ELITechGroup
EMPOWERING IVD

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NOTICE of CHANGE dated 13/10/2020

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

« MRSA/SA ELITe MGB Kit»
Ref. M800351

This new revision of the Instruction for Use (IFU) contains the following changes:

- *Introduction of the new product reference "ELITe InGenius Sonication tubes" (ref. INT032SON) to be used in combination with the product for sample sonication.*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS ALSO COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET EST PARFAITEMENT COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE TAMBIÉN CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU ÉTAMBÉM COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIESE FASSUNG DER GEBRAUCHSANLEITUNG IST KOMPATIBEL MIT DER VORHERIGEN VERSION DES TESTKITS

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MRSA/SA ELITe MGB® Kit

Reagent for DNA Real-Time amplification

REF M800351

CE

-20°C

TABLE OF CONTENTS

INTENDED USE

The **«MRSA/SA ELITe MGB® Kit»** product is part of a qualitative nucleic acids amplification assay for the **detection of** *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA, including the recently identified mecC strain) in DNA samples extracted from nasal swabs and Blood Culture.

The product is intended to aid in the prevention and control of MRSA infections in healthcare settings and is intended to aid in the diagnosis of MRSA infections, not to guide or monitor treatment for MRSA infections. A negative result does not preclude MRSA/SA nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

ASSAY EXPLANATION

Staphylococcus aureus is an opportunistic pathogen carried as a commensal organism on the skin and nares of approximately 30% of the normal population potentially causing a broad spectrum of diseases. SA and especially MRSA is consistently a leading cause of nosocomial infections and is associated with substantial morbidity, mortality, and cost. Emergence of community-associated MRSA infections calls for active surveillance of patients admitted to hospitals or other health care facilities for SA and MRSA to identify patients who may serve as a reservoir of infection for other patients.

The **«MRSA / SA ELITe MGB® Kit»** is a triplex real-time amplification-based assay that targets the conservative regions in a ***Staphylococcus aureus*-specific gene**, responsible for coagulase positive SA identification. The assay also targets the ***mecA gene***, including the ***mecC*** variant, which has been recently designated ***mecC* gene** (Ito T. et al.), responsible for resistance to methicillin and other beta-lactam antibiotics and an exogenous internal control, to control reaction inhibition and reagent integrity.

The *Staphylococcus aureus*-specific gene will unambiguously identify coagulase positive SA and the *mecA* genes will unambiguously identify methicillin resistance. Presence of both markers at the same relative quantity measured by a difference in cycle threshold value is indicative of MRSA; different relative quantities or presence of only *Staphylococcus aureus*-specific gene marker is indicative of SA.

MRSA/SA real-time amplification-based assays significantly reduce laboratory time compared with standard culture tests, improving the efficiency of the procedure. Current real-time PCR MRSA detection tests target the SCC*mec* (*mecA* carrying mobile genetic element called Staphylococcal Cassette Chromosome) insertion site, and /or the *mecA* gene and /or the *spa* gene. The **«MRSA / SA ELITe MGB® Kit»** targets conservative regions in MRSA and SA genetic markers, therefore minimizing false negative calls due to a natural SCC*mec* insertion site variability and minimizing false positive calls due to the "empty cassette" issue.

ASSAY PRINCIPLES

The assay consists of a real time amplification reaction with a programmable thermostat provided with a fluorescence detection optical system.

The probe specific to the SA-specific gene is based on ELITe MGB® technology, labelled with AP554 fluorophore (similar to TAMRA) and is activated when hybridisation occurs with the specific product of the *Staphylococcus aureus* amplification reaction.

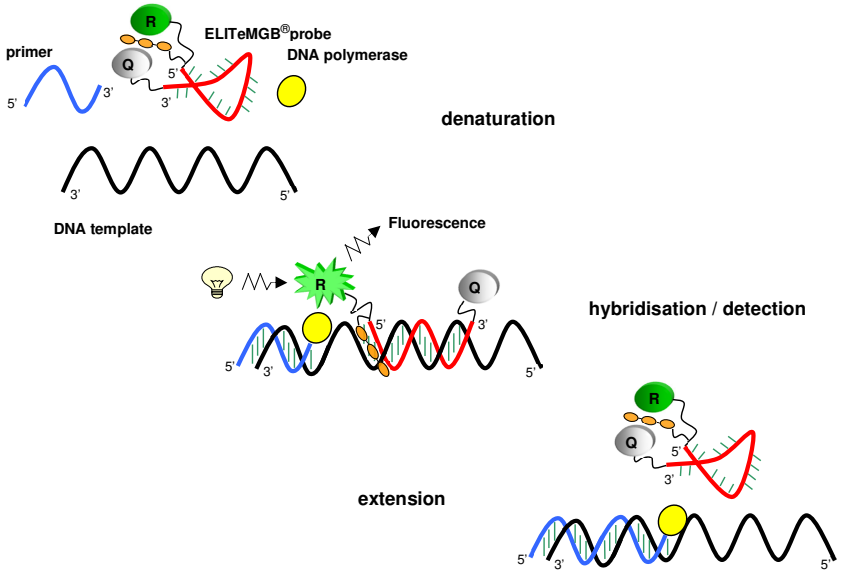
The probes specific to the *mecA* and the *mecC* genes are based on ELITe MGB® technology, labelled with FAM fluorophore and are activated when hybridisation occurs with the specific product of the antibiotic resistance gene amplification reactions.

The probe specific to the Internal Control is based on ELITe MGB® technology, labelled with AP642 fluorophore (similar toCy5) and is activated when hybridisation occurs with the specific product of the Internal Control amplification.

As the amount of specific product of the amplification increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data allows detecting the presence of MRSA or SA DNA in the starting sample.

The assay is validated with the systems described in this instruction for use.

In the following picture the mechanism of activation and fluorescence emission of ELITe MGB® technology probe is shown. Note that the probe is not hydrolysed during the amplification cycle.



PRODUCT DESCRIPTION

The **«MRSA/SA ELITe MGB® Kit»** product supplies the **ready to use** complete mixture for real time amplification in a stabilising solution. The mixture is aliquoted into **four ready-to-use test tubes**. Each tube contains **540 µL** of solution, sufficient for **24 tests** in association with **«ELITe InGenius®»** and **25 tests** in association with other systems.

The *Staphylococcus aureus*-specific gene-specific primers and probe (stabilised by MGB® group, labelled with AP554 (AquaPhluor® 554) fluorophore, similar to TAMRA, and quenched by a non-fluorescent molecule) are specific to a conservative region in the coagulase positive ***Staphylococcus aureus***.

The *mecA* and *mecC* gene specific primers and probes (stabilised by MGB® group, labelled with FAM fluorophore and quenched by a non-fluorescent molecule) are specific to conservative regions in the ***mecA*** and ***mecC* genes** that are responsible for resistance to methicillin and other beta-lactam antibiotics.

The Internal Control primers and probe (stabilised with MGB® group, labelled with AP642 fluorophore, similar to Cy5, and quenched by a non-fluorescent molecule) are specific to a non-infectious plasmid DNA containing **Internal Control** artificial sequences.

The reaction mixture also provides buffer, magnesium chloride, triphosphate nucleotides, AP593 fluorophore (similar to ROX) used as passive reference to normalise fluorescence, the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by an amplification product, and the "hot start" DNA polymerase.

The product is sufficient for **96 tests in association with ELITe InGenius**, including controls.

The product is sufficient for **100 tests in association with other systems**, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazard
MRSA/SA PCR Mix	Complete reaction mixture	**4 x 540 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable powderless nitrile or similar material gloves.
- Vortex mixer.
- Benchtop microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile aerosol barrier tips or sterile positive displacement tips (2 - 20 µL, 5 - 50 µL, 50 - 200 µL, 200 - 1000 µL).
- Molecular biology grade water.
- Trypticase Soy Broth.
- Programmable thermal cycler with optical fluorescence detection system 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems®) or 7500 Real-Time PCR System (Applied Biosystems®) calibrated following manufacturer's instructions.

OTHER PRODUCTS REQUIRED

The reagents for sample collection and for DNA extraction from the samples, the internal control, the positive control of the amplification and and the consumables **are not** included in this product.

The generic product, **«eSwab Collection Kit»** (Copan, ref. 480CE), is recommended to collect patient samples when the assay is used in combination with **«ELITe InGenius»**

The generic product, **«eNAT™ kit»** (Copan, ref. 608CS01R), is recommended to collect patient samples when the assay is used in combination with **«ELITe InGenius».**

The generic product, **«BBL CultureSwab Plus Amies Gel without Charcoal swabs»** (Becton-Dickinson, ref. 220116), is recommended to collect patient samples when the assay is used in combination with EasyMag extraction and 7500 Fast Dx Real-Time PCR thermal cycler.

For automatic DNA extraction, Real Time amplification and result interpretation of samples to be analyzed, the «**ELITe InGenius**» (ELITechGroup S.p.A., ref. INT030) instrument and the following specific Assay protocols are required:

- parameters for the amplification positive control «**MRSA-SA ELITe_PC**» (ELITechGroup S.p.A.),

- parameters for the amplification negative control «**MRSA-SA ELITe_NC**» (ELITechGroup S.p.A.),

- parameters for samples to be analyzed «**MRSA-SA ELITe_NS_200_50**» and «**MRSA-SA ELITe_BC_200_100**» (ELITechGroup S.p.A.).

For automatic sample analysis with the instrument «**ELITe InGenius**» (ELITechGroup S.p.A., ref. INT030) the following generic products are required:

- extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200),

- consumables for extraction and amplification **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS),

- consumables for sonication **«ELITe InGenius® Sonication tubes» (**ELITechGroup S.p.A, ref. INT032SON),

- amplification cartridges **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR),

- tips **«300 µL Universal Filter Tips»** (Axygen BioScience Inc., CA, ref. TF-350-L-R-S),

- boxes **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000).

For automatic DNA extraction from samples to be analyzed, the following generic products **«NucliSENS® easyMAG®Strip for Premix»** (bioMérieux SA, ref. 278303), **«bioHit Electronic Multichannel Pipettor»** (bioMérieux SA, ref. 280141), **«Filter tips for bioHit»**(bioMérieux SA, ref. 280146) and **«NucliSENS® easyMAG® Reagents»** (bioMérieux SA, ref. 280130, 280131, 280132, 280133, 280134, 280135) to be used with the instrument **«NucliSENS® easyMAG®»** (bioMérieux SA, ref. 200111) are also validated.

As template of extraction and inhibition internal control, the generic product **«CPE - Internal Control»** (ELITechGroup S.p.A., ref. CTRCPE), is required. This is a stabilised solution containing two plasmid DNAs and the genomic RNA of MS2 phage.

When 7500 Fast Dx Real-Time PCR System is used for DNA amplification, it is required the use of generic product **«Q - PCR Microplates Fast»** (ELITechGroup S.p.A., ref. RTSACC02), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification.

When 7500 Real-Time PCR System is used for DNA amplification, it is required the use of generic product **«Q - PCR Microplates»** (ELITechGroup S.p.A., ref. RTSACC01), microplates with 0.2 mL wells and adhesive sealing sheets for real time amplification.

The main product, **«MRSA/SA - ELITe Positive Control»** (ELITechGroup S.p.A., ref. M800356), plasmid DNA amplification positive control, is required as amplification positive control.

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification
REF M800351

WARNINGS AND PRECAUTIONS

This product is exclusively designed for *in-vitro* use.

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite or autoclaved for one hour at 121°C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were able to transmit infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralised before disposal.

Wear suitable protective clothes and gloves and protect eyes and face.
Never pipette solutions by mouth.
Do not eat, drink, smoke or apply cosmetic products in the work areas.
Carefully wash hands after handling samples and reagents.
Dispose of leftover reagents and waste in compliance with the regulations in force.
Carefully read all the instructions provided in the product before running the assay.
While running the assay, follow the instructions provided in the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided in the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

Molecular biology procedures, such as nucleic acids extraction, amplification and detection, require qualified and trained staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

When amplification session is manually setup, it is necessary to have available separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designated for extraction/preparation of amplification reactions.

When amplification session is manually setup, it is necessary to have available lab coats, gloves and tools which are exclusively used for the extraction/preparation of the amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designated for the amplification/detection of amplification products to the area designated for the extraction/preparation of the amplification reactions.

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases and free from DNA and RNA.

The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, free from DNA and RNA.

Amplification products must be handled in such a way as to reduce as much as possible dispersion into the environment in order to avoid the possibility of contamination. The pipettes used to handle amplification products must be exclusively used for this purpose.

Warnings and precautions specific for the components

The **MRSA/SA PCR Mix** must be stored at -20°C in the dark.
The **MRSA/SA PCR Mix** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

ELITe InGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with DNA extracted from the following clinical samples:

Nasal Swab

The nasal swab samples, intended for DNA extraction, should be collected with the following collection and transport systems:

«eNAT™ kit» (COPAN Italia S.p.A., ref. 608CS01R) for DNA extraction, identified according to laboratory guidelines. The nasal swab must be transported and stored at +2 / +8 °C for up to four week otherwise they must be frozen and stored at -20 °C for a maximum of six months. Before the analysis with this product 0.2 mL of sample in eNAT™ medium has to be transferred in the sonication tube provided with **«ELITe InGenius Sonication tubes»**.

«eSwab Collection Kit» (COPAN Italia S.p.A., ref. 480CE) for DNA extraction, identified according to laboratory guidelines, and transported preferably within 2 hours of collection. If immediate delivery or processing is delayed, then specimens should be transported and stored at +2 / +8 °C for up to 48 hours, otherwise they must be frozen and stored at -20 °C for a maximum of six months. Before the analysis with this product 0.2 mL of sample in eSwab medium has to be transferred in the sonication tube provided with « **ELITe InGenius Sonication tubes**».

N.B.: When DNA extraction from nasal swabs is carried out with the **ELITe InGenius** and **ELITe InGenius Software®** version **1.2** (or later equivalent versions), use the extraction protocol **MRSA-SA ELITe_NS_200_50**. This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 50 µL.

Blood culture

The blood culture samples for nucleic acid extraction must be collected and identified according to laboratory guidelines. The samples must be transported and stored at room temperature for a maximum of 24 hours.

Before the analysis with this product dilute the sample 1:1000 in ultrapure water (at least 10 µL of samples into 10 mL of ultrapure water), mix by vortexing and transfer 0.2 mL of the diluted samples in a sonicator tube provided with « **ELITe InGenius Sonication tubes**».

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

N.B.: when nucleic acid extraction from Blood Culture is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.2 (or later equivalent versions), use the extraction protocol **MRSA-SA ELITe_BC_200_100**. This protocol processes 200 µL of sample, adds the **CPE** at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Interfering substances

No data concerning inhibition caused by antiviral, antibiotic and chemotherapeutic or immunosuppressant drugs are available.

High quantity of human genomic DNA in the DNA extracted from the sample may inhibit the amplification reaction.

Amplification controls

Before analysis of any sample, it is absolutely mandatory to generate and to approve the amplification controls for each lot of amplification reagent:

as amplification Positive Control use the **MRSA/SA - ELITe Positive Control**, in association with the protocol **«MRSA-SA ELITe_PC»**,

as amplification Negative Control, use molecular grade water (not provided with this kit) in association with the protocol **«MRSA-SA ELITe_NC»**.

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification
REF M800351

N.B.: The **ELITe InGenius** system requires approved and valid results of amplification controls for each amplification reagent lot stored in its database.
The amplification control results, approved and stored in the database, will expire after **15 days**. At the expiration date it is necessary to re-run the positive and negative controls in association with the amplification reagent lot.

Furthermore the amplification controls must be re-run when:
- a new lot of amplification reagents is started,
- the results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance service is performed on the **ELITe InGenius** instrument.

Quality controls

It is recommended to validate the whole analysis procedure, extraction and amplification, by testing Process Controls, i.e. a negative tested sample and a positive tested sample or a calibrated reference material.

External controls shall be used in accordance with local, state, federal accrediting organizations, as applicable.

PROCEDURE

The procedure to use the **«MRSA/SA ELITe MGB® Kit »** with the system **ELITe InGenius** consists of three steps:

- Verification of the system readiness
- Set up of the session
- Review and approval of results

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the **ELITe InGenius** and select the mode "**CLOSED**";
- verify that the amplification Controls (**MRSA/SA - Positive Control**, **MRSA/SA Negative Control**) are run, approved and not expired (status) in association with the amplification reagent lot in use. If there are not amplification controls approved or valid, run them as described in the following paragraphs.
- choose the type of run, following the instructions Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by ELITechGroup S.p.A.. These IVD protocols were specifically validated with ELITe MGB kits, matrices and **ELITe InGenius** instrument and the cited matrix. The Assay protocols available for **«MRSA/SA ELITe MGB® Kit»** are described in the table below.

Assay protocol for MRSA/SA ELITe MGB Kit

Name	Matrix	Report unitage	Characteristics
MRSA-SA ELITe_NS_200_50	Nasal swab	Positive / Negative	Extraction Input Volume: 200 µL Extracted Elute Volume: 50 µL Internal Control: 10 µL Sonication: 1 cycle, 5 sec. ON PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
MRSA-SA ELITe_BC_200_100	Blood Culture	Positive / Negative	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume: 10 µL

If the assay protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service.

Setup of the session

The product **MRSA/SA ELITe MGB® Kit** can be used with the **ELITe InGenius** system in order to perform:

A. Integrated run (Extract + PCR),
B. Amplification run, (PCR only),
C. Amplification Positive and Negative Control run (PCR only),

All the parameters needed for the session are included in the Assay protocol available on the instrument and are automatically recalled when the Assay protocol is selected.

N.B.: the ELITe InGenius system can be linked to the "Location Information Server" (LIS) through which it is possible to send the work session information. Refer to the instrument user's manual for more details.

The main steps for the setup of the four types of runs are described here below.

A. Integrated run

To set up the integrated run carry on the steps below following the GUI:

1. Thaw MRSA/SA PCR Mix tubes in sufficient number for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home screen".
4. If Nasal Swab samples are processed, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 50 µL.
5. If Blood Culture samples are processed, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
6. For each Track of interest fill in the "SampleID" (SID) by typing or by scanning the sample barcode.
7. Select the assay protocol to be used in the "Assay" column (i.e. MRSA-SA ELITe_NS_200_50).
8. Ensure that the "Protocol" displayed is: "Extract + PCR".
9. Select the sample loading position in the "Sample Position" column and select "Sonication Tube". Click "Next" to continue the setup.
10. Load CPE and MRSA/SA PCR Mix on the Inventory Block selected by following the GUI instruction. Click "Next" button to continue the setup.
11. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click "Next" button to continue the setup.
12. Load the "PCR Cassettes", the "ELITe InGenius SP 200" extraction cartridges, all the required consumables and the samples to be extracted in the positions specified in step 8, following the GUI instruction. Click "Next" to continue the setup.
13. Close the instrument door.
14. Press "Start" to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

N. B.: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped, identified and stored at -20 °C. Avoid the spilling of the Extracted Sample.

N. B.: At the end of the run the PCR Cassettes with the reaction products and other consumables must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

N. B.: The PCR Mix can be kept on board in the refrigerated block up to **15 hours** (5 work sessions of 3 hours each).

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification
REF M800351

B. Amplification run

To set up the amplification run carry out the following steps as per GUI:

1. Thaw a sufficient number of MRSA/SA PCR Mix tubes for the session. Each tube is sufficient for 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Select "Perform Run" from the "Home screen".
3. Even if no extraction will be carried out and if Nasal Swab samples are processed, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 50 µL.
4. Even if no extraction will be carried out and if Blood Culture samples are processed, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
5. For each Track of interest type the SID by typing or by scanning the sample barcode.
6. Select the assay protocol to be used in the "Assay" column (i.e. MRSA-SA ELITe_NS_200_50).
7. Select "PCR Only" in the "Protocol" column.
8. Ensure the Eluted sample loading position in the "Sample Position" column is "ExtraTube (bottom row)". Click "Next" to continue the setup.
9. Load MRSA/SA PCR Mix on the Inventory Block selected by following the GUI instruction. Click "Next" to continue the setup.
10. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load the "PCR Cassettes" and the extracted Nucleic Acid samples following the GUI instruction. Click "Next" to continue the setup.
12. Close the instrument door.
13. Press "Start" to start the run.

After process completion, the **ELITe InGenius** allows to view, approve, store the results and to print and save the report.

N. B.: At the end of the run the remaining Extracted Sample can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Extracted Sample.

N. B.: At the end of the run the PCR Cassettes with the reaction products and other consumable must be removed from the instrument and disposed of without producing environmental contaminations. Avoid any spilling of the reaction products.

N. B.: The PCR Mix can be kept on board in the refrigerated block up to **15 hours** (5 work sessions of 3 hours each).

C. Amplification run for Positive Control and Negative Control

To set up the amplification Positive Control and Negative Control run, carry out the following steps as per GUI:

1. Thaw a sufficient number of MRSA/SA PCR Mix tubes for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.
2. Thaw the MRSA/SA Positive Control and LGA251/SA Positive Control tubes for the session. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 30 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP Consumable Set.
4. Select "Perform Run" from the "Home screen".
5. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
6. In the Track of interest, select the assay protocol to be used in the "Assay" column.
7. For the positive control, select MRSA-SA ELITe_PC in the "Assay" column and fill in the lot number and expiry date of MRSA/SA Positive Control.
8. For the negative control, select MRSA-SA ELITe_NC and fill in the lot number and expiry date of the molecular biology grade water.
9. Click "Next" to continue the setup.
10. Load MRSA/SA Q-PCR Mix on the Inventory Block selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load and check the Tip Racks in the Inventory Area selected by following the GUI instruction. Click "Next" to continue the setup.
12. Load "PCR Reaction Cassettes", the 2 Positive Control tubes (MRSA/SA and LGA251/SA) or the Negative Control tube, following the GUI instruction. Click "Next" to continue the setup.
13. Close the instrument door.
14. Press "Start" to start the run.

After process completion, the **ELITe InGenius** system allows users to view, approve, store the results and to print and save the report.

N.B.: The results of Positive Control and Negative Control amplification runs are used by the instrument software to set up the "Control Charts". Four Positive Control and Negative Control results, from four different runs, are requested to set up the control chart. After that, the results of Positive control and Negative Controls are used for monitoring the amplification step performances. Refer to the instrument user's manual for more details.

N. B.: At the end of the run the remaining Positive Controls can be removed from the instrument, capped and stored at -20 °C. The remaining Negative Control must be disposed.

N.B.: At the end of the run the PCR Cassettes with the reaction products and other consumables must be removed from the instrument and disposed of without producing environmental contaminations. Avoid any spilling of the reaction products.

N. B.: The PCR Mix can be kept on board in the refrigerated block up to **15 hours** (5 work sessions of 3 hours each).

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification
REF M800351

Review and approval of results

At the end of the run, the "Results Display" screen is automatically shown. In this screen the sample / Control results and the information regarding the run are shown. From this screen it is possible to approve the result, print or save the reports ("Sample Report" or "Track Report"). Refer to the instrument user's manual for more details.

N.B.: the ELITe InGenius system can be linked to the "Location Information Server" (LIS) through which it is possible send the work session results to the laboratory data center. Refer to the instrument user's manual for more details.

The **ELITe InGenius** system generates the results using the product **«MRSA/SA ELITe MGB® Kit»** through the following procedure:

A. Validation of amplification Positive Control and Negative Control results,
B. Validation of sample results,
C. Sample result reporting.

A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probe specific to the mecA and mecC genes (mecA) and by the probe specific to the SA gene (SA) in the Positive Controls and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocols "MRSA-SA ELITe_PC" and "MRSA-SA ELITe_NC".

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, are stored in the database (Controls) after the approval of the "Administrator" or "Analyst" personnel by following the GUI instruction.

The amplification Positive Control and Negative Control results, specific for the amplification reagent lot, will expire **after 15 days**.

Before analysing any sample it is absolutely mandatory to verify that amplification Positive Control and Negative Control were run with the lot of amplification reagent to be used and results are approved and valid. The availability of "Approved" (Status) amplification Positive Control and Negative Control results is shown in the "Controls" window of the GUI. If the amplification Positive Control and Negative Control results are missing, generate them as described above.

N.B.: When the Positive Control or Negative Control result does not meet the acceptance criteria, the "not passed" message is shown on the "Controls" screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reaction has to be repeated.

N.B.: When the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the entire session is invalid. In this case, the amplification of all samples must be repeated too.

B. Validation of Samples results

The fluorescence signals emitted by the probe specific to the mecA and mecC genes (mecA), by the probe specific to the SA gene (SA) and by the specific Internal Control probe (IC) in each sample amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol.

N.B.: Before analysing any sample, verify that amplification controls were run with the lot of amplification reagent to be used and results are approved and valid. The availability of "Approved" (Status) amplification control results is shown in the "Controls" window of the GUI. If the amplification control results are missing, generate them as described above.

Results are shown in the reports generated by the instrument ("Result Display").

The Sample run can be approved when the two conditions reported in the table below are met.

1) Positive Control	Status
MRSA/SA Positive Control	APPROVED
LGA251/SA Positive Control	APPROVED
2) Negative Control	**Status**
MRSA/SA - Negative Control	APPROVED

For each sample, the assay result is automatically interpreted by the system as established by the **ELITe InGenius software** algorithm and the Assay protocol parameters.

The possible result messages of a Sample are listed the table below.

Result of Sample run	Interpretation
MRSA Detected	**MRSA DNA was detected** in the sample.
MRSA/SA Not Detected or below LoD	**MRSA/SA DNA was not detected** in the sample. The sample is negative for these targets or its concentration is below the Limit of Detection of the assay.
MRSA Not Detected or below LoD, SA Detected	**MRSA DNA was not detected** in the sample. The sample is negative for this targets or its concentration is below the Limit of Detection of the assay, **SA was detected**
Invalid - Retest Sample	**Not valid assay result** due to Internal Control failure (Incorrect extraction or inhibitor carry-over).

Samples not suitable for result interpretation are reported as “Invalid - Retest Sample” by the **ELITe InGenius software**. In this case, the Internal Control DNA was not efficiently detected due to problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction or inhibitor in the extracted carry-over in the eluate), which may cause incorrect results and false negative.

When the eluate volume is sufficient, the extracted sample can be retested via an amplification run in “PCR Only” mode. In the case of a second invalid result, the sample must be retested starting from extraction of a new aliquot using “Extract + PCR” mode.

Samples suitable for analysis but in which it was not possible to detected MRSA/SA DNA are reported like: “MRSA/SA: DNA Not Detected or below LoD". In this case it cannot be excluded that the MRSA/SA DNA is present at a concentration below the limit of detection of the assay (see “performance and characteristic).

N.B.: The results obtained with this assay must be interpreted taking into consideration all the clinical data and the other laboratory test outcomes concerning the patient.

The Sample run results are stored in the database and, if valid, can be approved (Result Display) by personnel qualified as “Administrator” or “Analyst”, following the GUI instruction. From the Result Display” window it is possible to print and save the Sample run results as “Sample Report” and “Track Report”.

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification

REF M800351

C. Samples result reporting

The sample results are stored in the database and can be exported as “Sample Report” and “Track Report”.

The “Sample Report” shows the details of a work session sorted by selected sample (SID).

The “Track Report” shows the details of work session by selected Track.

The "Sample Report" and "Track Report" can be printed and signed by authorized personnel.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: Limit of Detection

The analytical sensitivity of this assay, as Limit of Detection (LoD) of the DNA amplification, allows detecting the presence of about 20 copies in 10 µL of DNA added to the amplification reaction.

The LoD of this assay was tested using plasmids DNA containing the amplification products whose initial concentration were measured by spectrophotometer. The plasmids DNA were diluted to a titre of about 20 copies / 10 µL in presence of 40,000 copies of Internal Control (IC) / 10 µL. These samples were tested in 18 replicates carrying out the amplification by ELITechGroup S.p.A. products on two different instruments.

The results are reported in the following table.

Samples	N	positive	negative	Mec A Ct mean	SA Ct mean
20 copies plasmid MRSA/SA DNA + 40,000 copies of IC	18	17	1	35.04	34.43
20 copies plasmid LGA251/SA DNA + 40,000 copies of IC	18	18	0	34.75	34.12

Analytical sensitivity: reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of value of a calibrated reference material, was evaluated using as reference material the QCMD 2014 Methicillin Resistant S. aureus EQA Panel (Qnostics Ltd, UK) a panel of MRSA/SA dilutions within the limit concentration. Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation, using «**ELITe InGenius**» and ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «ELITe InGenius»			
Sample	**Sample Content**	**Expected Result**	**Actual Result**
MRSADNA14-01	MRSA N315	MRSA Detected	MRSA Detected
MRSADNA14-02	MSSA ATCC 29213	MRSA Negative	MRSA Negative
MRSADNA14-03	MSSA 29213 + MRCoNS 634	MRSA Negative	MRSA Negative
MRSADNA14-04	E. coli ATCC 35218	MRSA Negative	MRSA Negative
MRSADNA14-05	MRSA N315	MRSA Frequently Detected	MRSA Detected
MRSADNA14-06	MHB only	MRSA Negative	MRSA Negative
MRSADNA14-07	MRSA N315	MRSA Infrequently Detected	MRSA Detected
MRSADNA14-08	MRSA mecC	MRSA Infrequently Detected	MRSA Detected
MRSADNA14-09	MRCoNS 634	MRSA Negative	MRSA Negative
MRSADNA14-10	MRSA ST398	MRSA Detected	MRSA Detected
MRSADNA14-11	MRSA N315	MRSA Frequently detected	MRSA Detected
MRSADNA14-12	MRSA N315	MRSA Detected	MRSA Detected

All samples were correctly detected.

The analytical sensitivity of the assay, as reproducibility of value of a calibrated reference material, was also evaluated using as reference material the NATtrol™ MRSA/SA Panel (Zeptometrix, US) a panel of S. aureus or S. epidermidis. Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation, using **«ELITe InGenius»** and ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «ELITe InGenius»		
Sample	**Expected Result**	**Actual Result**
S. aureus_MRSA Community Strain	MRSA Positive	MRSA Detected
S. aureus_MRSA Hospital Strain	MRSA Positive	MRSA Detected
S. aureus_MSSA	MSSA Positive	MSSA Detected
S. aureus_MSSA – empty cassette	MSSA Positive	MSSA Detected
S. epidermidis_MSSE HER 1292	Negative	Negative

All samples were correctly detected.

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification
REF M800351

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analysing clinical samples of nasal swab and blood culture positive for MRSA and MSSA.

The diagnostic sensitivity was evaluated using 60 samples of nasal swab positive for MSSA, 21 samples of nasal swab positive for MRSA and 20 nasal swabs, that were spiked for MRSA DNA adding MRSA BAA-1556 (ATCC) at a titre of 100,000 CFU/mL.

The diagnostic sensitivity was evaluated using 39 samples of blood culture positive for MSSA, 21 samples of blood culture positive for MRSA and 10 blood culture spiked with MRSA isolates, given the difficulty of finding a significant number of positive clinical samples for some MRSA target genes.

Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **«ELITe InGenius»**and ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Nasal swab samples positive for MSSA DNA	60	56	4
Nasal swab samples positive for MRSA DNA	41	40	1
Blood culture samples positive for MSSA DNA	39	39	0
Blood culture samples positive for MRSA DNA	31	31	0

For Nasal swab samples, 56 out of 60 MSSA samples were correctly detected. Four (4) samples resulted negative. 40 out of 41 MRSA samples were correctly detected. One sample resulted MSSA positive.

The diagnostic sensitivity of the assay in association to nasal swab was equal to 93% for MSSA and 98% for MRSA.

For Blood culture samples, all samples were correctly detected.

The diagnostic sensitivity of the assay in association to blood culture was equal to 100% for MSSA and 100% for MRSA.

The diagnostic sensitivity of the assay in this test was equal to 96% for MSSA and 99% for MRSA.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analysing 48 clinical samples of nasal swab and 34 clinical samples of blood culture negative for MRSA/SA.

Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **«ELITe InGenius»**and ELITechGroup S.p.A. products.
The results are summed up in the following table.

Samples	N	positive	negative
Nasal swab samples negative for MRSA/SA DNA	48	0	48
Blood culture samples negative for MRSA/SA DNA	34	0	34

All samples were correctly detected.

The diagnostic specificity of the assay in this test was equal to 100%.

OTHER SYSTEMS

SAMPLES AND CONTROLS

Samples

This product must be used with DNA extracted from clinical samples of nasal swabs.

The nasal swab samples, intended for DNA extraction, should be collected with BBL Culture Swab Plus Amies Gel without Charcoal swabs (Becton-Dickinson) and identified according to laboratory guidelines. The nasal swab samples must be transported and stored at +18 / +25 °C for a maximum of one day, otherwise they must be stored at +2 / +8 °C for up to seven days. The nasal swab samples must be immersed in 1 mL of Trypticase Soy Broth (TSB) and vortexed for 10 seconds before starting the extraction procedure.

N.B.: When you extract DNA with the **«NucliSENS® easyMAG®»** system, please use the following set up.

Define the extraction parameters as follows:
- Matrix = Other;
- Protocol = Generic 2.0.1;
- Volume (mL) = 1.0 mL;
- Eluate (µL) = 50 µL;
- Type = Primary.

Transfer **1 mL** of each TSB sample in the 8-well disposable sample vessel as established in the instrument worklist and dispense the lysis buffer. During the 10 minutes of incubation, prepare the magnetic silica suspension for 8 samples by mixing **550 µL** of **NucliSENS® easyMAG® Magnetic Silica**, **545 µL** of **molecular biology grade water** and **5 µL** of **CPE**. For each sample, use the BioHit pipettor to dispense 125 µL of the magnetic silica suspension into the NucliSENS easyMAG Strip for Premix. Use the BioHit pipettor to transfer 100 µL of the magnetic silica suspension into each sample in the 8-well disposable sample vessel, mix well by pipetting up and down three times, and then start the extraction procedure.

Interfering substances

Substances that may interfere with the detection of SA and MRSA by the **«MRSA/SA ELITe MGB® Kit»** and potentially generate invalid results include propylene glycol and excessive amounts of nasal secretions / mucus.

The exogenous substances listed below, which are components of decongestants and substances used to relieve nasal dryness and/or irritation, have been shown, with the exception of propylene glycol, not to interfere with the detection of MRSA / SA by the **«MRSA/SA ELITe MGB® Kit»**. Presence of human blood in the sample has been shown not to interfere with the detection of MRSA / SA by the **«MRSA/SA ELITe MGB® Kit»** used in association with **NucliSENS® easyMAG®**.

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification
REF M800351

Potentially Interfering Substance (Type)	Active Ingredient	Interferes?
Mucin, bovine submaxillary gland, type I-S	Purified mucin protein	No
Blood (Human)	Hemoglobin	No
Nasal sprays or drops	Phenylephrine	No
	Oxymetazoline	No
	Sodium chloride with preservatives	No
	Benzalkonium chloride	No
	Sodium Phosphate	No
	Phenylcarbinol	No
	Propylene glycol	**Yes**
	Sorbitol, benzyl alcohol	No
	disodium edetate, hypromellose	No
	phosphoric acid	No
Nasal corticosteroids	Dexamethasone	No
	Triamcinolone	No
	Beclomethasone	No
	Flunisolide	No
	Budesonide	No
	Mometasone	No
	Fluticasone	No
Nasal gel	Luffa opperculata, sulfur	No
Homeopathic allergy relief medicine	Galphimia glauca	No
	Histaminum hydrochloricum	No
Vaccine	Live intranasal influenza virus vaccine	No
Throat lozenges, oral anaesthetic and analgesic	Benzocaine, Menthol	No
Anti-viral drugs	Zanamivir, Oseltamivir phosphate	No
Antibiotic, nasal ointment	Mupirocin	No
Antibacterial, systemic	Tobramycin	No

Interference experimental data were obtained using NucliSENS® easyMAG® extraction and 7500 Fast Dx Real-Time PCR Instrument detection platform with an earlier version of the assay **«MRSA/SA ELITe MGB® Kit»**, which is identical to the current assay except that it lacks *mecC* specific oligonucleotides.

No data concerning inhibition caused by other antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs are available.

High quantity of human genomic DNA in the DNA extracted from the sample may inhibit the amplification reaction.

Amplification controls

It is mandatory to validate each amplification session with a negative control reaction and a positive control reaction.

For the negative control, use molecular biology grade water (not provided).

For the positive control, use the two solutions of **«MRSA/SA - ELITe Positive Control»** product (not provided).

Quality controls

It is recommended to validate the entire analysis procedure of each extraction and amplification session by processing a negative tested sample and a positive tested sample or a calibrated reference material.

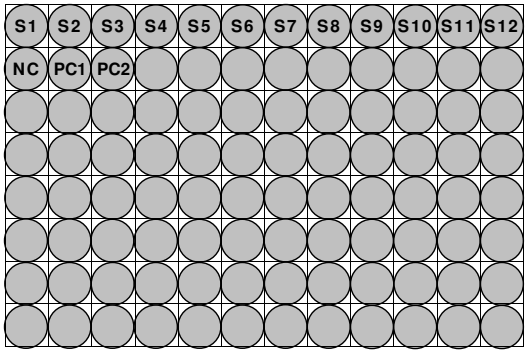
PROCEDURE

Setting of the real-time amplification session
(To perform in the amplification / detection of amplification products area)

Before starting the session, follow the manufacturer recommendations provided in the instrument documentation and:

- switch on the computer, switch on the real-time thermal cycler, run the dedicated software, open an "absolute quantification" session;
- when the **7500 Fast Dx Real-Time PCR Instrument** is used, choose “Run mode: Fast 7500”;
- create a new “detector” set or set the appropriate "detector" in the Tool menu by selecting the Detector Manager:
  - set the "detector" for the SA-specific gene probe with the "reporter" = "TAMRA" (AP554 is similar to TAMRA), the "quencher" = "none" (non fluorescent) and call it “SA”;
  - set the "detector" for the *mecA* and *mecC* gene probes with the "reporter" = "FAM", the "quencher" = "none" (non fluorescent) and call it “mecA”;
  - set the "detector" for the Internal Control probe with the "reporter" = "Cy5" (AP642 is similar to Cy5), the "quencher" = "none" (non fluorescent) and call it “IC”;
- go to View menu, select the Well Inspector and, for each well in use in the microplate, set the "detector" (type of fluorescence that is to be measured), the "passive reference" = "ROX" (AP593 is similar to ROX, normalisation of the measured fluorescence) and the type of reaction (sample, amplification negative control, amplification positive control). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

See below an example of how a qualitative analysis of 12 samples can be organised.



Legend: S1 - **S12**: Samples to be analysed; **NC**: amplification Negative Control;
PC1: amplification MRSA/SA Positive Control; **PC2**: amplification LGA251/SA Positive Control

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:

- add to the amplification stage (Add Step) an **extension step at 72 °C**;

N.B.: the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the hybridisation step at 56°C.

- modify timing as indicated in the table **"Thermal cycle"** below;
- set the number cycles to **45**;
- set the reaction volume to **30 µL**.

Thermal cycle		
Stage	**Temperatures**	**Timing**
Decontamination	50° C	2 min.
Initial denaturation	93° C	2 min.
Amplification and Detection (45 cycles)	93° C	10 sec.
	56° C (data collection)	30 sec.
	72° C	15 sec.

Amplification set-up
(To be performed in the extraction / preparation of the amplification reaction area)

Before starting the session, it is necessary to:

- take and thaw the tubes containing the samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **MRSA/SA PCR Mix** tubes required for the session, remembering that each tube is sufficient to prepare **25 reactions**. Mix gently, spin down the content for 5 seconds and keep them on ice for a maximum of four hours;
- take and thaw a **MRSA/SA Positive Control** tube (positive control of the real-time amplification reactions for the SA-specific gene and for the *mecA* gene). Mix gently, spin down the content for 5 seconds and keep on ice for a maximum of four hours;
- take and thaw a **LGA251/SA Positive Control** tube (positive control of the real-time amplification reactions for the *mecC* gene). Mix gently, spin down the content for 5 seconds and keep on ice for a maximum of four hours;
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powderless gloves and not to damage the wells.

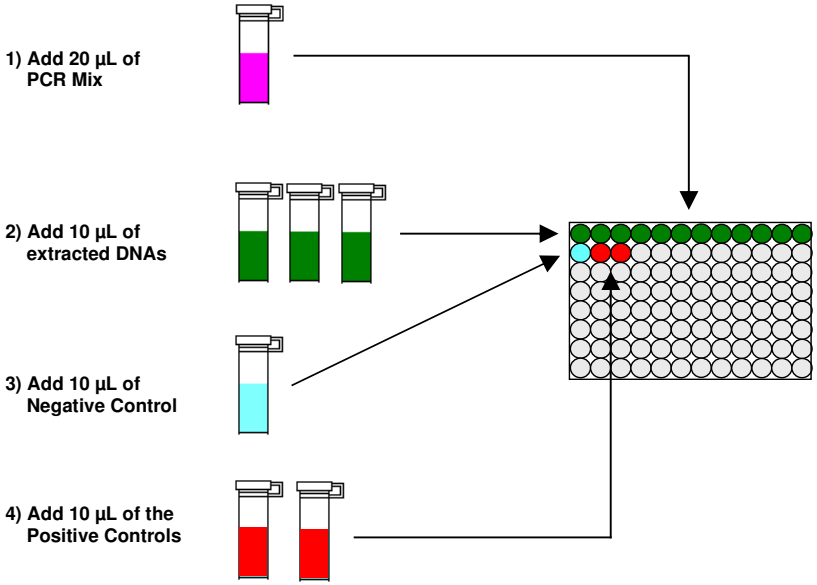
1. Accurately dispense **20 µL** of the **MRSA/SA PCR Mix into** the bottom of the **Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.

N.B.: If not all the reaction mixture is used, store the remaining volume in the dark at -20°C for no longer than one month. Freeze and thaw the reaction mixture up to **five times**.

2. Add to the reaction mixture **10 µL** of the first processed sample in the designated well, as previously established in the **Work Sheet**. Mix well the sample by pipetting the **extracted DNA** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other extracted samples.
3. Add to the reaction mixture **10 µL** of **molecular biology grade water** (not provided) in the negative control well, as previously established in the **Work Sheet**. Mix well the negative control by pipetting the **molecular biology grade water** three times into the reaction mixture. Avoid creating bubbles.
4. Add to the reaction mixture **10 µL** of **MRSA/SA Positive Control** in the designated well, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **MRSA/SA Positive Control** three times into the reaction mixture. Avoid creating bubbles.
5. Add to the reaction mixture **10 µL** of **LGA251/SA Positive Control** in the designated well, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **LGA251/SA Positive Control** three times into the reaction mixture. Avoid creating bubbles.
6. Accurately seal the **Amplification microplate** with the **Amplification Adhesive Sheet**.
7. Transfer the **Amplification microplate** into the real-time thermal cycler in the amplification / detection of amplification products area and start the thermal cycle for the amplification.Save the session setting with an univocal and recognisable file name (e.g. "year-month-day-MRSA/SA-EGSpA").

N. B.: At the end of the thermal cycle the **Amplification microplate** with the reaction products must be removed from the instrument and discarded without producing environmental contaminations. In order to avoid spilling the reaction products, the **Amplification Adhesive Sheet must not be removed from the Amplification microplate**.

The following figure illustrates the set up of the amplification reaction.



Qualitative analysis of the results

The recorded values of the fluorescence emitted by the SA-specific gene probe (TAMRA detector "SA"), by the *mecA* and *mecC* gene probes (FAM detector "mecA") and by the Internal Control probe (Cy5 detector "IC") during the amplification reactions must be analysed by the instrument software.

Before starting the analysis, follow the manufacturer recommendations provided in the instrument documentation and:

- set (Results > Amplification plot > delta Rn vs. Cycle) the **Analysis Settings** for all detectors to **Auto Baseline** and **Manual Ct**, with the **Threshold** set to **0.1.** Push the **Analyze** button and **save** the results.

The values of fluorescence emitted by the specific probes during the amplification reaction and the **Threshold** value of fluorescence allow determining the **Threshold cycle (Ct)**.The Ct is the cycle when the fluorescence reached the **Threshold** value and it is proportional to the initial target quantity.

In the **MRSA/SA Positive Control** and **LGA251/SA Positive Control** amplification reactions, the **Ct** values of SA and mecA detectors (Results > Report) are used to validate the amplification and detection, as described in the following table:

Positive Control reaction detector TAMRA "SA"	Assay result	Amplification / Detection
Ct ≤ 35	POSITIVE	CORRECT

Positive Control reaction detector FAM "mecA"	Assay result	Amplification / Detection
Ct ≤ 35	POSITIVE	CORRECT

If the result of the **Positive Controls** amplification is **Ct > 35** or **Ct Undetermined** for SA and for mecA detectors, then the target DNA has been incorrectly detected. It means that problems occurred during the amplification or the detection step (incorrect dispensing of the reaction mix or the positive controls, degradation of the reaction mix or the positive controls, incorrect setting of the positive control position, incorrect setting of the thermal cycle), which may lead to incorrect results. The session is not valid and has to be repeated starting from the amplification step.

In the **Negative control** amplification reaction, the **Ct** values of SA, mecA and IC detectors (Results > Report) are used to validate the amplification and the detection as described in the following table:

Negative Control reaction detector TAMRA "SA"	Assay result	Amplification / Detection
Ct Undetermined or Ct > 35	NEGATIVE	CORRECT

Negative Control reaction detector FAM "mecA"	Assay result	Amplification / Detection
Ct Undetermined or Ct > 35	NEGATIVE	CORRECT

Negative control reaction detector Cy5 "IC"	Assay result	Amplification / Detection
Ct Undetermined or Ct ≥ 34	NEGATIVE	CORRECT

If the result of the **Negative Control** amplification is **Ct ≤ 35**, for SA or mecA detectors, and **Ct < 34**, for IC detector, then the target DNA has been incorrectly detected. It means that problems have occurred during the amplification step (contamination), which may lead to incorrect results and false positives. The session is not valid and needs to be repeated starting from the amplification step.

In each **sample** amplification reaction, the **Ct** values of mecA and SA detectors are used to detect the target DNA while the Internal Control **Ct** value is used to validate extraction, amplification and detection.

N.B.: Verify on the instrument software (Results > Amplification plot > delta Rn vs. Cycle) that the **Ct** was determined by a prompt and regular increase of the fluorescence and not by peaks or an increase of the background (irregular or high background).

The amplification reactions **Ct** values of each **sample** (Results > Report) are used as described in the following table:

Sample reaction				Assay result	
detector TAMRA "SA" (Ct1)	**detector FAM "mecA" (Ct2)**	**ΔCt |Ct1 –Ct2|**	**detector Cy5 "IC"**	**SA Result**	**MRSA Result**
Undetermined or Ct >35	**Undetermined or Ct > 35**	**NA**	**Ct < 34**	**Negative**	**Negative**
		NA	**Undetermined or Ct ≥ 34**	**Invalid**	**Invalid**
Determined, Ct ≤ 35	**Undetermined or Ct > 35**	**NA**	**NA**	**Positive**	**Negative**
	Determined, Ct ≤ 35	**ΔCt ≥ 2**	**NA**	**Positive**	**Negative**
		ΔCt < 2	**NA**	**Positive**	**Positive**
Undetermined or Ct > 35	**Determined, Ct ≤ 35**	**NA**	**NA**	**Negative**	**Negative**

Assay result		Result interpretation
SA Result	**MRSA Result**	
Negative	**Negative**	**No SA, including MRSA, DNA detected. Presumed negative for all SA, including MRSA, or number of organisms may be below the detection limit.**
Invalid	**Invalid**	**Invalid result. Repeat run from extraction of the sample or of a new sample.**
Positive	**Negative**	**No MRSA DNA detected. Presumed negative for MRSA or number of MRSA may be below the detection limit. SA DNA detected. Presumed positive for SA.**
Positive	**Positive**	**MRSA DNA detected. Presumed positive for MRSA.**

NA = not applicable

The presence of both markers (SA gene and *mecA*) measured by Ct value at the same relative quantity (a difference in Ct less than 2) is indicative of MRSA (including the recently identified LGA251 strain); different relative quantities (a difference in Ct equal or greater than 2) or presence of only the *Staphylococcus aureus*-specific gene marker is indicative of SA.

If the result of the sample amplification reaction is **Ct Undetermined** or **Ct > 35** for SA and mecA detector and **Ct Undetermined** or **Ct ≥ 34** for the IC detector, it means that it was impossible to detect efficiently the Internal Control DNA. In this case problems have occurred during the amplification step (inefficient or no amplification) or during the extraction step (degradation of DNA, loss of DNA during extraction or presence of inhibitors in the extracted DNA) which may lead to incorrect results and false negatives. The sample is not suitable, the assay is invalid and it needs to be repeated starting from the extraction of the sample or of a new sample from the same patient.

If the result of the sample amplification is **Ct Undetermined** or **Ct > 35** for SA detector and **Ct < 34** for the IC detector, it means that the SA (including MRSA) DNA is not detected in the processed sample. The sample is presumed negative or number of organisms in the sample is below the detection limit of the product (see Performance Characteristics, page 15). In this case the result could be a false negative.

N.B.: When SA or MRSA DNA is detected in a sample, the IC detector may be **Ct Undetermined** or **Ct ≥ 34**. In fact, the high efficiency of the SA or MRSA amplification may compete with the low efficiency of the Internal Control amplification. In this case the sample is suitable and the positive result of the assay is valid.

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification
REF M800351

PERFORMANCE CHARACTERISTICS

Clinical Performance

Performance characteristics of the **«MRSA/SA ELITe MGB® Kit»** were determined by comparing the **«MRSA/SA ELITe MGB® Kit»** used in association with **NucliSENS® easyMAG®** with Remel Spectra™ MRSA and/or agglutination/susceptibility tests. A true MRSA culture-positive specimen was defined as a specimen where MRSA was identified by any of the culture techniques used. A true methicillin-sensitive SA culture-positive specimen was defined as a specimen negative for all culture techniques used except for the latex agglutination test.

One nasal swab was collected from each patient and used to inoculate a selective chromogenic MRSA screening agar plate (Remel Spectra™ MRSA). Then the swab was inserted into a tube with trypticase soy broth and thoroughly mixed before the entire volume of the cell suspension was processed as described above. Each swab was then subjected to enrichment in trypticase soy broth with 6.5% NaCl. The enriched culture samples were inoculated onto Trypticase Soy Blood Agar plates. Colonies from the Trypticase Soy Blood Agar plates were used for latex agglutination (Remel Staphaurex®) testing. Specimens positive for latex agglutination were used for the cefoxitin susceptibility test (BD BBL™ Sensi-Disc™ Susceptibility Test Disc Cefoxitin 20) as directed by the respective instructions for use.

Performance of the **«MRSA/SA ELITe MGB® Kit»** was calculated relative to the combination of direct chromogenic culture and the broth culture followed by latex agglutination and cefoxitin susceptibility test results.

Nasal swab specimens were obtained from a health care organization and from healthy donors and tested by a combination of culture methods as described above. 20 MRSA culture-positive, 20 MSSA culture-positive, and 40 SA culture-negative samples were thus identified. Out of 40 SA culture-negative samples 20 samples were spiked with MRSA BAA-2312 strain (bearing *mecC* gene) near LoD level.

Compared to the culture method of reference, **«MRSA/SA ELITe MGB® Kit»** identified 100% of the specimens positive for MRSA and MRSA *mecC* by the reference method (diagnostic sensitivity) and 97.5% of the negative specimens (diagnostic specificity). For the specimens tested, the MRSA positive predictive value (PPV) was 97,6% and the MRSA negative predictive value (NPV) was 100%.

MRSA results obtained with «MRSA/SA ELITe MGB® Kit» in comparison to the reference method.

	MRSA *mecA* Diagnostic sensitivity	**MRSA *mecC* Diagnostic sensitivity**	**MRSA Diagnostic specificity**
7500 Fast Dx Real Time PCR Instrument	**100%**	**100%**	**97.5%**
7500 Real Time PCR System	**100%**	**100%**	**97.5%**

Compared to the culture method of reference, the **«MRSA/SA ELITe MGB® Kit»** identified 95% of the specimens positive (diagnostic sensitivity) for SA by the reference method and 100% of the negative specimens (diagnostic specificity). For the specimens tested, the SA positive predictive value (PPV) was 100% and the SA negative predictive value (NPV) was 95%.

SA Results obtained with «MRSA/SA ELITe MGB® Kit» in comparison to the reference method.

	SA Diagnostic sensitivity	**SA Diagnostic specificity**
7500 Fast dx Real Time PCR Instrument	**95%**	**100%**
7500 Real Time PCR System	**95%**	**100%**

Limit of Detection

The Limit of Detection (LoD) of the **«MRSA/SA ELITe MGB® Kit»** used in association with **NucliSENS® easyMAG®** was determined using the strains shown below. Cultures of these strains were quantified, diluted in simulated nasal matrix to values spanning the range of approximately 5 to 1500 colony forming units (CFU) and absorbed onto swabs. All dilutions were tested, and the LoD was determined by Probit analysis. LoD for each strain represents the lowest number of CFU/swab at which a positive result will be obtained with 95% probability and with at least 95% confidence. LoD for each strain was then verified by testing at least 20 replicates.

List of Bacterial Strains for LoD Determination Studies

Strain No.	Designation	Description	Drug Resistance
ATCC 29213	Wichita	QC strain	MSSA
ATCC BAA-1556	MRSA252	hospital acquired, UK	MRSA
ATCC BAA-2312	M10/0061	LGA251	MRSA

Limit of Detection Results (CFU/swab)

	ATCC 29213	BAA-1556	BAA-2312
ABI 7500 Fast	210	159	237
ABI 7500 Standard	262	141	314

Genotype detection efficiency (inclusivity)

Performance of the **«MRSA/SA ELITe MGB® Kit»** used in association with **NucliSENS® easyMAG®** was tested with MRSA/SA QCMD proficiency panel. All the strains were correctly identified. In addition to that the assay[1] was tested against 75 well characterized MRSA and methicillin-sensitive SA isolates representative of the global genetic diversity, including clonal complexes and sequence types as well as various Pulse-Field Gel Electrophoresis (PFGE) types and MIC (Minimum Inhibitory Concentration) values. The strains were obtained through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) Program and from American Tissue Culture Collection (ATCC) or were a gift from Medical College of Wisconsin[2]. All strains were absorbed onto swabs at near detection limit and tested. In addition, all methicillin-sensitive SA strains were tested at $1x10^6$ CFU/swab. All methicillin-sensitive SA strains tested positive for SA and negative for MRSA. All MRSA strains tested positive for MRSA. Two BORSA (Borderline Oxacillin Resistant *Staphylococcus aureus*) isolates that lack the *mecA* gene tested SA positive and MRSA negative which yields an overall genotype detection efficiency (inclusivity) of 97.3%

The analysis of the regions chosen for the hybridisation of the primers and of fluorescent probes in the alignment of the sequences available in the database for the SSC *mec*A elements, including *mecC*, showed conservation and absence of significant mutations.

Analytical Specificity (cross-reactivity)

The analysis of the alignment of the sequences of the SA primers and of the fluorescent probe with the sequences of species phylogenetically related to *Staphylococcus aureus*, pathogenic microorganisms, and microorganisms commonly present in normal nasal micro flora available in databases for organisms other than SA, showed their specificity and the absence of significant homology for **«MRSA/SA ELITe MGB® Kit»**.

[1] Experimental data were obtained using NucliSENS® easyMAG® extraction system and 7500 Fast Dx Real-Time PCR Instrument with an earlier version of the assay, which is identical to the current except that it lacks *mecA*$_{LGA251}$ specific oligonucleotides.

[2] Gift from Dr. Nathan A. Ledeboer, Medical College of Wisconsin, WI; the strains are described in: Buchan, B.W, Ledeboer, N.A. Identification of Two Borderline Oxacillin-Resistant Strains of Staphylococcus aureus From Routine Nares Swab Specimens by One of Three Chromogenic Agars Evaluated for the Detection of MRSA, Microbiology and Infectious Disease.2010:134;921-927

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification

REF M800351

Species Tested for Cross-Reactivity by sequence database analysis

***Staphylococci* species**		**Other organisms**	**Viruses**
Staphylococcus arlettae	CoNS	*Acinetobacter haemolyticus*	*Adenovirus type 1, 7*
Staphylococcus capitis	CoNS	*Bacillus cereus*	*Human coronavirus 229E, OC 43*
Staphylococcus carnosus	CoNS	*Bordetella pertussis*	*Cytomegalovirus*
Staphylococcus chromogenes	CoNS	*Citrobacter freundii*	*Coxsackievirus A21*
Staphylococcus delphini	MSCoPS	*Citrobacter koseri*	*Epstein Barr Virus*
Staphylococcus epidermidis	MSCoNS	*Corynebacterium aquaticum*	*Human influenza virus A, B*
Staphylococcus epidermidis	MRCoNS	*Corynebacterium bovis*	*Human parainfluenza virus 1,2,3,4*
Staphylococcus equorum	CoNS	*Corynebacterium flavescens*	*Human metapneumovirus*
Staphylococcus felis	CoNS	*Corynebacterium genitalium*	*Measles virus*
Staphylococcus gallinarum	CoNS	*Enterobacter aerogenes*	*Mumps virus*
Staphylococcus hyicus	CoPS	*Enterococcus faecalis*	*Respiratory syncytial virus*
Staphylococcus intermedius	CoPS	*Enterococcus flavescens*	*Rhinovirus*
Staphylococcus kloosii	CoNS	*Enterococcus gallinarum*	
Staphylococcus lentus	CoNS	*Enterococcus hirae*	
Staphylococcus pulvereri	CoNS	*Escherichia coli*	
Staphylococcus simulans	CoNS	*Klebsiella oxytoca*	
Staphylococcus warneri	CoNS	*Klebsiella pneumoniae,*	
Staphylococcus xylosus	MSCoNS	*Listeria monocytogenes*	
		Micrococcus luteus	
		Moraxella catarrhalis	
		Pasteurella aerogenes	
		Proteus mirabilis	
		Proteus vulgaris	
		Pseudomonas aeruginosa	
		Salmonella typhimurium	
		Serratia marcescens	
		Shigella sonnei	
		Streptococcus mitis	
		Streptococcus salivarius	
		Yersinia enterocolitica	
		Candida albicans	
		Candida glabrata	
		Cryptococcus neoformans	
		Lactobacillus acidophilus	
		Legionella pneumophila	
		Mycobacterium tuberculosis	
		Mycoplasma pneumoniae	
		Neisseria meningitidis	
		Streptococcus mutans	
		Streptococcus pneumoniae	
		Streptococcus pyogenes	
		Homo sapiens	

CoNS = Coagulase Negative *Staphylococcus.*
MSCoNS= methicillin-sensitive Coagulase Negative *Staphylococcus.*
MRCoNS= methicillin-resistant Coagulase Negative *Staphylococcus.*
CoPS= Coagulase Positive *Staphylococcus.*

Rreproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of results compared with results obtained using other assays in different laboratories, was checked testing a panel of certified reference material.

The tests were carried out using as calibrated and certified reference material a panel of dilutions of MRSA (QCMD 2010 Methicillin Resistant *S. aureus* EQA Panel). The panel consists of six samples containing various concentrations of MRSA, three samples containing Methicillin sensitive *Staphylococcus aureus* (MSSA), one sample containing Methicillin resistant coagulase-negative Staphylococci (MRCoNS), one sample containing *Escherichia coli* (*E. coli)* and one true negative sample. Each sample of the panel was tested in 2 replicates carrying out the whole analysis procedure: extraction with **NucliSENS® easyMAG®** and amplification with ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with certified reference material				
Sample ID	**Content**	**Sample Conc. CFU/mL**	**Expected Result**	**Actual Result**
MRSADNA10-04	MRSA	1 x 10^8	Frequently detected	Detected
MRSADNA10-03	MRSA	5 x 10^7	Frequently detected	Detected
MRSADNA10-01	MRSA	5 x 10^6	Frequently detected	Detected
MRSADNA10-09	MRSA	5 x 10^5	Frequently detected	Detected
MRSADNA10-08	MRSA	5 x 10^5	Frequently detected	Detected
MRSADNA10-02	MRSA	5 x 10^5	Detected	Detected
MRSADNA10-05	MSSA	5 x 10^6	MRSA Negative	MRSA Negative SA Positive
MRSADNA10-06	MSSA	1 x 10^7	MRSA Negative	MRSA Negative SA Positive
MRSADNA10-07	MSSA	5 x 10^6	MRSA Negative	MRSA Negative SA Positive
MRSADNA10-12	MRCoNS	1 x 10^7	Negative	Negative
MRSADNA10-10	*E. coli*	5 x 10^6	Negative	Negative
MRSADNA10-11	MHBonly	-	Negative	Negative

All samples were correctly detected.

Carry-Over / Cross-Contamination

An analytical study was performed to evaluate the potential for cross-contamination between high MRSA (1×10^7 CFU/mL) specimens and negative specimens throughout the **«MRSA/SA ELITe MGB® Kit»** workflow. Two operators performed five 24 sample (11 high MRSA samples, 11 negative samples, 1 Positive Control sample, and 1 Negative Control sample per run) extraction runs in a checkerboard pattern (high MRSA samples interrupted by completely negative samples). The processed samples were then amplified in five separate runs using two different checkerboard patterns. The cross-contamination testing resulted in zero false negatives from fifty-five high MRSA positive samples and one false positive sample from fifty-five negative samples.

Carry-over / Cross-Contamination data were obtained using NucliSENS® easyMAG® extraction system and 7500 Fast Dx Real-Time PCR Instrument with an earlier version of the assay, **«MRSA / SA ELITe MGB® Kit»**, which is identical to the current except that it lacks *mecC* specific oligonucleotides.

N.B.: The complete data and results of the tests carried out to evaluate the product performance characteristics with instruments are recorded in the Section 7 of the Product Technical File "MRSA/SA ELITe MGB® Kit", FTP M800351.

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification

REF M800351

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Stegger, M. et al. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue $mecA_{LGA251}$. Clin Microbiol Infect 2012; 18:395-400.

Ito T. et al. Guidelines for reporting novel *mec*A gene homologues. Antimicrob Agents Chemother. 2012 October; 56(10): 4997-4999.

PROCEDURE LIMITATIONS

Use this product only in association with the recommended nucleic acid extraction system and Real-Time PCR instruments.

Use this product only with DNA extracted from human nasal swabs.

Do not use extracted DNA contaminated with mucoproteins, propylene glycol, ethanol or 2-propanol with this product. These substances inhibit the amplification of nucleic acids and may cause invalid results.

Do not use extracted DNA containing high quantity of human genomic DNA, which may inhibit the amplification reaction of nucleic acids, with this product.

Reliable results depend on adequate identification, collection, transport storage and processing of the samples. To avoid incorrect results, it is necessary to be careful during these steps and to carefully follow the instructions for use provided with the products.

Due to its high analytical sensitivity, the real-time amplification method used in this product is sensitive to cross-contamination from SA or MRSA positive clinical samples, from positive controls and from products of the same amplification. Cross-contamination causes false positive results. The product format is able to limit cross-contamination. However, cross-contamination can be avoided only by good laboratory practices and carefully following these instructions for use.

This product must be handled by qualified personnel trained in the processing of potentially infectious biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of work clothes and areas that are suitable for the processing of potentially infectious biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product must be handled by qualified personnel trained in molecular biology techniques, such as extraction, amplification and detection of nucleic acids, to avoid incorrect results.

It is necessary to have separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products to prevent false positive results.

This product requires the use of special clothing and instruments for extraction/preparation of amplification reactions and for amplification / detection of amplification products to avoid false positive results.

Due to inherent differences between technologies, it is recommended that users perform method correlation studies to estimate technology differences prior to switching to a new technology.

A positive result obtained with this product does not indicate the presence of viable SA or MRSA but is presumptive for the presence of SA or MRSA. Therefore, a positive result does not necessarily indicate intervention eradication failure since non-viable DNA may persist.

A negative result obtained with this product means that the SA or MRSA DNA is not detected in the DNA extracted from the sample, but it cannot be excluded that the SA or MRSA DNA has a lower titre than the product detection limit (see Performance Characteristics, page 15). In this case the result could be a false negative.

A negative result following a previously positive result may or may not indicate eradication success.

Results obtained with this product may sometimes be “Invalid” due to failed internal control and require retesting that can lead to a delay in obtaining final results.

Though rare, polymorphisms within the region of the bacterial genome covered by the product primers and probes may impair detection.

The detection of MRSA in the presence of excess amounts of methicillin-sensitive SA or coagulase-negative *mecA*-carriers might be impaired.

Borderline Oxacillin Resistant *Staphylococcus aureus* (BORSA) that do not carry the *mecA* gene are not detected by the product.

Results should be interpreted in conjunction with other laboratory and clinical data available to the clinician, and should be used as an adjunct to nosocomial infection control efforts to identify patients needing enhanced precautions.

TROUBLESHOOTING

Target DNA not detected in the Positive Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing in the microplate wells.	Take care when dispensing reagents in the microplate wells and comply with the work sheet. Check the volumes of reaction mix dispensed. Check the volumes of positive control dispensed.
Probe degradation.	Use a new aliquot of reaction mix.
Standard degradation.	Use a new aliquot of positive control.
Instrument setting error.	Check the instrument settings for the positive control reactions. Check the instrument settings for the thermal cycle.

Target DNA detected in the Negative Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing in the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between samples. Be careful when dispensing samples, negative control and positive control into the microplate wells and comply with the work sheet.
Error while setting the instrument.	Check the position settings for the samples, negative control and positive control on the instrument.
Microplate poorly sealed.	Be careful when sealing the microplate.
Contamination of the molecular biology grade water.	Use a new aliquot of molecular biology grade water.
Contamination of the reaction mix.	Use a new aliquot of reaction mix.
Contamination of the extraction/preparation area for amplification reactions.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing or inadequate mixing of sample.	Carefully pipette three times, when mixing samples, negative control and positive control into the reaction mixture. Avoid creating bubbles during sample dispensing step.

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination during pre-analytical steps	Avoid any contact between micropipette and tube wall. Clean the micropipette with fresh 3% sodium hypochlorite solution or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples in the last positions of the instruments, as indicated by the ELITe InGenius GUI. Follow the loading sequence indicated by the software
Laboratory environmental contamination	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of PCR Mix and / or CPE.

SYMBOLS

REF — Catalogue Number.


Upper limit of temperature.

LOT — Batch code.


Use by (last day of month).

IVD — *in vitro* diagnostic medical device.

CE — Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical devices.


Contains sufficient for "N" tests.


Attention, consult instructions for use.

CONT — Contents.


Manufacturer.

MRSA / SA ELITe MGB® Kit
Reagent for DNA Real-Time Amplification

REF M800351

NOTICE TO PURCHASER: LIMITED LICENSE

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ELITe® MGB detection reagents are covered by one or more of U.S. Patent numbers 6,127,121, 6,485,906, 6,660,845, 6,699,975, 6,727,356, 6,790,945, 6,949,367, 6,972,328, 7,045,610, 7,319,022, 7,368,549, 7,381,818, 7,662,942, 7,671,218, 7,715,989, 7,723,038, 7,759,126, 7,767,834, 7,897,736, 8,008,522, 8,067,177, 8,163,910, 8,389,745, 8,969,003, 8,980,855, 9,056,887, 9,085,800, 9,169,256 and EP patent numbers 1068358, 1144429, 1232157, 1261616, 1430147, 1781675, 1789587, 1975256, 2714939 as well as applications that are currently pending.

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