cobas® MRSA/SA Test

for use on the cobas® 4800 System

For in vitro diagnostic use

IVD

cobas® 4800 System Sample Preparation Kit	240 Tests 960 Tests	P/N 05235782190 P/N 05235804190
cobas® 4800 System Lysis Kit 1	240 Tests 960 Tests	P/N 06768253190 P/N 06768270190
cobas® 4800 System Wash Buffer Kit	240 Tests 960 Tests	P/N 05235863190 P/N 05235871190
cobas® 4800 System Internal Control Kit 1	20 Runs	P/N 06768318190
cobas® 4800 MRSA/SA Amplification/Detection Kit	80 Tests 240 Tests	P/N 06768113190 P/N 06768172190
cobas® 4800 MRSA/SA Controls and Cofactor Kit	10 Runs	P/N 06768288190

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Intended use

The **cobas**® MRSA/SA Test on the **cobas**® 4800 System is an automated, real-time PCR assay for the rapid *in vitro* qualitative detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus 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.

Summary and explanation of the test /principle of the procedure

Background: Screening of MRSA and SA

SA is an opportunistic pathogen carried as a commensal organism on the skin and nares of approximately 30% of the normal population. It has the potential to cause a broad spectrum of diseases.[1] SA can rapidly adapt to the selective pressure of antibiotics, which has resulted in the emergence and spread of MRSA strains. Resistance to methicillin, in addition to other β-lactam antibiotics, is mediated by the mecA gene product, which is located on a mobile genetic element, the Staphylococcal Cassette Chromosome mec (SCCmec). The mecA gene encodes the altered penicillin-binding protein (PBP) 2a. This prevents the normal binding of β-lactam antibiotics to the PBP in the cell wall, where they would have disrupted synthesis of the peptidoglycan layer, resulting in bacterial cell death. Multiple SCCmec types have been distinguished.[2] Numerous MRSA strains have emerged and spread worldwide, and SCCmec has been acquired by different lineages of SA.[2]

SA and MRSA strains are a major source of healthcare-acquired infections and have been responsible for bacterial outbreaks in healthcare settings worldwide for many years.[3,4] SA and MRSA infections are a tremendous burden for healthcare systems, for single hospitals, and are associated with significant healthcare costs.[5] Guidelines and recommendations[6] as well as hospital standard procedures recommend active screening and isolation and/or decolonization of patients as measures to control the spread of MRSA and SA.[7]

In outbreak situations, additional measures such as inpatient and healthcare worker screening and closure of wards may be implemented. Despite public guidelines, the standard operating procedures for infection control may vary widely from country to country and from hospital to hospital.

The sensitivity of the methods used and the time to result appear to be key factors for the success of screening and treatment strategies.[8] Conventional culture-based methods require several days for results to become apparent and do not allow rapid implementation of specific infection control measures, but instead require more general infection control measures to be used for all patients. Only rapid techniques, such as molecular methods, enable early detection of MRSA and SA in colonized patients and consequent implementation of appropriate barrier precautions.[9] A number of reports have shown the value of rapid molecular tests for the rapid detection of colonization with MRSA and SA.[10-13]

The **cobas**® MRSA/SA Test processes nasal swab specimens collected with the COPAN MSwab Collection, Transport and Preservation kit. The tubes containing the primary specimens are loaded on the **cobas**® 4800 System, and nucleic acid extraction and PCR reaction set up occurs by an automated process. The

subsequent real-time PCR process detects MRSA- and SA-specific DNA target in the sample, if present. The test can be run with the **cobas**® Cdiff and **cobas**® HSV 1 and 2 tests in mixed batch fashion in the same run. All three tests share the same automated specimen extraction process as well as PCR profile for amplification and detection.

Explanation of the test

The **cobas**® MRSA/SA Test contains two major processes: (1) automated sample preparation to extract nucleic acids from the nasal 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, 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.

Principles of the procedure

Sample preparation

Sample preparation for the **cobas**® MRSA/SA Test is automated with the use of the **cobas x** 480 instrument. Organisms 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.

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 three targets: the SCCmec cassette Right Extremity junction region which is specific to MRSA, a genomic target for all SA (including MRSA), and Internal Control. If the target nucleic acid sequences are present, amplification with the corresponding primers will occur by a thermostable DNA polymerase, generating PCR products (amplicon). 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.

Selective amplification

Selective amplification of target nucleic acid from the specimen is achieved in the **cobas**® MRSA/SA Test by the use of AmpErase (uracil-N-glycosylase) enzyme and deoxyuridine triphosphate (dUTP). The AmpErase enzyme recognizes and catalyzes the destruction of DNA strands containing deoxyuridine[11], but not DNA containing deoxythymidine. Deoxyuridine is not present in naturally occurring DNA, but is always present in amplicon due to the use of deoxyuridine triphosphate in place of thymidine triphosphate as one of the dNTPs in the Master Mix reagent; therefore, only amplicon contain deoxyuridine. Deoxyuridine renders contaminating amplicon susceptible to destruction by AmpErase enzyme prior to amplification of the target DNA. AmpErase enzyme, which is included in the Master Mix reagent, catalyzes the cleavage of deoxyuridine-containing DNA at the deoxyuridine residues by opening the deoxyribose chain at the C1-position. When heated in the first thermal cycling step at the alkaline pH of Master Mix, the amplicon DNA chain breaks at the position of the deoxyuridine, thereby rendering the DNA non-amplifiable.

AmpErase enzyme is inactive at temperatures above 55ºC, i.e., throughout the thermal cycling steps, and therefore does not destroy target amplicon. The **cobas**® MRSA/SA Test has been demonstrated to inactivate at least 10^3 copies of deoxyuridine-containing MRSA/SA amplicon per PCR.

Materials, reagents, and specimens

Materials and reagents provided

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Sample Preparation Kit 240 Tests (P/N: 05235782190)	**MGP** (**cobas**® 4800 System Magnetic Glass Particles) Magnetic Glass Particles 93% Isopropanol**	10 x 4.5 mL	 **DANGER** H225 Highly flammable liquid and vapour. H319 Causes serious eye irritation. H336 May cause drowsiness or dizziness. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 Keep container tightly closed. P261 Avoid breathing dust/fume/gas/ mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P370 + P378 In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish. 67-63-0 Propan-2-ol
	EB (**cobas**® 4800 System Elution Buffer) Tris buffer 0.09% Sodium azide	10 x 18 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Sample Preparation Kit 960 Tests (P/N: 05235804190)	**MGP** (**cobas**® 4800 System Magnetic Glass Particles) Magnetic Glass Particles 93% Isopropanol**	10 x 13.5 mL	 **DANGER** H225 Highly flammable liquid and vapour. H319 Causes serious eye irritation. H336 May cause drowsiness or dizziness. P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P233 Keep container tightly closed. P261 Avoid breathing dust/fume/gas/ mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P370 + P378 In case of fire: Use dry sand, dry chemical or alcohol-resistant foam to extinguish. 67-63-0 Propan-2-ol
	EB (**cobas**® 4800 System Elution Buffer) Tris buffer 0.09% Sodium azide	10 x 18 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Lysis Kit 1 240 Tests (P/N: 06768253190)	**LYS-1** (**cobas**® 4800 System Lysis Buffer 1) Sodium citrate 5% Polydocanol** 42.6% Guanidinium thiocyanate** Dithiothreitol**	10 x 10 mL	 **DANGER** H302 + H332 Harmful if swallowed or if inhaled. H314 Causes severe skin burns and eye damage. H412 Harmful to aquatic life with long lasting effects. EUH032 Contact with acids liberates very toxic gas. P261 Avoid breathing dust/fume/gas/ mist/vapours/spray. P273 Avoid release to the environment. P280 Wear protective gloves/protective clothing/eye protection/face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Lysis Kit 1 240 Tests (P/N: 06768253190)	**PK** (**cobas**® 4800 System Proteinase K) Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K* Glycerine	10 x 0.9 mL	 **DANGER** H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261 Avoid breathing dust/fume/gas/ mist/vapours/spray. P280 Wear protective gloves. P284 Wear respiratory protection. P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313 If skin irritation or rash occurs: Get medical advice/ attention. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor. 39450-01-6 Proteinase, Tritirachium album serine
	SDS (**cobas**® 4800 System SDS Reagent) Tris buffer Sodium dodecyl sulfate 0.09% Sodium azide	10 x 3 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Lysis Kit 1 960 Tests (P/N: 06768270190)	**LYS-1** (**cobas**® 4800 System Lysis Buffer 1) Sodium citrate 5% Polydocanol** 42.6% Guanidinium thiocyanate** Dithiothreitol**	10 x 36 mL	 **DANGER** H302 + H332 Harmful if swallowed or if inhaled. H314 Causes severe skin burns and eye damage. H412 Harmful to aquatic life with long lasting effects. EUH032 Contact with acids liberates very toxic gas. P261 Avoid breathing dust/fume/gas/ mist/vapours/spray. P273 Avoid release to the environment. P280 Wear protective gloves/ protective clothing/eye protection/face protection. P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water. P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor. P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor. 593-84-0 Guanidinium thiocyanate 9002-92-0 Polidocanol 3483-12-3 (R*,R*)-1,4-dimercaptobutane-2,3-diol

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 System Lysis Kit 1 960 Tests (P/N: 06768270190)	**PK** (**cobas**® 4800 System Proteinase K) Tris buffer EDTA Calcium chloride Calcium acetate < 2.0% Proteinase K** Glycerine	20 x 1.2 mL	 **DANGER** H317 May cause an allergic skin reaction. H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled. P261 Avoid breathing dust/fume/gas/ mist/vapours/spray. P280 Wear protective gloves. P284 Wear respiratory protection. P304 + P340 IF INHALED: Remove person to fresh air and keep comfortable for breathing. P333 + P313 If skin irritation or rash occurs: Get medical advice/attention. P342 + P311 If experiencing respiratory symptoms: Call a POISON CENTER/ doctor. 39450-01-6 Proteinase, Tritirachium album serine
	SDS (**cobas**® 4800 System SDS Reagent) Tris buffer Sodium dodecyl sulfate 0.09% Sodium azide	10 x 9 mL	N/A
cobas® 4800 System Wash Buffer Kit 240 Tests (P/N: 05235863190)	**WB** (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl	10 x 55 mL	N/A
cobas® 4800 System Wash Buffer Kit 960 Tests (P/N: 05235871190)	**WB** (**cobas**® 4800 System Wash Buffer) Sodium citrate dihydrate 0.05% N-Methylisothiazolone HCl	10 x 200 mL	N/A
cobas® 4800 System Internal Control Kit 1 20 Runs (P/N: 06768318190)	**IC-1** (**cobas**® 4800 IC-1) Tris buffer EDTA < 0.01% Poly rA RNA (synthetic) 0.05% Sodium azide < 0.01% Non-infectious, synthetic internal control DNA encapsulated in Lambda bacteriophage coat protein	20 x 0.5 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 MRSA/SA Amplification/Detection Kit 80 Tests (P/N: 06768113190)	**MRSA/SA MMX** (**cobas**® MRSA/SA Master Mix) Tricine buffer EDTA Potassium acetate Potassium hydroxide Tween 20 Glycerol 0.09% Sodium azide < 0.19% dATP, dCTP, dGTP, dUTP < 0.01% Upstream and downstream MRSA, SA and Internal Control primers < 0.01% Fluorescent-labeled MRSA, SA and labeled Internal Control probes < 0.01% Oligonucleotide aptamer < 0.01% Z05 DNA polymerase (microbial) < 0.02% AmpErase (uracil-N-glycosylase) enzyme (microbial)	10 x 0.3 mL	N/A
cobas® 4800 MRSA/SA Amplification/Detection Kit 240 Tests (P/N: 06768172190)	**MRSA/SA MMX** (**cobas**® MRSA/SA Master Mix) Tricine buffer EDTA Potassium acetate Potassium hydroxide Tween 20 Glycerol 0.09% Sodium azide < 0.19% dATP, dCTP, dGTP, dUTP < 0.01% Upstream and downstream MRSA, SA and Internal Control primers < 0.01% Fluorescent-labeled MRSA, SA and labeled Internal Control probes < 0.01% Oligonucleotide aptamer < 0.01% Z05 DNA polymerase (microbial) < 0.02% AmpErase (uracil-N-glycosylase) enzyme (microbial)	10 x 0.7 mL	N/A

Kit/Cassettes	Components and Reagent Ingredients	Quantity per Test	Safety Symbol and Warning*
cobas® 4800 MRSA/SA Controls and Cofactor Kit 10 Runs (P/N: 06768288190)	**MRSA/SA (+) C** (**cobas**® MRSA/SA Positive Control) Tris buffer EDTA < 0.01% Poly rA RNA (synthetic) 0.05% Sodium azide < 0.01% Non-infectious plasmid DNA (microbial) containing MRSA sequence < 0.01% Non-infectious plasmid DNA (microbial) containing SA sequence	10 x 0.5 mL	N/A
	(–) C (**cobas**® 4800 System Negative Control) Tris buffer EDTA < 0.01% Poly rA RNA (synthetic) 0.05% Sodium azide	10 x 0.5 mL	N/A
	Cofactor-1 (**cobas**® 4800 Cofactor-1) Manganese acetate Magnesium acetate 0.09% Sodium azide	10 x 1.7 mL	N/A

* Product safety labeling primarily follows EU GHS guidance

**Hazardous substance

Reagent storage and handling

Reagent	Storage Temperature	Storage Time
cobas® 4800 System Sample Preparation Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Lysis Kit 1	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Internal Control Kit 1	2–8°C	Stable until the expiration date indicated
cobas® 4800 MRSA/SA Amplification/Detection Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 MRSA/SA Controls and Cofactor Kit	2–8°C	Stable until the expiration date indicated
cobas® 4800 System Wash Buffer Kit	15–25°C	Stable until the expiration date indicated

Do not freeze reagents.

Reagent expiry date is based on the Coordinated Universal Time (UTC). Local time for reagent expiry could be offset by plus or minus 12 hours, depending on the local time zone relative to UTC.

Additional materials required

Materials	P/N
CORE Tips, 1000 µL, rack of 96	04639642001
50 mL Reagent Reservoir	05232732001
200 mL Reagent Reservoir	05232759001
cobas® 4800 System Extraction (deep well) Plate	05232716001
cobas® 4800 System AD (microwell) Plate 0.3 mL and Sealing Film	05232724001
Sealing foil applicator	04900383001
32-position carrier	04639529001
Solid waste bag	05530873001 (small) or 04691989001 (large)
Hamilton STAR Plastic Chute	Roche 04639669001
MSwab Collection, Transport and Preservation System	07007248190 or COPAN P/N 404C.R or 404C
Disposable gloves, powderless	Any powderless disposable gloves are acceptable.
Vortex Mixer (single tube)	Any vortex mixer is acceptable.

For more information regarding the materials sold separately, contact your local Roche representative.

Optional materials

Materials	P/N
Sealing mat or deep well plate cover	Roche 04789288001 or Hamilton 6474-01
Caps, white color (for recapping post-run primary specimens)	07033893001 or COPAN 2U008N100.R or 2U008N100

For more information regarding the optional materials, contact your local Roche representative.

Instrumentation and software required but not provided

Required Instrumentation and Software, Not Provided
cobas® 4800 System **cobas x** 480 instrument **cobas z** 480 analyzer Control Unit
cobas® 4800 System **cobas**® MRSA/SA AP Software Version 1.0.0 or higher
cobas® 4800 System Application Software (Core) Version 2.2.0 or higher

For more information regarding the materials sold separately, contact your local Roche representative.

Precautions and handling requirements

Warnings and precautions

As with any test procedure, good laboratory practice is essential to the proper performance of this assay. Due to the high analytical sensitivity of this test, care should be taken to keep reagents, specimens and amplification mixtures free of contamination.

- For in vitro diagnostic use only.
- Avoid microbial and DNA contamination of reagents and specimens. SA is carried by ~ 30% of the population and may reside in the nares or skin. Be extra alert when handling samples and reagents to avoid potential contamination by SA from the operator.
- Safety Data Sheets (SDS) are available upon request from your local Roche office.
- LYS-1 reagent contains guanidine thiocyanate. Do not allow direct contact between guanidine thiocyanate and sodium hypochlorite (bleach) or other highly reactive reagents such as acids or bases. These mixtures can release a noxious gas.
- MGP contains isopropanol and is highly flammable. Keep away from open flames and potential spark producing environments.
- EB, MRSA/SA MMX, SDS, Cofactor-1, (–)C, MRSA/SA (+)C and IC-1 contain sodium azide.
- For additional warnings, precautions and procedures to reduce the risk of contamination for the **cobas x** 480 instrument or **cobas z** 480 analyzer, consult the **cobas**® 4800 System - User Assistance. If contamination is suspected, perform cleaning and weekly maintenance as described in the **cobas**® 4800 System - User Assistance.

Note: For specific instructions, see “Specimen collection, transport, and storage”.

Good laboratory practice

- Do not pipette by mouth.
- Do not eat, drink or smoke in laboratory work areas.
- Wash hands thoroughly after handling specimens and kit reagents.
- Wear eye protection, laboratory coats and disposable gloves when handling any reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated. If spills occur, dilute with water before wiping dry.
- Thoroughly clean and disinfect all laboratory work surfaces with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10). Follow by wiping the surface with 70% ethanol.

Contamination

- Gloves must be worn and must be changed between handling specimens and **cobas**® MRSA/SA reagents to prevent contamination. Avoid contaminating gloves when handling specimens and controls. Wear lab gloves, laboratory coats, and eye protection when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of reagents.
- False positive results may occur if carryover of specimens is not prevented during specimen handling.
- Specimens should be handled as infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[14] and in the CLSI Document M29-A4.[15]

Integrity

- Do not use kits after their expiration dates.
- Do not pool reagents.
- Do not use disposable items beyond their expiration date.
- All disposable items are for one-time use. Do not reuse.
- All equipment should be properly maintained according to the manufacturer's instructions.

Disposal

- **cobas**® 4800 reagents and the **cobas**® MRSA/SA Test specific reagents contain sodium azide (see "**Warnings and precautions**"). Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. While disposing of solutions containing sodium azide down laboratory sinks, flush the drains with a large volume of cold water to prevent azide buildup.
- Dispose of unused reagents and waste in accordance with country, federal, state and local regulations.

Note: For disposal of liquid waste, refer to the cobas® 4800 System – User Assistance.

Spillage and cleaning

- LYS-1 reagent contains guanidine thiocyanate. If liquid containing guanidine thiocyanate is spilled, clean with suitable laboratory detergent and water. If the spilled liquid contains potentially infectious agents, FIRST clean the affected area with laboratory detergent and water, and then with 0.5% sodium hypochlorite.
- If spills occur on the **cobas**® 4800 instrument, follow the instructions in the **cobas**® 4800 System - User Assistance to clean.
- Do not use sodium hypochlorite solution (bleach) for cleaning the **cobas x** 480 instrument or the **cobas z** 480 analyzer. Clean the **cobas x** 480 instrument or the **cobas z** 480 analyzer according to procedures described in the **cobas**® 4800 System - User Assistance.

Specimen collection, transport, and storage

Note: Handle all specimens as if they are capable of transmitting infectious agents.

Specimen collection

Nasal swab specimens collected with the MSwab Collection, Transport and Preservation System have been validated for use with the **cobas**® MRSA/SA Test. Specimens should be collected following the procedure detailed in the Specimen Collection Procedure section and according to your institution's standard operating procedures.

Specimen transport storage and stability

Nasal swab specimens collected with the MSwab Collection, Transport and Preservation System are stable for transport and storage at 2-30°C for 4 days, or 2-8°C for 9 days, or frozen at -20°C for 30 days before testing on the **cobas**® 4800 System (this was demonstrated by testing specimens after consecutive storage at 15 $\pm$ 1°C and 31 $\pm$ 1°C for 4 days, followed by 2-8°C for 5 days, followed by -20 $\pm$ 5°C for 30 days).

Transportation of MRSA/SA specimens must comply with country, federal, state and local regulations for the transport of etiologic agents.

Instructions for use

Running the test

Workflow

Figure 1 **cobas**® MRSA/SA workflow

1	Start up the system.
2	Perform instrument maintenance.
3	Remove samples and reagents from storage.
4	Start run: • Load carriers with samples.
5	With LIS: confirm work order Without LIS: create work order
6	Load consumables (deepwell plate, microwell plate, tip racks) and reagents
7	Start sample preparation run
8	Unload and seal microwell plate
9	Remove samples, used reagents, and deepwell plate.
10	Load microwell plate into analyzer
11	Review results
12	With LIS: send results to LIS
13	Unload analyzer

Test procedure

Specimen collection procedure

1. Please use the flocked swab provided in the MSwab collection kit. Use swab dry or pre-moisten with two drops of sterile physiological saline.
2. Carefully insert the swab into the patient's nostril (swab tip should be inserted up to 2.5 cm (1 inch) from the edge of the nares).
3. Roll the swab along the mucosa inside the nostril 3 times.
4. Repeat Steps 2 and 3 in second nostril with the same swab.
5. Replace the swab in its transport tube. Leverage the swab shaft against the edge of the tube to break at pre-scored point.
6. Close the cap firmly while ensuring that the upper end of the swab shaft is in the center of the cap.
7. Label the sample and transport to testing laboratory according to your institution's standard operating procedures (refer to "**Specimen transport storage and stability**" section). Refer to the "**Workflow**" section for notes on specimens.

All reagents except MRSA/SA MMX and Co-factor 1 must be at ambient temperature prior to loading on the **cobas x** 480 instrument. The MRSA/SA MMX and Co-factor 1 reagents may be taken directly from

2-8°C storage as they will equilibrate to ambient temperature on board the **cobas x** 480 instrument by the time they are used in the process.

Note: Refer to the cobas® 4800 System - User Assistance for detailed operating instructions.

Run size

The **cobas**® 4800 System is designed to support mixed-batch runs between the **cobas**® MRSA/SA, **cobas**® Cdiff and **cobas**® HSV 1 and 2 tests. The generic **cobas**® 4800 System Sample Preparation Kit, generic **cobas**® 4800 System Lysis Kit 1 and generic **cobas**® 4800 System Wash Buffer Kit are available in two kit sizes, each sufficient for 10 runs of up to either 24 or 96 samples, which include the controls and specimens for all assays to be run. The **cobas**® 4800 MRSA/SA Amplification/Detection Kit is available in two sizes, each sufficient to test up to either 80 or 240 samples, which include MRSA/SA controls and specimens to be run. Multiple vials of the **cobas**® 4800 MRSA/SA Master Mix reagent can be used as appropriate in one run, as long as they are the same kit size. The generic **cobas**® 4800 System Internal Control Kit 1 and the **cobas**® 4800 MRSA/SA Controls and Cofactor Kit are available in a single kit size, which is sufficient for 20 and 10 runs, respectively, and can support all run configurations. For each run containing MRSA/SA specimens, one **cobas**® 4800 MRSA/SA Positive Control and one **cobas**® 4800 System Negative Control must be run (see "**Quality control**"). For a single test run, the maximum number of samples allowed is 94 specimens and two controls.

Note: Although not an optimal use of reagents, a 96-Test generic reagent can be used for a run containing 1-22 specimens. However, different sizes of the cobas® 4800 System Wash Buffer (WB) Kit, cobas® 4800 System Sample Preparation Kit and cobas® 4800 System Lysis Kit 1 cannot be mixed. For example, if a 96-Test WB reagent bottle is scanned at the start of the run, 96-Test size reagents from the other two kits must also be used.

Note: Although not an optimal use of reagents, a 24-Test cobas® 4800 MRSA/SA MMX can be used for a run containing 1-6 MRSA/SA specimens. See the cobas® 4800 System - User Assistance for details on how to change kit size.

Workflow

The **cobas**® MRSA/SA Test is run using the full workflow within the **cobas**® 4800 Software. It consists of sample preparation on the **cobas x** 480 instrument followed by amplification/detection on the **cobas z** 480 analyzer. The run can be MRSA/SA only, or mixed-batched with the **cobas**® Cdiff and/or **cobas**® HSV 1 and 2 tests. Refer to the **cobas**® 4800 System - User Assistance for details.

Specimens

Note: The cobas® MRSA/SA Test has been validated for use with the MSwab Collection, Transport and Preservation System. Do not use other swab collection devices or media types.

Note: A properly collected nasal swab specimen should have a single FLOQ swab with the shaft captured by the cap. Incoming specimens with no swabs or with more than one swab have not been collected according to the instructions, and should not be tested.

Note: Do not process nasal swab specimens that appear bloody or have a dark brown color.

Note: Specimens must be in the primary specimen containers with a proper barcode for processing on the cobas x 480 instrument. Consult the cobas® 4800 System – User Assistance for proper barcoding procedures and the list of acceptable barcodes for the cobas® 4800 System.

Note: To avoid cross-contamination, it is recommended that primary tubes be processed on the cobas® 4800 System prior to other processing and testing.

Note: To avoid cross-contamination of processed specimens, additional caps for MSwab specimen container in an alternate color (white; see "Optional materials") should be used to recap specimens after processing.

Note: Nasal swab specimens collected in MSwab media contain sufficient volume to be assayed twice on the cobas® 4800 System, and can also be used for any necessary culture processing (see "Culturing of clinical specimens"), provided that no spillage has occurred during sample handling prior to the test. Refer to the package insert of the MSwab Collection, Transport and Preservation System for culture inoculation instruction. Minimum sample volume to conduct a cobas® MRSA/SA run is 700 µL in the primary MSwab specimen container.

Performing the cobas® MRSA/SA Test

Note: Mixed batch runs between cobas® MRSA/SA and cobas® Cdiff and/or cobas® HSV 1 and 2 tests can be conducted. Refer to the cobas® 4800 System – User Assistance for more information.

1. Perform the system startup and maintenance procedures by following the instructions in the **cobas**® 4800 System – User Assistance.
2. Collect all reagents and consumables needed. Reagents must be at room temperature by the time the run is started with the exception of **cobas**® MRSA/SA MMX and Cofactor-1 reagents.

Note: All reagents and reagent reservoirs are barcoded and designed for one-time use. The cobas® 4800 Software tracks the use of the reagents and reagent reservoirs and rejects previously used reagents or reagent reservoirs.

3. Check the appearance of nasal swab specimens collected in MSwab media to make sure they meet the requirements in the "**Specimens**" section. Ensure that all caps have been tightened. Vortex the specimen for a minimum of 10 seconds. Uncap the tube (top of the swab should be captured by the cap) and swirl the swab around the inside wall of the tube to drain excess liquid. Discard the cap with the swab just before loading on the **cobas**® 4800 System. Make sure swab is taken out with the cap. Swab left in the sample vial will interfere with the **cobas**® MRSA/SA Test.
4. Start a new run and define the work order for the run. There are three ways to create a work order:
   - By using the sample editor before sample rack is loaded into **cobas x** 480 instrument ("Editor" button on the right of the main menu). Work orders can be saved, edited and reloaded if necessary.
   - By following the software wizard for the new run and loading specimens into **cobas x** 480 instrument when prompted. The specimen barcodes will be automatically scanned, and the requested results for each specimen must be defined.
   - By using your institution's LIS system.

   Refer to the **cobas**® 4800 System – User Assistance for more details. When selecting the requested results, check "MRSA" only, both "MRSA" and "SA", or "SA" only depending on the tests that need to be performed. For example, if only "SA" is selected, MRSA results will not be available.

5. Load samples and define/select workorder or use LIS as appropriate. The “Unload sample carriers after transferring to deep well plate” option is selected by default. This allows the operator to retrieve the remaining specimens as soon as possible after they are aliquoted for processing by **cobas x** 480 instrument. Specimen containers should be re-capped with fresh closure (see “**Optional materials**”) if storage is needed.
6. Follow the software wizard guide and load consumables. Do not load or remove individual tips into a partially used tip rack, as the software tracks the number of tips that are left. If there are not enough tips for the run to be conducted, the software will alert the user.
7. Load the sample preparation reagents into the barcoded reagent reservoirs. The reagent reservoirs are available in two sizes: 200 mL and 50 mL. Follow the software wizard guide to select the correct reagent reservoir size. The reagent reservoir barcodes must face to the right of the carrier. Use the “scan-scan-pour-place” method to load sample preparation reagents:
   - Scan the reagent bottle barcode
   - Scan the reagent reservoir barcode
   - Pour the reagent into the reservoir
   - Place the filled reagent reservoir into the designated position on the reagent carrier

Note: The cobas® 4800 System has an internal clock to monitor the length of time the reagents are on-board. Once the WB is scanned, 1 hour is allowed to complete the loading process and click on the Start button. A countdown timer is displayed on the Workplace Tab. The system will not allow the run to start if the on-board timer has expired.

Note: To assure the accurate transfer of MGP, vortex or vigorously shake the MGP vial immediately prior to dispensing into the reagent reservoir.

8. Load amplification/detection reagents (MRSA/SA MMX and Co-factor 1), Proteinase K (PK) and controls [MRSA/SA (+) C, IC and (–) C] directly onto the reagent carriers. In order to prevent contamination, it is required to change gloves after handling positive controls.

Note: The software wizard will calculate the optimal number and size of cobas® MRSA/SA MMX reagent to use. This will be reflected in the “Kit size” column on the MMX and Co-factor loading screen. To use a different size of cobas® MRSA/SA MMX reagent, click the “Change kit size” button.

9. Start sample preparation by clicking on “Start run”.
10. After a successful sample preparation run, the “Sample Preparation results” button and the Unload button become available. If desired, select "Sample Preparation results" button to review the results then select "Unload" to unload the plate carrier. Alternatively, select "Unload" to unload the plate carrier without reviewing the results. See the **cobas**® 4800 System - User Assistance.
11. Follow the instructions in the **cobas**® 4800 System - User Assistance to seal the microwell plate, transport the plate to the **cobas z** 480 analyzer and start the amplification and detection run.

Note: The cobas® 4800 System has an internal clock to monitor the length of time after addition of the prepared samples to activated master mix. Amplification and detection should be started as soon as possible but no later than 90 minutes after the end of the cobas x 480 instrument run. A countdown timer is displayed on the Workplace Tab. The system will abort the run if the timer has expired.

12. When the amplification and detection run is completed, unload the microwell plate from the **cobas z** 480 analyzer.
13. Follow the instructions in the **cobas**® 4800 System - User Assistance to review and accept results.

Results

Quality control and validity of results

One set of **cobas**® MRSA/SA Test Positive and Negative Controls are included in each run. For any run, valid results must be obtained for both the Positive and Negative Control for the **cobas**® 4800 Software to display the reportable **cobas**® MRSA/SA Test results from that run.

Positive control

The MRSA (+) Control contains non-infectious DNA plasmids of both MRSA and *Staphylococcus aureus*. The MRSA/SA (+) Control monitors the nucleic acid extraction, amplification, and detection steps in a given run of the test. The MRSA/SA (+) Control result must be 'Valid'. If the MRSA/SA (+) Control results are consistently invalid, contact your local Roche office for technical assistance.

Negative control

The (–) Control result must be 'Valid'. If the (–) Control results are consistently invalid, contact your local Roche office for technical assistance.

Internal control

The Internal Control is a lambda phage molecule that contains randomized sequences and targets for internal control-specific primers and probe. The Internal Control is added to all specimens and the Positive and Negative Controls during sample preparation on the **cobas x** 480 instrument. The Internal Control monitors nucleic acid extraction, amplification, and detection steps for a given specimen. The Internal Control is also required for validation of the run controls.

Interpretation of results

Note: All assay and run validation is determined by the cobas® 4800 Software.

Note: A valid run may include both valid and invalid specimen results.

For a valid run, specimen results are interpreted as shown in Table 1.

Table 1 Result interpretation of the cobas® MRSA/SA Test

cobas® MRSA/SA Test	**Result Report and Interpretation**
Requested Result "MRSA/SA"	
POS MRSA, POS SA	**MRSA Positive, SA Positive** Specimen is positive for the presence of both MRSA and SA.
NEG MRSA, NEG SA	**MRSA Negative*, SA Negative*** Neither MRSA nor SA, if present, could be detected.
NEG MRSA, POS SA	**MRSA Negative*, SA Positive** MRSA, if present, could not be detected. Specimen is positive for the presence of SA.
Invalid MRSA, POS SA	**MRSA Invalid, SA Positive** **MRSA result is Invalid. Original specimen should be re-tested to obtain valid MRSA result.** Specimen is positive for the presence of SA.
Invalid MRSA, NEG SA	**MRSA Invalid, SA Negative*** MRSA result is Invalid. Original specimen should be re-tested to obtain valid MRSA results. SA, if present, could not be detected.
NEG MRSA, Invalid SA	**MRSA Negative***, **SA Invalid** MRSA, if present, could not be detected. SA result is Invalid. Original specimen should be re-tested to obtain valid SA result.
Invalid MRSA, Invalid SA	**MRSA Invalid, SA Invalid** Both MRSA and SA results are Invalid. Original specimen should be re-tested to obtain valid MRSA and SA results.
Failed	**No Result for Specimen** Consult the **cobas**® 4800 System - User Assistance for instructions to review run flags and recommended actions. If a clot was detected and sufficient volume remains, original specimen should be vortexed for a minimum of 10 seconds and re-tested to obtain valid MRSA and SA results.

Table 1 Result interpretation of the cobas® MRSA/SA Test (continued)

cobas® MRSA/SA Test	**Result Report and Interpretation**
Requested Result "MRSA"	
POS MRSA	**MRSA Positive** Specimen is positive for the presence of MRSA.
NEG MRSA	**MRSA Negative*** MRSA, if present, could not be detected.
Invalid MRSA	**MRSA Invalid** MRSA result is Invalid. Original specimen should be re-tested to obtain valid MRSA result.
Failed	**No Result for Specimen** Consult the **cobas**® 4800 System - User Assistance for instructions to review run flags and recommended actions. If a clot was detected and sufficient volume remains, original specimen should be vortexed for a minimum of 10 seconds and re-tested to obtain valid MRSA results.
Requested Result "SA"	
POS SA	**SA Positive** Specimen is positive for the presence of SA.
NEG SA	**SA Negative*** SA, if present, could not be detected.
Invalid SA	**SA Invalid** SA result is Invalid. Original specimen should be re-tested to obtain valid SA result.
Failed	**No Result for Specimen** Consult the **cobas**® 4800 System - User Assistance for instructions to review run flags and recommended actions. If a clot was detected and sufficient volume remains, original specimen should be vortexed for a minimum of 10 seconds and re-tested to obtain valid SA results.

* A negative result does not preclude the presence of MRSA and/or SA because results depend on adequate specimen collection, absence of inhibitors, and sufficient DNA to be detected.

Invalid results may be obtained if the specimen contains inhibitory substances that prevent nucleic acid target extraction and/or amplification and detection. See "**Procedural limitations**" for known interference substances.

Failed results may be obtained if the specimen contains clots that interfere with the sample preparation procedure on the **cobas**® 4800 instrument.

List of result flags

The following table lists flags which are relevant for result interpretation.

Table 2 List of flags for the **cobas**® MRSA/SA Test

cobas® MRSA/SA Test	cobas® MRSA/SA Test	Result Report and Interpretation
R20	The positive control is invalid.	An external control is invalid. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
R21	The negative control is invalid.	An external control is invalid. 1. Repeat entire run with fresh reagents. 2. If the problem persists, contact Roche Service.
X3	Error: Clot was detected Sample was not processed.	Make sure that the samples were handled according to the workflow description. 1. Check the sample for clots. 2. Rerun the sample.
X4	Error: Pipetting error occurred. Sample was not processed.	Insufficient sample volume or mechanical error during pipetting is the most likely reason. 1. Make sure that there is enough sample volume. 2. Check whether the tip eject plate is placed correctly. 3. Rerun the sample.

Culturing of clinical specimens

In order to perform antimicrobial susceptibility testing or epidemiological typing, clinical specimens may be cultured from the collection media. Refer to the COPAN MSwab Collection, Transport and Preservation System Package Insert for culture processing instructions.

Note: The minimum sample volume to conduct a single cobas® MRSA/SA Test is 700 µL in the primary MSwab specimen container. To avoid cross-contamination, it is recommended that primary tubes be processed on the cobas® 4800 System prior to removing aliquots for bacterial culture. If aliquots of the specimen must be taken for culture purpose prior to the cobas® MRSA/SA Test, make sure at least 700 µL of the specimen is still present and exercise utmost caution while handling the specimen. Testing specimens with less than 700 µL volume may result in false negative results.

Procedural limitations

1. The **cobas**® MRSA/SA Test has only been validated for use with nasal swab specimens collected with the MSwab Collection, Transport and Preservation System.
2. Reliable results are dependent on adequate specimen collection, transport, storage and processing. Follow the procedures in this Instructions-For-Use document (also referred to as a Package Insert), Package Inserts for the MSwab Collection, Transport and Preservation System and the **cobas**® 4800 System - User Assistance.

3. Detection of MRSA and SA is dependent on the number of organisms present in the specimen and may be affected by specimen collection methods, patient factors (i.e., stage of colonization, history of hospitalization, antibiotic treatment regime, proximity with MRSA carrier) and/or MRSA/SA strains.
4. False negative or invalid results may occur due to interference from various substances. The Internal Control is included in the **cobas**® MRSA/SA Test to help identify the specimens containing substances that may interfere with nucleic acid isolation and PCR amplification. Known interference includes, but is not limited to the following:
   - Specimens containing greater than 75% (v/v) blood per swab may generate false negative results. Do not test samples that appear dark red or brown in color.
   - Specimens containing greater than 10% (w/v) mucin per swab may generate false negative results.
   - Specimens containing greater than 15% (w/v) Rhinaris® Nasal Gel per swab may generate false negative results.
   - Specimens containing greater than 25% Releev (v/v) per swab may generate false negative results.
5. A positive result is indicative of the presence of MRSA DNA and not necessarily viable organisms. Therefore, a positive result does not necessarily mean eradication treatment failure. A negative result following a previously positive test result may indicate eradication treatment success or may occur due to intermittent colonization.
6. The **cobas**® MRSA/SA Test does not detect the mecA gene directly nor the penicillin-binding protein (PBP 2a) encoded by this gene. A false positive MRSA result may occur if an "empty cassette variant" *Staphylococcus aureus* is present.
7. Mutations or polymorphisms in primer- or probe-binding regions may affect detection of new or unknown variants, resulting in a false negative result with the **cobas**® MRSA/SA Assay.
8. The predictive value of an assay depends on the prevalence of the disease in any particular population.
9. The addition of AmpErase enzyme into the **cobas**® 4800 MRSA/SA Master Mix enables selective amplification of target DNA; however, good laboratory practices and careful adherence to the procedures specified in this Instructions-For-Use document are necessary to avoid contamination of reagents and amplification mixtures.
10. Use of this product must be limited to personnel trained in the techniques of PCR and the use of the **cobas**® 4800 System.
11. Only the **cobas x** 480 instrument and **cobas z** 480 analyzer have been validated for use with this product. No other sample preparation instrument or PCR System can be used with this product.
12. Due to inherent differences between technologies, it is recommended that, prior to switching from one technology to the next, users perform method correlation studies in their laboratory to qualify technology differences. One hundred percent agreement between the results should not be expected due to aforementioned differences between technologies.
13. Cross-contamination can cause false positive results. The sample to sample cross-contamination rate of the **cobas**® MRSA/SA Test on the **cobas**® 4800 System has been determined in a non-clinical study to be 0%. Run to run cross-contamination has not been observed.

Non-clinical performance evaluation

Analytical sensitivity

The analytical sensitivity (Limit of Detection or LOD) for the **cobas**® MRSA/SA Test was determined by analyzing quantified MRSA and SA culture isolates at multiple levels with at least 61 replicates per level. The test samples were prepared by spiking culture into the FLOQ swab, and then incubating the swab in a simulated nasal swab matrix. The simulated matrix composed of mucin and human cells mimics the effect of the clinical nasal specimen background on the **cobas**® MRSA/SA Test. All panel members were tested using the **cobas**® MRSA/SA Test across three lots of **cobas**® MRSA/SA Test reagents. LOD for this test is defined as the target concentration which can be detected as positive in ≥ 95% of the replicates tested, based on results generated by the worst performing reagent lot.

Two MRSA isolates and one SA isolate were tested in the analytical sensitivity study. The **cobas**® MRSA/SA Test LOD on these isolates are shown in Table 3.

Table 3 **cobas**® MRSA/SA Test Limit of Detection (LOD)

Organism	Origin	Origin ID	RE Type	SCCmec Type	Spa Type	PFGE	MIC Value	Levels Tested	LOD (CFU/swab)
MRSA	NARSA	NRS384	2	IVa	t008	USA300-0114	32	9	650
MRSA	ATCC	43300	2	II	t007	Sac-15	N/A	8	700
SA	NARSA	NRS164	N/A	N/A	t084	N/A	N/A	8	700

N/A = not applicable

Detection of MRSA and SA genotypes

The limits of detection of the **cobas**® MRSA/SA Test on 35 MRSA isolates and five SA isolates representing common genotypes (including RE types 1, 2, 3, 4, 6, MRSA SCCmec types I, II, III, IV, V, VI, and VIII, and MRSA pulse-field gel electrophoresis (PFGE) types USA 100 to 1000) were verified by testing 40 replicates per level at multiple levels. MRSA/SA genetic diversity of this collection is covered by different SCCmec types, MREJ types and spa types found in the species *Staphylococcus aureus* based on its phylogenetic structure and representative strains of various PFGE types. Dilutions and testing samples were prepared in a similar fashion as in the Limit of Detection (LOD) study described previously. The lowest level that had at least 95% observed hit rate are summarized in Table 4 and Table 5. The results have shown that the **cobas**® MRSA/SA Test detects all 35 MRSA and five SA strains correctly with LOD ranging from 175 to 750 CFU/Swab. Strains detected represent at least eight SCCmec types (I, II, III, IV, V, VI, VIII, and new), 10 MREJ types, 21 spa-types, nine PFGE types, and cefoxitin MIC values of 8 to greater than 32.

Table 4 **cobas**® MRSA/SA Test Limit of Detection (LOD) on MRSA genotypes

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

Table 5 **cobas**® MRSA/SA Test Limit of Detection (LOD) on SA genotypes

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

Geographical inclusivity

In addition to the 37 MRSA and six SA isolates included in the analytical sensitivity and genotype inclusivity studies shown above, 281 MRSA isolates and 85 SA isolates collected from diverse geographical locations were tested at concentrations near the detection limit of the **cobas**® MRSA/SA Test. The collection of 281 MRSA isolates from 16 countries contained MRSA isolates of different SCCmec types (I, II, III, IV, IVa, V, VI, VII, and new), 71 spa types and cefoxitin MIC values from 6 to greater than 256. The collection of 85 SA isolates from geographically diverse locations within US contained SA isolates of 75 different spa types. The **cobas**® MRSA/SA Test detected all 85 SA isolates. Of the 281 MRSA isolates, 277 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. The geographical sources of the MRSA collection are shown in Table 6.

Table 6 Geographical inclusivity of the **cobas**® MRSA/SA Test

Geographical Origin	Total Number of MRSA Isolates	Detected by cobas® MRSA/SA Test
UK	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**

Precision

In-house precision study was conducted with two MRSA and one SA isolates diluted in a simulated nasal swab matrix to concentration levels below Limit of Detection (LOD), near LOD and above LOD of the **cobas**® MRSA/SA Test. A negative level composed of only the simulated nasal swab matrix was also tested. The study used three unique lots of **cobas**® MRSA/SA Test reagents and three instruments for a total of 36 runs over 12 days. A description of the precision panels and the study performance hit rate is shown in Table 7. An analysis of the variance of the Ct values from tests performed on positive panel members above the LOD level (see Table 8 and Table 9) yielded overall CV (%) ranges from 0.8% to 1.3% for MRSA Ct and 1.2% for SA Ct.

Table 7 In-house precision study hit rate analysis

Panel Member	Isolate	Target Concentration	N Tested	N Positive	Hit Rate	95% CI	
						Lower	Upper
1	NRS164 (SA)	< 1 x LOD	71	67	94.4%	86.2%	98.4%
2	NRS164 (SA)	~ 1 x LOD	72	72	100.0%	95.0%	100.0%
3	NRS164 (SA)	~ 3 x LOD	72	72	100.0%	95.0%	100.0%
4	NRS384 (MRSA)	< 1 x LOD	72	57	79.2%	68.0%	87.8%
5	NRS384 (MRSA)	~ 1 x LOD	72	72	100.0%	95.0%	100.0%
6	NRS384 (MRSA)	~ 3 x LOD	72	72	100.0%	95.0%	100.0%
7	ATCC43300 (MRSA)	< 1 x LOD	72	63	87.5%	77.6%	94.1%
8	ATCC43300 (MRSA)	~ 1 x LOD	72	72	100.0%	95.0%	100.0%
9	ATCC43300 (MRSA)	~ 3 x LOD	72	72	100.0%	95.0%	100.0%
10	None	Negative	72	0	0.0%	0.0%	5.0%

Table 8 Variance components analysis for precision panel members above the LOD

Strain	Mean Ct	Variance Components/Percent Contribution to Total					Total
		Lot	Kit Size	Instrument	Run	Random	
NRS 164 (SA)	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%
NRS 384 (MRSA)	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%
ATCC 43300	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%

Table 9 Standard deviations and coefficients of variation (%) analysis for precision panel members above the LOD

Strain	Mean Ct	SD Components/ CV (%)					Total
		Lot	Kit Size	Instrument	Run	Random	
NRS164 (SA)	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%
NRS 384 (MRSA)	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%
ATCC 43300	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%

Competitive inhibition

Panels were constructed with two MRSA isolates 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 10).

Table 10 Competitive inhibition study for MRSA by SA (Ct values)

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

Analytical specificity

To assess the analytical specificity of the **cobas**® MRSA/SA Test, the following panels were tested: 1) 92 bacteria, fungi and viruses that may be found in nasal swab specimens (Table 11); 2) human cells (Table 11); 3) 43 Coagulase-Negative *Staphylococcu*s (CoNS) and Methicillin-Resistant Coagulase-Negative *Staphylococcus* (MR-CoNS) (Table 12); 4) 10 Borderline Methicillin-Resistant SA (BORSA) isolates and two SA isolates for MRSA specificity only (Table 13).

All bacteria and human cells were spiked to 1 x 10^6 Units*/mL or higher except for *Chlamydia pneumonia*, and all viruses were spiked to the highest concentration allowed by the respective stocks (1 x 10^5 Units*/mL except for adenovirus 1 and Influenza A/H1N1). Testing was performed with the organisms alone or with two MRSA and one SA isolates present at 3 x Limit of Detection (LOD) of the **cobas**® MRSA/SA Test. Results indicated that none of these organisms interfered with detection of intended MRSA or SA targets. None produced false positive results when there was no intended MRSA/SA target present.

*All bacteria were quantified as Colony Forming Units (CFU) except *Chlamydia pneumoniae* which was quantified as DNA copies. Human metapneumovirus was quantified in viral particles. Adenovirus 1, 7 and 40, Human enterovirus, HSV1, Influenza A/H3N2A/Hong Kong/8/68, Measles virus, Mumps virus, parainfluenza 1, parainfluenza 2 and parainfluenza 3, Corona Virus 229E, Corona Virus OC43, Cytomegalovirus, and Rhinovirus were all quantified in plaque-forming unit (PFU). RSV A, and RSV B were quantified in $TCID_{50}$ units. Influenza A/H1N1 and Influenza B were quantified in EID_{50} units. EBV was quantified in copies.

Table 11 Microorganisms commonly found in nasal flora tested for analytical specificity

Acinetobacter baumannii	*Haemophilus parainfluenzae*	*Streptococcus anginosus*
Acinetobacter haemolyticus	*Issatchenkia orientalis*	*Streptococcus mitis*
Bacillus cereus	*Klebsiella oxytoca*	*Streptococcus mutans*
Bordetella bronchiseptica	*Klebsiella pneumoniae (KPC producing) ATCC # 700603*	*Streptococcus pneumoniae*
Bordetella parapertussis	*Klebsiella pneumoniae (KPC producing) ATCC # BAA1900*	*Streptococcus pyogenes*
Bordetella pertussis	*Lactobacillus crispatus*	*Streptococcus salivarius*
Burkholderia cepacia	*Lactobacillus delbrueckii*	*Streptococcus sanguinis*
Candida albicans	*Legionella pneumophila*	*Streptococcus suis*
Candida glabrata	*Leifsonia aquatica*	*Yersinia enterocolitica*
Candida parapsilosis	*Listeria monocytogenes*	*Adenovirus 40*
Candida tropicalis	*Microbacterium testaceum*	*Coronavirus 229E*
*Chlamydia pneumoniae**	*Micrococcus luteus*	*Coronavirus OC43*
Citrobacter freundii	*Moraxella catarrhalis*	*Cytomegalovirus*
Citrobacter koseri	*Mycobacterium tuberculosis avirulent*	*Epstein Barr Virus*
Corynebacterium amycolatum	*Mycoplasma pneumoniae*	*HSV 1*
Corynebacterium bovis	*Mycoplasma salivarium*	*Human Adenovirus type 1**
Corynebacterium flavescens	*Neisseria meningitidis*	*Human Adenovirus type 7A*
Corynebacterium genitalium	*Parvimonas micra*	*Human enterovirus 71*
Corynebacterium glutamicum	*Pasteurella aerogenes*	*Human metapneumovirus*
Corynebacterium jeikeium	*Planococcus maritimus*	Influenza A/H1N1
Cryptococcus neoformans	*Proteus mirabilis*	Influenza A/H3N2 A/Hong Kong/8/68
Eikenella corrodens	*Proteus vulgaris*	Influenza B
Enterobacter aerogenes	*Providencia stuartii*	Measles virus
Enterobacter cloacae	*Pseudomonas aeruginosa*	Mumps virus
Enterococcus flavescens	*Pseudomonas fluorescens*	Parainfluenza 1
Enterococcus gallinarum	*Rhodococcus equi*	Parainfluenza 2
Enterococcus hirae	*Rothia mucilaginosa*	Parainfluenza 3
Escherichia coli	*Salmonella enterica subsp. Enterica*	Rhinovirus type 1A
Finegoldia magna	*Serratia marcescens*	RSV A
Haemophilus aphrophilus	*Shigella sonnei*	RSV B
Haemophilus influenzae	*Streptococcus agalactiae*	HCT-15 cells (human genomic DNA)

* *Chlamydia pneumoniae* was tested at 1.0 x 10^5 copies/mL and Adenovirus type 1 at 1.0 x 10^4 PFU/mL.

Table 12 Closely related CoNS and MR-CoNS organisms tested for specificity

Staphylococcus arlettae	*Staphylococcus epidermidis ATCC27676 (Methicillin-resistant)*	*Staphylococcus pasteuri*
Staphylococcus auricularis (Methicillin-resistant)	*Staphylococcus equorum*	*Staphylococcus pseudointermedius*
Staphylococcus caprae (Methicillin-resistant)	*Staphylococcus felis*	*Staphylococcus pulvereri*
Staphylococcus capitis	*Staphylococcus gallinarum*	*Staphylococcus saprophyticus*
Staphylococcus carnosus	*Staphylococcus haemolyticus ATCC29970*	*Staphylococcus schleiferi*
Staphylococcus chromogenes	*Staphylococcus haemolyticus ATCC29968 (Methicillin-resistant)*	*Staphylococcus sciuri*
Staphylococcus cohnii	*Staphylococcus haemolyticus ATCC43252*	*Staphylococcus simulans ATCC27848 (Methicillin-resistant)*
Staphylococcus delphini	*Staphylococcus hominis ATCC25615*	*Staphylococcus simulans ATCC11631*
Staphylococcus epidermidis ATCC14990 (Methicillin-resistant)	*Staphylococcus hominis ATCC35982*	*Staphylococcus warneri ATCC27836 (Methicillin-resistant)*
Staphylococcus epidermidis ATCC35547 (Methicillin-resistant)	*Staphylococcus hominis ATCC27844*	*Staphylococcus warneri ATCC27839 (Methicillin-resistant)*
Staphylococcus epidermidis ATCC35983 (Methicillin-resistant)	*Staphylococcus hominis ATCC27845*	*Staphylococcus warneri RMSCC1224*
Staphylococcus epidermidis ATCC35984 (Methicillin-resistant)	*Staphylococcus intermedius*	*Staphylococcus xylosus ATCC35663*
Staphylococcus epidermidis ATCC51624 (Methicillin-resistant)	*Staphylococcus kloosii*	*Staphylococcus xylosus ATCC29971*
Staphylococcus epidermidis ATCC51625 (Methicillin-resistant)	*Staphylococcus lentus*	-
Staphylococcus epidermidis ATCC700583	*Staphylococcus lugdunensis*	-

Table 13 SA and BORSA isolates tested for MRSA specificity

Staphylococcus aureus 10851	*Staphylococcus aureus 10323 (BORSA)*
Staphylococcus aureus 10852	*Staphylococcus aureus 10324 (BORSA)*
Staphylococcus aureus 10319 (BORSA)	*Staphylococcus aureus 10325 (BORSA)*
Staphylococcus aureus 10320 (BORSA)	*Staphylococcus aureus 10326 (BORSA)*
Staphylococcus aureus 10321 (BORSA)	*Staphylococcus aureus 10327 (BORSA)*
Staphylococcus aureus 10322 (BORSA)	*Staphylococcus aureus 10328 (BORSA)*

Interference

Twenty-five commonly used nasal or throat medications, as well as whole blood and mucin, were tested for potential interference effects with the **cobas**® MRSA/SA Test. All substances were tested at levels above what could be reasonably expected to be collected by a swab in a nasal specimen. The amount of interference substance is expressed as the percentage of the maximum amount that a swab can absorb or carry. Two MRSA isolates and one SA isolate were spiked to 3 x Limit of Detection (LOD) of the **cobas**® MRSA/SA Test and used as targets in the tests. No interference was observed up to 100% of the swab capacity for exogenous substances, except for Relenza® (no interference up to 6.25% of swab capacity), Rhinaris® Nasal Gel (no interference up to 15% of swab capacity) and Releev (no interference up to 25% of swab capacity). Note that Relenza® was tested only up to 6.25% of the swab capacity because this already represents the whole amount of a normal application of the drug according to prescription. For whole blood, no interference was observed up to 75% of the swab capacity, and for mucin, no interference was observed up to 10% of the swab capacity. These results are summarized in Table 14.

Table 14 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.

Correlation

The performance of the **cobas**® MRSA/SA Test was compared to a FDA-cleared and CE-marked State-of-the-Art comparator NAT, using combined direct/enrichment bacterial culture as reference method. Two nasal swab specimens were collected from each subject enrolled in the study. The specimen for the **cobas**® MRSA/SA Test was collected with MSwab Collection, Transport and Preservation System, and the specimen for the comparator test was collected with Liquid Stuart swabs. The MSwab specimen was used to inoculate one plate each of selective and differential chromogenic media for MRSA and for SA (direct culture), as well as a tube containing Trypticase Soy Broth (TSB) with 6.5% NaCl for enrichment over-night (enrichment culture). The enrichment culture was then plated on chromogenic media. Presumptive SA positive colonies on chromogenic plates were confirmed by a latex agglutination test (Staphraurex®, Remel Microbiology Products, Thermo Scientific, Inc.). Presumptive MRSA colonies were further confirmed by cefoxitin disc diffusion test.

A total of 383 subjects were enrolled from two sites in the EU. Four subjects were excluded due to incomplete results for one of the tests. There were 27 MRSA positive and 144 SA positive specimens by combined direct/enrichment culture (prevalence: MRSA 7.1%, SA 38.0%). The performance of the **cobas**® MRSA/SA Test and the comparator NAT against direct culture is shown in Table 15.

Table 15 **cobas**® MRSA/SA and comparator Nucleic Acid Tests (NAT) against direct culture

MRSA		**Direct Culture**	
		Positive	**Negative**
cobas® MRSA/SA	**Positive**	**15**	**18**
	Negative	**1**	**345**
	Estimate	95% CI LL	95% CI UL
Sensitivity	94%	70%	100%
Specificity	95%	92%	97%

SA		**Direct Culture**	
		Positive	**Negative**
cobas® MRSA/SA	**Positive**	**121**	**29**
	Negative	**5**	**224**
	Estimate	95% CI LL	95% CI UL
Sensitivity	96%	91%	99%
Specificity	89%	84%	92%

MRSA		**Direct Culture**	
		Positive	**Negative**
Comparator NAT	**Positive**	**14**	**16**
	Negative	**2**	**347**
	Estimate	95% CI LL	95% CI UL
Sensitivity	88%	62%	98%
Specificity	96%	93%	97%

SA		**Direct Culture**	
		Positive	**Negative**
Comparator NAT	**Positive**	**122**	**31**
	Negative	**4**	**222**
	Estimate	95% CI LL	95% CI UL
Sensitivity	97%	92%	99%
Specificity	88%	83%	92%

The performance of the **cobas**® MRSA/SA Test and the comparator NAT against direct/enrichment culture is shown in Table 16. In this analysis, a positive result by either direct or enrichment culture is considered positive. A negative result can only be obtained if both direct and enrichment culture results are negative.

Table 16 **cobas**® MRSA/SA and comparator Nucleic Acid Tests (NAT) against direct/enrichment culture

MRSA		Direct/Enrichment Culture	
		Positive	**Negative**
cobas® MRSA/SA	**Positive**	**25**	**8**
	Negative	**2**	**344**
	Estimate	95% CI LL	95% CI UL
Sensitivity	93%	76%	99%
Specificity	98%	96%	99%

SA		Direct/Enrichment Culture	
		Positive	**Negative**
cobas® MRSA/SA	**Positive**	**137**	**13**
	Negative	**7**	**222**
	Estimate	95% CI LL	95% CI UL
Sensitivity	95%	90%	98%
Specificity	94%	91%	97%

MRSA		Direct/Enrichment Culture	
		Positive	**Negative**
Comparator NAT	**Positive**	**24**	**6**
	Negative	**3**	**346**
	Estimate	95% CI LL	95% CI UL
Sensitivity	89%	71%	98%
Specificity	98%	96%	99%
NPV	99%	98%	100%
PPV	80%	61%	92%

SA		Direct/Enrichment Culture	
		Positive	**Negative**
Comparator NAT	**Positive**	**138**	**15**
	Negative	**6**	**220**
	Estimate	95% CI LL	95% CI UL
Sensitivity	96%	91%	98%
Specificity	94%	90%	96%
NPV	97%	94%	99%
PPV	90%	84%	94%

Finally, the performance of the **cobas**® MRSA/SA Test in direct comparison to a FDA-cleared and CE-marked State-of-the-Art comparator NAT is shown in Table 17.

Table 17 **cobas**® MRSA/SA Test against comparator Nucleic Acid Test (NAT)

MRSA		Comparator NAT	
		Positive	**Negative**
cobas® MRSA/SA	**Positive**	**28**	**5**
	Negative	**2**	**344**
	Estimate	95% CI LL	95% CI UL
Positive Agreement	93%	78%	99%
Negative Agreement	99%	97%	100%

SA		Comparator NAT	
		Positive	**Negative**
cobas® MRSA/SA	**Positive**	**144**	**6**
	Negative	**9**	**220**
	Estimate	95% CI LL	95% CI UL
Positive Agreement	94%	89%	97%
Negative Agreement	97%	94%	99%

Additional information

Key assay features

Sample type	Nasal swab
Amount of sample required	1.6 mL of MSwab media in primary vial, a minimum of 700 µL is required for a **cobas**® MRSA/SA Test.
Test duration	Results are available within 2.5 hours after loading the specimen on the system (1-22 specimens).
Analytical sensitivity	From 175 CFU/swab to 750 CFU/swab depending on isolate.
Specificity	No cross-reactivity with 147 closely related organisms or organisms typically found in nasal specimens.
Inclusivity	314 MRSA isolates and 91 SA isolates from 16 countries, representing at least 7 SCCmec types, 10 RE types and 71 spa types were tested and detected in total.

Symbols

The following symbols are used in labeling for Roche PCR diagnostic products.

Table 18 Symbols used in labeling for Roche PCR diagnostic products

	Ancillary Software		*In Vitro* diagnostic medical device
	Authorized representative in the European community		Lower Limit of Assigned Range
	Barcode Data Sheet		Manufacturer
	Batch code		Store in the dark
	Biological risks		Contains sufficient for <*n*> tests
	Catalogue number		Temperature limit
	Consult instructions for use		Test Definition File
	Contents of kit		Upper Limit of Assigned Range
	Distributed by		Use-by date
	For IVD performance evaluation only		Global Trade Item Number
Rx Only	US Only: Federal law restricts this device to sale by or on the order of a physician.		

This product fulfills the requirements of the European Directive 98/79 EC for *in vitro* diagnostic medical devices.

US Customer Technical Support 1-800-526-1247

Manufacturer and distributors

Table 19 Manufacturer and distributors

Roche Molecular Systems, Inc.
1080 US Highway 202 South
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Roche Diagnostics (Schweiz) AG
Industriestrasse 7
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Roche Diagnostics GmbH
Sandhofer Strasse 116
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Roche Diagnostics, SL
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Roche Diagnostica Brasil Ltda.
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Roche Diagnostics
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(For Technical Assistance call:
Pour toute assistance technique,
appeler le: 1-877-273-3433)

Roche Diagnostics
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38240 Meylan, France

Distributore in Italia:
Roche Diagnostics S.p.A.
Viale G. B. Stucchi 110
20052 Monza, Milano, Italy

Distribuidor em Portugal:
Roche Sistemas de Diagnósticos Lda.
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Trademarks and patents

See http://www.roche-diagnostics.us/patents

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Document revision

Document Revision Information	
Doc. Rev. 3.0 12/2018	Updated Software (Core) version to 2.2.0 or higher. Added reference to **cobas**® 4800 System - User Assistance. Removed reference to **cobas**® 4800 System - System Manual. Removed reference to **cobas**® 4800 System – Operator's Manual for **cobas**® MRSA/SA Test. Changed "Tris-HCl buffer" to "Tris buffer" as a reagent component. Added a Procedural Limitation that 100% agreement between results should not be expected in correlation studies. Updated descriptions of and added Rx Only symbol and description to the harmonized symbol page at the end of the package insert. Added Roche web address www.roche.com. Updated Mannheim, Germany address format per current standard. Please contact your local Roche Representative if you have any questions.