Instructions for use

MRSA/SA ELITe MGB® Kit

reagents for DNA Real-Time PCR

ELITechGroup
A BRUKER COMPANY

REF M800351

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CE IVD

CHANGE HISTORY

Rev.	Notice of change	Date (dd/mm/yy)
10	Extended use of the product in association with «ELITe BeGenius®» instrument (REF INT040) and nasal swabs and blood culture matrices. Removal of sonication protocol during the extraction step Update of the paragraph “Symbols” with the symbol “Consult instructions for use” New graphics and content setting of the IFU	27/03/25
09	Introduction of the new product reference "ELITe InGenius Sonication tubes" (ref. INT032SON) to be used in combination with the product for sample sonication.	13/10/20
08	Formal corrections.	06/02/19
00–07	New product development and subsequent changes	

NOTE

The revision of this IFU is also compatible with the previous versions of the kit

TABLE OF CONTENT

1 INTENDED USE

The **MRSA/SA ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative nucleic acids Real-Time PCR assay for the detection of the DNA of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA, including the mecC strain), extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of nasal swabs and blood culture.

The assay is also validated in association with the **7500 Real-Time PCR Instrument**, using human specimens of nasal swabs and blood culture.

The product is intended for use as an aid in 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.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

2 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 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.

3 ASSAY PRINCIPLE

The assay is a qualitative Real-Time PCR detecting SA and MRSA DNA isolated from specimens and amplified using the assay reagent MRSA/SA PCR Mix, that contains primers and probes with ELITe MGB Kit technology.

The ELITe MGB Kit probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycles (Ct) and the melting temperatures (Tm).

In the ELITe MGB Kit probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore.

Note that the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

4 PRODUCT DESCRIPTION

The **MRSA/SA ELITe MGB Kit** provides the assay reagent **MRSA/SA PCR Mix**, an optimized and stabilized PCR Mixture that contains the specific primers and probes for:

- The SA-specific gene (specific to a conservative region in the coagulase positive ***Staphylococcus aureus***), detected in Channel **SA**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor® 554 (AP554) dye,

- *mecA* and the *mecC* genes (specific to conservative regions in the ***mecA*** and ***mecC*** **genes** that are responsible for resistance to methicillin and other beta-lactam antibiotics) detected in Channel **MecA**; the probes are stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye,

- Internal Control (**IC**), specific for artificial sequence IC2**,** detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by AquaPhluor 642 (AP642) dye.

The **MRSA/SA PCR Mix** also contains buffer, magnesium chloride, triphosphates nucleotides, AP593 fluorophore (analogue of ROX or Cy5) as passive reference for fluorescence normalisation, the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by the amplification product, the "hot start" DNA Polymerase.

The **MRSA/SA ELITe MGB Kit** contains sufficient reagents for **96 tests** on the **ELITe InGenius** and **ELITe BeGenius (24 tests each tube)** and for **100 tests** on **other systems (25 tests each tube),** with 20 µL used per reaction.

The **MRSA/SA ELITe MGB Kit** can be also used in association with equivalent instruments.

5 MATERIALS PROVIDED IN THE PRODUCT

Table 1

Component	Description	Quantity	Classification of hazards
MRSA/SA PCR Mix ref. M800351	Mixture of reagents for Real-Time PCR tube with WHITE cap	**4 x 540 µL**	**-**

6 MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench centrifuge (~5,000 RPM).
- Bench microcentrifuge (~13,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).
- Molecular biology grade water.
- Trypticase Soy Broth

7 OTHER PRODUCTS REQUIRED

The reagents for the extraction of sample DNA, the extraction and inhibition Internal Control, the amplification positive and negative controls and the consumables **are not** provided with this product.

For automated extraction of nucleic acids, Real-Time PCR and result interpretation of samples, the following products are required:

Table 2

<table>
<tr><th>Instruments and Software</th><th>Products and Reagents</th></tr>
<tr><td>ELITe InGenius (ELITechGroup S.p.A., EG SpA ref. INT030)
ELITe InGenius Software version 1.3.0.19 (or later)
MRSA-SA ELITe_PC_200_100 or MRSA-SA ELITe_PC_200_50, Assay Protocol with parameters for Positive Control analysis
MRSA-SA ELITe_NC_200_100 or MRSA-SA ELITe_NC_200_50, Assay Protocol with parameters for Negative Control analysis
MRSA-SA ELITe_NS_200_50, Assay Protocol with parameters for nasal swabs specimen analysis
MRSA-SA ELITe_BC_200_100, Assay Protocol with parameters for blood culture specimen analysis</td><td rowspan="2">ELITe InGenius SP200 (EG SpA, ref. INT032SP200)
ELITe InGenius SP 200 Consumable Set (EG SpA, ref. INT032CS)
ELITe InGenius PCR Cassette (EG SpA, ref. INT035PCR),
ELITe InGenius Waste Box (EG SpA, ref. F2102-000)
300 µL Filter Tips Axygen (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only
1000 µL Filter Tips Tecan (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only
CPE – Internal Control (EG SpA, ref. CTRCPE)
MRSA-SA — ELITe Positive Control (EG SpA, ref. M800356)
eNAT™ kit (Copan, ref. 608CS01R),
eSwab Collection Kit (Copan, ref. 480CE),</td></tr>
<tr><td>ELITe BeGenius (EG SpA ref. INT040)
ELITe BeGenius Software version 2.2.1. (or later)
MRSA-SA ELITe_Be_PC_200_100 or MRSA-SA ELITe_Be_PC_200_50, Assay Protocol with parameters for Positive Control analysis
MRSA-SA ELITe_Be_NC_200_100 or MRSA-SA ELITe_Be_NC_200_50, Assay Protocol with parameters for Negative Control analysis
MRSA-SA ELITe_Be_NS_200_50, Assay Protocol with parameters for nasal swabs specimen analysis
MRSA-SA ELITe_Be_BC_200_100, Assay Protocol with parameters for blood culture specimen analysis</td></tr>
<tr><td>7500 Fast Dx Real-Time PCR Instrument (ThermoFisher Scientific, ref. 4406985)
NucliSENS® easyMAG (bioMérieux SA, Ref. 200111)</td><td>MicroAmp Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL (Life Technologies, ref. 4346906)
CPE – Internal Control (EG SpA, ref. CTRCPE)
MRSA-SA — ELITe Positive Control (EG SpA, ref. M800356)
NucliSENS easyMAG Reagents (bioMérieux SA, Ref. 280130, 280131, 280132, 280133, 280134, 280135)
NucliSENSeasyMAGStrip for Premix (bioMérieux SA, ref. 278303)
bioHit Electronic Multichannel Pipettor (bioMérieux SA, ref. 280141)
Filter tips for bioHit (bioMérieux SA, ref. 280146)
BBL CultureSwab Plus Amies Gel without Charcoal swabs (Becton-Dickinson, ref. 220116)</td></tr>
</table>

8 WARNINGS AND PRECAUTIONS

This product is designed for in-vitro use only.

8.1 General warnings and precautions

Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) 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 infectious. 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 neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

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 before running the assay.

While running the assay, follow the product instructions provided.

Do not use the product after the indicated expiry date.

Only use reagents provided with the product and those recommended by the manufacturer.

Do not use reagents from different batches.

Do not use reagents from other manufacturers.

8.2 Warnings and precautions for molecular biology

Molecular biology procedures require qualified and trained staff to avoid the risk of erroneous results, especially due to sample nucleic acid degradation or sample contamination by PCR 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.

Laboratory coats, gloves and tools dedicated to work session setup are needed.

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 reagents must be handled under a laminar airflow hood. 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 and free from DNA and RNA.

The extraction products must be handled in such a way as to minimize dispersion into the environment in order to avoid the possibility of contamination.

The PCR Cassette must be handled carefully and never opened to avoid PCR product diffusion into the environment and sample and reagent contamination.

8.3 Warnings and precautions specific for the components

Table 3

Component	Storage temperature	Use from first opening	Freeze / thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
MRSA/SA PCR Mix	-20°C or below (protected from light)	one month	up to five	up to 15 hours (5 work sessions of 3 hours each)

9 SPECIMENS AND CONTROLS for ELITe InGenius and ELITe BeGenius

9.1 Specimens

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified and handled according to laboratory guidelines, and collected, transported, and stored under the following conditions:

Table 4

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2° / +8°C**	**-20 ± 10 °C**	**-70 ± 15 °C**
nasal swab	collected with **eNAT™ kit**		≤ 4 weeks	≤ 6 months	-
nasal swab	collected with **eSwab Collection Kit**	≤ 2 hours	≤ 48 hours	≤ 6 months	-
blood culture	-	≤ 24 hours	-	-	-

Before the analysis dilute the blood culture 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 into an Extraction tube (for ELITe InGenius instrument) or into a 2 mL Sarstedt tube (for ELITe BeGenius instrument).

It is recommended to divide the specimens into aliquots before freezing to prevent repeated freeze / thaw cycles. When using frozen samples, thaw the samples just before the extraction to avoid possible nucleic acid degradation.

To perform samples testing on the **ELITe InGenius** and **ELITe BeGenius**, the following Assay Protocols must be used. These IVD protocols were specifically validated with ELITe MGB Kits and the **ELITe InGenius** or **ELITe BeGenius** with the indicated matrices.

Table 5 Assay Protocols for MRSA/SA ELITe MGB Kit

Specimen	Instrument	Assay Protocol Name	Report	Characteristics
Nasal swab	**ELITe InGenius**	**MRSA-SA ELITe_NS_200_50**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe BeGenius	**MRSA-SA ELITe_Be_NS_200_50**	Positive / Negative	
blood culture	**ELITe InGenius**	**MRSA-SA ELITe_BC_200_100**	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO PCR Mix volume: 20 µL Sample PCR input volume: 10 µL
	ELITe BeGenius	**MRSA-SA ELITe_Be_BC_200_100**	Positive / Negative	

For all protocols, 200 µL of sample must be transferred into an Extraction tube (for ELITe InGenius) or 2 mL Sarstedt Tube (for ELITe BeGenius).

NOTE

Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the 8 WARNINGS AND PRECAUTIONS page 6section.

Purified nucleic acids can be left at room temperature for 16 hours and stored at -20 °C or below for no longer than one month.

Refer to “Potentially Interfering Substances” in the 12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 18 section to check data concerning interfering substances.

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

9.2 PCR controls

PCR control results must be generated and approved for each lot of PCR reagent.

- For the Positive Control, use the product **MRSA/SA - ELITe Positive Control** (not provided with this kit) with the **MRSA-SA ELITe_PC_200_50** or **MRSA-SA ELITe_PC_200_100** and **MRSA-SA ELITe_Be_PC_200_50** or **MRSA-SA ELITe_Be_PC_200_100** Assay Protocols.
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **MRSA-SA ELITe_NC_200_50** or **MRSA-SA ELITe_NC_200_100** and **MRSA-SA_ELITe_Be_NC_200_50** or **MRSA-SA ELITe_Be_NC_200_100** Assay Protocols.

NOTE

The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the PCR control validation for each lot of PCR reagent.PCR control results expire after **15 days**, at which time it is necessary to re-run the Positive and Negative Controls. The PCR controls must be re-run if any of the following events occur:

- a new lot of reagents is used,
- results of quality control analysis (see following paragraph) are out of specification,
- any major maintenance or service is performed on the **ELITe InGenius** or **ELITe BeGenius**.

9.3 Quality controls

Verification of the extraction and PCR procedure is recommended. Archived samples or certified reference material may be used. External controls should be used in accordance with local, state, and federal accrediting organizations, as applicable.

10 ELITe InGenius PROCEDURE

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

Table 6

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only)
		C) Positive Control and Negative Control run (PCR Only)
STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		1) Validation of sample results
		3) Sample result reporting

10.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Controls" menu on the Home page, verify the PCR Controls (**MRSA/SA - Positive Control** and **LGA251/SA Positive Control**, **MRSA/SA Negative Control**) are approved and valid (Status) for the **MRSA/SAPCR Mix** lot to be used. If no valid PCR Controls are available for the **MRSA/SA PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see 9 SPECIMENS AND CONTROLS for ELITe InGenius and ELITe BeGenius page 8).
- If the Assay Protocol of interest is not loaded in the system, contact your local ELITechGroup Customer Service S.p.A.

10.2 STEP 2 - Session Setup

The **MRSA/SA ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Positive Control and Negative Control run (PCR Only).

All required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe InGenius** can be connected to the "Laboratory Information System" (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the three types of run follow the steps below while referring to the GUI

Table 7

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature. Transfer **200 µL of sample** in an Extraction tube previously labelled.	**Thaw Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control** tubes (MRSA/SA Positive Control and LGA251/SA Positive Control) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. (Each tube is sufficient for 4 reactions.)
2	**Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	Not applicable	**Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an “Elution tube”, provided with ELITe InGenius SP 200 Consumable Set.
3	Select “Perform Run” from the “Home” screen.	Select “Perform Run” from the “Home” screen.	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. If Blood Culture samples are processed, ensure that the Extraction Input Volume is 200 µL andthe Extracted Elute Volume is 100 µL	If Nasal Swab samples are processed, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 50 µL. If Blood Culture samples are processed, ensure that the Extraction Input Volume is 200 µL andthe Extracted Elute Volume is 100 µL.	Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 50 µL" (with NS) or "100 µL" (with BC).
5	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the “SampleID” (SID) by typing or by scanning the sample barcode.	Not applicable
6	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”).	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”).	**Select the Assay Protocol** in the “Assay” column (see “Specimens and Controls”). Enter the lot number and expiry date of the Positive Control and of the molecular biology grade water.
7	Ensure the “Protocol” displayed is: “Extract + PCR”.	Select “PCR Only” in the “Protocol” column.	Ensure “PCR Only” is selected in the “Protocol” column.
8	Select the sample loading position as ”Primary tube” or “Extraction Tube” in the “Sample Position” column.	Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”.	Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”.
9	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
10	**Load CPE and PCR Mix** on the “Inventory Block” referring to the “Load List” and enter CPE and PCR Mix lot number, expiry date and number of reactions for each tube.	**Load PCR Mix** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.	**Load PCR Mix** on the “Inventory Block” referring to the “Load List” and enter PCR Mix lot number, expiry date and number of reactions for each tube.
11	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
12	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.
13	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.

Table 7 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
14	**Load** PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted	**Load** PCR Cassette and Elution tubes with samples extracted	**Load** PCR Cassette, Positive Control and Negative Control tubes.
15	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
16	Close the instrument door.	Close the instrument door.	Close the instrument door.
17	Press “Start”.	Press “Start”.	Press “Start”.

When the session is finished, the **ELITe InGenius** allows users to view, approve, store the results, print and save the report.

NOTE

At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified and stored at -20 ± 10 °C for no longer than one month. Avoid spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block up to 5 work sessions of 3 hours each, mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the **Positive Control** .The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run, the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

10.3 STEP 3 - Review and approval of results

The **ELITe InGenius** monitors target and Internal Control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen, results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe InGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe InGenius** generates results with the **MRSA/SA ELITe MGB Kit** through the following procedure:

1. Validation of Positive Control and Negative Control results,

2. Validation of sample results,
3. Sample result reporting.

10.3.1 Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius Software** interprets the PCR results for the targets of the Positive Control and Negative Control reaction with the **ELITe_PC** and **ELITe_NC** Assay Protocols parameters. The resulting Ct values are used to verify the system (reagents lot and instrument).

The Positive Control and Negative Control results, specific for the PCR reagent lot, are recorded in the database (Controls).They can be viewed and approved by “Administrator” or “Analyst” users, following the GUI instructions.

The Positive Control and Negative Control results expire after **15 days**.

The results of the Positive Control and Negative Control amplification are used by the **ELITe InGenius software** to set up the Control Charts monitoring the amplification step performances. Refer to the instrument manual for more details.

NOTE

If the Positive Control or Negative Control result does not meet the acceptance criteria, the “Failed” message is shown on the “Controls” screen. In this case, the results cannot be approved, and the Positive Control or Negative Control runs must be repeated.

NOTE

If the Positive Control or Negative Control result is not valid and samples were included in the same run, the samples can be approved but their results are not validated. In this case, the failed Control(s) and samples must all be repeated.

10.3.2 Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the targets (channels **mecA** and **SA**) and the Internal Control (channel **IC**) with the **MRSA-SA ELITe_NS_200_50** and **MRSA-SA ELITe_BC_200_100** Assay Protocols parameters.

Results are shown in “Results Display” screen.

The sample results can be approved when the two conditions in the table below are true.

Table 8

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

The sample results are automatically interpreted by the **ELITe InGenius software** using Assay Protocol parameters. The possible result messages are listed in the table below.

For each sample the system reports a combination of the following messages specifying if the pathogen DNAs are either detected or not detected.

Table 9

Result of sample run	Interpretation
MRSA:detected.	**MRSA DNA was detected** in the sample.
MRSA/SA:not detected or below the LoD	**MRSA/SA DNA was not detected** in the sample. The sample is a **valid** negative or the target concentrations are below the assay Limit of Detection.
MRSA:not detected or below LoD, SA detected	**MRSA DNA was not detected** in the sample. The sample is negative for this target or its concentration is below the Limit of Detection of the assay, **SA was detected**
Invalid-Retest Sample	**Not valid assay result** caused by Internal Control failure (incorrect extraction, inhibitors carry-over). The test should be repeated.

Samples reported as: "MRSA/SA:not detected or below the LoD" are suitable for analysis but it was not possible to detect MRSA/SA DNA. In this case it cannot be excluded that MRSA/SA DNA is present at a concentration below the limit of detection of the assay (see “12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius page 18”).

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, extraction or PCR steps (e. g. incorrect sampling, degradation or loss of DNA, during the extraction or inhibitors in the eluate), which may cause incorrect results. If sufficient eluate volume remains, the eluate can be retested by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see “18 TROUBLESHOOTING page 37”).

NOTE

The results obtained with this assay must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

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

10.3.3 Sample result reporting

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

The “Sample Report” shows the results details by selected sample (SID).

The “Track Report” shows the results details by selected Track.

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

11 ELITe BeGenius PROCEDURE

The procedure to use the **MRSA/SA ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

Table 10

STEP 1	Verification of the system readiness	
STEP 2	Session setup	A) Sample run (Extract + PCR)
		B) Eluted sample run (PCR Only),
		C) Positive Control and Negative Control run (PCR Only).

Table 10 (continued)

STEP 3	Review and approval of results	1) Validation of Positive Control and Negative Control results
		2) Validation of sample results
		3) Sample result reporting

11.1 STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in "**CLOSED**" mode,
- in the "Controls" menu on the Home page, verify the PCR Controls (**MRSA/SA - Positive Control** and **LGA251/ SA Positive Control**, **MRSA/SA Negative Control**) are approved and valid (Status) for the **MRSA/SA PCR Mix** lot to be used. If no valid PCR Controls are available for the **MRSA/SA PCR Mix** lot, run the PCR Controls as described in the following sections
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "9 SPECIMENS AND CONTROLS for ELITe InGenius and ELITe BeGenius page 8").

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

11.2 STEP 2 - Session Setup

The **MRSA/SA ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),

B. Eluted sample run (PCR Only),

C. Positive Control and Negative Control run (PCR Only).

All the required parameters are included in the Assay Protocols available on the instrument and are loaded automatically when the Assay Protocol is selected.

NOTE

The **ELITe BeGenius** can be connected to the "Laboratory Information System" (LIS) which enables downloading the session information. Refer to the instrument manual for more details.

Before to setup a run:

Thaw the needed **PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

NOTE

Protect the **PCR Mix** from light while thawing because this reagent is photosensitive.

To set up one of the three types of run follow the steps below while referring to the GUI:

Table 11

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature). Transfer **200 µL of sample** in a 2 mL Sarstedt tube previously labelled.	If needed, **thaw the Elution tubes** containing the extracted nucleic acids at room temperature. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control** tubes (MRSA/SA Positive Control and LGA251/SA Positive Control) at room temperature for 30 minutes. Each tube is sufficient for 4 reactions. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.
2	**Thaw** the needed **CPE** tubes at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	Not applicable	Prepare the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an “Elution tube”, provided with the ELITe InGenius SP 200 Consumable Set.
3	Select “**Perform Run**” from the “Home” screen.	Select “**Perform Run**” from the “Home” screen	Select “**Perform Run**” from the “Home” screen.
4	Remove all the Racks from the “Cooler Unit” and place them on the preparation table.	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) of the “Cooler Unit” and place them on the preparation table	Remove the “Racks” from “Lane 1, 2 and 3” (L1, L2, L3) from the “Cooler Unit” and place them on the preparation table.
5	Select the “Run mode”: “**Extract + PCR”.**	Select the “Run mode”: “**PCR Only**”.	Select the “Run mode”: “**PCR Only**”.
6	**Load the samples** into the “Sample Rack”. When secondary tubes “2 mL Tubes” are loaded, use the blue adaptors for the “Sample Rack”.	**Load the samples** into the “Elution Rack”.	**Load the Positive Control and Negative Control** tubes into the “Elution Rack”.
7	**Insert** the “**Sample Rack**” into the “Cooler Unit” starting from the “Lane 5” (L5). If needed, insert the “Sample ID” (SID) for each “Position” used (If secondary tubes are loaded, flag “2 mL Tube”. If secondary tubes are not barcoded, type manually the “Sample ID”).	**Insert** the “**Elution Rack**” into the “Cooler Unit” starting from “Lane 3” (L3). If needed, for each “Position” enter the “Sample ID”, the “Sample matrix”, the “Extraction kit” and the “Extracted eluate vol.” (eluate volume).	**Insert** the “**Elution Rack**” into the “Cooler Unit” starting from the “Lane 3” (L3). If needed, for each “Position” enter the “Reagent name” and the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
8	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
9	If Nasal Swab samples are processed, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 50 µL. If Blood Culture samples are processed, ensure that the Extraction Input Volume is 200 µL andthe Extracted Elute Volume is 100 µL.	If Nasal Swab samples are processed, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 50 µL. If Blood Culture samples are processed, ensure that the Extraction Input Volume is 200 µL andthe Extracted Elute Volume is 100 µL.	Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 50 µL" (with NS) or "100 µL" (with BC).
10	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).	Select the Assay Protocol in the “Assay” column (see “Specimens and Controls”).
11	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.

Table 11 (continued)

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)	C. Positive and Negative Control run (PCR Only)
	NOTE Note: When more than 12 samples are processed, repeat the procedure from point 6.		-
12	Load the “Elution tubes” into the “Elution Rack” (Elution tubes can be labelled with barcode to improve traceability).	Not applicable	Not applicable
13	Insert the “Elution Rack” into the “Cooler Unit” starting from “Lane 3” (L3). When more than 12 samples are processed, repeat using “Lane 2” (L2).	Not applicable	Not applicable
14	Click “Next” to continue.	Not applicable	Not applicable
15	**Load CPE and PCR Mix** into the “Reagent/Elution Rack”.	**Load the PCR Mix** into “Reagent/Elution Rack”.	**Load the PCR Mix** into “Reagent/Elution Rack”.
16	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent and / or CPE enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).	Insert the “Reagent/Elution Rack” into the “Cooler Unit” in “Lane 2” (L2) if available or in “Lane 1” (L1). If needed, for each PCR Mix reagent enter the “S/N” (serial number), the “Lot No.” (lot number), the “Exp. Date” (expiry date) and the “T/R” (number of reactions).
17	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
18	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.	Verify the tips in the “Tip Racks” in the “Inventory Area” and replace Tip Racks if necessary.
19	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
20	Load the “**PCR Rack**” with “PCR Cassette” in the Inventory Area.	Load the “**PCR Rack**” with “PCR Cassette” in the Inventory Area.	Load the **“PCR Rack**” with “PCR Cassette” in the Inventory Area.
21	Click “Next” to continue.	Click “Next” to continue.	Click “Next” to continue.
22	Load the “Extraction Rack” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables.	Not applicable	Not applicable
23	Close the instrument door.	Close the instrument door.	Close the instrument door.
24	Press “Start”.	Press “Start”.	Press “Start”.

When the session is finished, the **ELITe BeGenius** allows users to view, approve, store the results, print and save the report.

NOTE

At the end of the run the remaining Extracted Sample in the **Elution tube** must be removed from the instrument, capped, identified and stored at -20 ± 10 °C for no longer than one month. Avoid the spilling of the Extracted Sample.

NOTE

At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 5 work sessions of 3 hours each, mix gently and spin down the content for 5 seconds before starting the next session.

NOTE

At the end of the run the remaining **Positive Control** can be removed from the instrument, capped and stored at -20 °C or below. Avoid the spilling of the Positive Control. The remaining **Negative Control** must be discarded.

NOTE

The **Positive Control** can be used for 4 separate sessions of 3 hours each.

NOTE

At the end of the run the **PCR Cassette** and the other consumables must be disposed of following all governmental and environmental regulations. Avoid spilling the reaction products.

11.3 STEP 3 - Review and approval of results

The **ELITe BeGenius** monitors target and Internal Control fluorescence signals for each reaction and automatically applies the Assay Protocol parameters to generate PCR curves which are then interpreted into results.

At the end of the run, the “Results Display” screen is automatically shown. In this screen the results and the run information are shown. From this screen results can be approved, and reports printed or saved (“Sample Report” or “Track Report”). Refer to the instrument manual for more details.

NOTE

The **ELITe BeGenius** can be connected to the “Laboratory Information System” (LIS) which enables uploading the session results to the laboratory data center. Refer to the instrument manual for more details.

The **ELITe BeGenius** generates the results with the **MRSA/SA ELITe MGB Kit** through the following procedure:

1. Validation of Positive Control and Negative Control results,
2. Validation of sample results,
3. Sample result reporting.

NOTE

Please, refer to the same paragraph of the **ELITe InGenius** Procedure for the details.

12 PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius

12.1 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 on ELITe InGenius 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.

Table 12

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

The theoretical LoD value was confirmed on ELITe InGenius and on ELITe BeGenius by testing 20 replicates of plasmid MRSA/SA and 20 replicates of plasmid LGA251/SA at claimed concentration (20 copies / reaction).

The LoD of the method was verified on ELITe InGenius and on ELITe BeGenius by testing nasal swabs samples collected in eSwab, nasal swabs samples collected in eNat and Blood Culture samples, spiked with MRSA/SA - ELITe Positive Control (both plasmid MRSA/SA and plasmid LGA251/SA) at 1000 copies/mL for nasal swabs and 2000 copies/mL for Blood Culture.

The results are reported in the following table.

Table 13 Limit of Detection (copies / mL) for nasal swabs and blood culture samples on ELITe InGenius and on ELITe BeGenius

Sample	LoD (copies / mL)
Nasal Swab	1000
Blood Culture	2000

The results obtained confirmed the claimed concentration for the target MRSA/SA on both ELITe InGenius and ELITe BeGenius.

12.2 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.

Table 14 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

Table 14 Tests with calibrated reference materials and ELITe InGenius (continued)

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.

Table 15 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.

12.3 Repeatability

The Repeatability of the assay was evaluated on **ELITe InGenius** and **ELITe BeGenius** instruments by analysis of a panel of nasal eSwab samples, including one negative sample and positive samples spiked with MRSA/SA - ELITe Positive Control.

An example of Intra-Session Repeatability (on one day) results are shown in the tables below.

Table 16 Intra - Session Repeatability on ELITe InGenius

Sample	N	MecA target			SA target			% Agree-ment
		Mean Ct	SD	% CV	Mean Ct	SD	% CV	
Negative	8	-	-	-	-	-	-	100%
3X LoD	8	33.10	0.24	0.73	32.89	0.31	0.93	100%
10X LoD	8	31.14	0.09	0.28	30.96	0.17	0.54	100%

Table 17 Intra - Session Repeatability on ELITe BeGenius

Sample	N	MecA target			SA target			% Agree-ment
		Mean Ct	SD	% CV	Mean Ct	SD	% CV	
Negative	8	-	-	-	-	-	-	100%
3X LoD	8	32.55	0.29	0.90	31.73	0.29	0.90	100%
10X LoD	8	31.01	0.23	0.75	30.17	0.31	1.03	100%

An example of Inter-Session Repeatability (on two days) results are shown in the tables below.

Table 18 Inter - Session Repeatability on ELITe InGenius

Sample	N	MecA target			SA target			% Agree-ment
		Mean Ct	SD	% CV	Mean Ct	SD	% CV	
Negative	16	-	-	-	-	-	-	100%
3X LoD	16	33.24	0.37	1.10	32.95	0.40	1.22	100%
10X LoD	16	31.51	0.69	2.20	31.38	0.74	2.37	100%

Table 19 Inter - Session Repeatability on ELITe BeGenius

Sample	N	MecA target			SA target			% Agree-ment
		Mean Ct	SD	% CV	Mean Ct	SD	% CV	
Negative	16	-	-	-	-	-	-	100%
3X LoD	16	32.69	0.36	1.10	31.86	0.34	1.07	100%
10X LoD	16	30.90	0.29	0.94	30.11	0.30	1.01	100%

In the Repeatability test, the MRSA/SA ELITe MGB Kit detected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

12.4 Reproducibility

The Reproducibility of the assay was evaluated on **ELITe InGenius**and **ELITe BeGenius**instruments by analysis of a panel of nasal eSwab samples, including one negative sample and positive samples spiked with MRSA/SA - ELITe Positive Control.

An example of Inter-Batch Reproducibility (on two lots) is shown in the tables below.

Table 20 Inter-Batch Reproducibility on ELITe InGenius

Sample	N	MecA target			SA target			% Agree-ment
		Mean Ct	SD	% CV	Mean Ct	SD	% CV	
Negative	16	-	-	-	-	-	-	100%
3X LoD	16	33.37	0.34	1.01	33.17	0.42	1.27	100%
10X LoD	16	31.58	0.67	2.11	31.61	0.64	2.01	100%

Table 21 Inter-Batch Reproducibility on ELITe BeGenius

Sample	N	MecA target			SA target			% Agree-ment
		Mean Ct	SD	% CV	Mean Ct	SD	% CV	
Negative	16	-	-	-	-	-	-	100%
3X LoD	16	32.72	0.30	0.92	31.94	0.33	1.04	100%
10X LoD	16	31.01	0.21	0.67	30.25	0.25	0.83	100%

An example of Inter-Instrument Reproducibility (on two instruments) is shown in the tables below.

Table 22 Inter-Instrument Reproducibility on ELITe InGenius

Sample	N	MecA target			SA target			% Agree-ment
		Mean Ct	SD	% CV	Mean Ct	SD	% CV	
Negative	16	-	-	-	-	-	-	100%
3X LoD	16	32.94	0.47	1.44	33.04	0.39	1.18	100%
10X LoD	16	30.97	0.38	1.23	31.09	0.43	1.37	100%

Table 23 Inter-Instrument Reproducibility on ELITe BeGenius

Sample	N	MecA target			SA target			% Agree-ment
		Mean Ct	SD	% CV	Mean Ct	SD	% CV	
Negative	16	-	-	-	-	-	-	100%
3X LoD	16	32.83	0.21	0.65	32.18	0.26	0.82	100%
10X LoD	16	30.92	0.25	0.81	30.32	0.20	0.68	100%

In the Reproducibility test, the MRSA/SA ELITe MGB Kitdetected all the samples as expected and showed a maximum variability of target Ct values as %CV lower than 5%.

12.5 Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as confirmation of negative samples, was evaluated in association with **ELITe InGenius** by analyzing clinical samples of nasal swab and blood culture negative for MRSA/SA.

As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Specificity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius.**

The results are summarized in the following table.

Table 24

Samples	N	positive	negative	% Diagnostic Specificity
Nasal swab samples negative for MRSA/ SA DNA	48	0	48	100
Blood culture samples negative for MRSA/ SA DNA	34	0	34	100

The IC Ct cut-off value is set at 29 for nasal swabs and blood culture samples when tested with ELITe InGenius and ELITe BeGenius.

12.6 Diagnostic Sensitivity: confirmation of positive samples

The Diagnostic Sensitivity of the assay, as confirmation of positive clinical samples, was evaluated in association with **ELITe InGenius** by analyzing clinical samples of nasal swab, positive for MRSA or MSSA, or spiked for MRSA DNA adding MRSA BAA-1556 (ATCC) at a titre of 100,000 CFU/mL, and blood culture positive for MRSA and MSSA or spiked with MRSA isolates, given the difficulty of finding a significant number of positive clinical samples for some MRSA target genes.

As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent.Therefore, the Diagnostic Sensitivity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius.**

The results are summarized in the following table.

Table 25

Samples	N	positive	negative	% Diagnostic Sensitivity
Nasal swab samples positive for MSSA DNA	60	56	4	93
Nasal swab samples positive for MRSA DNA	41	40	1	98
Blood culture samples positive for MSSA DNA	39	39	0	100
Blood culture samples positive for MRSA DNA	31	31	0	100

13 SPECIMENS AND CONTROLS FOR OTHER SYSTEMS

13.1 Samples

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

NOTE

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 125µL 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.

13.2 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®**.

Table 26

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 or drops	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

Table 26 (continued)

Potentially Interfering Substance (Type)	Active Ingredient	Interferes?
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.

13.3 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 with this kit).

For the Positive Control, use the **MRSA/SA - ELITe Positive Control** product (not provided with this kit).

13.4 Quality controls

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

14 OTHER SYSTEMS PROCEDURE

14.1 Setting of the real time amplification session

(To perform in the amplification / detection of amplification products area)

When a **7500 Fast Dx Real-Time PCR Instrument** is used.

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

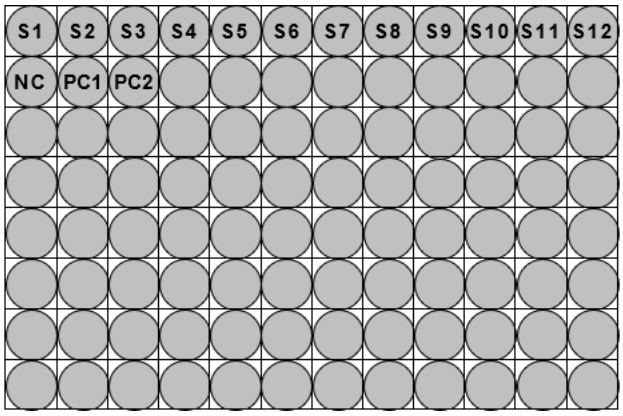
- switch on the computer, switch on the real-time thermal cycler, run the dedicated software, open an "absolute quantification" session
- when the **7500 Fast Dx Real-Time PCR Instrument** is used, choose “Run mode: Fast 7500”
- create a new “detector” set or set the appropriate "detector" in the Tool menu by selecting the Detector Manager:

1. 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”;
2. 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”;
3. set the "detector" for the *mecA* and *mecC* gene probes with the "reporter" = "FAM", the "quencher" = "none" (non fluorescent) and call it “mecA”;
4. 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**: Negative Control of amplification;

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**;

NOTE
The fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization 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**.

Table 27

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.

14.2 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 for preparing **25 reactions**. Mix gently, spin down the content for 5 seconds and keep them on ice;
- 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

NOTE

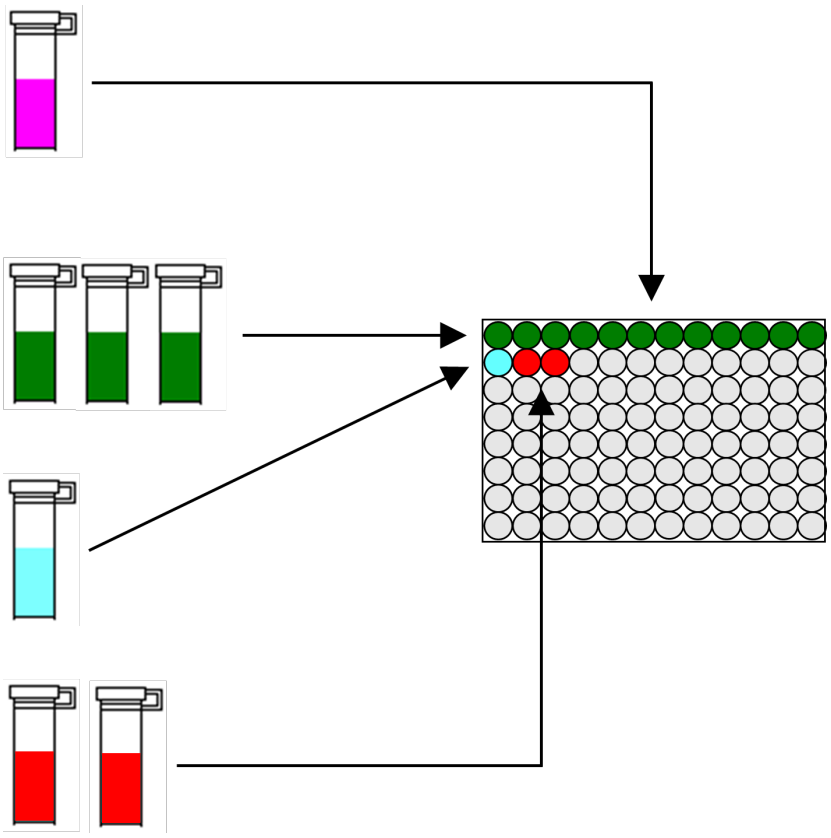
If not all the reaction mixture is used, store the remaining volume in the dark at -20°C for no longer than one month. Freeze and thaw the reaction mixture up to **five times**.

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").

NOTE

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 shows synthetically the preparation of the amplification reaction



1. **Add 20 µL of PCR Mix**
2. **Add 10 µL of extracted DNAs**
3. **Add 10 µL of Negative Control**
4. **Add 10 µL of Positive Controls**

14.3 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:

Table 28

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

Table 29

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:

Table 30

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

Table 31

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

Table 32

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.

NOTE

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:

Table 33

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

Table 34

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 mecC 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 15 PERFORMANCE CHARACTERISTICS WITH OTHER SYSTEMS page 31). In this case the result could be a false negative.

NOTE

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.

15 PERFORMANCE CHARACTERISTICS WITH OTHER SYSTEMS

15.1 Clinical Performance

The performance characteristics of the assay 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** as 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%.

Table 35 MRSA results obtained with MRSA/SA ELITe MGB Kit in comparison to the reference method.

	MRSA *mec*A Diagnostic sensitivity	**MRSA *mecC* Diagnostic sensitivity**	**MRSADiagnostic 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%.

Table 36 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%

15.2 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.

Table 37 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

Table 38 Limit of Detection Results (CFU/swab)

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

15.3 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.

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 *mec*A**LGA251** specific oligonucleotides.
2. Gift from Dr. Nathan A. Ledebouer, 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

15.4 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**.

Table 39 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	

Table 39 Species Tested for Cross-Reactivity by sequence database analysis (continued)

Staphylococci species		Other organisms	Viruses
		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.*

15.5 Reproducibility 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.

Table 40 Tests with certified reference material

Sample ID	Content	Sample Conc. CFU/mL	Expected Result	Actual Result
MRSADNA10-04	MRSA	1×10^8	Frequently detected	Detected
MRSADNA10-03	MRSA	5×10^7	Frequently detected	Detected

Table 40 Tests with certified reference material (continued)

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.

15.6 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.

NOTE

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.

16 REFERENCES

Centers for Disease Control and Prevention. National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1992 through June 2004. Am J Infect Control 2004; 32:470-485.

Clinical and Laboratory Standards Institute (CLSI). Surveillance for Methicillin-Resistant *Staphylococcus aureus:* Principles, Practices, and Challenges; A Report. CLSI Document X07-R (ISBN 1-56238-719-7) Wayne, PA:CLSI, 2010.

Jernigan, J. A. et al. Prevalence of and risk factors for colonization with methicillin-resistant *Staphylococcus aureus* at the time of hospital admission. Infect Control Hosp Epidemiol. 2003; 24:409-414.

Garcia-Alvarez, L. et al. Methicillin-resistant Staphylococcus aureus with a novel *mec*A homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect Dis 2011; 11:595-603.

Stegger, M. et al. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new *mecA* homologue *mecALGA251*. 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.

17 PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: nasal swab and blood culture.

Currently there are no data available concerning product performance with other clinical samples.

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.

The results obtained with this product depend on proper identification, collection, transport storage and processing of the samples. To avoid incorrect results, it is therefore necessary to take care during these steps and to carefully follow the instructions for use provided with the product.

Owing to its high analytical sensitivity, the Real Time PCR method used in this product is sensitive to contamination from positive clinical samples, Positive Controls and PCR products. Cross-contamination cause false positive results. The product format is designed to limit cross-contamination. However, cross-contamination can only be avoided by good laboratory practices and following these instructions for use.

This product must be handled by qualified personnel trained in the processing of potentially infective 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 personal protective equipment and areas that are suitable for the processing of potentially infective 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 personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

To avoid incorrect results, this product must be handled by professional personnel, qualified and trained in molecular biology techniques such as extraction, PCR and detection of nucleic acids.

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.

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.

As with any other diagnostic medical device, the results obtained with this product must be interpreted in combination with all relevant clinical observations and laboratory results.

As with any other diagnostic medical device, there is a residual risk of obtaining invalid, or erroneous results with this product. This residual risk cannot be eliminated or further reduced. In some cases, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient. However, this residual risk associated to the intended use of the product has been weighed against the potential benefits to the patient and it has been assessed acceptable.

18 TROUBLESHOOTING

ELITe InGenius and ELITe BeGenius

Table 41

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Positive Control. Check the volumes of PCR Mix and Positive Control.
PCR Mix degradation.	Do not use the PCR Mix for more than 5 independent sessions (3 hours each in the Inventory Area Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 5 consecutive sessions (in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Positive Control degradation.	Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use a new aliquot of Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Table 42

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix and Negative Control. Check the volumes of PCR Mix and Negative Control.
Contamination of the Negative Control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the PCR Mix.	Use a new aliquot of PCR Mix.
Contamination of the extraction area, Racks, Inventory Block or Cooler Unit	Clean surfaces with aqueous detergents, wash lab coats, replace tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Table 43

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control, and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 5 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the PCR Mix for more than 5 consecutive sessions (in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

Table 44

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but Tm different from that of the other samples and that of the positive control.	Check for target Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target with a possible mutation. The target in the sample should be sequenced to confirm mutation.

Table 45

Error in Ct calculation	
Possible Causes	**Solutions**
Too high concentration of target in the sample or sample with anomalous fluorescence signal.	If significant amplification is observed in PCR plot select the track related to the sample and manually approve the result as positive. If no amplification is observed in PCR plot select the track related to the sample and manually approve the result as negative or leave it as invalid. If a Ct value is required: - repeat the amplification of eluted sample with a 1:10 dilution in molecular biology grade water in a “PCR Only” session. - repeat the extraction of the sample with a 1:10 dilution in molecular biology grade water in an “Extract + PCR” session.

Table 46

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination in preanalytical steps.	Clean the micropipette with fresh 3% sodium hypochlorite solution (bleach) 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 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 (bleach) or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of PCR Mix and / or CPE.

Open Platform:

Table 47

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

Table 48

Target DNA detected in the Negative Control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples, Negative Controls, positive controls into the microplate wells and comply with the work sheet.
Error while setting the instrument	Check the position settings of the samples, Negative Controls, Positive Controls on the instrument
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of molecular biology grade water.	Use a new aliquot of sterile water.
Contamination of the PCR Mix.	Use a new aliquot of PCR 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.

Table 49

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing or inadequate mixing of sample.	Take care, by pipetting three times, when mixing samples, Negative Controls and Positive Controls into the reaction mixture. Avoid creating bubbles.
Baseline setting error.	Set the baseline calculation range within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase, e.g from cycle 6 to cycle 15. Use the automatic baseline calculation by setting the "Auto Baseline" option.

Table 50

Anomalous dissociation curve	
Possible causes	**Solutions**
Absence of a defined peak. Defined peak but different from that of the other samples and of the Positive Control.	Check for detector FAM Ct lower than 30. High quantity of amplification product at the end of the reaction may interfere with the melting curve analysis. Repeat the sample amplification to confirm the presence of target DNA with a possible mutation. The target DNA of the sample should be sequenced to confirm mutation.

19 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 device.
UDI	Unique Device Identification
	Contains sufficient for "N" tests.
	Consult instructions for use.
CONT	Contents.

Keep away from sunlight.

Manufacturer.

20 NOTICE TO PURCHASER: LIMITED LICENSE

This product contains reagents manufactured by Thermo Fisher Scientific and are sold under licensing arrangements between ELITechGroup S.p.A. and its Affiliates and Thermo Fisher Scientific. The purchase price of this product includes limited, nontransferable rights to use only this amount of the product solely for activities of the purchaser which are directly related to human diagnostics. For information on purchasing a license to this product for purposes other than those stated above, contact Licensing Department, Thermo Fisher Scientific. Email: outlicensing@thermofisher.com.

ELITe MGB ® detection reagents are covered by one or more of U. S. Patent numbers 7319022, 7348146, 7381818, 7541454, 7671218, 7723038, 7767834, 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

ELITe InGenius® and ELITe BeGenius® technologies are covered by patents and pending applications.

This limited license allows the person or entity to whom the product has been provided to use the product and data generated by the use of the product, solely for human diagnostics. Neither ELITechGroup S. p. A. nor its licensors grant any other licenses, expressed or implied for any other purposes.

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Appendix A MRSA/SA ELITe MGB Kit used in association with Genius series® platforms

CAUTION

This document is a simplified version of the official instruction for use. Please refer to the complete document before use: www.elitechgroup.com

INTENDED USE

The **MRSA/SA ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative nucleic acids Real-Time PCR assay for the detection of the DNA of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA, including the mecC strain), extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of nasal swabs and blood culture.

The assay is also validated in association with the **7500 Real-Time PCR Instrument**, using human specimens of nasal swabs and blood culture.

The product is intended for use as an aid in 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.

The results must be interpreted in combination with all relevant clinical observations and laboratory outcomes.

Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target 1	conservative region in the coagulase positive *Staphylococcus aureus* gene	AP554	SA
Target 2	conservative regions in the mecA and mecC genes (responsible for resistance to methicillin and other beta-lactam antibiotics)	FAM	MeCA
Internal Control	artificial sequence IC2	AP642	IC

Validated matrix

- Nasal swab collected in eNAT™ kit
- Nasal swabs collected in eSwab Collection Kit
- Blood Culture

Kit content and related products

MRSA/SA ELITe MGB Kit (M800351)		MRSA/SA ELITe - Positive Control (M800356)	
 X 4		 X 2  X 2	
MRSA/SA PCR Mix 4 tubes of 540 µL 24 reactions per tube 96 reactions per kit 4 freeze-thaw cycles per tube		**MRSA/SA - Positive Control** and **LGA251/ SA Positive Control** 2 tubes of 160 µL for MRSA/SA 2 tubes of 160 µL for LGA251/SA 5 reactions per tube 10 reactions per kit 12 freeze-thaw cycles	
Maximum shelf-life:	**24 months**	Maximum shelf-life	**24 months**
Storage temperature	**≤ -20°C**	Storage temperature	**≤ -20°C**

Other products required not provided in the kit

- ELITe InGenius instrument: INT030.
- ELITe BeGenius instrument: INT040.
- ELITe InGenius SP 200: INT032SP200.
- ELITe InGenius SP1000: INT033SP1000
- ELITe InGenius SP 200 Consumable Set: INT032CS.
- ELITe InGenius PCR Cassette: INT035PCR.
- ELITe InGenius Waste Box: F2102-000.
- CPE - Internal Control: CTRCPE
- 300 µL Filter Tips Axigen: TF-350-L-R-S.
- 1000 µL Filter Tips Tecan: 30180118.

ELITe InGenius and ELITe BeGenius protocol

› Sample volume › CPE volume › Total elution volume	200 µL 10 µL 100 µL (with BC) or 50 µL (with NS)	› Eluate PCR input volume › Q—PCR Mix volume › Frequency of controls	10 µL 20 µL 15 days

ELITe InGenius and ELITe BeGenius Performances

Matrix	Limit of Detection	Sensitivity	Specificity
Nasal Swab	1000 copies / mL	MSSA: 93% (56/60) MRSA: 98% (40/41)	100 % (48/48)
Blood Culture	2000 copies / mL	MSSA: 100% (39/39) MRSA: 100% (31/31)	100 % (34/34)

Sample preparation

This product is intended for use on the **ELITe InGenius** and **ELITe BeGenius** with the following clinical specimens identified according to laboratory guidelines, and collected, transported, and stored under the following conditions.

Table 51

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	**+2° / +8°C**	**-20 ± 10 °C**	**-70 ± 15 °C**
nasal swab	collected with **eNAT™ kit**	-	≤ 4 weeks	≤ 6 months	-
nasal swab	collected with **eSwab Collection Kit**	≤ 2 hours	≤ 48 hours	≤ 6 months	-
blood culture	-	≤ 24 hours	-	-	-

ELITe InGenius Procedures

The user is guided step-by-step by the Graphic User Interface of ELITe InGenius software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR), or PCR Only.

Before analysis

1. Switch on ELITe InGenius. Log in with username and password. Select the mode "**CLOSED**".	**2.** Verify controls: **Positive Control** and **Negative Control** in the "Controls" menu. Note: Both must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extraction + PCR (e.g., samples)

1. Select "Perform Run" on the touch screen	**2.** Verify the extraction volumes: Input: "200 µL", elution: "50 µL" (with NS) or "100 µL" (with BC)	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the "Assay Protocol" of interest: MRSA-SA ELITe_NS_200_50 or MRSA-SA ELITe_BC_200_100.	**5.** Select the method "Extract + PCR" and the sample position: Extraction Tube	**6.** Load the PCR Mix and the Internal Control in the Inventory Block
7. Load: PCR Cassette, Extraction cartridge, Elution tube, Tip Cassette, Extraction Tube racks	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE

If an Extract Only mode is needed, refer to the instrument user's manual for procedure.

Procedure 2: PCR only (e.g., eluates, controls)

1. Select “Perform Run” on the touch screen	**2.** Verify the extraction volumes: Input: “200 µL”, elution: “50 µL” (with NS) or “100 µL” (with BC)	**3.** Scan the sample barcodes with hand-barcode reader or type the sample ID
4. Select the “Assay Protocol” of interest: MRSA-SA ELITe_NS_200_50 or MRSA-SA ELITe_BC_200_100, MRSA-SA ELITe_PC_200_100 or MRSA-SA ELITe_PC_200_50, MRSA-SA ELITe_NC_200_100 or MRSA-SA ELITe_NC_200_50	**5.** Select the method “PCR Only” and the sample position “Elution Tube”	**6.** Load the PCR Mix in the Inventory Block
7. Load: PCR Cassette rack and the Elution tube rack with the extracted nucleic acid	**8.** Close the door. Start the run	**9.** View, approve and store the results

ELITe BeGenius Procedures

The user is guided step-by-step by the Graphic User Interface of ELITe BeGenius® software to setup the run. All the steps: extraction, Real-Time PCR and result interpretation are automatically performed. Two operational modes are available: complete run (Extract + PCR), or PCR Only.

Before analysis

1. Switch on ELITe BeGenius. Log in with username and password. Select the mode “**CLOSED**”.	**2.** Verify controls: **Positive Control** and **Negative Control** in the “Controls” menu. Note: Both must have been run, approved and not expired.	**3.** Thaw the **PCR Mix** and the **CTRCPE** tubes. Vortex gently. Spin down 5 sec.

Procedure 1 - Complete run: Extraction + PCR (e.g., samples)

1. Select “Perform Run” on the touch screen and then click on the run mode «Extract and PCR»	**2.** Insert the Sample Rack with the barcoded samples in the Cooler Unit. The barcode scan is already active	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “50 µL” (with NS) or “100 µL” (with BC)
4. Select the “Assay Protocol” of interest: MRSA-SA ELITe_Be_NS_200_50 or MRSA-SA ELITe_Be_BC_200_100. **Note**: if a second extraction is performed repeat steps from 2 to 4	**5.** Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the Cooler Unit	**6.** Load the PCR Mix and the Internal Control in the Reagent/Elution Rack and insert it in the Cooler Unit
7. Load “PCR Rack” with “PCR Cassette” and the “Extraction Basket” with the “ELITe InGenius SP 200” extraction cartridges and the required extraction consumables	**8.** Close the door. Start the run	**9.** View, approve and store the results

NOTE
If an Extract Only mode is needed, refer to the instrument user’s manual for procedure.

Procedure 2: PCR only (e.g., eluates, controls)

1. Select “Perform Run” on the touch screen and then click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid or controls barcoded tubes in the Elution Rack and insert it in the Cooler Unit”	**3.** Verify the extraction volumes: Input: “200 µL”, Eluate: “50 µL” (with NS) or “100 µL” (with BC)
4. Select the “Assay Protocol” of interest: MRSA-SA ELITe_Be_NS_200_50 or MRSA-SA ELITe_Be_BC_200_100, MRSA-SA ELITe_Be_PC_200_100 or MRSA-SA ELITe_Be_PC_200_50 MRSA-SA ELITe_Be_NC_200_100 or MRSA-SA ELITe_Be_NC_200_50	**5.** Load the PCR-Mix in the Reagent/ Elution Rack and insert it in the Cooler Unit	**6.** Load “PCR Rack” with “PCR Cassette”
7. Close the door. Start the run	**8.** View, approve and store the results	

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