ELITechGroup
MOLECULAR DIAGNOSTICS

MRSA/SA ELITe MGB®

For use with the bioMérieux NucliSENS® easyMAG® automated DNA isolation Instrument and Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument

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IVD   **For *in vitro* Diagnostic Use**

Rx only

REF **M800346**

-20°C

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Intended Use

MRSA/SA ELITe MGB is a qualitative *in vitro* diagnostic test for the direct detection of *Staphylococcus aureus* (SA) and methicillin-resistant *Staphylococcus aureus* (MRSA) using DNA purified from nasal swabs. MRSA/SA ELITe MGB is intended to aid in the prevention and control of MRSA infections in healthcare settings. It is not intended to diagnose, guide or monitor MRSA infections, or provide results of susceptibility to oxacillin/methicillin. A negative result does not preclude MRSA/SA (*Staphylococcus aureus)* nasal colonization. Concomitant cultures are necessary to recover organisms for epidemiological typing or for further susceptibility testing.

Summary and Explanation of the Test

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[1]. *S. aureus* and especially MRSA is consistently a leading cause of nosocomial infections and is associated with substantial morbidity, mortality, and cost[2]. 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 test is a triplex real-time amplification-based assay that targets the conserved regions in a *Staphylococcus aureus*-specific gene, which is responsible for SA identification, *mecA* gene, which is responsible for resistance to methicillin and other beta-lactam antibiotics, and an exogenous internal control to monitor reaction inhibition and reagent integrity. The *Staphylococcus aureus*-specific gene will unambiguously identify SA, and the *mecA* gene will unambiguously identify the methicillin resistance gene. Presence of both markers at the same relative quantity measured by a difference in cycle threshold (Ct) value is indicative of MRSA; different relative quantities or presence of only *Staphylococcus aureus*-specific gene marker is indicative of SA.

Real-time PCR detection of MRSA/SA significantly reduces laboratory time compared with standard culture tests, improving the efficiency of the procedure. Current real-time PCR MRSA detection tests[3,4,5] 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 test targets conservative regions in MRSA/SA genetic markers, thereby minimizing false negative calls due to a natural SCC*mec* insertion site variability and minimizing false positive calls due to the "empty cassette" issue.

Principles of the Procedure

Detection of MRSA/SA with the MRSA/SA ELITe MGB relies on two major processes:

- Specimen preparation by automated DNA extraction from nasal swabs;
- Real-time PCR amplification and detection of target DNA by specific hybridization probes.

Specimen preparation

Each nasal swab is inserted into a tube with trypticase soy broth and thoroughly mixed to create a cell suspension which is then subjected to automated DNA extraction using the NucliSENS easyMAG instrument. Internal control plasmid template is added to the silica solution to act as a control for the extraction process and monitor for PCR inhibitors. One batch of twenty-four specimens can be processed in less than 50 minutes.

PCR Amplification and Detection

The processed specimens and the MRSA/SA ELITe MGB PCR Mix containing hot-start non-Taq thermostable DNA polymerase are placed in the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument PCR plate for amplification and detection. The primers and probes in the MRSA/SA ELITe MGB PCR Mix detect proprietary conservative regions in *Staphylococcus aureus*-specific gene (*Staphylococcus aureus* marker) and mecA (methicillin-resistance marker) genes. The MRSA/SA ELITe MGB PCR Mix contains internal control probe and primers which detect the internal control template in the processed specimen to control for inhibition and reagent integrity. The MRSA/SA ELITe MGB PCR Mix contains a passive reference labeled with AP593 fluorophore (similar to ROX) to control well-to-well

variation in amplification and detection. The Uracil-DNA-glycosylase enzyme, included in the MRSA/SA ELITe MGB PCR Mix, recognizes and catalyzes the destruction of DNA strands containing deoxyuridine, but not DNA containing deoxythymidine. Since amplicons produced with the MRSA/SA ELITe MGB contain deoxyuridine, potential amplicon contaminants are eliminated during a heating step performed prior to the start of PCR amplification.

Specific detection of PCR products by 5'- MGB Hybridization Probes

The ELITe MGB Probes detect amplified target through a hybridization-triggered fluorescent mechanism (Figure 1). When the probe is unbound in solution, the three-dimensional conformation brings the quencher and MGB in close proximity to the fluorescent label, quenching the fluorescence. When the probe anneals to a target sequence, the probe unfolds, spatially separating the quencher from the fluorescent label to allow a strong fluorescent signal. ELITe MGB Probes are not degraded during the amplification. The probe specific to the SA-specific gene is labeled with AP554 fluorophore (similar to TAMRA).The probe specific to the *mecA* gene is labeled with FAM fluorophore. The probe specific to the Internal Control is labeled with AP642 fluorophore (similar to Cy5). The Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument monitors simultaneously the fluorescence emitted by each probe. Following PCR, the results are interpreted (see Interpretation of Results) to provide a final call.
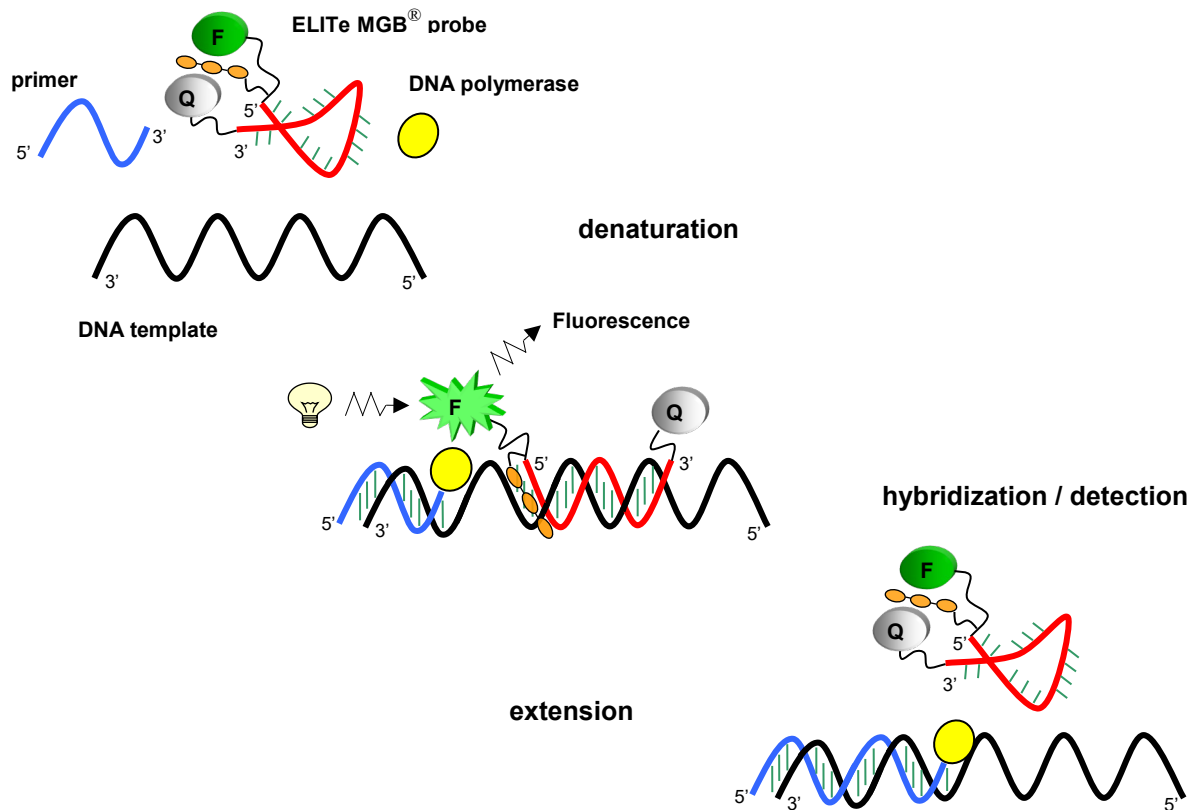

Figure 1: Hybridization-triggered fluorescent mechanism

Materials Provided

MRSA/SA ELITe MGB   (REF M800346)   **100 Tests**

Component	Quantity	Cap Color
MRSA/SA ELITe MGB PCR Mix	4 × 540 µL	Yellow
MRSA/SA Internal Control	4 × 270 µL	Natural (Colorless)
MRSA/SA Positive Control	4 × 65 µL	Natural (Colorless)

MRSA/SA ELITe MGB PCR Mix   4 × 540 µL
       Tfi PCR Master Mix[6]
       <0.01% MRSA/SA primers
       <0.01% Internal Control primers
       <0.01% MRSA/SA Fluorescent-labeled oligonucleotide probes
       <0.01% Internal Control Fluorescent-labeled oligonucleotide probe
       <0.01% Fluorescent Passive Reference dT(8)-AP593

MRSA/SA Internal Control   4 × 270 µL
       Tris buffer
       <0.01% EDTA
       0.01% total yeast RNA
       <0.001% Non-infectious plasmid DNA (recombinant) containing Internal Control sequences

MRSA/SA Positive Control   4 × 65 µL
       Tris buffer
       <0.01% EDTA
       0.01% total yeast RNA
       <0.001% Non-infectious plasmid DNA (microbial) containing MRSA sequences

Materials Required But Not Provided

Instrumentation and Software

- bioMérieux NucliSENS easyMAG (REF 280140) with software v 2.0.

  NOTE: Use of any software other than the specified will violate the safety, effectiveness and design controls of this medical device and may result in an increase risk to users and patients. ELITechGroup Inc. Molecular Diagnostics (EGI MDx) will evaluate the performance of MRSA/SA ELITe MGB with new software releases and will notify users and issue a new or revised manual if the software revision can be used.

- Biohit eLINE 8-channel Electronic Pipettor (bioMérieux REF 280141).

- Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument with SDS Software v1.4 (Applied Biosystems REF 4406984 or 4406985).

  NOTE: Use of any software other than the specified will violate the safety, effectiveness and design controls of this medical device and may result in an increase risk to users and patients. EGI MDx will evaluate the performance of MRSA/SA ELITe MGB with new software releases and will notify users and issue a new or revised manual if the software revision can be used.

Reagents and Disposables

- bioMérieux NucliSENS easyMAG Extraction Buffer 1 (REF 280130)

- bioMérieux NucliSENS easyMAG Extraction Buffer 2 (REF 280131)

- bioMérieux NucliSENS easyMAG Extraction Buffer 3 (REF 280132)

- bioMérieux NucliSENS easyMAG Magnetic Silica (REF 280133)

- bioMérieux NucliSENS easyMAG Lysis Buffer (REF 280134)

- bioMérieux NucliSENS easyMAG Disposables (REF 280135)

- bioMérieux disposable strips for Magnetic Silica pre-mix (REF 278303)

- Filter tips for the Biohit eLINE 8-channel Electronic Pipettor (bioMérieux REF 280146)

- ABI MicroAmp Fast Optical 96-well Plates (Applied Biosystems REF 4346906 or 4366932)

- ABI MicroAmp Fast Optical Adhesive Film (Applied Biosystems REF 4311971)

Other Materials Required But Not Provided

- Becton-Dickinson BBL CultureSwab Plus Amies Gel without Charcoal swabs (BD REF 220116)

- Trypticase Soy Broth, (Becton-Dickinson Trypticase® Soy Broth, 1mL/tube, BD REF 295634 or equivalent)

- Bench microcentrifuge (VWR® Galaxy Mini REF 37001-594 or equivalent)

- Bench microcentrifuge with PCR plate adaptor (Peqlab PerfectSpin P REF 91-PSPINP-US or equivalent)

- Vortex mixer (VWR REF 58815-121 or equivalent)

- Disposable gloves

- Adjustable Pipettors: (capacities: 20 µL, 200 µL and 1000 µL) with sterile, DNase-free, filter-blocked or positive displacement micropipettor tips

- Sterile tubes

Other Materials Recommended But Not Provided

- Negative Specimen Processing Control (See **Quality Control**)

- Positive Specimen Processing Control

Optional Materials Available But Not Provided

- ELITe MGB Software, ELITech Molecular Diagnostics PN M800476– for automated results generation . Consult with your sales representative if you are interested in this software.

Warnings and Precautions

IVD

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

General Warnings and Precautions

1. Use of this product should be limited to personnel trained in PCR.

2. This test is for use with nasal swab specimens only.

3. Do not pipette by mouth.

4. Do not eat, drink or smoke in laboratory work areas. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens and product reagents. Wash hands thoroughly after handling specimens and reagents.

5. Avoid microbial contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipette tips is recommended.

6. Dispose of leftover reagents and waste in compliance with the regulations in force.

7. Read all the instructions provided in the kit before running the assay.

8. While running the assay, follow the instructions provided in the kit.

9. Do not pool reagents from different lots or from different bottles of the same lot.

10. Do not mix reagents from different kit lots.

11. Do not use a kit after its expiration date.

12. Material Safety Data Sheets (MSDS) are available upon request.

13. Handle and dispose of all biological specimens as if they were able to transmit infective agents. Use safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories*[7] and in the CLSI Document M29-A3[8]. Avoid direct contact with the biological specimens. Avoid producing spills or aerosol. Any material coming in contact with the biological specimens must be treated for at least 30 minutes with a 10% bleach solution (0.5% sodium hypochlorite) or autoclaved for 30 min at 121º C before disposal. Be sure that disposal of materials is conducted per the prevailing laws and regulations in the area.

Warnings and Precautions for Molecular Biology

1. Molecular biology procedures, such as nucleic acid extraction, reverse transcription, amplification and detection, require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the specimens or specimen contamination by amplification products.

2. 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 designed for extraction/preparation of amplification reactions.

3. It is necessary to have available lab coats, gloves and tools, which are exclusively employed 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 designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.

4. Only the specimen type indicated herein may be employed for this type of analysis. Specimens must be handled under a laminar flow hood. Tubes containing different specimens must never be opened at the same time. Pipettes used to handle specimens must be exclusively employed for this specific purpose only. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases and free of RNA and DNA.

5. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be exclusively used for this purpose only. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be free from DNases and RNases and free from DNA and RNA.

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

Storage and Handling Requirements

1. Store the MRSA/SA ELITe MGB reagents at -20°C upon arrival. Unopened, these reagents are stable until the expiration date indicated. Once opened, store the remaining reagents at -20°C until the expiration date indicated. All reagents can sustain up to 8 freeze-thaw cycles.

2. Protect the MRSA/SA ELITe MGB PCR Mix from unnecessary light exposure.

3. Prior to usage, vials must be thawed and utilized within 4 hours. Open vials must be securely recapped prior to storage. All vials must be returned to -20°C after the required volumes are removed.

4. Processed specimens are stable for up to 7 days when stored at 20-25°C or 4°C.

5. Amplification should be started immediately after addition of the processed specimens and controls to the MRSA/SA ELITe MGB PCR Mix. If this is not possible, store the filled PCR plates away from light for a maximum of 24 hours at 4°C.

Indication of Instability or Deterioration of Reagents

When a positive or negative control value is out of the expected range (see Interpretation of Results), it may indicate deterioration of the reagents. Associated test results are invalid and specimens must be retested.

Specimen Collection, Transport and Storage

Precaution: All specimens have to be treated as potentially infectious material.

A.   **Specimen Collection**

1. Moisten the swab with two drops (approximately 50 µL) of sterile saline or use dry.

2. Gently insert the swab inside the nostril approximately 0.5 inch or 1.3 cm and rotate against the mucosa five times while applying light pressure on the outside of the nose (to help ensure contact of the swab head with the inside of the nose).

3. Place the swab into its collection tube, cover and label appropriately.

B.   **Specimen Transport and Storage**

Specimens should be transported in a shatterproof transport container to avoid a potential infection due to a leakage of specimen. The specimens should be transported following the local and national instructions for the transport of pathogenic material[9].

• Specimens taken with the BBL CultureSwab Plus Amies Gel without Charcoal swabs may be stored before processing (including time needed for transport) for up to one day or less at 20-25°C or up to 7 days at 4°C.

Instructions for Use

Specimen and Control Preparation

A.   Specimen Preparation

1. In a biological safety cabinet, label one Trypticase Soy Broth 1 mL tube appropriately for each specimen.

2. Remove the swab from the transport container.

3. Place the swab in a Trypticase Soy Broth 1mL tube and mix thoroughly in a Vortex mixer for 10-15 sec at high speed while pressing the swab stem to the tube wall. Fresh gauze may be used to cover the tube to minimize contamination.

4. Place the swab back into the transport container and keep refrigerated in case a retest is needed (see Interpretation of Results, p. 12). Swabs are stable up to 7 days if kept at 4ºC. Discard the swab in the appropriate waste container when no longer needed.

B.   Internal Control Preparation

1. Remove one tube of MRSA/SA Internal Control Template from the boxed kit, and thaw at room temperature. Ensure the reagent is completely thawed before use.

2. Vortex for 3-5 seconds, and centrifuge briefly to collect material at the base of the tube.

C.   DNA Extraction Using the bioMérieux NucliSENS easyMAG

   Note: 24 samples may be processed in each extraction run.

1. Start both the NucliSENS easyMAG instrument and software.

2. Scan or manually enter the reagent barcodes into the NucliSENS easyMAG.

3. Define sample extraction parameters in the NucliSENS easyMAG software

   3.1.   Click the **Daily Use** button on the main menu bar to display the sub-menu items.

   3.2.   Click on the **Define Extraction Requests** button, and define the following parameters:

      3.2.1.   Sample ID: Enter the sample ID, either by keyboard or barcode reader.

      3.2.2.   Matrix = Other

      3.2.3.   Protocol = Generic 2.0.1

      3.2.4.   Volume (mL) = 1.0 mL

      3.2.5.   Eluate (µL) = 50 µL

      3.2.6.   Type = Primary

   3.3.   Click on the **Create Run** button, name the run appropriately, and then click on OK.

      3.3.1.   After creating the run, load 1.0 mL of each prepared sample from step A. 3 into a sample vessel.

      3.3.2.   Prepare (if required) the positive (PSPC) and negative (NSPC) specimen processing controls that are used to monitor reagent integrity through the entire process (see Quality Control on , p.13) and load 1.0 mL of each into a sample vessel.

   3.4.   Insert the loaded sample vessel into the easyMAG instrument.

   3.5.   Close the process door of the NucliSENS easyMAG instrument and touch the **Dispense Lysis** button, and the addition of the lysis buffer will start for all installed strip positions that contain primary samples. This will start the 10 minute lysis incubation. During the lysis incubation, prepare the premix Magnetic Silica / Internal Control (enough for eight samples).

      3.5.1.   Briefly vortex the magnetic silica.

      3.5.2.   Using the Biohit pipettor set to program P1 and a single tip or a manual pipettor, add 550µL of the magnetic silica suspension to a separate tube (not provided).

     3.5.3.   Using a pipettor, add 540 µL of molecular biology grade water.

     3.5.4.   Using a pipettor, add 10 µL of the MRSA/SA Internal Control DNA template.

     3.5.5.   Briefly vortex the magnetic silica / Internal Control mixture.

     3.5.6.   Using the Biohit pipettor set to program P2 and a single tip or a manual pipettor, dispense the magnetic silica / Internal Control mixture into ELISA strip wells in 8 × 125µL aliquots.

     3.5.7.   After the 10 minute lysis incubation is over, open the process door and add the magnetic silica / Internal Control mixture using the Biohit pipettor set to program P3 and up to eight tip(s). Transfer 100 µL of the magnetic silica / Internal Control mixture to the sample vessels.

     3.5.8.   Touch the **Start** button to begin the run

   3.6.   Eluates will be ready within 34-40 minutes (depending on number of vessels loaded) and should be transferred to labeled Eppendorf tubes and capped securely within 30 minutes of extraction completion to prevent magnetic silica contamination of the processed specimen. The processed specimens are stable for up to 7 days when stored at 20-25°C or 4°C or for approximately three years at -20°C.

D.   **APPLIED BIOSYSTEMS 7500 FAST DX REAL-TIME PCR AMPLIFICATION PROCEDURE:**

   1.   Thaw the MRSA/SA ELITe MGB PCR Mix on ice in a biological safety cabinet, in a template-free area.

   2.   Create a "plate map" that depicts the locations of the reaction wells where controls and processed specimens will be located.

     NOTE: It is important to label the plate map accurately so the results can be matched to the controls and specimens on the reaction plate.

   3.   Once the solution is thawed, briefly vortex the tube, and then spin it down in a centrifuge.

   4.   In a new MicroAmp Fast Optical 96-well plate, pipette 20 µL of the thawed MRSA/SA PCR Mix into the wells where the processed specimens and controls will be placed. You have to include at least one positive (PC and/or PSPC) and one negative (NTC and/or NSPC) control.

     4.1.   Add 10 µL of molecular biology grade water to the NTC well if required, and loosely cover the plate with an optical adhesive cover.

   5.   Transport the plate to a template addition area and add the remaining controls and processed specimens.

     5.1.   Thaw the MRSA/SA Positive Control Template and any previously extracted specimens, PSPC, or NSPC controls to be analyzed. Briefly vortex each tube, and then spin it down in a centrifuge.

     5.2.   Add 10 µL of the appropriate controls and processed specimen to the designated well.

     5.3.   Store the unused remainder of the control and specimen eluates in individual tubes or sealed annotated plates at -20°C for repeat testing in case of an invalid result.

   6.   Carefully seal the plate with an optical adhesive cover and spin the plate in a centrifuge. If running the plate within one hour, proceed to the instrument; otherwise place the plate at 4°C for up to 24 hours.

     NOTE: Following centrifugation, visually inspect the liquid levels in each reaction well to ensure they contain the same volume. Discard the plate and repeat Steps 1 – 5 above if wells appear to have different volumes.

   7.   Move the plate to the 7500 Fast Dx instrument, turn on the instrument and start the SDS software following Applied Biosystems 7500 Fast Dx Real Time PCR System Operation & Maintenance manual[10] .

   8.   Open a new SDS document, and then in the **New Document Wizard**, use the following settings, and then click on **Finish**:

     8.1.   Assay: Standard Curve (Absolute Quantitation)

     8.2.   Container: 96-well Clear

     8.3.   Template: Blank Document

8.4. Run Mode: Fast 7500

8.5. Operator: List the name of the current operator

8.6. Comments: List any additional comments, as necessary

8.7. Plate Name: Name appropriately

8.8. Well ID: Enter a sample ID for each reaction well containing a control or processed specimen.

IMPORTANT: Do not enter an ID for empty wells. Empty wells should never be included or analyzed as part of a run. Including empty wells may generate erroneous results.

9. The new document will start on the **Setup** tab, shown at the top left of the screen, just under the menu bar. Follow the manufacturer recommendations provided in the ABI 7500 Fast Dx user manual[11] to set up the appropriate detectors using the FAM (mecA), TAMRA (SA-specific marker), and CY5 (IC) dye calibrations.

9.1. In the Tools menu select the Detector Manager and create the detectors with the following settings:

9.1.1. Name: mecA, SA, and IC

9.1.2. Reporter Dye: FAM (mecA), TAMRA (SA), and CY5 (IC)

9.1.3. Quencher Dye: (none)

9.2. In the View menu select the Well Inspector and set ROX as the passive reference.

10. After the detectors have been added, click on the Instrument tab to change the default PCR cycling times and temperature settings in the **Thermal Profile** tab as follows:

Step	Temp. (°C)	Time
UNG Reaction	50°C	2 Min.
Initial Activation & Denaturation		
Polymerase Activation	93°C	2 Min.
3-Step PCR Cycling Profile (45 Cycles)		
Denaturation	93°C	10 Sec.
Annealing /Detection	56°C	30 Sec.
Extension	72°C	15 Sec.

11. Click **File** on the menu, and then select **Save**.

11.1. Assign an appropriate filename.

12. Open the instrument, place the sealed plate into the instrument and click on the **Start** button.

13. After the run is completed, click on the **Results** tab and then the **Amplification Plot** tab.

13.1. On the 96-well plate layout at the bottom of the screen, select all of the wells.

13.2. On the menu bar, select Analysis, and then click on Analysis Settings.

13.3. In the Analysis Settings window, set **Detector** to **All**, and then click on the **Manual Ct** check box. Set the **Threshold** to **0.05**, and select **Automatic Baseline**.

13.4. Click on **OK & Reanalyze**, to close the window and reanalyze the data.

13.5. Click on **File → Save**, to save the data file.

NOTE: Results may be interpreted manually using the procedure described below or generated automatically by analyzing the information in the data file using ELITe MGB Software, ELITech Molecular Diagnostics PN M800476. Contact your Sales Representative to obtain a copy of the software.

13.6. In the report from the ABI 7500 Fast Dx, select one well at a time and interpret the results ("Make calls") for <u>each</u> <u>specimen</u> according to this table:

Results Interpretation[1] Table

SA = C_{T1}	mecA = C_{T2}	ΔC_T $\|C_{T1} - C_{T2}\|$	IC	MRSA Result	SA Result
Undetermined or C_T >35.0	Undetermined or C_T > 35.0	NA	C_T < 34.0	Negative	Negative
			Undetermined or $C_T \geq$ 34.0	Invalid	Invalid
Determined, $C_T \leq$ 35.0	Undetermined or C_T > 35.0	NA	NA	Negative	Positive
	Determined, $C_T \leq$ 35.0	$\Delta C_T \geq$ 2		Negative	Positive
		ΔC_T < 2		Positive	Positive
Undetermined or C_T > 35.0	Determined, $C_T \leq$ 35.0	NA	NA	Negative	Negative

Here is the results interpretation algorithm:

Situation 1:   IF C_{T1} >35.0 **AND** C_{T2} >35.0 **AND** IC C_T **< 34.0**, then the result is "**MRSA-negative/SA-negative.**"

Situation 2:   IF C_{T1} >35.0 **AND** C_{T2} >35.0**AND** IC $C_T \geq$ 34.0, then the result is "**Invalid.**"

Situation 3:   IF $C_{T1} \leq$35.0 **AND** $C_{T2} \leq$35.0 **AND** $\|C_{T1} - C_{T2}\|$ < 2, then the result is "**MRSA-positive.**"

Situation 4:   IF $C_{T1} \leq$35.0, **AND** $\|C_{T1} - C_{T2}\| \geq$ 2, then the result is "**MRSA-negative/SA-positive.**"

Situation 5:   IF C_{T1} >35.0 **AND** $C_{T2} \leq$35.0, then the result is "**MRSA-negative/SA-negative.**"

NOTE: See graphic examples of each situation on the next pages.

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[1] Each well will have three (3) C_T values: one for SA that is called C_{T1}, one for mecA, C_{T2}, and one for Internal Control (IC). For a valid test the presence of both SA and mecA markers at the same relative quantity (that is the absolute value of $C_{T1} - C_{T2,}$ a "difference in C_T called "ΔC_T less than 2") is indicative of MRSA; different relative quantities (a difference in C_T equal or greater than 2) or presence of only the *Staphylococcus aureus*-specific gene marker is indicative of SA
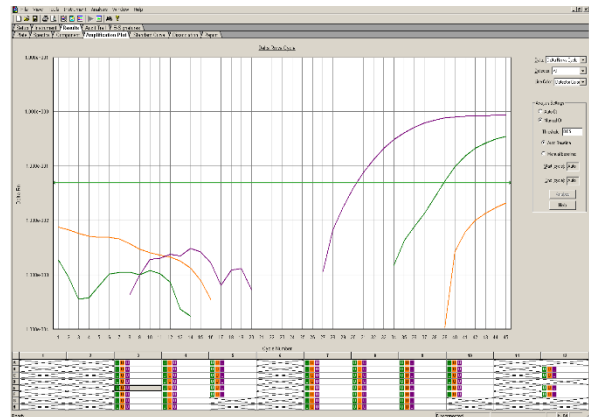
14. Situation 1 Results Calling

Example: The following example will result in a **MRSA/SA-negative (Situation 1)** call.

14.1. Select a filled well in the Results Plate window.
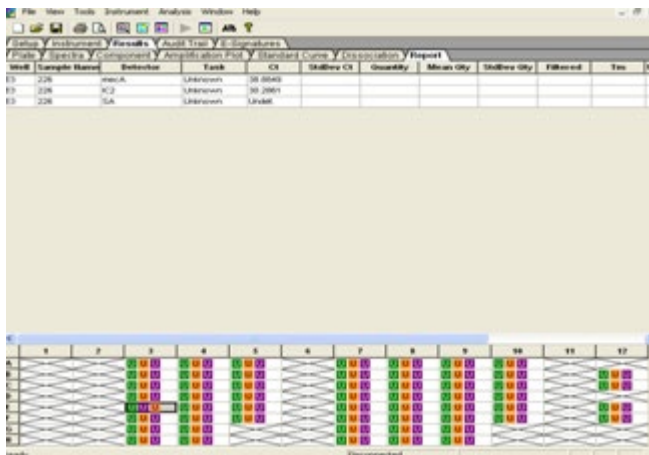(In this example, sample # is 226.)
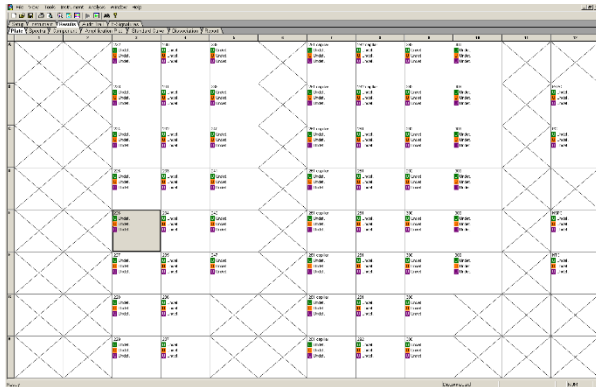
14.2. Click on Amplification Plot to see the picture if desired.

14.3. Click on Report and print if necessary.

Note that there are three (3) different C_T values for sample #226: one for mecA (green), one for SA (orange) and another for Internal Control (purple).

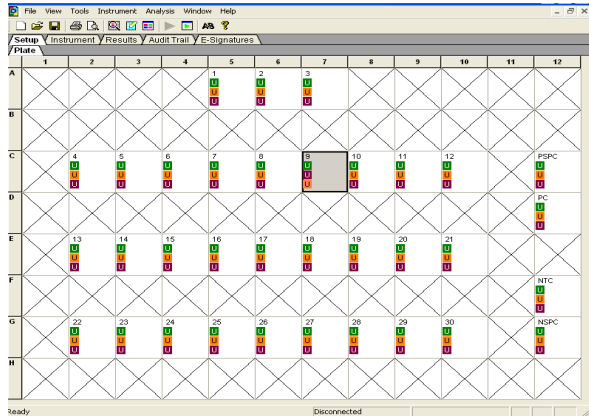14.4. Interpret the results using to the Clinical Cut Off table (see 12.6)

mecA C_{T2} =   38.8849   (Is it >35? **Yes**)

SA C_{T1} =   "Undetermined" ((Is it >35? **NA)**

ΔC_T =   NA   (≥2)

IC C_T =   30.2861   (Is it <34? Yes) →Means that the test is VALID

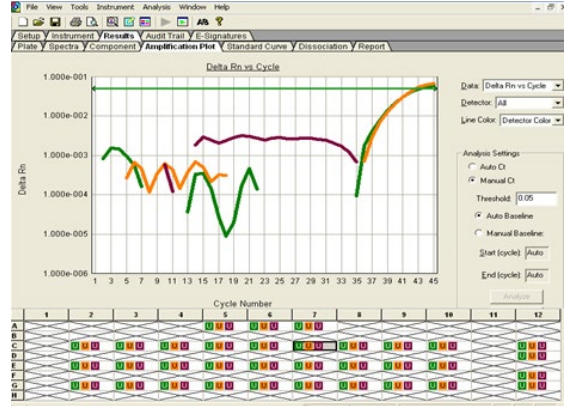**Therefore, sample #226 is MRSA Negative/SA Negative.**

15. Situation 2 Results Calling

Example: The following example will result in a **'Invalid" (Situation 2**) call**.

15.1. Select a filled well in the Results Plate window. (In this example, sample # is 9.)
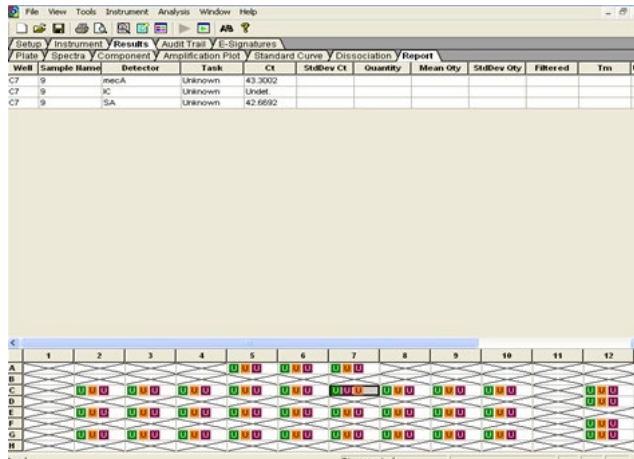
15.2. Click on Amplification Plot to see the picture if desired.

15.3. Click on Report and print if necessary.

Note that there are three (3) different C_T values for sample #9: one for mecA (green), one for SA (orange) and another for Internal Control (purple).

15.4. Interpret the results using to the Clinical Cut Off table (see 12.6)

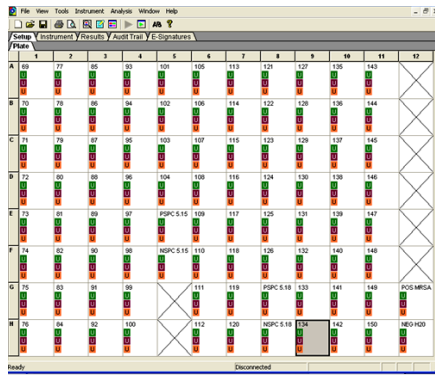| mecA C_{T2} =	43.3002	(Is it >35? **Yes**)
SA C_{T1} =	42.6692	(Is it >35? **Yes**)
ΔC_T =	NA	(Is it ≥2? **NA**)
IC C_T =	"Undetermined"	

Therefore, sample # 9 is "Invalid".
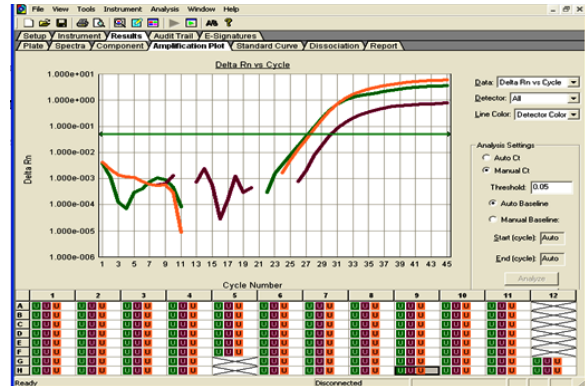
16. Situation 3 Results Calling

Example: The following example will result in a **MRSA/SA Positive (Situation 3)** call

16.1. Select a filled well (highlight by clicking on

the well) in the Results Plate window. (In this example, sample # 134.)

16.2. Click on Amplification Plot to see the picture if desired.

16.3. Click on Report and print if necessary.

Note that there are three (3) different C_T values for sample #134: one for mecA (green), one for SA (orange) and another for Internal Control (purple).

16.4. Interpret the results using to the Clinical Cut Off table (see 12.6)

mecA C_T =   27.1656   (Is it ≤35? **Yes**)

SA C_T =   27.3698   (Is it ≤35? **Yes**)

ΔC_T =   0.2   (Is it <2? **Yes**)

Therefore, sample #134 is MRSA/SA Positive.

17. Situation 4 Results calling

Example: This example is for **MRSA-Negative/SA-Positive (Situation 4)** call

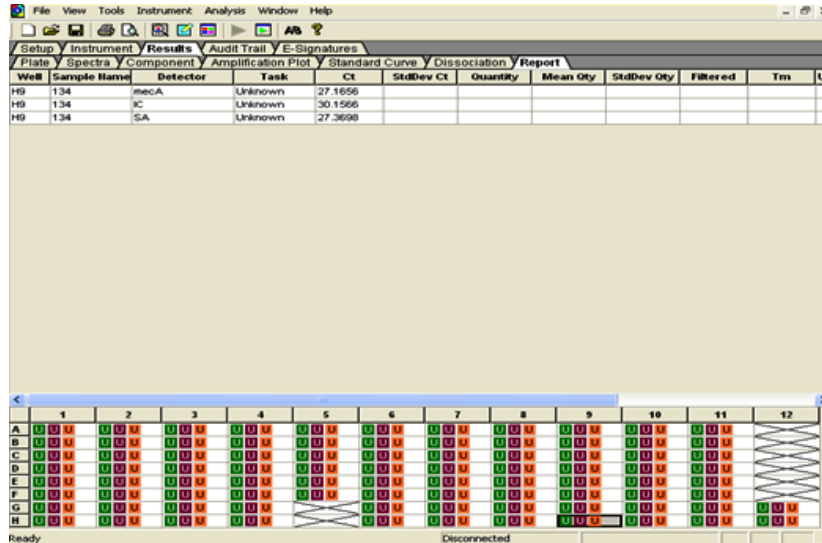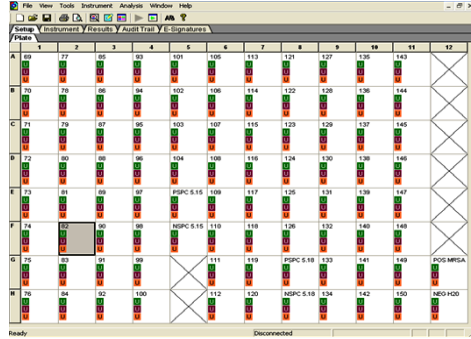17.1. Select a filled well in the Results Plate window. (In this example, the sample #82]

17.2. Click on Amplification Plot to see the picture if desired.

17.3. Click on Report and print if necessary. Again, note that there are three (3) C_T results.

17.4. Interpret the results using to the Clinical Cut Off table (see 12.6))

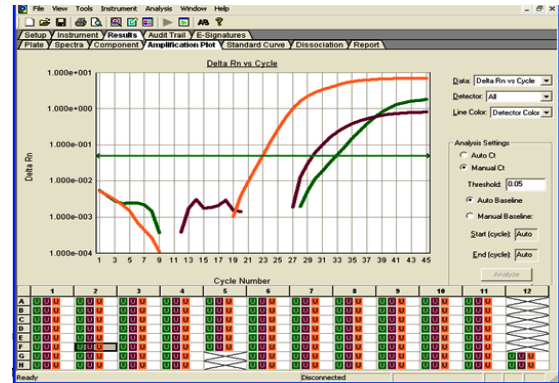| mecA C_T | = | 32.8225 | (Is it ≤35? **Yes**) |
| SA C_T | = | 22.9133 | (Is it ≤35? **Yes**) |
| ΔC_T | = | 9.9 | (Is it <2? NO) |

Therefore, sample #82 is MRSA-Negative/SA-Positive.

18. Situation 5 Result calling

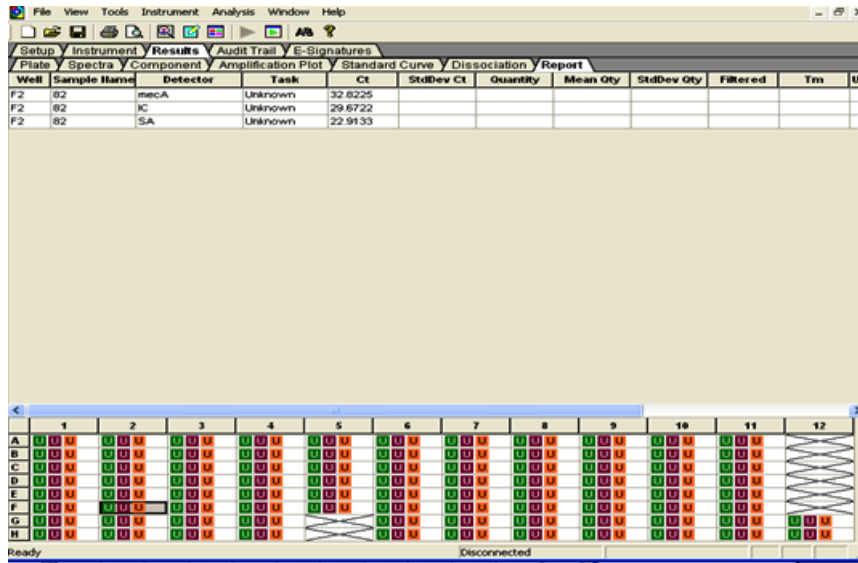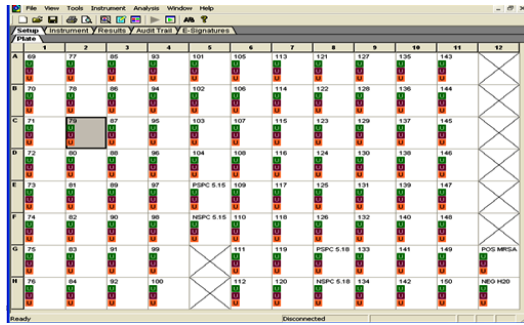Example: This example is for **MRSA/SA-Negative (Situation 5)** call

18.1. Select filled well in the Results Plate window. (In this example, the sample #79.)

18.2. Click on Amplification Plot to see the picture if desired

18.3. Click on Report and print if necessary.

Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity	Mean Qty	StdDev Qty	Filtered	Tm	U(
C2	79	mecA	Unknown	29.9693							
C2	79	IC	Unknown	31.3505							
C2	79	SA	Unknown	Undet.							
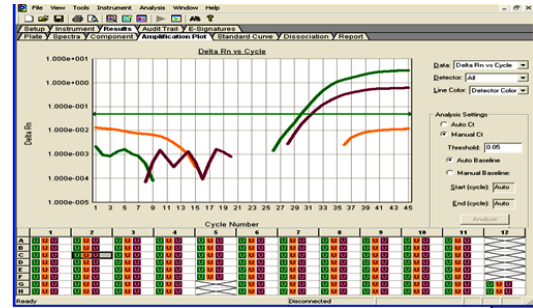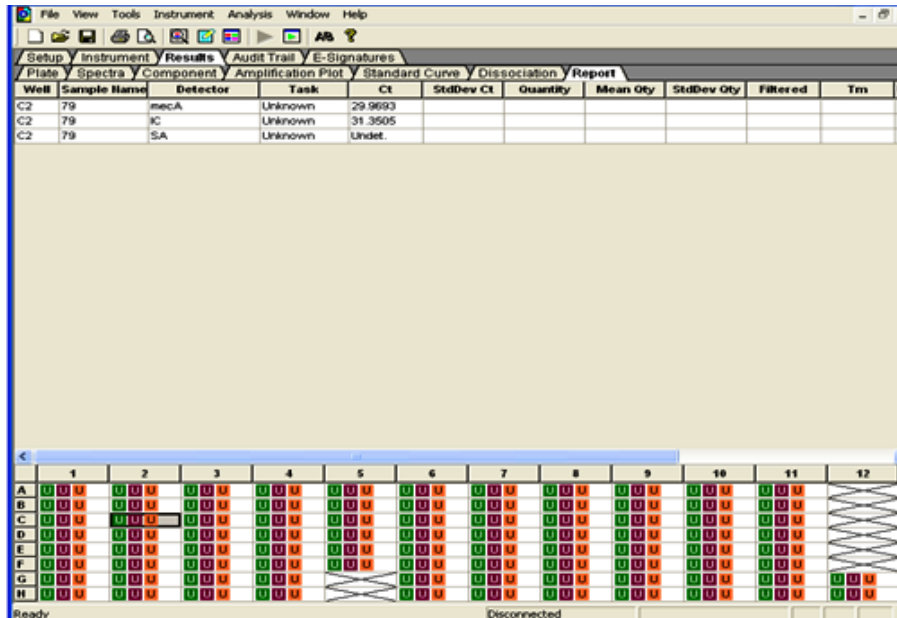
18.4. Result Calling according to the Clinical Cut Off table (see 12.6)

mecA C_T	=	29.9693	(Is it ≤35? **Yes**)
SA C_T	=	"Undetermined"	(Is it ≤35? NO)
ΔC_T	=	NA	(Is it <2? NO)

Therefore, this sample, #79, is MRSA-Negative/SA-Negative.

Interpretation of Results

1. For a valid run the control results should be as follows:

    1.1.   No Template Control (NTC) – must be IC, MRSA and SA negative (IC undetermined or $C_T \geq 34.0$; mecA and SA must both be undetermined or $C_T > 35.0$).

    1.2.   Positive Control (PC) – must be positive for mecA and SA (mecA and SA C_T must both be ≤ 35.0, "NA" for IC (Note: IC is not added to the PCR containing PC; therefore, no IC signal is expected).

    1.3.   Negative Specimen Processing Control (NSPC) – must be positive for IC, negative for MRSA and SA (IC $C_T < 34.0$; mecA "NA"; SA must be undetermined or > 35.0).

    1.4.   Positive Specimen Processing Control (PSPC) – must be positive for MRSA, "NA" for IC (mecA and SA C_T must both be ≤ 35.0, $\Delta C_T < 2$).

2.   For a valid run, the specimen results are interpreted as follows.

Results Reported	Interpretation
MRSA/SA Positive	**SA and mecA target DNA detected. Presumed positive for MRSA.**
MRSA Negative, SA Positive	**SA target DNA detected. No mecA target DNA detected. Presumed negative for MRSA or number of organisms may be below the detection limit. Test result does not preclude MRSA nasal colonization.** **SA DNA detected. Presumed positive for SA.**
MRSA/SA Negative	**No SA, including MRSA, DNA detected. Presumed negative for all SA, including MRSA or number of organisms may be below the detection limit. The test result does not preclude MRSA or SA nasal colonization.**
Invalid	**Invalid. Repeat test from processed specimens (or new specimens).**

Quality Control

1.   Each MRSA/SA ELITe MGB reaction except No Template Control (NTC) and Positive Control (PC) contains MRSA/SA Internal Control Template (IC) to control for specimen inhibition and monitor reagent integrity. At least one positive and one negative control should be included in each PCR run.

    1.1.   Internal Control (IC)

        The IC must be positive in all MRSA/SA negative specimens except NTC. If the IC is negative in a negative specimen, the specimen test result is invalid due to Internal Control failure. Test should be repeated from processed specimen (or new specimen). If the repeat PCR from the frozen processed specimen is still invalid, contact a local ELITech office. The IC result is ignored in MRSA/SA positive specimens since MRSA/SA target amplification may compete with this control. IC template is not included in the PC reaction.

    1.2.   Positive Control (PC)

        The PC monitors for reagent failure during PCR and is not processed with the bioMérieux NucliSENS easyMAG instrument. The PC must be positive for SA and mecA. If the PC does not meet these criteria, the entire run is invalid due to a positive control failure and must be repeated from the frozen specimen

eluates or new specimens.

1.3. No Template Control (NTC)

The NTC is an optional PCR contamination control and must be negative for MRSA/SA and IC. If the NTC does not meet these criteria, the entire PCR run is invalid due to No Template Control failure and must be repeated from the frozen specimen eluates or new specimens.

2. Specimen Processing Controls

The Positive Specimen Processing Control (PSPC) is intended to monitor the entire process. The Negative Specimen Processing Control (NSPC) detects reagent or environmental contamination by MRSA/SA DNA. Positive and negative control strains should be tested routinely in each laboratory according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. A swab inoculated with a $\sim 5 \times 10^3$ CFU of MRSA (e.g. American Type Culture Collection, ATCC BAA-1556) or a well characterized clinical isolate of MRSA may be used as a Positive Specimen Processing Control while a swab inoculated with a $\sim 1 \times 10^7$ CFU of *Staphylococcus epidermidis* (e.g. ATCC 12228) or of any other non-*Staphylococcus aureus* may be used as a Negative Specimen Processing Control.

The PSPC must be MRSA/SA positive (with IC either positive or negative). If the PSPC does not meet this criterion, the entire bioMérieux NucliSENS easyMAG run is invalid due to Positive Specimen Control failure and must be repeated from new specimen.

The NSPC must be MRSA/SA negative and IC positive. If the NSPC does not meet these criteria, the entire bioMérieux NucliSENS easyMAG run is invalid due to Negative Specimen Control failure and must be repeated from new specimen.

Procedural Precautions

1. As with any test procedure, good laboratory technique is essential to the proper performance of this assay.

Procedural Limitations

1. This product can only be used with the bioMérieux NucliSENS easyMAG and Applied Biosystems 7500 Fast Dx Real-Time PCR Instruments.

2. This assay has been validated for use with only human nasal specimens.

3. Reliable detection of MRSA/SA require adequate specimen collection, transport, storage and processing procedures.

4. Use of this product should be limited to trained personnel.

5. A positive MRSA/SA ELITe MGB result does not indicate the presence of viable MRSA/SA but is presumptive for the presence of MRSA/SA. Therefore, a positive result does not necessarily indicate intervention eradication failure since non-viable DNA may persist. A negative result following a previously positive result may or may not indicate eradication success. Results should be interpreted in conjunction with other laboratory and clinical data available to the clinician, and should be used as an adjunct to nosocomial infection control efforts to identify patients needing enhanced precautions. Results should not be used to guide or monitor treatment for MRSA or SA infections.

6. The performance characteristics were not established for patients ≤21 years of age.

7. While the presence of Uracil-DNA-glycosylase enzyme in the MRSA/SA ELITe MGB PCR Mix reduces the risk of amplicon contamination, contamination from positive control and clinical specimens can be avoided only by utilizing good laboratory practices and carefully adhering to the procedures specified in this Product Insert.

8. MRSA/SA ELITe MGB results may sometimes be "Invalid" due to failed internal control and require retesting that can lead to a delay in obtaining final results.

9. The detection of MRSA in the presence of excess amounts of MSSA or coagulase-negative mecA-carriers might be impaired. MRSA/SA ELITe MGB may generate a false positive MRSA result in nasal specimen containing both methicillin-resistant coagulase negative *Staphylococcus* and SA.

10. Though rare, polymorphisms within the region of the bacterial genome covered by the MRSA/SA ELITe MGB primers and probes may impair detection. Methicillin-resistant *S. aureus* strains that carry the $mecA_{LGA251}$ gene mutation, a novel mecA variant, were not evaluated. Therefore the performance of MRSA/SA ELITe MGB with these strains is unknown.

11. As with all PCR based *in vitro* diagnostic assays, levels of target below the limit of detection may be detected, but results may not be reproducible (refer to "Reproducibility" section for further details).

12. Borderline Oxacillin Resistant *Staphylococcus aureus* (BORSA) or modified *S. aureus* (MOD-SA) that do not carry mecA are not detected by the MRSA/SA ELITe MGB.

13. Excessive amounts of nasal secretions/mucus as well as propylene glycol in some nasal gels have been shown to interfere with the results of the MRSA/SA ELITe MGB.

14. When MRCoNS and MSSA are co-present at the same concentration, the sample will be falsely called MRSA-positive by MRSA/SA ELITe MGB.

15. As with all in vitro diagnostic tests, positive and negative predictive values are highly dependent on prevalence. The MRSA/SA ELITe MGB performance may vary depending on the prevalence.

16. The following substances have been shown to interfere with the performance of the assay: AYR® saline nasal gel and excessive amounts of nasal secretions/mucus and blood.

17. MRCoNS *S. epidermidis* and MSSA, when tested in a mix with near-detection-limit of MRSA, resulted in "SA positive, MRSA negative" call by MRSA/SA ELITe MGB.

Interfering Substances

Substances that may interfere with the detection of MRSA/SA by the MRSA/SA ELITe MGB and potentially generate invalid results include AYR saline nasal gel 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 AYR saline nasal gel, not to interfere with the detection of MRSA/SA by the MRSA/SA ELITe MGB reagents. Presence of human blood in sample has been shown not to interfere with the detection of MRSA/SA by the MRSA/SA ELITe MGB reagents.

Potentially Interfering Substances

Substance (Type)	Active Ingredient	Medication, Containing an Active Ingredient	Interferes?
Mucin, bovine submaxillary gland, type I-S	Purified mucin protein		No
Blood (Human)	Hemoglobin		No
Nasal sprays or drops	Phenylephrine	Neo-synephrine	No
	Oxymetazoline	Dristan nasal spray, Zicam nasal gel	No
	Sodium chloride with preservatives	Saline	No
	Benzalkonium chloride	Dristan nasal spray, Zicam nasal gel	No
	Sodium Phosphate	Dristan nasal spray, Zicam nasal gel	No
	Phenylcarbinol	Saline	No

Substance (Type)	Active Ingredient	Medication, Containing an Active Ingredient	Interferes?
	Propylene glycol	AYR saline nasal gel	**Yes**
	Sorbitol, benzyl alcohol	Dristan nasal spray, Zicam nasal gel	No
	disodium edetate, hypromellose	Dristan nasal spray	No
	phosphoric acid	Dristan nasal spray, Zicam nasal gel	No
Nasal corticosteroids	Dexamethasone	Dexamethasone sodium phosphate	No
	Triamcinolone	Nasacort	No
	Beclomethasone	Beconase AQ	No
	Flunisolide	Flunisolide nasal solution	No
	Budesonide	Rhinocort Aqua	No
	Mometasone	Nasonex	No
	Fluticasone	Flonase nasal spray	No
Nasal gel	Luffa opperculata, sulfur	Zicam nasal gel	No
Homeopathic allergy relief medicine	Galphimia glauca	Zicam nasal gel	No
	Histaminum hydrochloricum	Zicam nasal gel	No
Vaccine	Live intranasal influenza virus vaccine	FluMist©	No
Throat lozenges, oral anesthetic and analgesic	Benzocaine, Menthol	Cepacol sore throat lozenges	No
Anti-viral drugs	Zanamivir, Oseltamivir phosphate	Relenza, Tamiflu	No
Antibiotic, nasal ointment	Mupirocin	Mupirocin ointment USP	No
Antibacterial, systemic	Tobramycin	Tobramycin inhalation solution	No

Expected Values

Humans are a natural reservoir for *S. aureus*. Colonization may be transient or persistent and can last for years. *S. aureus* nasal carriage rates of 25 to 30% have been reported for the general population and rates of 10 to 40% have been reported for outpatient population or on admission[12]. In the investigational study for MRSA/SA ELITe MGB, the overall *S. aureus* nasal carriage rate determined by culture followed by Staphaurex latex agglutination test was 24%. Of the *S. aureus* isolates, 29% were methicillin resistant by agglutination/cefoxitin susceptibility test for an overall MRSA nasal carriage rate of 7%. With MRSA/SA ELITe MGB overall *S. aureus* nasal carriage rate was 27% where 41 % of all *S. aureus* were methicillin resistant for an overall MRSA nasal carriage rate of 11%.

Performance Characteristics

Clinical Performance

Performance characteristics of the MRSA/SA ELITe MGB were determined in a prospective investigational study at three (3) sites by comparing the MRSA/SA ELITe MGB with agglutination/susceptibility tests. Specimens were collected from three (3) unique geographies at institutions having MRSA culture-based screening programs in place. To be enrolled in the study, patients had to be eligible for MRSA screening according to the policies of the respective facilities. A true MRSA culture-positive specimen was defined as a specimen where MRSA was identified by the latex agglutination and cefoxitin disk (30mcg/disk) susceptibility test after a broth enrichment (both positive). A true MSSA culture-positive specimen was defined as a specimen negative for cefoxitin susceptibility testing and positive for the latex agglutination test.

One nasal swab was collected from each patient and used to inoculate a selective chromogenic MRSA screening agar plate. Then the swab was inserted into a tube with trypticase soy broth and thoroughly mixed. The entire volume of the cell suspension was tested using MRSA/SA ELITe MGB. All swabs were subjected to enrichment in trypticase soy broth with 6.5% NaCl. The enriched culture samples were inoculated onto Trypticase Soy Blood Agar plates. Grown overnight cultures were used for latex agglutination. Specimens positive for latex agglutination were used for the cefoxitin susceptibility test.

Performance of the MRSA/SA ELITe MGB was calculated relative to the broth culture followed by latex agglutination and cefoxitin susceptibility test results.

A total of 3271 nasal swab specimens were collected and tested. Of the 3271 specimen tested, 3174 specimens were eligible to be included in statistical analyses: 72 specimens were considered to be ineligible due to a duplicating error during samples collection and preparation; 21 specimens failed the initial extraction due to an operator error. Those samples were retested from the original swabs. 25 specimens failed the extraction and have been removed from the study.

Compared to the reference culture method, MRSA/SA ELITe MGB identified 92% of the specimens testing positive for MRSA and 95% of the negative specimens.

Compared to the reference culture method, MRSA/SA ELITe MGB identified 96% of the specimens testing positive for SA and 95% of the negative specimens.

Summary Performance[1] (for all data combined) of MRSA/SA ELITe MGB :

Combined Data		Reference Culture			
		MRSA+	SA+/MRSA-	Neg/No Growth	Total
MRSA/SA ELITe MGB	MRSA+	**205**	**111**	**32**	**348**
	SA+/MRSA-	17	405	86	508
	SA-	0	30	2288	2318
	Total	222	546	2406	3174
	MRSA: (also see Discrepancy Analysis results below) Sensitivity: 92.3% (88.08%-95.16%) Specificity: 95.2% (94.32%-95.87%) PPV: 58.9% (53.67%-63.95%) NPV: 99.4% (99.04%-99.62%) **SA:** Sensitivity: 96.1% (94.48%-97.25%) Specificity: 95.1% (94.16%-95.89%) PPV: 86.2% (83.74%-88.36%) NPV: 98.7% (98.16%-99.09%) Note: * The statistics shown are the calculated values with the 95% confidence interval in the parentheses.				

Non-Clinical Performance Evaluation

A. Analytical Sensitivity

The analytical sensitivity of the MRSA/SA ELITe MGB was determined using 5 strains of MRSA and one MSSA strain. Cultures of these strains were quantified, diluted in simulated nasal matrix to values spanning the range of approximately 5 to 1500 colonies forming units (CFU) and absorbed onto swabs. All dilutions were tested, and the limit of detection (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 at least 95% confidence. LoD for each strain was then verified by testing at least 20 replicates. Results indicate that the MRSA/SA ELITe MGB average LoD is 165 CFU/mL of a swab eluate.

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[1] Discrepant Analysis:

Further investigation (testing for MRSA by sequencing of SCCmec right extremity junction) was performed on all specimens that gave discordant MRSA results between the reference culture method and MRSA/SA ELITe MGB®.

- 16 of the 17 specimens that were MRSA-positive by culture but MRSA-negative by MRSA/SA ELITe MGB® were found to be MRSA positive by SCCmec right extremity junction sequencing.

- 22 of the 143 specimens that were MRSA-negative (111 SA+/MRSA- and 32 Neg/No growth) by culture but MRSA-positive by MRSA/SA ELITe MGB® were found to be MRSA positive by SCCmec right extremity junction sequencing.

Thus, the positive and negative agreements for MRSA were each increased by 1% to achieve 93% and 96% respectively. Positive Predictive Value for MRSA was also increased by 6% to achieve 65%.

B. Analytical Reactivity

Inclusivity

Performance of the MRSA/SA ELITe MGB was tested on 75 well characterized MRSA and MSSA 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, with the emphasis on the USA epidemiologic clones. 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[15]. All strains were absorbed onto swabs at near detection limit and tested with MRSA/SA ELITe MGB. In addition to that all MSSA strains were tested at 10^6 CFU/swab. All MSSA 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 mecA[19] tested SA positive and MRSA negative.

C. Analytical Specificity

Exclusivity

The specificity of the MRSA/SA ELITe MGB was evaluated by testing for cross-reactivity to species phylogenetically related to *S. aureus,* pathogenic microorganisms and to microorganisms commonly present in normal nasal microflora. The test panel consisted of 17 viral, 3 fungal, 1 mycoplasma, and 41 bacterial species. The microorganisms were tested as cultures in concentrations of $1x10^6$ CFU ($1x10^5$ PFU)/swab. In addition human cells in a concentration of 10^6 cells /mL were tested. Human cells and all tested species were found negative for MRSA and SA with the MRSA/SA ELITe MGB. The analytical specificity was 100%.

Species Tested for Cross-Reactivity and Microbial Interference

Staphylococci Species	Other Organisms	Viruses
CoNS* *Staphylococcus arlettae,* *Staphylococcus capitis,* *Staphylococcus carnosus,* *Staphylococcus chromogenes,* *Staphylococcus equorum,* *Staphylococcus felis,* *Staphylococcus gallinarum,* *Staphylococcus hominis* *subsp. hominis,* *Staphylococcus kloosii,* *Staphylococcus lentus,* *Staphylococcus pulvereri,* *Staphylococcus simulans,* *Staphylococcus warneri* **MSCoPS*** *Staphylococcus delphini,* **MSCoNS*** *Staphylococcus epidermidis,* *Staphylococcus xylosus* **MRCoNS*** *Staphylococcus epidermidis* **CoPS*** *Staphylococcus hyicus,* *Staphylococcus intermedius*	*Acinetobacter haemolyticus, Bacillus cereus, Bordetella pertussis, Citrobacter freundii, Citrobacter koseri, Corynebacterium aquaticum, Corynebacterium bovis, Corynebacterium flavescens, Corynebacterium genitalium, Enterobacter aerogenes, Enterococcus faecalis, Enterococcus faecium, Enterococcus flavescens, Enterococcus gallinarum, Enterococcus hiraem, Escherichia coli, ESBL producer, Klebsiella oxytoca, Klebsiella pneumoniae, ESBL producer, Listeria monocytogenes, Moraxella catarrhalis, Pasteurella aerogenes, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens, Shigella sonnei, Streptococcus mitis, Streptococcus salivarius, Yersinia enterocolitica, Candida albicans, Candida glabrata, Cryptococcus neoformans, Lactobacillus acidophilus, Legionella pneumophila, Mycobacterium tuberculosis avirulent, Mycoplasma pneumoniae, Neisseria meningitides, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Homo sapiens, Human Cells* HT1080	*Adenovirus Type 1,* *Adenovirus Type 7A,* *Human coronavirus (229E),* *Human coronavirus (OC43),* *Cytomegalovirus,* *Coxsackievirus Type A21,* *Epstein Barr Virus,* *Human influenza virus A,* *Human influenza virus B,* *Human parainfluenza Type 2,* *Human parainfluenza Type 3,* *Human metapneumovirus 3 Type B1,* *Measles,* *Mumps virus,* *Respiratory syncytial virus Type B,* *Rhinovirus Type 1A*

* **CoNS:** coagulase-negative *Staphylococci*,
  MSCoPS: methicillin susceptible coagulase positive *Staphylococci*,
  MSCoNS: methicillin susceptible coagulase negative *Staphylococci*,
  MRCoNS: methicillin resistant coagulase negative *Staphylococci*,
  CoPS: coagulase positive *Staphylococci*

MRCoNS *Staphylococcus epidermidis,* strain NRS34, is considered to interfere with MRSA/SA ELITe MGB. MRCoNS ***Staphylococcus epidermidis,* strain NRS34,** when tested in a mix with near-detection-limit -MRSA resulted in "SA positive, MRSA negative" call.

The same species and MSSA were also tested for microbial interference. The microorganisms were spiked at 1×10^6 CFU/mL (1×10^5 PFU/mL) or higher together with MRSA strains at near detection limit and tested. MRCoNS *Staphylococcus epidermidis* and MSSA when tested in a mix with near detection limit of MRSA resulted in "SA positive, MRSA negative" call. None of the other tested species interfered with MRSA/SA detection.

D. Reproducibility

A 10-member panel of specimens with varying concentrations of MRSA and MSSA in a simulated nasal matrix was tested. Two MRSA strains (ATCC BAA-1556 and BAA-1720) and one MSSA strain (BAA-12600) were used. Simulated matrix contained human genomic DNA and mucin to imitate a normal human nasal matrix. For each MRSA/MSSA strain the panel included negative member, specimen below the LoD (expected to yield a positivity rate of between 20 to 80%), low positive (at LoD, expected to yield a 95% positivity rate), and moderate positive (three times LoD, expected to have 100% positivity rate). Each of the two operators performed one run per day for 12 days on three reagent lots at one site. In two other sites two runs per day on one reagent lot were performed for 5 days (10 specimens x 3 replicates x 5 days x 2 runs). The negative panel member yielded negative results 100%, the below LoD specimens positivity rate was 77%, the low positive specimen positivity rate was 98%, and the moderate positive panel members positivity rate was 100%.

Cumulative data of reproducibility study

Specimen Type	Lot 1	Lot 2	Lot 3	Total Agreement (%)
Negative (R1)	14/14	14/14	30/30	58/58 (100%)
Below LoD (R2,R5,R8)	33/42	31/42	70/90	134/174 (77%)
Low Positive (R3,R6,R9)	40/42	42/42	88/90	170/174 (98%)
Moderate Positive (R4,R7,R10)	42/42	42/42	90/90	174/174 (100%)

The numerical results based on Ct values follow.

ldh1

Panel	N	Mean	Within-Run		Between-Run		Between-Day		Between-Operator		Between-Lot		Between-System		Total	
#		Ct	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
R1	118	38.12	NA1	NA1	0.67	1.75	0.45	1.18	0.29	0.76	0.39	1.03	0.15	0.40	0.39	1.02
R2	118	36.22	0.65	1.79	0.99	2.74	0.79	2.18	0.82	2.26	0.32	0.89	0.86	2.38	0.74	2.04
R3	118	35.39	0.62	1.73	0.96	2.72	0.86	2.42	0.94	2.65	0.43	1.23	1.08	3.06	0.82	2.30
R4	118	34.35	0.41	1.20	0.67	1.94	0.53	1.54	0.42	1.22	0.48	1.40	0.35	1.03	0.48	1.39
R5	118	36.73	0.71	1.94	0.87	2.37	0.79	2.16	0.56	1.52	0.50	1.37	0.45	1.23	0.65	1.77
R6	118	33.66	0.42	1.24	0.69	2.05	0.64	1.92	0.56	1.66	0.48	1.44	0.58	1.72	0.56	1.67
R7	118	31.81	0.17	0.54	0.76	2.38	0.67	2.09	0.73	2.28	0.43	1.36	0.83	2.60	0.60	1.88
R8	118	37.68	0.77	2.05	0.74	1.97	0.43	1.15	0.39	1.03	0.12	0.33	0.40	1.06	0.48	1.26
R9	118	34.65	0.56	1.60	0.64	1.84	0.51	1.49	0.54	1.56	0.08	0.23	0.60	1.74	0.49	1.41
R10	118	32.82	0.58	1.76	0.48	1.45	0.42	1.28	0.37	1.13	0.17	0.53	0.40	1.21	0.40	1.23
PSPC	44	34.25	NA2	NA2	0.71	2.08	0.41	1.21	0.41	1.18	0.18	0.52	0.59	1.72	0.46	1.34
PC	44	28.12	NA2	NA2	1.08	3.86	1.18	4.20	0.58	2.07	1.21	4.24	0.50	1.79	0.91	3.23
NSPC	44	38.24	NA2	NA2	0.63	1.64	0.65	1.69	0.85	2.24	0.11	0.30	0.82	2.15	0.61	1.60
NTC	44	38.14	NA2	NA2	NA3	NA3	NA3	NA3	NA3	NA3	NA3	NA3	NA3	NA3	NA3	NA3

mecA

Panel #	N	Mean Ct	Within-Run SD	Within-Run CV%	Between-Run SD	Between-Run CV%	Between-Day SD	Between-Day CV%	Between-Operator SD	Between-Operator CV%	Between-Lot SD	Between-Lot CV%	Between-System SD	Between-System CV%	Total SD	Total CV%
R1	118	38.53	NA1	NA1	1.08	2.81	1.31	3.42	0.76	1.97	0.97	2.54	0.32	0.83	0.89	2.31
R2	118	36.87	0.53	1.43	0.93	2.52	0.68	1.84	0.68	1.85	0.40	1.09	0.74	2.02	0.66	1.79
R3	118	36.40	0.59	1.62	1.13	3.12	0.99	2.73	1.15	3.15	0.66	1.83	1.27	3.51	0.97	2.66
R4	118	35.17	0.37	1.05	0.68	1.94	0.54	1.54	0.46	1.32	0.43	1.24	0.41	1.17	0.48	1.38
R5	118	37.31	0.60	1.62	0.64	1.71	0.50	1.35	0.44	1.17	0.47	1.26	0.46	1.24	0.52	1.39
R6	118	34.45	0.43	1.24	0.74	2.15	0.70	2.04	0.58	1.68	0.61	1.78	0.60	1.74	0.61	1.77
R7	118	32.50	0.19	0.58	0.87	2.68	0.76	2.34	0.85	2.61	0.52	1.61	0.95	2.91	0.69	2.12
R8	118	39.12	NA4	NA4	0.55	1.42	0.52	1.35	0.45	1.16	0.25	0.65	0.33	0.86	0.42	1.09
R9	118	38.83	NA4	NA4	0.62	1.59	0.46	1.17	0.38	0.97	0.14	0.35	0.32	0.83	0.38	0.98
R10	118	38.74	NA4	NA4	0.77	1.98	0.57	1.46	0.40	1.05	0.36	0.94	0.31	0.79	0.48	1.25
PSPC	44	35.03	NA2	NA2	0.73	2.08	0.56	1.60	0.42	1.20	0.28	0.80	0.56	1.60	0.51	1.45
PC	44	29.18	NA2	NA2	1.11	3.82	1.23	4.21	0.51	1.74	1.24	4.22	0.40	1.37	0.90	3.07
NSPC	44	39.03	NA2	NA2	0.48	1.22	0.48	1.22	0.48	1.22	0.58	1.48	NA5	NA5	0.50	1.28
NTC	44	39.53	NA2	NA2	0.24	0.61	0.24	0.61	0.24	0.61	NA6	NA6	0.24	0.61	0.24	0.61

IC2

Panel #	N	Mean Ct	Within-Run SD	Within-Run CV%	Between-Run SD	Between-Run CV%	Between-Day SD	Between-Day CV%	Between-Operator SD	Between-Operator CV%	Between-Lot SD	Between-Lot CV%	Between-System SD	Between-System CV%	Total SD	Total CV%
R1	118	30.26	0.15	0.50	0.27	0.89	0.17	0.55	0.18	0.59	0.05	0.18	0.23	0.75	0.17	0.58
R2	118	30.24	0.18	0.58	0.30	1.00	0.24	0.79	0.18	0.60	0.03	0.10	0.24	0.78	0.19	0.64
R3	118	30.30	0.17	0.57	0.25	0.81	0.18	0.60	0.17	0.57	0.04	0.14	0.20	0.66	0.17	0.56
R4	118	30.26	0.12	0.39	0.27	0.88	0.16	0.53	0.17	0.57	0.11	0.36	0.18	0.58	0.17	0.55
R5	118	30.23	0.22	0.72	0.33	1.09	0.24	0.78	0.23	0.75	0.10	0.34	0.27	0.88	0.23	0.76
R6	118	30.38	0.22	0.74	0.43	1.40	0.31	1.01	0.14	0.47	0.06	0.20	0.09	0.30	0.21	0.69
R7	118	30.31	0.15	0.50	0.30	0.98	0.21	0.68	0.13	0.42	0.04	0.12	0.14	0.45	0.16	0.53
R8	118	30.39	0.23	0.76	0.38	1.24	0.24	0.79	0.25	0.81	0.21	0.68	0.20	0.66	0.25	0.82
R9	118	30.34	0.14	0.46	0.32	1.06	0.23	0.74	0.28	0.93	0.12	0.38	0.26	0.87	0.23	0.74
R10	118	30.16	0.24	0.80	0.51	1.70	0.27	0.89	0.31	1.04	0.13	0.42	0.33	1.09	0.30	0.99
PSPC	44	30.26	NA2	NA2	0.47	1.55	0.36	1.18	0.32	1.05	0.33	1.09	0.28	0.94	0.35	1.16
PC	44	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7
NSPC	44	30.27	NA2	NA2	0.63	2.09	0.51	1.68	0.48	1.59	0.35	1.15	0.32	1.05	0.46	1.51
NTC	44	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7	NA7

NA1: For R1 (Negative Samples) only in a few runs, just one sample in triplicates shown a weak signal in ldh channel. Therefore, calculations of within-run SD and %CV for these (R1) samples were not possible.

NA2: For the Controls (PSPC, PC, NSPC, NTC), just one replicate was used for each run. Therefore, it was impossible to calculate within-run SD and %CV for these samples. See between-run values instead

NA3: In NTCs, there was only one single observation of ldh1 detection throughout the study. Therefore, SD & %CV calculations were not possible for this category of samples.

NA4: In R8-R10 samples (MSSA), due to none or rare (one per triplicate) occurrence of mecA signal detection it was not possible to calculate SD & %CV for this target.

NA5: For NSPC samples, only testing with one System (Instrument-Site) gave a positive signal in mecA channel. Therefore, SD & %CV calculations are not possible for this category of samples.

NA6: For NTC samples, only testing with one Lot gave a positive signal in mecA channel. Therefore, SD & %CV calculations are impossible for this category of samples.

NA7: Internal Control (IC2) was not added to Positive (PC) and Negative (NTC) amplification controls.

E. Carry-Over / Cross-Contamination

An analytical study was performed to evaluate the potential for cross-contamination between high MRSA (1×10^7 CFU per mL) specimens and negative specimens throughout the MRSA/SA ELITe MGB workflow. Two operators performed five 24-sample extraction runs (11 high MRSA samples, 11 negative samples, 1 PSPC sample, and 1 NSPC sample per run) in a checkerboard pattern (high MRSA samples interrupted by completely negative samples). The processed samples were then PCR 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.

Symbol Glossary

REF	Catalog number	**IVD**	*In vitro* diagnostic medical device
(thermometer symbol)	Upper temperature limit	Σ (triangle symbol)	Contains sufficient material for \<n> tests
LOT	Batch code	(book symbol)	Consult instructions for use
(hourglass symbol)	Use by	(factory symbol)	Manufacturer
Rx only	For Prescription Use Only	(exclamation mark symbol)	Caution
CONTROL +	Positive Control	**CONTROL**	Control

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