas vivoDx MRSA test was evaluated using the same panel of organisms as for the Cross-reactivity Study described above (**Table 10**), including those that initially produced false positive results (**Table 11**). Each species was tested in triplicate at a concentration of between 3.4×10^5 and 2.1×10^7 CFU/mL in the presence of each of two strains of MRSA at approximately 4X LoD (i.e., ~1.2×10^3 CFU/mL). No interference in the detection of MRSA strain NRS 725 was observed in the presence of any other any species; however, false negative results were obtained when testing was performed with MRSA strain NRS 642 (**Table 12**), although in each case, the expected positive results for MRSA were produced when the interfering organism was tested at a lower level. The potential for false negative results in the presence of high levels of the species listed in **Table 12** is noted as a Limitation in the device labeling (refer to **Section H2**).

Table 12. Species that interfered with detection of MRSA strain NRS 642 by cobas vivoDx MRSA test

Interfering Species	CFU/mL (MRSA positive/tested)	
	Interference [1]	No Interference
Citrobacter koseri	(b) (4)	1.5×10^6 (3/3)
Corynebacterium jeikeium		1.9×10^5 (3/3)
Enterobacter cloacae		1.1×10^6 (3/3)
Klebsiella pneumoniae		3.0×10^4 (3/3)

[1] As determined by reporting of false negative results for MRSA
[2] Highest level tested at which no interference observed (i.e., no false negative results obtained with low levels of MRSA)

For the five species that were found to cross-react in the cobas vivoDx MRSA test when present in high concentration, additional testing was performed to assess the potential for interference with the detection of low levels of MRSA (~1.2×10^3 CFU/mL) when the organisms were at levels below the threshold for cross-reactivity. The expected MRSA positive and negative assay results were obtained when the cross-reactive organisms were tested at the levels indicated in **Table 11**.

Interfering Substances

The potential for interference with the cobas vivoDx MRSA test by endogenous and exogeneous substances that may be present in nasal swab specimens was evaluated. Two strains of MRSA were tested at approximately 4X LoD ((b) (4) CFU/mL) in the presence and absence of 24 substances at different concentrations. The highest concentration each substance at which no interference was observed is shown in **Table 13**. Eight substances did not interfere at any of the concentrations tested. However, false negative results were obtained in the presence of the topical antibacterial agent mupirocin at every concentration that was evaluated. The remaining 15 substances were shown to interfere when present at high concentration but the inhibitory affect was alleviated at lower levels. Because of the potential for interference, subjects with visibly bloody swabs (n = 23) were excluded from the clinical performance evaluation described in **Section L(3)(a)**. The potential for false negative results with the cobas vivoDx MRSA in the presence of mupirocin and blood is noted as a Limitation in the device labeling (refer to **Section H2**).

Table 13. Substances evaluated for potential interference with the cobas vivoDx MRSA test

Substance Type	Substance	Highest Quantity at which No Interference Observed	
		% Swab Capacity	Concentration (mg/mL)
Antibacterial/antiviral	Bepanthen [1]	(b) (4)	40.4
	Mupirocin		Not applicable [2]
	Relenza [1]		73.1
	Tobramycin and Dexamethasone		4.9
Decongestant/allergy relief	Afrin		39.7
	Afrin Menthol		36.1
	Azelastine HCL		38.3
	Beconase AQ		2.3
	ClearLife [1]		74.4
	Dristan		34.6
	Flonase		38.8
	Flunisolide [1]		81.4
	Nasacort		38.5
	NasalCrom [1]		76.3
	Nasonex		39.9
	Neo-Synephrine		37.7
	Nostrilla		37.5
	Otrivine [1]		76.7
	Rhinocort [1]		85.0
	Saline Nasal Spray		19.4
	Zicam		47.5
Endogenous	Bovine Mucin [1, 3]		77.9
	Human Blood [4]		21.3
Topical Analgesic	Anbesol		42.2

(b) (4)

g. *Assay Cut-off:*

The result algorithm for the cobas vivoDx MRSA test uses a series of decision trees to evaluate features of the kinetic luminescent curve obtained after addition of substrate to the test sample. The algorithm was developed using data from known MRSA positive and negative analytical and clinical samples and the cutoff was selected based on Receiver Operating Curve (ROC) analysis to optimize sensitivity and specificity.

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable.

b. *Matrix comparison:*

To provide a sufficient quantity of *S. aureus*-negative material for testing, a simulated throat matrix comprised of human DNA and porcine mucin was used for most of Analytical Studies with the cobas vivoDx MRSA test. A comparison study was therefore conducted to verify that the device performed similarly in the presence of natural and simulated nasal matrix. The study was performed with two strains of MRSA that were each diluted in both matrices to approximately the same concentrations prior to testing. The results of the study are summarized in **Table 14** and demonstrate that the analytical sensitivity of the cobas vivoDx MRSA test for each strain was approximately the same in both types of matrix. Because of this, the use of simulated matrix in Analytical Studies to characterize the performance of the cobas vivoDx MRSA test was determined to be acceptable.

Table 14. Summary of results from comparison of cobas vivoDx MRSA test performance with natural and simulated nasal swab matrix

MRSA Strain	PFGE Type	SCC*mec* Type	Cefoxitin MIC (µg/mL)	Target Level (Multiple of LoD)	Positive/Tested (%)	
					Natural	Simulated
NRS 659	USA 300	IV	>16	(b) (4)		
NRS 663	USA 100	II	>16	(b) (4)		

LoD: Limit of Detection; PFGE: Pulsed Field Gel Electrophoresis; SCC*mec*: Staphylococcal Cassette Chromosome *mec*; MIC Minimal Inhibitory Concentration

3. Clinical studies:

a. Clinical Sensitivity:

The performance of the cobas vivoDx MRSA test was evaluated in a prospective Clinical Study that was conducted at seven geographically diverse locations in the U.S. Subjects aged ≥18 years of age who were eligible for MRSA screening according to the policies of each respective institution were enrolled under informed consent. Each subject provided two nasal swabs: one for the cobas vivoDx MRSA test and one for reference culture. The order of collection of the swabs was alternated between patients.

Of 4,198 paired specimens enrolled in the study, 3,963 were considered evaluable. Forty-two (42) pairs of specimens were excluded for the following reasons: bloody swab (23), subject previously enrolled (15) or met predefined exclusion criteria (3), subject identity unknown (1). The results from a further 193 subjects were excluded for failure to obtain valid reference and/or cobas vivoDx MRSA test results (specimen not processed or processing delayed for the reference method (94), vivoDx system failure (28), laboratory error (26), delay in cobas vivoDx MRSA testing (21), absence of cobas vivoDx MRSA controls (11), cobas vivoDx MRSA result invalid (7), insufficient sample (4), specimens not tested or lost (2)).

Of the 3,963 subjects with evaluable results, 182 (4.6%) were positive for MRSA by direct culture on chromogenic medium. A further 28 subjects were positive (b) (4) resulting in a total of 210 (5.3%) MRSA positive subjects. The identity of suspected colonies of MRSA on (b) (4) was confirmed following (b) (4) The performance of the cobas vivoDx MRSA test in comparison to direct/enriched reference culture and direct culture alone is shown in **Table 15**. The performance stratified by study site is shown in **Table 16**.

Table 15. Performance of the vivoDx MRSA test in comparison to culture

		Direct & Enriched Culture (reference)		
		Positive	**Negative**	**Total**
cobas vivoDx MRSA	**Positive**	189	51 [2]	**240**
	Negative	21 [1]	3702	**3723**
	Total	**210**	**3753**	**3963**
Sensitivity		90.0% (189/210); 85.2-93.4%		
Specificity		98.6% (3702/3753); 98.2-99.0%		
Positive Predictive Value		78.8% (189/240)		
Negative Predictive Value		99.4% (3702/3723)		
		Direct Culture alone		
		Positive	**Negative**	**Total**
cobas vivoDx MRSA	**Positive**	176	64 [3]	**240**
	Negative	6	3717	**3723**
	Total	**182**	**3781**	**3963**
Positive Percent Agreement		96.7% (176/182); 93.0-98.5%		
Negative Percent Agreement		98.3% (3717/3781); 97.8-98.7%		

(b) (4)

Table 16. Performance of the cobas vivoDx MRSA compared to culture, stratified by study site

Study Site	Direct & Enriched Culture (reference)		Direct Culture Alone	
	Sensitivity	Specificity	PPA	NPA
(b) (4)				
All Sites	**90.0% (189/210)** [1]	**98.6% (3702/3753)** [2]	**96.7% (176/182)**	**98.3% (3717/3781)** [3]

PPA: Positive Percent Agreement; NPA: Negative Percent Agreement

[1] 15/21 subjects with false negative cobas vivoDx MRSA test results were MRSA positive only by enriched culture, suggesting the presence of low level colonization (<10 CFU/mL based on the theoretical limit of detection of the direct culture method)

[2] 13/51 subjects with false positive cobas vivoDx MRSA test results compared to the Reference Method were MRSA positive by alternative methods (i.e., PCR and/or nonselective direct and enrichment culture)

[3] 26/64 subjects with false positive cobas vivoDx MRSA test results compared to Direct Culture were MRSA positive by alternative methods (i.e., PCR and/or nonselective direct and enrichment culture)

b. Clinical specificity:

Refer to **Section L(3)(a)**, above.

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:

The performance of the cobas vivoDx MRSA assay was evaluated in a multicenter prospective Clinical Study that was performed with nasal swab specimens collected at seven geographically diverse sites in the U.S., as described in **Section L(3)(a)**, above. An analysis of the prevalence of MRSA at each study site as determined by the reference

direct and enriched culture method and the vivoDx MRSA test is shown in **Table 17**. Overall, the prevalence of MRSA nasal colonization was determined to be 6.1% by the vivoDx MRSA test and 5.1% by the reference culture method.

Table 17. Prevalence of MRSA as determined by the reference culture method and vivoDx MRSA test, stratified by study site

Study Site	Number of Specimens	Number Positive (% Prevalence)	
		Reference Culture	cobas vivoDx MRSA
(b) (4)			
All Sites Combined	**3963**	**210 (5.3)**	**240 (6.1)**

M. Instrument Name:

cobas vivoDx system

N. System Descriptions:

1. Modes of Operation:

   Manual loading/unloading of samples, followed by automated analysis and compilation of the test report.

2. Software:

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

   Yes ___X___ No _____

3. Specimen Identification:

   The identity of the patient is entered manually using the computer keyboard. Additional information may be uploaded from the Laboratory Information System (if connected). The Primary Tube, Test Tube and Reagent Cap are identified by a unique patient-specific barcode that is printed automatically and affixed by the operator.

4. Specimen Sampling and Handling:

   Nasal swab specimens for evaluation with the cobas vivoDx MRSA test must be collected and transported to the laboratory using the cobas vivoDx MRSA Collection and Transport Kit, comprised of the cobas vivoDx Nasal Swab and a Primary Tube

containing a specifically formulated transport medium. Upon receipt of the specimen in the laboratory, the test operator vortexes the Primary Tube to elute the sample from the swab and transfers an aliquot of the transport medium to a cobas vivoDx MRSA Test Tube. After applying a cobas vivoDx MRSA Reagent Cap, the assembled test cartridge is loaded into the cobas vivoDx Instrument for automated processing.

5. Calibration:

   User calibration of the cobax vivoDx Instrument is not required. Preventative maintenance by trained service personnel is required twice a year.

6. Quality Control:

   Refer to **Section L(1)(c)**.

O. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable.

P. Proposed Labeling:

The labeling supports the decision to grant the De Novo request for this device.

Q. Identified Potential Risks and Required Mitigation Measures:

Identified Potential Risks	Mitigation Measures
Failure to use the device correctly	Certain labeling information identified in special controls (1) and (3) Certain design verification and validation identified in special control (4)(vii)
False positive or negative results	Certain labeling information identified in special controls (1) and (3) Use of certain specimen collection and transport devices identified in special control (2) Certain design verification and validation identified in special control (4)
Failure to interpret results correctly	Certain labeling information identified in special controls (1) and (3) Certain design verification and validation identified in special control (4)(vii)

R. Benefit/Risk Analysis:

Summary of the Assessment of Benefit

Healthcare-associated infections caused by MRSA are associated with high morbidity and mortality. Asymptomatic colonization is relatively common and serves as a reservoir for transmission. MRSA decolonization has been shown to reduce carriage, transmission and subsequent infection among inpatients.

Several *in vitro* diagnostic devices for the detection of MRSA colonization are commercially available. These include growth-based devices that require prolonged incubation, as well as molecular devices that provide results within a few hours. The cobas vivoDx MRSA test provides an alternative to conventional culture for phenotypic detection of viable MRSA in nasal swab specimens with the benefit of reduced time-to-result. Although urgent action is not required in response to testing for MRSA colonization, such timely provision of results will support effective response by healthcare facilities attempting to control the spread of MRSA.

In a prospective Clinical Study, the performance of the cobas vivoDx MRSA test for the detection of nasal colonization with viable MRSA bacteria was shown to be comparable to that of the reference culture method.

Summary of the Assessment of Risk

The risks associated with this device are primarily those associated with false positive and false negative results.

False positive results may lead to unnecessary or inappropriate treatment for MRSA colonization with potential harm to the patient due to complications or adverse events associated with the decolonization regimen. Complications of MRSA decolonization therapy include the potential for development of antimicrobial resistance to the agents used for treatment (e.g., mupirocin and chlorhexidine) and drug-related toxicities. False positive results may also lead to unnecessary implementation of infection control measures, while at the same time diminishing the quality of care by decreasing the frequency and duration of interaction between patients and healthcare workers, which induces adverse psychological effects on patients.

False negative results may lead to failure to detect MRSA colonization and prevent implementation of appropriate decolonization procedures and/or infection control measures. Unrecognized colonization with MRSA increases the likelihood of its spread to other body sites, within the environment, among patients and healthcare workers, and thereby increases the potential for infection.

Nasal colonization with MRSA may be transient in some patients and failure to detect the organisms in nasal samples cannot rule out the potential for colonization of other body sites or the presence of MRSA at levels below the limit of detection of the test method. Therefore, a negative cobas vivoDx MRSA test result cannot definitively preclude colonization. In addition, although acquisition of the *mecA/mecC* gene and expression of a mutated form of penicillin binding protein, PBP2a, is currently the predominant mechanism of oxacillin resistance in *S. aureus*, other resistance mechanisms exist or may evolve. Changes in the

preponderance of these mechanisms over time may affect the performance of the cobas vivoDx MRSA test, as well as that of other methods used to detect MRSA.

Summary of the Assessment of Benefit-Risk

General controls are insufficient to mitigate the risks associated with the device. However, the probable clinical benefits outweigh the probable risks for the assay, considering the mitigation of the risks provided for in the special controls. The proposed intended use and assay labeling will facilitate accurate implementation of the assay and interpretation of results. Use of appropriate specimen collection devices will help ensure the accuracy of test results by maintaining microbial viability prior to testing. Design verification and validation will confirm that the device functions as intended and that appropriate quality control procedures have been implemented to enable monitoring of performance.

S. Conclusion:

The De Novo request is granted and the device is classified under the following, subject to the special controls identified in the letter granting the De Novo request:

Device Type: System for detection of microorganisms and antimicrobial resistance using reporter expression.

Class: II (special controls)

Regulation: 21 CFR 866.1655

Product Code: QIV