DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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Silver Spring, MD 20993-0002

ROCHE MOLECULAR SYSTEMS, INC.
WILK VON GUSTEDT, PH.D.
SR. REGULATORY SPECIALIST II
4300 HACIENDA DRIVE
PLEASANTON CA 94588-2722

December 17, 2014

Re: K142721
Trade/Device Name: cobas MRSA/SA Test
Regulation Number: 21 CFR 866.1640
Regulation Name: Antimicrobial susceptibility test powder
Regulatory Class: II
Product Code: NQX, OOI
Dated: September 22, 2014
Received: September 23, 2014

Dear Dr. von Gustedt:

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

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

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

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

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

Sincerely yours,

Uwe Scherf -S for

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

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

510(k) Number *(if known)*
k142721

Device Name
cobas® MRSA/SA Test

Indications for Use *(Describe)*
The cobas® MRSA/SA Test on the cobas® 4800 system, is a qualitative in vitro diagnostic real-time PCR assay, for the direct detection of methicillin-resistant Staphylococcus aureus (MRSA) and S.aureus (SA) DNA from nasal swabs to aid in the prevention and control of MRSA and SA infections in healthcare settings. The cobas® MRSA/SA Test is not intended to diagnose, guide or monitor treatment for MRSA or SA infections, or provide results of susceptibility to methicillin. A negative result does not preclude MRSA/SA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiology typing or for further susceptibility testing.

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

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

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

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510(k) Summary

Submitter Name	Roche Molecular Systems, Inc.
Address	4300 Hacienda Drive Pleasanton, CA 94588-2722
Contact	Wilk von Gustedt Phone: (925) 730-8046 FAX: (925) 225-0207
Date Prepared	September 18, 2014
Proprietary Name	cobas® MRSA/SA Test
Common Name	MRSA/SA Test
Classification Name	Antimicrobial susceptibility test powder. (21 CFR 866.1640) Real Time Nucleic Acid Amplification System (21 CFR 862.2570)
Product Codes	NQX: System, Nucleic Acid amplification Test, DNA, Methicillin Resistant Staphylococcus aureus, Direct Specimen OOI: Real Time Nucleic Acid Amplification System
Predicate Devices	Roche Lightcycler MRSA, k091409
Establishment Registration	Branchburg: 2243471 Pleasanton: 3004141078 Indianapolis: 1823260

1. DEVICE DESCRIPTION

The Roche Molecular Systems (RMS) **cobas**® MRSA/SA Test utilizes real-time polymerase chain reaction (PCR) for the detection of Methicillin resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus aureus* (SA) DNA from nasal swab specimens collected in MSwab medium to aid in the prevention and control of MRSA and SA infections in healthcare settings.

The **cobas**® MRSA/SA Test contains two major processes: (1) automated sample preparation to extract nucleic acids from the nasal swab specimens; (2) PCR amplification of target DNA sequences using MRSA and SA specific primers, and real-time detection of cleaved fluorescent-labeled MRSA and SA specific oligonucleotide detection probes. An Internal Control (IC), containing unrelated randomized DNA sequence, is added to all samples prior to automated sample preparation and is amplified and detected simultaneously with each sample to monitor the entire process.

The MSwab Collection, Transport and Preservation System (Copan Flock Technologies S.r.l.) is used for specimen collection, transportation and storage of specimen for the **cobas**® MRSA/SA Test.

The **cobas**® MRSA/SA Test utilizes six reagent kits**:**

1. **cobas**® 4800 MRSA/SA Amplification/Detection Kit
2. **cobas**® 4800 MRSA/SA Controls and Cofactor Kit
3. **cobas**® 4800 System Wash Buffer Kit
4. **cobas**® 4800 System Lysis Kit 1
5. **cobas**® 4800 System Internal Control Kit 1
6. **cobas**® 4800 System Sample Preparation Kit

1.1. Target Selection

The well-characterized Right Extremity (RE) junction between the Staphylococcus aureus orfX gene and SCCmec cassette carrying the mecA drug-resistant gene was chosen to be the target to specifically detect MRSA. The capsular polysaccharide enzyme CAP5N (CPE) gene was chosen as the target for Staphylococcus aureus identification. This gene is conserved in Staphylococcus aureus and present in both methicillin resistant and methicillin sensitive organisms. Internal Control (IC) DNA sequence is unique and unrelated to either MRSA or SA target sequences.

1.2. Test Principle

1.1.1. Sample Preparation

Sample preparation for the **cobas**® MRSA/SA Test is automated with the use of the **cobas x** 480 instrument. Organisms in nasal swab specimens collected in MSwab medium are lysed with chaotropic agent, proteinase K, and SDS reagents. Released nucleic acids, along with added Internal Control DNA, are bound by magnetic glass particles. They are washed and then eluted into a small volume of buffer. The instrument then takes an aliquot of the eluted material and sets up the PCR reaction with an activated Master Mix.

1.1.2. PCR Amplification and TaqMan® Detection

The PCR cycling steps and detection of target signal occurs in the **cobas z** 480 Analyzer. The Master Mix reagent contains primer pairs and probes for orfX (MRSA), CPE (SA) and IC targets. If the target nucleic acid sequences are present, amplification with the corresponding primers will occur by a thermostable DNA polymerase, generating PCR products (amplicons). These products are detected by specific TaqMan probes containing a fluorescent dye and a quencher. Normally, the quencher suppresses the fluorescence of the dye. However, if the PCR product is present, the probe hybridizes to the product and gets cleaved by the 5’ to 3’ nuclease activity of the polymerase. This reaction allows the fluorescence to be emitted from the dye, and the signal is recorded in real time during each PCR cycle by the **cobas z** 480 analyzer. The signal is interpreted by the **cobas**® 4800 System Software and reported as final results.

1.3. cobas® 4800 System Description

The **cobas**® 4800 System uses the **cobas x** 480 Instrument for sample preparation, and the **cobas z** 480 Analyzer for amplification and detection. Both the **cobas x** 480 Instrument and the **cobas z** 480 Analyzer are controlled by a computer workstation running the **cobas**® 4800 System Software.

The system hardware is unchanged from that originally approved for IVD use in PMA P100020 (**cobas**® HPV Test, April 19, 2011). The software version has been updated to software release 2.1 in order to support the expanded test menu. The updated software was cleared for other currently available tests on the **cobas**® 4800 System per Special 510(K) 140887.

2. INDICATIONS FOR USE

The **cobas**® MRSA/SA Test on the **cobas**® 4800 system, is a qualitative *in vitro* diagnostic real-time PCR assay, for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and *S.aureus* (SA) DNA from nasal swabs to aid in the prevention and control of MRSA and SA infections in healthcare settings. The **cobas**® MRSA/SA Test is not intended to diagnose, guide or monitor treatment for MRSA or SA infections, or provide results of susceptibility to methicillin. A negative result does not preclude MRSA/SA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiology typing or for further susceptibility testing.

3. TECHNOLOGICAL CHARACTERISTICS

As indicated in Table 1, the RMS **cobas**® MRSA/SA Test has the same general intended use as the predicate device. The subject device is substantially equivalent from its technological characteristics to the currently legally marketed predicate device.

Differences reside in sample collection and preparation. The candidate test utilizes an automated extraction process while the predicate uses a manual process. However both devices are similar in the method used for extraction of nucleic acids from specimens, using glass beads together with Lysis buffer. The additional (SA) target detected by the **cobas**® MRSA/SA test uses the same technological methodology as used for detection of the MRSA target.

Table 1: Similarities and Differences between the cobas® MRSA/SA Test and the Predicate Device

	Submitted Device: RMS cobas® MRSA/SA Test	**Predicate Device: Lightcycler® MRSA Advanced Test K091409**
Similarities		
Intended Use	The **cobas®** MRSA/SA Test on the **cobas®** 4800 system, is a qualitative *in vitro* diagnostic real-time PCR assay, for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and *S.aureus* (SA) DNA from nasal swabs to aid in the prevention and control of MRSA and SA infections in healthcare settings. The **cobas®** MRSA/SA Test is not intended to diagnose, guide or monitor treatment for MRSA or SA infections, or provide results of susceptibility to methicillin. A negative result does not preclude MRSA/SA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiology typing or for further susceptibility testing.	The LightCycler® MRSA Advanced Test is a qualitative in vitro diagnostic test for the direct detection of nasal colonization with methicillin-resistant (MRSA) to aid in the prevention and control of MRSA infections in healthcare settings. The test is performed on the LightCycler® 2.0 Instrument with nasal swab specimens from patients suspected of colonization, uses swab extraction and mechanical lysis for specimen preparation followed by polymerase chain reaction (PCR) for the amplification of MRSA DNA, and fluorogenic target specific hybridization probes for the detection of the amplified DNA. The LightCycler® MRSA Advanced Test is not intended to diagnose, guide or monitor treatment for MRSA infections. Concomitant cultures are necessary to recover organisms for epidemiology typing or for further susceptibility testing.
Conditions for use	For prescription use	Same
Sample Types	Nasal swab	Same
Amplification Technology	Real-time PCR	Same
Detection Chemistry	paired target-specific hybridization probes using Förster resonance energy transfer (FRET)	Same
Controls used	Sample processing control (IC) Positive and negative control	Same
Analyte Target	SCC*mec* cassette Right Extremity (RE) junction of methicillin-resistant *Staphylococcus aureus* (MRSA)	Same target region for MRSA detection

	Submitted Device: RMS cobas® MRSA/SA Test	Predicate Device: Lightcycler® MRSA Advanced Test K091409
Differences		
Sample Collection Devices	Copan MSwab Collection, Transport and Preservation System	Liquid Stuart Swabs, Amies Gel with Charcoal Swabs, Amies Gel without Charcoal Swabs
Analyte Target	wt *Staphylococcus aureus* (SA)	methicillin-resistant *Staphylococcus aureus* (MRSA) only
Sample Preparation Procedure	**Automated by cobas® x480**	Lysis of the nasal swab specimens is performed by using the LightCycler® Advanced Lysis Kit and the MagNA Lyser Instrument.
Result Analysis	Based on PCR cycle threshold analysis	Melting peak analysis

In summary, the intended use and technological characteristics of the **cobas**® MRSA/SA Test as compared to the predicate device do not raise any new types of safety or effectiveness questions and are substantially equivalent.

4. NON-CLINICAL PERFORMANCE EVALUATION

4.1. Analytical Sensitivity

The analytical sensitivity (Limit of Detection or LOD) for the **cobas**® MRSA/SA Test was determined by analyzing 2 MRSA culture isolates and 1 SA culture isolate. Quantified cultures were diluted to 8-9 concentration levels into a simulated nasal swab background matrix to determine the LOD. All levels were analyzed using **cobas**® MRSA/SA Test with 3 unique lots of MRSA/SA specific reagents. The LOD of the test was determined as the lowest concentration exhibiting at least 95% positive rate for which all higher concentrations were greater than or equal to 95% positive rate.

The highest LOD among 3 reagent lots are shown in Table 2. The highest claimed LOD between the 2 MRSA strains tested was 700 CFU/swab and for SA it was 700 CFU/swab based on 95% positive rate.

Table 2: cobas® MRSA/SA Test Limit of Detection

Isolate Name	Source ID	RE type	SCCmec type	spa type	PFGE type	MIC value	LOD (CFU/swab)
MRSA 10364	NARSA 384	2	IVa	t008	USA300-0114	32	650
MRSA 8065	ATCC 43300	2	II	t007	Sac-15	n/a	700
SA 10851	NARSA 164	n/a	n/a	t084	n/a	n/a	700

4.2. Genotype LOD verification

The sensitivity of the **cobas®** MRSA/SA Test was determined for 35 additional MRSA culture isolates and 5 additional SA culture isolates. The genotype LOD panel consisted of at least three concentrations per isolate in simulated nasal swab background matrix. The LOD was calculated as the lowest concentration level with ≥ 95 % positive rate for which all higher concentration levels show ≥ 95 % positive rate. A minimum of one lot of reagents was used for this study.

The genotype LOD study results are listed in Table 3 and Table 4. As shown in Table 3, the LOD is ranging from 175 to 750 CFU/swab for the 35 MRSA isolates tested. The cobas MRSA/SA Test detected the RE types 1, 2, 3, 4, 6, 9, 11, 14, 24 and 25. The cobas MRSA/SA Test detected MRSA SCC*mec* types I, II, III, IV, V, VI and VIII, as well as MRSA PFGE types USA 100 to 1000. Five SA isolates tested represent 5 different *spa* types, and the LOD is 175 CFU/swab for all isolates (Table 4).

Table 3: MRSA isolate LOD Results

Isolate #	RE Type	SCCmec Type	*spa* Type	Cefoxitin MIC Value* (µg/mL)	PFGE Type	LOD (CFU/Swab)
1	11	new	t002	≥8	Unknown	485
2	6	II	t242	≥8	Unknown	720
3	9/11	new	t024	≥8	Unknown	175
4	14	Unknown	Unknown	Unknown	Unknown	700
5	25	Unknown	t003	≥8	Unknown	175
6	6	II	t216	≥8	USA100	720
7	2	IV	t008	≥8	USA300	350
8	2	II	t037	≥8	USA200	700
9	2	IV	t1578	≥8	USA300	700
10	2	II	t002	≥8	USA100	720
11	2	IV	t008	≥8	USA800	750
12	2	IV	t008	≥8	USA300	266
13	2	IV	t064	≥8	USA500	260
14	2	IV	t148	≥8	USA700	700
15	2	IV	t688	≥8	USA800	271
16	2	IV	t688	≥8	USA300	700
17	2	II	t042	≥8	USA100	463
18	2	II	t018	≥8	USA200	350
19	2	IV	t008	≥8	USA300	410
20	2	IV	t008	≥8	USA300	175
21	2	IV	t5576	≥8	USA800	202
22	2	II	t004	≥8	USA600	350
23	2	IV	t216	≥8	USA1000	350
24	2	IV	t064	≥8	Iberian	175
25	2	II	t266	≥8	USA600	700
26	2	IV	t008	≥8	USA300	700
27	2	IV	t008	≥8	USA300	350
28	2	IV	t002	≥8	USA800	350
29	3	V	t242	≥8	USA1000	350
30	24	new	t476	≥8	Unknown	350
31	1	I	t149	≥8	Unknown	175
32	3	VIII	Unknown	≥8	Unknown	700
33	4	IV	Unknown	≥8	Unknown	350
34	2	III	t030	≥8	Unknown	700
35	25	VI	Unknown	Unknown	Unknown	175

*Isolates 1,2,5-10, 12-29, 31 and 34 have Cefoxitin MIC values ≥32 µg/mL; isolates 3,11 and 32 have Cefoxitin MIC values of ≥16 µg/mL; isolate 33 has an Cefoxitin MIC value of 12 µg/mL; isolate 30 has an Cefoxitin MIC value of 8 µg/mL.

Table 4: SA Isolates LOD Results

SA Isolate #	spa Type	LOD (CFU/Swab)
1	t238	175
2	t018	175
3	t008	175
4	t002	175
5	t088	175

4.3. Geographical Inclusivity

In addition to 37 MRSA isolates and 6 SA isolates tested in analytical LOD and genotype LOD studies shown above, the inclusivity was also examined by testing a broad collection of MRSA and SA isolates from diverse geographical locations. Two hundred and eighty-one (281) MRSA from 16 European countries or regions, US, Japan and Australia and 85 SA isolates from diverse locations within US were tested. The MRSA collection contained MRSA isolates of different SCCmec types (I, II, III, IV, IVa, V, VI, VII, and new), and 71 *spa* types and cefoxitin MIC value from 6 to greater than 256. All 85 SA isolates sourced from the US contains 75 *spa* types were detected by the cobas® MRSA/SA Test. The geographical inclusivity study results for MRSA isolates are listed in Table 5. Of the two hundred eighty-one MRSA isolates, two hundred seventy-seven of the MRSA isolates were detected. The four MRSA isolates not detected by the **cobas**® MRSA/SA Test were sequenced, and the results suggested that the target regions contained sequences not recognized by the primers and probes in the **cobas**® MRSA/SA Test. One of the four isolates was a mec ALGA251 (also known as mec C) strain.

Table 5: Geographical Coverage of the cobas® MRSA/SA Test for MRSA Isolates

Geographical Origin	Total Number of MRSA Isolates	Detected by cobas® MRSA/SA Test
England	58	58
Germany	51	51
Denmark	37	36
France	33	31
US	20	20
Spain	20	20
Switzerland	18	18
Japan	15	15
Sweden	7	7
Australia	6	5
Netherlands	5	5
Italy	4	4
Belgium	3	3
Scotland	2	2
Ireland	1	1
Norway	1	1
Total	**281**	**277**

4.4. Precision

In-house precision study was conducted with 2 MRSA culture isolates and 1 SA culture isolate a concentrations below, near and above Limit of Detection (LOD) of the **cobas**® MRSA/SA Test. The study used three unique lots of **cobas**® MRSA/SA Test reagents and three instruments for a total of 36 runs over 12 days (3 runs per day). A description of the precision panels, the study results, and variance components are shown in Table 6. An analysis of the variance of the Ct values from valid tests was performed on positive panel members above LOD concentrations. Overall, most of the variation in the **cobas**® MRSA/SA Test was attributed to random factors (41.2% - 44.9%) with contribution from reagent lot, kit size and run/day of testing (Table 7). The overall CV% of Ct values for **cobas**® MRSA/SA Test was less than or equal to 1.3% indicating good reproducibility (Table 8).

Table 6: In-House Precision Study Positive Rate Analysis

		Result interpretation						
Target	**Target Concentration**	**NEG MRSA; NEG SA**	**NEG MRSA; POS SA**	**POS MRSA; POS SA**	**Total Sample Tested**	**%Detected**	**2-sided 95% Lower CL**	**2-sided 95% Upper CL**
Negative	No analyte	72	0	0	72	0.0%	0.0%	5.0%
MRSA Culture Isolate 10364 (NARSA 384)	< 1.0 x LOD	0	15	57	72	79.2%	68.0%	87.8%
	~ 1.0 x LOD	0	0	72	72	100.0%	95.0%	100.0%
	~ 3.0 x LOD	0	0	72	72	100.0%	95.0%	100.0%
MRSA Culture Isolate 8065 (ATCC 43300)	< 1.0 x LOD	0	9	63	72	87.5%	77.6%	94.1%
	~ 1.0 x LOD	0	0	72	72	100.0%	95.0%	100.0%
	~ 3.0 x LOD	0	0	72	72	100.0%	95.0%	100.0%
SA Culture Isolate 10851 (NARSA 164)	< 1.0 x LOD	4	67	0	71*	94.4%	86.2%	98.4%
	~ 1.0 x LOD	0	72	0	72	100.0%	95.0%	100.0%
	~ 3.0 x LOD	0	72	0	72	100.0%	95.0%	100.0%

*One of 72 samples tested could not be processed due to sample pipetting error on the instrument.

Table 7: Variance Components Analysis of MRSA and SA Ct for cobas® MRSA/SA Test

			Variance Components/Percent Contribution to Total					
Target	**N**	**Mean Ct**	**Lot**	**Kit Size**	**Instrument**	**Run/Day**	**Random**	**Total**
MRSA Culture Isolate 10364 (NARSA 384)	72	36.9	0.057	0.003	0.027	0.057	0.101	0.244
			23.2%	1.2%	11.1%	23.3%	41.2%	100.0%
MRSA Culture Isolate 8065 (ATCC 43300)	72	38.0	0.003	0.024	0.007	0.010	0.037	0.082
			4.1%	29.6%	8.9%	12.5%	44.9%	100.0%
SA Culture Isolate 10851 (NARSA 164)	72	35.6	0.050	0.023	0.007	0.032	0.082	0.193
			25.9%	11.7%	3.6%	16.5%	42.3%	100.0%

Table 8: Standard Deviation Components/CV Percent for cobas® MRSA/SA Test

			Standard Deviation Components/CV Percent					
Target	**N**	**Mean Ct**	**Lot**	**Kit Size**	**Instrument**	**Run/Day**	**Random**	**Total**
MRSA Culture Isolate 10364 (NARSA 384)	72	36.9	0.238	0.054	0.165	0.239	0.317	0.494
			0.6%	0.1%	0.4%	0.6%	0.9%	1.3%
MRSA Culture Isolate 8065 (ATCC 43300)	72	38.0	0.058	0.156	0.086	0.102	0.192	0.287
			0.2%	0.4%	0.2%	0.3%	0.5%	0.8%
SA Culture Isolate 10851 (NARSA 164)	72	35.6	0.224	0.150	0.084	0.178	0.286	0.440
			0.6%	0.4%	0.2%	0.5%	0.8%	1.2%

4.5. Analytical Specificity

Analytical specificity of the cobas® MRSA/SA Test was examined by testing non-MRSA/SA microorganisms commonly present in nasal flora, including coagulase negative and positive Staphylococcus species, as well as human cells in the presence or absence of MRSA and SA targets. For the MRSA target, SA organisms including BORSA strains and “empty cassette variants” (also known as “mec A drop outs”) were also tested at high concentration to examine any cross-reactivity and/or interference with MRSA detection.

Table 9 shows the result for Coagulase-Negative Staphylococcus (CoNS) and Methicillin-Resistant Coagulase-Negative Staphylococcus (MR-CoNS) Organisms. Table 10 summarizes results of microorganisms commonly found in nasal flora including human cell line HCT-15.

All target negative samples generated negative MRSA and SA results, demonstrating that the cobas® MRSA/SA Test does not cross-react with these microorganisms and human DNA. None of the 135 organisms and the human cells interfered with the detection of both MRSA and SA targets by the **cobas**® MRSA/SA Test.

Table 11 presents the analytical specificity results in presence of 2 SA isolates, 10 BORSA isolates and 16 mecA dropouts.

The **cobas**® MRSA/SA Test did not generate false negative and false positive results for MRSA in the presence of 2 SA isolates, 10 BORSA, and 13 of 16 mecA dropouts.

Table 9: Analytical Specificity for Coagulase-Negative Staphylococcus (CoNS) and Methicillin-Resistant Coagulase-Negative Staphylococcus (MR-CoNS) Organisms

	Organisms	Strain/ATCC ID	No target		MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		SA 10851 (NARSA 164)	
			MRSA	SA	MRSA	SA	MRSA	SA	MRSA	SA
1	*Staphylococcus arlettae*	ATCC43957	NEG	NEG	POS	POS	POS	POS	NEG	POS
2	*Staphylococcus auricularis* *(Methicillin-resistant)*	ATCC33753	NEG	NEG	POS	POS	POS	POS	NEG	POS
3	*Staphylococcus caprae* *(Methicillin-resistant)*	ATCC35538	NEG	NEG	POS	POS	POS	POS	NEG	POS
4	*Staphylococcus captis*	ATCC35661	NEG	NEG	POS	POS	POS	POS	NEG	POS
5	*Staphylococcus carnosus*	ATCC51365	NEG	NEG	POS	POS	POS	POS	NEG	POS
6	*Staphylococcus chromogenes*	ATCC43764	NEG	NEG	POS	POS	POS	POS	NEG	POS
7	*Staphylococcus cohnii*	ATCC35662	NEG	NEG	POS	POS	POS	POS	NEG	POS
8	*Staphylococcus delphini*	MayoClinicH18859**	NEG	NEG	POS	POS	POS	POS	NEG	POS
9	*Staphylococcus epidermidis* *(Methicillin-resistant)*	ATCC14990	NEG	NEG	POS	POS	POS	POS	NEG	POS
10	*Staphylococcus epidermidis* *(Methicillin-resistant)*	ATCC35547	NEG	NEG	POS	POS	POS	POS	NEG	POS
11	*Staphylococcus epidermidis* *(Methicillin-resistant)*	ATCC35983	NEG	NEG	POS	POS	POS	POS	NEG	POS
12	*Staphylococcus epidermidis* *(Methicillin-resistant)*	ATCC35984	NEG	NEG	POS	POS	POS	POS	NEG	POS
13	*Staphylococcus epidermidis* *(Methicillin-resistant)*	ATCC51624	NEG	NEG	POS	POS	POS	POS	NEG	POS

	Organisms	Strain/ATCC ID	No target		MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		SA 10851 (NARSA 164)	
			MRSA	SA	MRSA	SA	MRSA	SA	MRSA	SA
14	*Staphylococcus epidermidis (Methicillin-resistant)*	ATCC51625	NEG	NEG	POS	POS	POS	POS	NEG	POS
15	*Staphylococcus epidermidis*	ATCC700583	NEG	NEG	POS	POS	POS	POS	NEG	POS
16	*Staphylococcus epidermidis (Methicillin-resistant)*	ATCC27676	NEG	NEG	POS	POS	POS	POS	NEG	POS
17	*Staphylococcus equorum*	ATCC43958	NEG	NEG	POS	POS	POS	POS	NEG	POS
18	*Staphylococcus felis*	ATCC49168	NEG	NEG	POS	POS	POS	POS	NEG	POS
19	*Staphylococcus gallinarum*	ATCC35539	NEG	NEG	POS	POS	POS	POS	NEG	POS
20	*Staphylococcus haemolyticus (Methicillin-resistant)*	ATCC29968	NEG	NEG	POS	POS	POS	POS	NEG	POS
21	*Staphylococcus haemolyticus*	ATCC29970	NEG	NEG	POS	POS	POS	POS	NEG	POS
22	*Staphylococcus haemolyticus*	ATCC43252	NEG	NEG	POS	POS	POS	POS	NEG	POS
23	*Staphylococcus hominis*	ATCC25615	NEG	NEG	POS	POS	POS	POS	NEG	POS
24	*Staphylococcus hominis*	ATCC35982	NEG	NEG	POS	POS	POS	POS	NEG	POS
25	*Staphylococcus hominis subsp. Hominis*	ATCC27844	NEG	NEG	POS	POS	POS	POS	NEG	POS
26	*Staphylococcus hominis subsp. Hominis*	ATCC27845	NEG	NEG	POS	POS	POS	POS	NEG	POS
27	*Staphylococcus intermedius*	ATCC29663	NEG	NEG	POS	POS	POS	POS	NEG	POS
28	*Staphylococcus kloosii*	ATCC43959	NEG	NEG	POS	POS	POS	POS	NEG	POS
29	*Staphylococcus lentus*	ATCC29070	NEG	NEG	POS	POS	POS	POS	NEG	POS
30	*Staphylococcus lugdunensis*	ATCC49576	NEG	NEG	POS	POS	POS	POS	NEG	POS
31	*Staphylococcus pasteuri*	ATCC51129	NEG	NEG	POS	POS	POS	POS	NEG	POS
32	*Staphylococcus pseudointermedius*	DSMZ21284**	NEG	NEG	POS	POS	POS	POS	NEG	POS
33	*Staphylococcus pulvereri*	ATCC51698	NEG	NEG	POS	POS	POS	POS	NEG	POS

	Organisms	Strain/ATCC ID	No target		MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		SA 10851 (NARSA 164)	
			MRSA	SA	MRSA	SA	MRSA	SA	MRSA	SA
34	*Staphylococcus saprophyticus*	ATCC15305	NEG	NEG	POS	POS	POS	POS	NEG	POS
35	*Staphylococcus schleiferi* *(subspecies coagulans)*	ATCC43808	NEG	NEG	POS	POS	POS	POS	NEG	POS
36	*Staphylococcus sciuri*	ATCC49575	NEG	NEG	POS	POS	POS	POS	NEG	POS
37	*Staphylococcus simulans* *(Methicillin-resistant)*	ATCC27848	NEG	NEG	POS	POS	POS	POS	NEG	POS
38	*Staphylococcus simulans*	ATCC11631	NEG	NEG	POS	POS	POS	POS	NEG	POS
39	*Staphylococcus warneri*	ATCC27836	NEG	NEG	POS	POS	POS	POS	NEG	POS
40	*Staphylococcus warneri* *(Methicillin-resistant)*	ATCC27839	NEG	NEG	POS	POS	POS	POS	NEG	POS
41	*Staphylococcus warneri*	RMSCC 1224**	NEG	NEG	POS	POS	POS	POS	NEG	POS
42	*Staphylococcus xylosus*	ATCC35663	NEG	NEG	POS	POS	POS	POS	NEG	POS
43	*Staphylococcus xylosus*	ATCC29971	NEG	NEG	POS	POS	POS	POS	NEG	POS

*RMSCC indicates Roche Culture Collection ID, MayoClinic indicates MayoClinic collection ID, DSMZ indicates DSMZ Penzburg collection ID

Table 10: Analytical Specificity for Non MRSA/SA Microorganisms and Human Cells

	Organisms	Strain/ATCC ID	No target		MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		SA 10851 (NARSA 164)	
			MRSA	SA	MRSA	SA	MRSA	SA	MRSA	SA
1	*Acinetobacter baumannii*	ATCC19606	NEG	NEG	POS	POS	POS	POS	NEG	POS
2	*Acinetobacter haemolyticus*	ATCC17906	NEG	NEG	POS	POS	POS	POS	NEG	POS
3	*Bacillus cereus*	ATCC13472	NEG	NEG	POS	POS	POS	POS	NEG	POS
4	*Bordetella bronchioseptica*	ATCC19395	NEG	NEG	POS	POS	POS	POS	NEG	POS
5	*Bordetella parapertussis*	ATCC15311	NEG	NEG	POS	POS	POS	POS	NEG	POS
6	*Bordetella pertussis*	ATCC9797	NEG	NEG	POS	POS	POS	POS	NEG	POS
7	*Burkholderia cepacia*	ATCC25416	NEG	NEG	POS	POS	POS	POS	NEG	POS
8	*Candida albicans*	ATCC10231	NEG	NEG	POS	POS	POS	POS	NEG	POS
9	*Candida glabrata*	ATCC2001	NEG	NEG	POS	POS	POS	POS	NEG	POS
10	*Candida parapsilosis*	ATCC22019	NEG	NEG	POS	POS	POS	POS	NEG	POS
11	*Candida tropicalis*	ATCC750	NEG	NEG	POS	POS	POS	POS	NEG	POS
12	*Chlamydia pneumoniae*	CDC-CWL-011 strain	NEG	NEG	POS	POS	POS	POS	NEG	POS
13	*Citrobacter freundii*	ATCC8090	NEG	NEG	POS	POS	POS	POS	NEG	POS
14	*Citrobacter koseri*	ATCC27028	NEG	NEG	POS	POS	POS	POS	NEG	POS
15	*Corynebacterium amycolatum*	ATCC49368	NEG	NEG	POS	POS	POS	POS	NEG	POS
16	*Corynebacterium bovis*	ATCC7715	NEG	NEG	POS	POS	POS	POS	NEG	POS
17	*Corynebacterium flavescens*	ATCC10340	NEG	NEG	POS	POS	POS	POS	NEG	POS
18	*Corynebacterium genitalium*	ATCC33030	NEG	NEG	POS	POS	POS	POS	NEG	POS
19	*Corynebacterium glutamicum*	ATCC13032	NEG	NEG	POS	POS	POS	POS	NEG	POS
20	*Corynebacterium jeikeium*	ATCC43734	NEG	NEG	POS	POS	POS	POS	NEG	POS
21	*Cryptococcus neoformans*	ATCC32719	NEG	NEG	POS	POS	POS	POS	NEG	POS

	Organisms	Strain/ATCC ID	No target		MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		SA 10851 (NARSA 164)	
			MRSA	SA	MRSA	SA	MRSA	SA	MRSA	SA
22	*Eikenella corrodens*	ATCC23834	NEG	NEG	POS	POS	POS	POS	NEG	POS
23	*Enterobacter aerogenes*	ATCC13048	NEG	NEG	POS	POS	POS	POS	NEG	POS
24	*Enterobacter cloacae*	ATCC13047	NEG	NEG	POS	POS	POS	POS	NEG	POS
25	*Enterococcus flavescens*	ATCC49996	NEG	NEG	POS	POS	POS	POS	NEG	POS
26	*Enterococcus gallinarum*	ATCC49573	NEG	NEG	POS	POS	POS	POS	NEG	POS
27	*Enterococcus hirae*	ATCC8043	NEG	NEG	POS	POS	POS	POS	NEG	POS
28	*Escherichia coli*	ATCC11775	NEG	NEG	POS	POS	POS	POS	NEG	POS
29	*Finegoldia magna*	RMSCC 974**	NEG	NEG	POS	POS	POS	POS	NEG	POS
30	*Haemophilus aphrophilus*	ATCC19415	NEG	NEG	POS	POS	POS	POS	NEG	POS
31	*Haemophilus influenzae*	ATCC33391	NEG	NEG	POS	POS	POS	POS	NEG	POS
32	*Haemophilus parainfluenzae*	ATCC33392	NEG	NEG	POS	POS	POS	POS	NEG	POS
33	*Issatchenkia orientalis*	ATCC6258	NEG	NEG	POS	POS	POS	POS	NEG	POS
34	*Klebsiella oxytoca*	ATCC33496	NEG	NEG	POS	POS	POS	POS	NEG	POS
35	*Klebsiella pneumoniae (KPC producing)*	ATCC700603	NEG	NEG	POS	POS	POS	POS	NEG	POS
36	*Klebsiella pneumoniae (KPC producing)*	ATCC BAA1900	NEG	NEG	POS	POS	POS	POS	NEG	POS
37	*Lactobacillus crispatus*	ATCC33820	NEG	NEG	POS	POS	POS	POS	NEG	POS
38	*Lactobacillus delbrueckii*	ATCC12315	NEG	NEG	POS	POS	POS	POS	NEG	POS
39	*Legionella pneumophila*	ATCC33152	NEG	NEG	POS	POS	POS	POS	NEG	POS
40	*Leifsonia aquatica (formerly known as Corynebacterium aquaticum)*	ATCC14665	NEG	NEG	POS	POS	POS	POS	NEG	POS
41	*Listeria monocytogenes*	ATCC15313	NEG	NEG	POS	POS	POS	POS	NEG	POS
42	*Microbacterium testaceum*	ATCC15829	NEG	NEG	POS	POS	POS	POS	NEG	POS

	Organisms	Strain/ATCC ID	No target		MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		SA 10851 (NARSA 164)	
			MRSA	SA	MRSA	SA	MRSA	SA	MRSA	SA
43	*Micrococcus luteus*	ATCC4698	NEG	NEG	POS	POS	POS	POS	NEG	POS
44	*Moraxella catarrhalis*	ATCC8176	NEG	NEG	POS	POS	POS	POS	NEG	POS
45	*Mycobacterium tuberculosis avirulent*	ATCC25177	NEG	NEG	POS	POS	POS	POS	NEG	POS
46	*Mycoplasma pneumoniae*	ATCC15531	NEG	NEG	POS	POS	POS	POS	NEG	POS
47	*Mycoplasma salivarium*	ATCC23064	NEG	NEG	POS	POS	POS	POS	NEG	POS
48	*Neisseria meningitidis*	ATCC13077	NEG	NEG	POS	POS	POS	POS	NEG	POS
49	*Parvimonas micra*	ATCC33270	NEG	NEG	POS	POS	POS	POS	NEG	POS
50	*Pasteurella aerogenes*	ATCC27883	NEG	NEG	POS	POS	POS	POS	NEG	POS
51	*Planococcus maritimus*	RMSCC11454**	NEG	NEG	POS	POS	POS	POS	NEG	POS
52	*Proteus mirabilis*	ATCC29906	NEG	NEG	POS	POS	POS	POS	NEG	POS
53	*Proteus vulgaris*	RMSCC204**	NEG	NEG	POS	POS	POS	POS	NEG	POS
54	*Providencia stuartii*	ATCC22914	NEG	NEG	POS	POS	POS	POS	NEG	POS
55	*Pseudomonas aeruginosa*	ATCC33584	NEG	NEG	POS	POS	POS	POS	NEG	POS
56	*Pseudomonas fluorescens*	ATCC11250	NEG	NEG	POS	POS	POS	POS	NEG	POS
57	*Rhodococcus equi*	ATCC6939	NEG	NEG	POS	POS	POS	POS	NEG	POS
58	*Rothia mucilaginosa*	ATCC25296	NEG	NEG	POS	POS	POS	POS	NEG	POS
59	*Salmonella enterica subsp. Enterica (formerly known as Salmonella typhimurium)*	RMSCC374*	NEG	NEG	POS	POS	POS	POS	NEG	POS
60	*Serratia marcescens*	ATCC8100	NEG	NEG	POS	POS	POS	POS	NEG	POS
61	*Shigella sonnei*	ATCC29930	NEG	NEG	POS	POS	POS	POS	NEG	POS
62	*Streptococcus agalactiae*	RMSCC983**	NEG	NEG	POS	POS	POS	POS	NEG	POS
63	*Streptococcus anginosus*	ATCC12395	NEG	NEG	POS	POS	POS	POS	NEG	POS

	Organisms	Strain/ATCC ID	No target		MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		SA 10851 (NARSA 164)	
			MRSA	SA	MRSA	SA	MRSA	SA	MRSA	SA
64	*Streptococcus mitis*	ATCC33399	NEG	NEG	POS	POS	POS	POS	NEG	POS
65	*Streptococcus mutans*	ATCC25175	NEG	NEG	POS	POS	POS	POS	NEG	POS
66	*Streptococcus pneumoniae*	ATCC33400	NEG	NEG	POS	POS	POS	POS	NEG	POS
67	*Streptococcus pyogenes*	ATCC12344	NEG	NEG	POS	POS	POS	POS	NEG	POS
68	*Streptococcus salivarius*	ATCC7073	NEG	NEG	POS	POS	POS	POS	NEG	POS
69	*Streptococcus sanguinis*	ATCC10556	NEG	NEG	POS	POS	POS	POS	NEG	POS
70	*Streptococcus suis*	ATCC43765	NEG	NEG	POS	POS	POS	POS	NEG	POS
71	*Yersinia enterocolitica*	ATCC9610	NEG	NEG	POS	POS	POS	POS	NEG	POS
72	*Adenovirus Type 7*	VR-7	NEG	NEG	POS	POS	POS	POS	NEG	POS
73	*Adenovirus Type 40*	Dugan (VR-40)	NEG	NEG	POS	POS	POS	POS	NEG	POS
74	*Coronavirus 229E*	VR-740	NEG	NEG	POS	POS	POS	POS	NEG	POS
75	*Coronavirus OC43*	VR-1558	NEG	NEG	POS	POS	POS	POS	NEG	POS
76	*Cytomegalovirus*	AD-169 (VR-538)	NEG	NEG	POS	POS	POS	POS	NEG	POS
77	*Epstein Barr Virus*	B95-8	NEG	NEG	POS	POS	POS	POS	NEG	POS
78	*HSV 1*	MacIntyre (VR-539)	NEG	NEG	POS	POS	POS	POS	NEG	POS
79	*Human Adenovirus type 1*	VR-1	NEG	NEG	POS	POS	POS	POS	NEG	POS
80	*Human enterovirus 71*	VR-1775	NEG	NEG	POS	POS	POS	POS	NEG	POS
81	*Human metapneumovirus*	Peru6-2003	NEG	NEG	POS	POS	POS	POS	NEG	POS
82	*Influenza A/H1N1*	A/PR/8/34 (VR-95)	NEG	NEG	POS	POS	POS	POS	NEG	POS
83	*Influenza A/H3N2 A/HongKong/8/68*	H3N2	NEG	NEG	POS	POS	POS	POS	NEG	POS
84	*Influenza B*	N/A	NEG	NEG	POS	POS	POS	POS	NEG	POS
85	*Measles virus*	N/A	NEG	NEG	POS	POS	POS	POS	NEG	POS
86	*Mumps virus*	Enders (VR-106)	NEG	NEG	POS	POS	POS	POS	NEG	POS

	Organisms	Strain/ATCC ID	No target		MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		SA 10851 (NARSA 164)	
			MRSA	SA	MRSA	SA	MRSA	SA	MRSA	SA
87	*Parainfluenza 1*	C-35 (VR-94)	NEG	NEG	POS	POS	POS	POS	NEG	POS
88	*Parainfluenza 2*	Greer (VR-92)	NEG	NEG	POS	POS	POS	POS	NEG	POS
89	*Parainfluenza 3*	C-243 (VR-93)	NEG	NEG	POS	POS	POS	POS	NEG	POS
90	*Rhinovirus type 1A*	VR-1559	NEG	NEG	POS	POS	POS	POS	NEG	POS
91	*RSV A*	VR-1540	NEG	NEG	POS	POS	POS	POS	NEG	POS
92	*RSV B*	VR-1400	NEG	NEG	POS	POS	POS	POS	NEG	POS
93	*HCT-15 cells (human genomic DNA)*	RMSCC3515**	NEG	NEG	POS	POS	POS	POS	NEG	POS

**RMSCC indicates Roche Culture Collection ID, MayoClinic indicates MayoClinic collection ID, DSMZ indicates DSMZ Penzburg collection ID

Table 11: Analytical Specificity Results on MSSA, BORSA and Empty Cassette Variants

		RMS Culture Collection ID	MRSA 10364 (NARSA 384)		MRSA 8065 (ATCC 43300)		MRSA 10364 (NARSA 384)	
			MRSA	SA	MRSA	SA	MRSA	SA
1	*Staphylococcus aureus*	10851	NEG	POS	POS	POS	POS	POS
2	*Staphylococcus aureus*	10852	NEG	POS	POS	POS	POS	POS
3	*Staphylococcus aureus (BORSA)*	10319	NEG	POS	POS	POS	POS	POS
4	*Staphylococcus aureus (BORSA)*	10320	NEG	POS	POS	POS	POS	POS
5	*Staphylococcus aureus (BORSA)*	10321	NEG	POS	POS	POS	POS	POS
6	*Staphylococcus aureus (BORSA)*	10322	NEG	POS	POS	POS	POS	POS
7	*Staphylococcus aureus (BORSA)*	10323	NEG	POS	POS	POS	POS	POS
8	*Staphylococcus aureus (BORSA)*	10324	NEG	POS	POS	POS	POS	POS
9	*Staphylococcus aureus (BORSA)*	10325	NEG	POS	POS	POS	POS	POS
10	*Staphylococcus aureus (BORSA)*	10326	NEG	POS	POS	POS	POS	POS
11	*Staphylococcus aureus (BORSA)*	10327	NEG	POS	POS	POS	POS	POS
12	*Staphylococcus aureus (BORSA)*	10328	NEG	POS	POS	POS	POS	POS
13	*Staphylococcus aureus (mec A drop out)*	10332	NEG	POS	POS	POS	POS	POS
14	*Staphylococcus aureus (mec A drop out)*	10335	POS	POS	POS	POS	POS	POS
15	*Staphylococcus aureus (mec A drop out)*	10336	NEG	POS	POS	POS	POS	POS
16	*Staphylococcus aureus (mec A drop out)*	10339	NEG	POS	POS	POS	POS	POS
17	*Staphylococcus aureus (mec A drop out)*	10340	NEG	POS	POS	POS	POS	POS
18	*Staphylococcus aureus (mec A drop out)*	10341	NEG	POS	POS	POS	POS	POS
19	*Staphylococcus aureus (mec A drop out)*	10342	NEG	POS	POS	POS	POS	POS
20	*Staphylococcus aureus (mec A drop out)*	10343	NEG	POS	POS	POS	POS	POS
21	*Staphylococcus aureus (mec A drop out)*	10344	NEG	POS	POS	POS	POS	POS
22	*Staphylococcus aureus (mec A drop out)*	10348	NEG	POS	POS	POS	POS	POS
23	*Staphylococcus aureus (mec A drop out)*	10349	NEG	POS	POS	POS	POS	POS
24	*Staphylococcus aureus (mec A drop out)*	10354	NEG	POS	POS	POS	POS	POS
25	*Staphylococcus aureus (mec A drop out)*	10355	NEG	POS	POS	POS	POS	POS
26	*Staphylococcus aureus (mec A drop out)*	10356	POS	POS	POS	POS	POS	POS
27	*Staphylococcus aureus (mec A drop out)*	10359	POS	POS	POS	POS	POS	POS
28	*Staphylococcus aureus (mec A drop out)*	10360	NEG	POS	POS	POS	POS	POS

4.6. Competitive Inhibition

Panels were constructed with two MRSA isolates and one SA isolate as targets at 3 x Limit of Detection (LOD) of the **cobas**® MRSA/SA Test, and competing Staphylococcus aureus and Methicillin-Resistant Staphylococcus epidermidis (MRSE) isolates at increasing concentrations. The increasing concentration of SA or MRSE did not affect the detection of MRSA/SA targets, as shown by their relatively stable Ct value (Table 12).

Table 12: Competitive Inhibition Study Results with Methicillin Sensitive SA and MRSE

Competing Organism		Target		
Name	**Concentration**	**MRSA 10364 (NARSA 384)**	**MRSA 8065 (ATCC 43300)**	**SA 10851 (NARSA 164)**
Staphylococcus aureus 10851	1 x target	38.2	38.8	N/A
	100 x target	38.1	39.1	N/A
	10000 x target	38.4	38.8	N/A
Staphylococcus aureus 10852	1 x target	38.1	39.0	N/A
	100 x target	38.5	39.3	N/A
	10000 x target	37.4	39.0	N/A
Staphylococcus epidermidis 5649	1 x target	37.9	39.5	36.5
	100 x target	38.6	38.6	36.5
	10000 x target	38.1	39.7	37.0
Staphylococcus epidermidis 5657	1 x target	39.0	40.1	36.9
	100 x target	38.4	39.1	36.5
	10000 x target	38.3	39.7	36.7

N/A: Not applicable

4.7. Interference

Twenty five commonly used OTC products and antibiotic medicines, as well as whole blood and mucin were tested for potential interference effects with the **cobas**® MRSA/SA Test. All products were tested at or above the levels reasonably expected to be in a nasal swab specimen. Two MRSA isolates and 1 SA isolate were spiked to ~ 3 x LOD of the **cobas**® MRSA/SA Test and used as targets.

No interference was observed for most OTC products. Only Rhinaris® Nasal gel and Releev™ interfered with the performance of the cobas® MRSA/SA Test when present above 15% (Rhinaris® Nasal gel) and above 25% (ReleevTM) of the swab capacity. The **cobas**® MRSA/SA Test was able to tolerate up to 75% whole blood and 10% mucin (Table 13).

Table 13: Results from Interference Substances Testing

Substance	Results
Whole blood	No interference up to 75% of swab capacity
Mucin	No interference up to 10% of swab capacity
Afrin Nasal Spray	No interference
Beconase Nasal Spray	No interference
Bepanthen® nasal ointment	No interference
Chloraseptic Max Sore Throat Lozenges	No interference
Fluticasone Propionate (50 mcg) Nasal Spray	No interference
FluMist© (Afluria, Influenza virus vaccine)	No interference
Flunisolide Nasal Solution USP, 0.025%	No interference
Mupirocin Ointment	No interference
Dristan™ Nasal Mist	No interference
Luffeel™	No interference
Triamcinolone Acetonide Nasal spray	No interference
NasalCrom Nasal Spray	No interference
Nasonex Nasal Spray	No interference
Neo–Synephrine	No interference
Otrivine Nasal Spray	No interference
Relenza®	No interference up to 6.25% of swab capacity*
Budesonide Inhalation Suspension 0.25 mg/2 mL	No interference
Azelastin HCL Nasal Solution	No interference
Equate Saline Nasal Moisturizing Spray	No interference
Rhinaris® Nasal gel	No interference up to 15% of swab capacity
Tobramycin and Dexamethasone Ophthalmic Solution	No interference
Releev (for cold sores)	No interference up to 25% of swab capacity
Zicam Nasal Gel	No interference
QVAR (40 mcg) Inhalation Aerosol	No interference
Nostrilla	No interference

*This concentration represents the whole amount of Relenza® that would be applied in a single use according to prescription information.

4.8. Cross Contamination

The cross contamination rate for the **cobas**® MRSA/SA Test was assessed by testing high titer MRSA and negative samples that were processed in a checkerboard configuration on the cobas® 4800 system. High titer samples were prepared by spiking MRSA culture to simulated nasal swab matrix to generate a Ct that exceeded 95% of signal from specimens of infected patients of the clinical specimen population.

Five runs were performed on each of the three **cobas**® 4800 systems (total: 15 runs). The first run on each system contained only the negative samples to confirm the instrument was clean. The three subsequent runs on each system had alternating positive and negative samples in checkerboard configurations to assess the cross contamination rate. The last run on each system contained only the negative samples to assess the carry-over contamination rate.

Results from this study are summarized in Table 14. There were no cross-contamination events in any of the nine checkerboard runs across the three **cobas**® 4800 systems (a total of 423 MRSA negative samples) for an observed cross-contamination rate of 0%. All results in the last 3 runs containing only the negative samples were negative, suggesting that there was no carry-over run-to-run contamination.

Table 14: Cross Contamination and Carry-over Contamination Rate

Run category	No. of Runs	Total Negative Samples	Number of Positive Results in Negative Samples	Contamination Rate
Checkerboard run **(Cross Contamination)**	9	423	0	0%
Last run with all NEG **(Carryover Contamination)**	3	282	0	0%

5. CLINICAL PERFORMANCE EVALUATION

5.1. Reproducibility

The reproducibility of the **cobas**® MRSA/SA Test on the cobas 4800 System was established in a multi-site, investigation using contrived clinical samples evaluated across lot, site/instrument, operator, day, and run.

The MRSA/SA reproducibility test panels were prepared by seeding MRSA strains NRS384 (MRSA-384) and ATCC 43300 (MRSA-43300), or SA strain RMSCC 10851(NRS-164) into contrived sample matrix (simulated clinical MSwab nasal specimens with mucin and human epithelial cells) at 1 of 4 concentrations (Negative, Below LOD, 1 × LOD, and 3 × LOD). In all, there were 10 members per test panel with 3 replicates per panel member included in each run. Panels were tested at 3 sites by 2 operators per site with 1 run per operator per day, for 5 days per lot, over 2 lots for a total of 1,800 tests (180 tests/panel member or 90 tests/panel member/lot). Overall, 60 runs were performed and all were valid runs. There were no failed/invalid tests

5.1.1. MRSA Reproducibility Results

Table 15 summarizes the MRSA reproducibility results for Ct values and the percent agreement (two-sided 95% exact CI) by site and panel member. The positive percent agreement for the MRSA-positive panel members, “Below LOD MRSA-384” and “Below LOD MRSA-43300,” were 85.6% (95% CI: 79.6% to 90.3%) and 87.2% (95% CI: 81.4% to 91.7%), respectively; the positive percent agreement for all other MRSA-positive panel members was 100.0% (95% CI: 98.0% to 100.0%). The total SD and total CV (%) of Ct values across all MRSA-positive panel members were ≤ 0.51% and ≤1.4%, respectively.

Table 15: Summary of MRSA Reproducibility Results- Ct Values and Percent Agreement by Site and Panel Member

Panel Member	Valid Tests (n)	Ct			Percent Agreement by Site (n/N)			Total Agreement	
		Mean	SD	CV (%)	1	2	3	Percent (n/N)	(95% CI)
Negative	180	N/A	N/A	N/A	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0% (180/180)	(98.0%, 100.0%)
Below LOD MRSA-384	180	40.3	0.43	1.1	95.0 (57/60)	83.3 (50/60)	78.3 (47/60)	85.6% (154/180)	(79.6%, 90.3%)
1 x LOD MRSA-384	180	38.0	0.49	1.3	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0% (180/180)	(98.0%, 100.0%)
3 x LOD MRSA-384	180	36.3	0.44	1.2	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0% (180/180)	(98.0%, 100.0%)
Below LOD MRSA-43300	180	40.4	0.40	1.0	91.7 (55/60)	81.7 (49/60)	88.3 (53/60)	87.2% (157/180)	(81.4%, 91.7%)
1 x LOD MRSA-43300	180	38.9	0.45	1.1	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0% (180/180)	(98.0%, 100.0%)
3 x LOD MRSA-43300	180	37.4	0.51	1.4	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0% (180/180)	(98.0%, 100.0%)

Table 16 presents the SD and CV (%) of Ct values for MRSA-positive panel members overall and attributable to lot, site/instrument, operator, day and within-run.

Table 16: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Ct Values from Valid Results for MRSA Positive Panel Members

MRSA			Standard Deviation and Percent Coefficient of Variation											
			Lot		Site/Inst.		Operator		Day		Within-Run		Total	
Panel Member	N	Mean Ct	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Below LOD MRSA-384	154	40.3	0.00	0.0	0.06	0.2	0.00	0.0	0.23	0.6	0.36	0.9	0.43	1.1
1 x LOD MRSA-384	180	38.0	0.07	0.2	0.18	0.5	0.00	0.0	0.17	0.5	0.41	1.1	0.49	1.3
3 x LOD MRSA-384	180	36.3	0.12	0.3	0.20	0.6	0.02	0.1	0.15	0.4	0.35	1.0	0.44	1.2
Below LOD MRSA-43300	157	40.4	0.03	0.1	0.07	0.2	0.06	0.1	0.02	0.0	0.39	1.0	0.40	1.0
1 x LOD MRSA-43300	180	38.9	0.00	0.0	0.11	0.3	0.00	0.0	0.19	0.5	0.39	1.0	0.45	1.1
3 x LOD MRSA-43300	180	37.4	0.13	0.4	0.24	0.6	0.12	0.3	0.10	0.3	0.40	1.1	0.51	1.4

5.1.2. SA Reproducibility Results

Table 17 summarizes the SA reproducibility results for Ct values and the percent agreement (two-sided 95% exact CI) by site and panel member. The positive percent agreement for the SA-positive panel members "Below LOD SA," "1 × LOD SA" and "3 × LOD SA" were 50.0% (95% CI: 42.5% to 57.5%), 99.4% (95% CI: 96.9% to 100.0%), and 100.0% (95% CI: 98.0% to 100.0%), respectively. The total SD and total CV (%) of Ct values across all SA-positive panel members were ≤ 0.49 and ≤ 1.3%, respectively. The negative percent agreement for the MRSA/SA-negative panel members was 100.0% (95% CI: 98.0% to 100.0%).

Table 17: Summary of SA Reproducibility Results- Ct Values and Percent Agreement by Site and Panel Member.

Panel Member	Valid Tests (n)	Ct			Percent Agreement by Site (n/N)[a]			Total Agreement	
		Mean	SD	CV (%)	1	2	3	Percent (n/N)	(95% CI)[b]
Negative	**180**	**N/A**	**N/A**	**N/A**	**100.0 (60/60)**	**100.0 (60/60)**	**100.0 (60/60)**	**100.0% (180/180)**	**(98.0%, 100.0%)**
Below LOD SA	180	38.6	0.46	1.2	23.3 (14/60)	60.0 (36/60)	66.7 (40/60)	50.0% (90/180)	(42.5%, 57.5%)
1 x LOD SA	180	36.8	0.49	1.3	100.0 (60/60)	98.3 (59/60)	100.0 (60/60)	99.4% (179/180)	(96.9%, 100.0%)
3 x LOD SA	180	35.1	0.38	1.1	100.0 (60/60)	100.0 (60/60)	100.0 (60/60)	100.0% (180/180)	(98.0%, 100.0%)

Table 18 presents the SD and CV (%) of Ct values for SA-positive panel members overall and attributable to lot, site/instrument, operator, day and within-run.

Table 18: Overall Mean, Standard Deviations, and Coefficients of Variation (%) for Ct Values from Valid Results for SA Positive Panel Members

SA			Standard Deviation and Percent Coefficient of Variation											
			Lot		Site/Inst.		Operator		Day		Within-Run		Total	
Panel Member	N	Mean Ct	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Below LOD SA	90	38.6	0.00	0.0	0.00	0.0	0.00	0.0	0.00	0.0	0.46	1.2	0.46	1.2
1 x LOD SA	179	36.8	0.14	0.4	0.29	0.8	0.13	0.3	0.16	0.4	0.31	0.8	0.49	1.3
3 x LOD SA	180	35.1	0.11	0.3	0.14	0.4	0.12	0.3	0.02	0.1	0.31	0.9	0.38	1.1

5.2. Clinical Performance

The clinical performance of the **cobas**® MRSA/SA Test was established in an IRB-approved, prospective, multi-site, investigation comparing the results with direct chromogenic culture, and direct chromogenic culture combined with enrichment culture (reference method), using nasal swabs from eligible male and female subjects.

Specimens were collected at six geographically diverse sites across the US. One or two swab specimens were collected from each subject; one swab was collected for Standard-of-Care testing (if applicable) and one swab, an MSwab (Copan Flock Technologies S.r.1., Brescia, Italy), was collected for the **cobas**® MRSA/SA Test and for direct and enrichment culture.

The **cobas**® MRSA/SA Test was performed at three sites and the direct and enrichment culture was performed at a reference laboratory that specialized in culture and molecular detection of methicillin-resistant MRSA and SA. Briefly, aliquots of MSwab sample from each subject was transferred directly to one plate each of selective and differential chromogenic media for MRSA and SA (direct culture), and to a tube containing tryptic soy broth (TSB) with 6.5% NaCl (enrichment culture). Suspect isolates and positive enrichment broth cultures were subcultured to 5% sheep blood agar and isolates identified as SA by Gram-stain and latex agglutination testing. Putative MRSA isolates were confirmed using a Kirby-Bauer cefoxitin disc diffusion test for methicillin resistance.

A MRSA/SA culture positive specimen was defined as a specimen positive for MRSA/SA by either direct or enrichment culture technique. A MRSA/SA culture negative specimen was defined as a specimen negative for MRSA/SA for both direct and enrichment culture.

Discrepant analysis was performed on all samples with discordant results between the cobas® MRSA/SA Test and combined direct and enrichment culture (reference culture), using a second, FDA-cleared nucleic acid amplification test and a non-selective direct and enrichment culture; a randomly selected subset of samples with concordant results were included as controls. Briefly, an aliquot of remnant (leftover) MSwab sample was transferred to a chocolate agar plate (non-selective direct culture) and to TSB without NaCl (non-selective enrichment culture). Isolates recovered from non-selective direct and enrichment culture were characterized as described. In addition, the identification of suspicious, atypical isolates were confirmed using a laboratory developed PCR assay for femA and mecA genes according to the reference laboratory's established practice.

5.2.1. Results

Specimens were collected from 2528 subjects with 2504 (99.1%) evaluable results from 1372 males (54.8%) and 1132 (45.2%) female subjects. The majority of subjects were > 50 years of age (67.2%) and ranged in age from 18 to 101 (median age = 57). There were a total of 160 MRSA-positive and 660 SA-positive specimens.

5.2.1.1. Performance of the cobas® MRSA/SA Test Compared to Combined Direct and Enrichment Culture (Reference Method)

The performance of the **cobas®** MRSA/SA test compared to combined direct and enrichment culture from 2500 evaluable results for MRSA, and from 2501 evaluable results for SA, is shown in Table 19.

The sensitivity and specificity for MRSA compared to combined direct and enrichment culture was 93.1% (149/160) and 97.5% (2281/2340), respectively; and the prevalence, PPV and NPV was 6.4%, 71.6% and 99.5%, respectively.

The sensitivity and specificity for SA compared to combined direct and enrichment culture was 93.9% (620/660) and 94.2% (1734/1841), respectively, and the prevalence, PPV and NPV was 26.4%, 85.3% and 97.7%, respectively.

Table 19: Comparison of Results from the cobas® MRSA/SA Test with Direct and Enrichment Culture (Reference Method)

		Direct and Enrichment Culture (Reference Method)					
		MRSA			SA		
		Positive	Negative	Total	Positive	Negative	Total
cobas® MRSA/SA Test	Positive	149	59	208	620	107	727
	Negative	11	2281	2292	40	1734	1774
	Total	160	2340	2500	660	1841	2501

MRSA
Sensitivity: 93.1% (149/160) (95% CI: 88.1-96.1%)
Specificity: 97.5% (2281/2340) (95% CI: 96.8-98.0%)
Prevalence: 6.4%
PPV: 71.6%
NPV: 99.5%

SA
Sensitivity: 93.9% (620/660) (95% CI: 91.9-95.5%)
Specificity: 94.2% (1734/1841) (95% CI: 93.0-95.2%)
Prevalence: 26.4%
PPV: 85.3%
NPV: 97.7%

5.2.1.2. Comparison of Results of the cobas® MRSA/SA Test with Direct Culture

Results of the **cobas**® MRSA/SA test were compared to direct culture from 2504 evaluable results is shown in Table 20.

The overall, positive and negative percent agreement of the **cobas**® MRSA/SA Test for MRSA compared to direct culture was 96.9% (2427/2504), 97.1% (135/139) and 96.9% (2292/2365); and the prevalence was 5.6%.

The overall, positive and negative percent agreement of the **cobas**® MRSA/SA Test for SA compared to direct culture was 93.3% (2336/2504), 97.0% (577/595) and 92.1% (1759/1909), respectively; and the prevalence was 23.8%.

Table 20: Comparison of Results from the cobas® MRSA/SA Test with Direct Culture

		Direct Culture					
		MRSA			SA		
		Positive	Negative	Total	Positive	Negative	Total
cobas® MRSA/SA Test	Positive	135	73	208	577	150	727
	Negative	4	2292	2296	18	1759	1777
	Total	139	2365	2504	595	1909	2504

MRSA

Positive Percent Agreement: 97.1% (135/139) (95% CI: 92.8-98.9%)
Negative Percent Agreement: 96.9% (2292/2365) (95% CI: 96.1-97.5%)
Overall Percent Agreement: 96.9% (2427/2504) (95% CI: 96.2-97.5%)
Prevalence: 5.6%

SA

Positive Percent Agreement: 97.0% (577/595) (95% CI: 95.3-98.1%)
Negative Percent Agreement: 92.1% (1759/1909) (95% CI: 90.8-93.3%)
Overall Percent Agreement: 93.3% (2336/2504) (95% CI: 92.2-94.2%)
Prevalence: 23.8%

5.2.1.3. Discrepant Analysis of Discordant and Concordant Samples

Discrepant analysis was performed on all discordant samples, and a random subset of concordant samples included as controls, between the **cobas®** MRSA/SA Test and combined direct and enrichment culture, using a second, FDA-cleared nucleic acid amplification test (NAAT) and a non-selective direct and enrichment culture.

There were a total of 70 specimens with MRSA discordant results (11 MRSA False-negative results and 59 MRSA False-positive results). Of the 11 MRSA False-negative specimens, five tested MRSA-negative by a second NAAT method and non-selective direct and enrichment culture. Of the 59 MRSA False-positive specimens, 20 tested MRSA-positive by a second NAAT method or non-selective direct and/or enrichment culture.

There were a total of 147 specimens with SA discordant results (40 SA False-negative results and 107 SA False-positive results). Of the 40 SA False-negative specimens, 31 tested SA-negative by a second NAAT method and non-selective direct and enrichment culture. Of the 107 SA False-positive specimens, 24 tested SA-positive by a second NAAT method or non-selective direct and/or enrichment culture.

There were 74 randomly selected concordant samples included as controls in the discrepant analysis (25 MRSA-positive, 25 SA-negative and 24 SA-positive/MRSA-negative concordant samples). Of the 74 controls, all 25 MRSA-positive specimens tested MRSA-positive by a second NAAT method or non-selective direct and/or enrichment culture; all 25 SA-negative specimens tested SA-negative by a second NAAT method and non-selective direct and enrichment culture; and, of the 24 SA-positive specimens, 21 tested SA-positive by a second NAAT method or non-selective direct and/or enrichment culture, 1 tested MRSA-positive by nonselective enrichment culture, and two tested SA-negative by a second NAAT method and non-selective direct and enrichment culture.

5.3. Summary

Based on the clinical performance evaluation as documented in the reproducibility and clinical study, the **cobas**® MRSA/SA test was found to have a safety and effectiveness profile that is similar to the predicate device.

6. CONCLUSIONS

A comparison of the intended use, technological characteristics, and the results of non-clinical and clinical performance studies support that the **cobas**® MRSA/SA Test is substantially equivalent to the predicate device.