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NOTICE of CHANGE dated 19/01/2023

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«COLISTIN-R ELITe MGB Kit»
Ref. RTS202ING-48

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

- *Update to be in compliance with the Regulation (EU) 2017/746 and the Standard ISO 15223-1:2021 requirements.*
- *Composition, use and performance of the product remain unchanged.*
- *The following lot numbers still on the market have been commercialized as IVDD. According to Article 110 of the IVDR they will not be recalled and they will still be commercialized as per their expiration dates:*

PRODUCT REF	Lot Number	Expiry date
RTS202ING-48	U1222-035	December 2024
CTR202ING	U0123-014	January 2025
CTR202ING	U0821-030	March 2023

PLEASE NOTE

	LA REVISIONE DI QUESTO IFU E' COMPATIBILE ANCHE CON LA VERSIONE PRECEDENTE DEL KIT
	THE REVIEW OF THIS IFU IS COMPATIBLE WITH THE PREVIOUS VERSION OF THE KIT
	CET IFU MIS A JOUR ANNULE ET REMPLACE ET C'EST COMPATIBLE AVEC LA VERSION PRECEDENTE DU KIT
	LA REVISIÓN DE ESTE IFU ES COMPATIBLE CON LA VERSIÓN ANTERIOR DEL KIT
	A REVISÃO DO ESTE IFU É COMPATÍVEL COM A VERSÃO ANTERIOR DO KIT
	DIE REVIEW VON DIESER IFU IST _KOMPATIBLE MIT DER VORIGE VERSION VON DEM KIT

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EMPOWERING IVD

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COLISTIN-R ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS202ING-48

CE 0344

IVD

-20°C

UDI 08033891486662

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INTENDED USE

The product **COLISTIN-R ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as qualitative nucleic acids amplification assay for the detection and identification of the transmissible Colistin-resistance mcr-1 and mcr-2 gene DNA of *Enterobacteriaceae* in clinical samples.

The assay is validated in association with the **ELITe InGenius®** instrument, an automated integrated system for extraction, real time PCR and results interpretation, starting from rectal swabs.

The product is intended for use as an aid in the diagnosis of infections of *Enterobacteriaceae* positive for Colistin transmissible resistance genes, together with the patient's clinical data and other laboratory test results.

ASSAY PRINCIPLE

The assay consists of a multiplex real time amplification reaction performed by **ELITe InGenius**, an automated and integrated system for extraction, amplification and detection of nucleic acids and result interpretation.

Starting from DNA extracted from each sample under test, amplification reactions specific for the following Colistin-resistance genes are performed in the PCR Cassette:

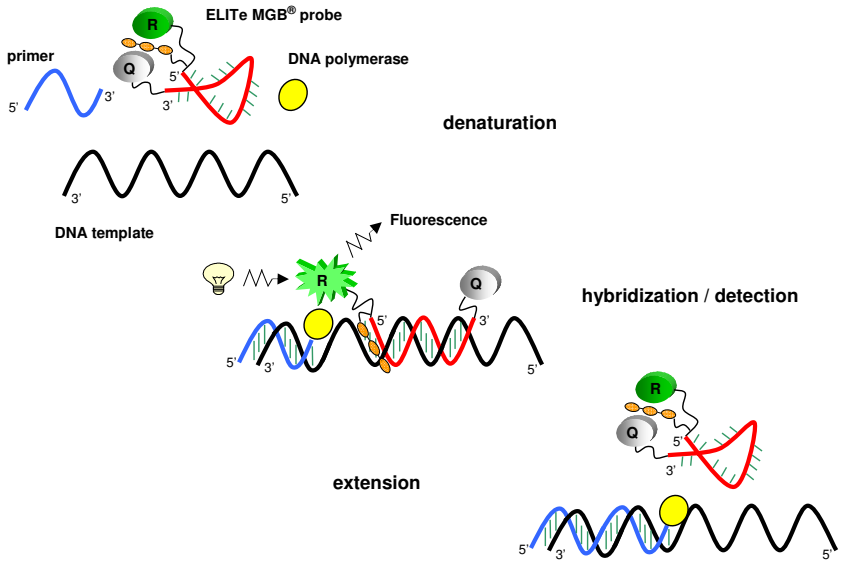
- mcr-1 gene, detected by a specific probe read in channel **mcr1** (Channel 5),
- mcr-2 gene, detected by a specific probe read in channel **mcr2** (Channel 1).

Furthermore, amplification reaction specific for the extraction and inhibition exogenous Internal Control, based on an artificial sequence (IC2), is carried out and detected by a specific probe read in channel **IC** (Channel 2).

The probes with ELITe MGB® technology are activated when they hybridize with the specific product of the amplification reaction. As the specific product of the amplification reaction increases, the fluorescence emission increases and it is measured and recorded by the instrument. At the end of amplification cycle, the fluorescence plots are analysed to identify the threshold cycles (Ct). The result interpretation allows to detect the presence of the transmissible Colistin-resistance mcr-1 and mcr-2 gene in the starting sample.

The assay has been validated with **ELITe InGenius** instrument.

In the following picture is shortly showed the mechanism of activation and fluorescence emission of ELITe MGB® technology probe. Note that the probe is not hydrolyzed during the amplification cycle so as it can be utilized for the dissociation curve analysis.



PRODUCT DESCRIPTION

The **COLISTIN-R ELITe MGB Kit** product supplies the **COL PCR Mix**, a **ready to use** complete mixture for Real Time amplification, aliquoted into four test tubes. Each tube contains **280 µL** of solution, sufficient for **12 tests** in optimal reagent consumption conditions (at least 2 tests per session) when used with **ELITe InGenius** system.

The COL PCR Mix contains:

- the specific primers and probe for the **mcr-1** gene. The probe **mcr1** is labelled by AP639 fluorophore, stabilized by the MGB® group and quenched by a non-fluorescent moiety. The probe is detected in channel **mcr-1** (channel 5) of **ELITe InGenius** system,
- the specific primers and probe for the **mcr-2** gene. The probe **mcr2** is labelled by FAM fluorophore, stabilized by the MGB group and quenched by a non-fluorescent moiety. The probe is detected in channel **mcr-2** (Channel 1) of **ELITe InGenius** system,
- the specific primers and probe for the **IC2** artificial sequence of exogenous Internal Control. The probe **IC** is labelled with AP525 fluorophore, stabilized by the MGB group and quenched by a non-fluorescent moiety. The probe is detected in channel **IC** (Channel 2) of **ELITe InGenius** system.

The COL PCR Mix also contains the buffer, the magnesium chloride, the nucleotide triphosphates, the stabilizers and the enzyme Taq DNA polymerase with thermic activation (hot start).

The product is sufficient for **48 tests in association with ELITe InGenius**, including controls.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
COL PCR Mix	Complete reaction mixture WHITE cap	**4 x 280 µL**	-

MATERIALS REQUIRED BUT NOT PROVIDED IN THE PRODUCT

- Laminar airflow hood.
- Disposable nitrile powder-free gloves or similar material.
- Vortex mixer.
- Bench microcentrifuge (12,000 - 14,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

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

For automatic analysis of samples, the **ELITe InGenius** instrument (ELITechGroup S.p.A., ref. INT030) and the following specific Assay Protocols (ELITechGroup S.p.A.) are required:

- parameters for the positive control amplification **«COL-R ELITe_PC»**,
- parameters for the negative control amplification **«COL-R ELITe_NC»**,
- parameters for samples to be analyzed **«COL-R ELITe_RcS_200_100»**.

With the **ELITe InGenius** instrument the following generic products are required:

- extraction cartridges **ELITe InGenius® SP 200** (ELITechGroup S.p.A., ref. INT032SP200),
- consumables for extraction **ELITe InGenius® SP 200 Consumable Set** (ELITechGroup S.p.A., ref. INT032CS),
- amplification cartridges **ELITe InGenius® PCR Cassette** (ELITechGroup S.p.A., ref. INT035PCR),
- tips **300 µL Filter Tips Axygen** (Axygen BioScience Inc., CA, ref. TF-350-L-R-S),
- boxes **ELITe InGenius® Waste Box** (ELITechGroup S.p.A., ref. F2102-000).

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As template of extraction and inhibition internal control, the generic product **CPE - Internal Control** (EG SpA, ref. CTRCPE), is required. This is a stabilised solution containing plasmid DNAs and the genomic RNA of MS2 phage.

As template of amplification positive control, the specifc product **COLISTIN-R - ELITe Positive Control** (EG SpA, ref. CTR202ING), is required. This is a stabilised solution of plasmid DNAs.

As collection device for rectal swab samples, the following generic product is required:

- **eSWAB®** kit (COPAN Italia S.p.A., ref. 480CE), swab and vial with 1 mL of transport medium or an equivalent device

For dilution of rectal swab samples, the following generic product is required:

- **eNAT®** kit (COPAN Italia S.p.A., ref.606CS01R), swab and vial with 2 ml of medium.

WARNINGS AND PRECAUTIONS

This product is designed for *in vitro* diagnostics use.

General warnings and precautions

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

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

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

Warnings and precautions for molecular biology

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

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 PCR Cassettes must be handled in such a way to avoid amplification product diffusion into the environment and sample and reagent contamination.

Warnings and precautions specific for the components

The **COL PCR Mix** must be stored at -20 °C in the dark.

The **COL PCR Mix** must be used within one month from the first tube opening.

The **COL PCR Mix** can be frozen and thawed for no more than **seven times**: further freezing / thawing cycles may cause a loss of product performance.

SAMPLES AND CONTROLS

Samples

This product is validated for use with the following clinical samples:

Rectal swabs collected in eSwab (COPAN Italia S.p.A., ref. 480CE)

The rectal swabs for DNA extraction must be collected in eSwab and identified according to laboratory guidelines, transported and stored at room temperature (~+25 °C) for a maximum of 48 hours or transported and stored at +2 / +8 °C for a maximum of 48 hours. Before analysis with this product 0.25 mL of sample in eSwab medium has to be transferred in a fresh eNAT tube with 2.0 mL of medium, mixed by vortexing. eNat preserves nucleic acids for four weeks at room temperature and six months at -20°C and -70°C. After addition of 0.25 mL of sample in eSwab medium, the eNAT tube can be directly loaded in the **ELITe InGenius** system as a primary tube.

Note: to the DNA extraction from rectal swab by the **ELITe InGenius** system with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the Assay protocol **COL-R ELITe_RcS_200_100**. This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Extracted DNA can be stored at +2 / +8 °C for 16 hours or at -20 °C for one month.

Note: samples with high turbidity must be treated as indicated in the troubleshooting chapter.

Interfering substances

Data available concerning inhibition caused by drugs and other substances are reported in "Interfering substances" paragraph of "Performance characteristics" chapter.

Note that a high content of faecal matrix collected with the rectal swab (sample with high turbidity) can inhibit the assay.

Amplification controls

Before analysis of any sample with the **ELITe InGenius** system, it is absolutely mandatory to generate and to approve the amplification controls for the amplification reagent lot that will be used in testing:

as amplification Positive Control, use the **COLISTIN-R - ELITe Positive Control** product reagent (not provided with this kit) in association with Assay Protocol **COL-R ELITe_PC**,

as amplification Negative Control, use molecular grade water (not provided with this kit) in association with Assay Protocol **COL-R ELITe_NC**.

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

Furthermore, the amplification controls must be re-run when:

- a new lot of amplification reagents is started,
- the results of quality controls (see following paragraph) are out of specification,
- any major maintenance service is performed on the **ELITe InGenius** instrument.

Certified Reference Material

The Metrological traceability of calibrators and control material for mcr-1 values is not fully applicable for **COLISTIN-R ELITe MGB Kit** as no WHO reference material is available.

The Metrological traceability of calibrators and control material for mcr-2 values is not fully applicable for **COLISTIN-R ELITe MGB Kit** as no WHO reference material is available.

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used.

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PROCEDURE

The procedure to use the **COLISTIN-R ELITe MGB Kit** with the **ELITe InGenius** system consists of three steps:

- Verification of the system readiness,
- Setup of the session,
- Review and export of results.

Verification of the system readiness

Before starting the session, referring to the instrument documentation, it is necessary to:

- switch on the **ELITe InGenius** and select the login mode "**CLOSED**",
- verify that the amplification controls (Controls, COL Positive Control, COL Negative Control) were run in association with the amplification reagent lot to be used and the results are approved and valid (Status). If there are not amplification control results approved or valid, generate them as described in the following paragraphs,
- 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 ELITechGroup S.p.A. These IVD protocols were specifically validated with **COLISTIN-R ELITe MGB Kit**, the **ELITe InGenius** instrument and the cited matrix.

The Assay Protocol available for sample testing with the product **COLISTIN-R ELITe MGB Kit** is described in the table below.

Assay protocol for COLISTIN-R ELITe MGB® Kit

Name	Matrix	Report	Characteristics
COL-R ELITe_RcS_200_100	Rectal swab	Positive / Negative	Extraction Input Volume: 200 µL Extraction Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Setup of the session

The product **COLISTIN-R ELITe MGB Kit** can be used with the **ELITe InGenius** system in order to perform:

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

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

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

The main steps for the setup of the three types of run are described here below.

A. Integrated run

To setup an integrated run with sample extraction and amplification, carry out the following steps as per the GUI:

1. Thaw COL PCR Mix tubes for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw COL PCR Mix in the dark because this reagent is sensitive to the light.

2. Thaw the CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home” screen.
4. Ensure that the “Extraction Input Volume” is 200 µL and the “Extracted Elute Volume” is 100 µL.
5. For each Track of interest fill in the “Sample ID” (SID) by typing or by scanning the sample barcode.
6. Select the Assay protocol to be used in the “Assay” column (i.e. COL-R ELITe_RcS_200_100).
7. Ensure that the “Protocol” displayed is: “Extract + PCR”.
8. Select the sample loading position in the “Sample Position” column:
   - if a primary tube is used, select “Primary Tube”,
   - if a secondary tube is used, select “Extraction Tube”.

   Click “Next” to continue the setup.
9. Load CPE and COL PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” button to continue the setup.
10. Load and check the “Tip Racks” in the “Inventory Area” selected by following the GUI instruction. Click “Next” button to continue the setup.
11. Load the “PCR Cassettes”, the “ELITe InGenius SP 200” extraction cartridges, all the required consumables and the samples to be extracted in the positions specified in step 8, following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

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

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 °C for one month. Avoid spilling the Extracted Sample.

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

Note: The PCR Mix can be kept on board in the refrigerated block up to 7 work sessions of 3 hours each.

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B. Amplification run

To set up the amplification run starting from extracted DNA, carry out the following steps as per GUI:

1. Thaw COL PCR Mix tubes for the session. Each tube is sufficient for 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw COL PCR Mix in the dark because this reagent is sensitive to the light.

2. Select “Perform Run” from the “Home” screen.
3. Even if no extraction will be carried out, ensure that the Extraction Input Volume is 200 µL and the Extracted Elute Volume is 100 µL.
4. For each Track of interest fill in the SID by typing or by scanning the sample barcode.
5. Select the Assay protocol to be used in the “Assay” column (i.e. COL-R ELITe_RcS_200_100).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
8. Load COL PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the “PCR Cassettes” and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.
12. Press “Start” to start the run.

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

Note: At the end of the run the remaining Extracted Sample must be removed from the instrument, capped and stored at -20 °C for one month. Avoid the spilling of the Extracted Sample.

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

Note: The PCR mix can be kept on board in the refrigerated block up to 7 work sessions of 3 hours each.

C. Amplification run for Positive Control and Negative Control

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

1. Thaw COL PCR Mix tubes for the session. Each tube is sufficient for preparing 12 reactions in optimal reagent consumption conditions (at least 2 tests per session). Mix gently, spin down the content for 5 seconds.

Note: Thaw COL PCR Mix in the dark because this reagent is sensitive to the light.

2. Thaw the COL - Positive Control tube for the session. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
4. Select "Perform Run" from the "Home" screen.
5. In the Track of interest, select the Assay Protocol to be used in the "Assay" column.
6. Even if no extraction will be carried out, ensure that the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.
7. For the positive control, select the Assay Protocol "COL-R ELITe_PC" in the "Assay" column and fill in the lot number and expiry date of COL - Positive Control,
8. For the negative control, select the Assay Protocol "COL-R ELITe_NC" and fill in the lot number and expiry date of the molecular biology grade water.
9. Click "Next" to continue the setup.
10. Load COL PCR Mix on the "Inventory Block" selected by following the GUI instruction. Click "Next" to continue the setup.
11. Load / check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
12. Load the "PCR Cassettes", the COL - Positive Control tube and the negative control tube following the GUI instruction. Click "Next" to continue the setup.
13. Close the instrument door.
14. Press "Start" to start the run.

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

Note: At the end of the run the remaining Positive Control must be removed from the instrument, capped and stored at -20 °C. Avoid spilling the Extracted Sample. The remaining Negative Control must be disposed.

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

Note: The PCR mix can be kept on board in the refrigerated block up to 7 work sessions of 3 hours each.

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Review and export of results

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

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

The **ELITe InGenius** system generates the results with the product **COLISTIN-R ELITe MGB Kit** through the following procedure:

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

A. Validation of amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probes of resistance genes (channels **mcr1** and **mcr2**) in the Positive Control and Negative Control amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the Assay protocols "COL-R ELITe_PC" and "COL-R ELITe_NC".

The amplification Positive Control and Negative Control results, specific for the lot of amplification reagent used, are recorded in the database (Controls). They can be viewed and approved by personnel qualified as "Administrator" or "Analyst", following the GUI instructions.

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

The results of Positive Control and Negative Control amplification runs are used by the instrument software to setup the "Control Charts" monitoring the amplification step performances. Refer to the instrument user's manual for more details.

Note: If the amplification Positive Control or Negative Control result does not meet the acceptance criteria, the "Failed" message is shown on the "Controls" screen and it is not possible to approve it. In this case, the amplification Positive Control or Negative Control reactions have to be repeated.

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

B. Validation of Sample results

The fluorescence signals emitted by the probes of resistance genes (channels **mcr1** and **mcr2**) and by the probe of Internal Control (channel **IC**) in the sample amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay protocol COL-R ELITe_RcS_200_100.

Results are shown in the reports generated by the instrument ("Result Display").
The sample run can be approved when the two conditions reported in the table below are met.

1) Positive Control	**Status**
COL Positive Control	APPROVED
2) Negative Control	**Status**
COL Negative Control	APPROVED

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

The possible result messages are listed in the table below. For each sample the system reports a combination of messages specifying if the Colistin-resistance genes are either detected or not detected.

Result of sample run	Interpretation
mcr1: DNA detected.	The **DNA of mcr1 gene was detected** in the sample. The sample could be **resistant to Colistin**.
mcr2: DNA detected.	The **DNA of mcr2 gene was detected** in the sample. The sample could be **resistant to Colistin**.
mcr1: DNA not detected or below the LoD.	The **DNA of mcr1 gene was not detected** in the sample **or** is **below the Limit of Detection of the assay**. The sample is negative for this gene or its concentration is below the Limit of Detection of the assay. If also the mcr-2 gene is not detected, the sample could be **sensitive to Colistin**.
mcr2: DNA not detected or below the LoD.	The **DNA of mcr2 gene was not detected** in the sample **or** is **below the Limit of Detection of the assay**. The sample is negative for these genes or their concentration is below the Limit of Detection of the assay. If also the mcr-1 gene is not detected, the sample could be **sensitive to Colistin**.
Invalid - Retest sample.	**Invalid assay result** caused by Internal Control failure (incorrect extraction, inhibitors carry-over). The Internal Control Ct resulted Not Determined or higher than 34 (IC cut-off = 34). The test should be repeated.

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

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

Samples reported as “mcr1 DNA Not Detected or below LoD” or “mcr2 DNA Not Detected or below LoD" are suitable for analysis but it was not possible to detect resistance gene DNA. In this case it cannot be excluded that the resistance gene DNA is present at a concentration below the limit of detection of the assay (see “Performance characteristics”).

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

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

C. Sample result export

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

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

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

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

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PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

The Limit of Detection (LoD) of the assay was defined in association with samples of rectal swabs and ELITe InGenius system.

Six levels of dilutions of an mcr-1 positive *E. coli* reference material (strain DSM 105182, equal to NCTC 13846, from DSMZ, Germany) were prepared in negative rectal swabs starting from the concentration above the expected LoD. Twelve replicates of each dilution level were processed on ELITe InGenius system in “Extract + PCR” mode. The LoD for mcr-1 target was calculated by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call. The calculated LoD was confirmed by analysis of 30 replicates of a mcr-1 target dilution at the corresponding concentration.

The final result is reported in the following table.

Limit of Detection of mcr-1 gene with rectal swabs and ELITe InGenius system (CFU / mL)		
LoD	95% confidence interval	
	lower bound	**upper bound**
32	19	472

As no mcr-2 positive strain or isolate was available, the LoD for mcr-2 target was estimated at 50 copies/mL (corresponding to 50 CFU / mL), similar to the LoD value of mcr-1 target. The estimated LoD was confirmed by analysis of 30 replicates of a mcr-2 target (plasmid DNA) dilution in negative rectal matrix at the corresponding concentration.

Efficiency of detection (inclusivity)

The efficiency of detection of the assay for mcr-1 and mcr-2 genes (inclusivity) was evaluated by comparison of sequences with nucleotide database.

The regions chosen for the hybridization of the primers and of the fluorescent probes were checked on the alignment of the sequences available in the database for the mcr-1 and mcr-2 genes. The analysis showed their conservation and absence of significant mutations.

The efficiency of detection the assay for mcr-1 gene was also verified for a set of 27 Colistin-resistant cultured isolates characterized as mcr-1 positive *Enterobacteriaceae*.

The final results are reported in the following table.

Efficiency of detection (inclusivity) of the product COLISTIN-R ELITe MGB® Kit					
Samples	**N**	**mcr-1 Positive**	**mcr-2 Positive**	**Negative**	**Invalid**
mcr-1 positive cultural isolates	27	27	0	0	0

This product is able to detect the transmissible (plasmid-mediated) Colistin resistance through mcr-1 and mcr-2 genes detection, while is not able to detect mcr types from 3 to 5 and from 7 to 10. Mcr-6 type may be detected within the mcr-2 detector.

Potential interfering markers

Potential cross-reactivity of the assay with other unintended targets was evaluated by *in silico* analysis of sequences in nucleotide database.

The regions chosen for the hybridisation of the primers and the fluorescent probes were checked on the alignment of the sequences from organisms that might reasonably be expected to be present in rectal swab samples. The analysis showed absence of significant homologies and indicated no potential interference.

The absence of cross-reactivity with other organisms potentially found in rectal swab samples was also verified by testing a panel of certified strains at concentration of about 10^4 CFU / mL in association with ELITe InGenius system in “Extract + PCR” mode.

The final results are reported in the following table.

Potential cross-reactivity		
Organism	**Strain**	**Outcome**
K. pneumoniae	NCTC 13439 (VIM)	No cross-reactivity
E. coli	NCTC 13476 (IMP)	No cross-reactivity
S. marcescens	UCLA 14-13-A11	No cross-reactivity

To be continued at next page.

Potential cross-reactivity		
Organism	**Strain**	**Outcome**
A. baumannii	NCTC 13301	No cross-reactivity
A. lwoffii	ATCC 15309	No cross-reactivity
B. adolescentis	ATCC 15703	No cross-reactivity
B. longum	ATCC 15707	No cross-reactivity
C. jejuni	ATCC 33292	No cross-reactivity
C. albicans	Zeptometrix Z006	No cross-reactivity
C. freundii	UCLA-14-13-A2 (KPC)	No cross-reactivity
C. difficile	ATCC 43593	No cross-reactivity
C. perfringens	ATCC 13124	No cross-reactivity
P. mirabilis	ATCC 12453	No cross-reactivity
P. aeruginosa	ATCC 27853	No cross-reactivity
S. enterica	ATCC 700720	No cross-reactivity
K. pneumoniae	DICON-126	No cross-reactivity
E. coli	DICON-055	No cross-reactivity
E. coli	DICON-045	No cross-reactivity
E. coli	NCTC13400	No cross-reactivity

All the strains resulted negative when tested with the assay.

The absence of interference by other organisms that can be found in rectal swab samples was also verified by testing a panel of certified strains at the concentration of about 10^4 CFU / mL or higher spiked with mcr-1 positive certified material to a final concentration of about 3x LoD in association with ELITe InGenius system in “Extract + PCR” mode.

The final results are reported in the following table.

Potential interference		
Organism	**Strain**	**Result**
K. pneumoniae	ATCC 700603	No interference
E. coli	ATCC BAA-201	No interference
S. marcescens	UCLA 14-13-A11	No interference
A. baumannii	NCTC 13301	No interference
A. lwoffii	ATCC 15309	No interference
B. adolescentis	ATCC 15703	No interference
B. longum	ATCC 15707	No interference
C. jejuni	ATCC 33292	No interference
C. albicans	Zeptometrix Z006	No interference
C. freundii	ATCC 8090	No interference
C. difficile	ATCC 43593	No interference
C. perfringens	ATCC 13124	No interference
P. mirabilis	ATCC 12453	No interference
P. aeruginosa	ATCC 27853	No interference
S. enterica	ATCC 700720	No interference
K. pneumoniae	DICON-126	No interference
E. coli	DICON-055	No interference
E. coli	DICON-045	No interference
E. coli	NCTC13400	No interference

All the strains did not interfere with the amplification of the targets when tested by the assay.

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Interfering substances

A panel of potentially interfering substances at relevant concentrations was tested with the assay. The substances tested were: human Whole Blood, Mucin, enemas (Vaseline Oil), antibiotic (Vancomycin), antiacids (Alginic Acid/Sodium Bicarbonate), anti-diarrheal medication (Loperamide Hydrochloride) and laxatives (Sennosides).

The substances were individually added to negative rectal matrix spiked with mcr-1 positive reference materials at concentration of 3x LoD. Samples were processed in three replicates on ELITe InGenius system in “Extract + PCR” mode.

The results are reported in the following tables.

Interfering substances			
Substance	**Tested Concentration**	**Pos. / Rep.**	**Outcome**
Whole Blood	5% v/v	3 / 3	Mcr-1 DNA detected
Mucin	10 mg/mL	3 / 3	Mcr-1 DNA detected
Vaseline Oil	20 mg/mL	3 / 3	Mcr-1 DNA detected
Vancomycin	12.5 mg/mL	3 / 3	Mcr-1 DNA detected
Alginic Acid/Sodium Bicarbonate	0.1 mg/mL	3 / 3	Mcr-1 DNA detected
Loperamide Hydrochloride	7 µg/mL	3 / 3	Mcr-1 DNA detected
Sennosides	0,1mg/mL	3 / 3	Mcr-1 DNA detected
Ampicillin	18 µg / mL	3 / 3	Mcr-1 DNA detected
Cefpodoxime	4.5 µg / mL	3 / 3	Mcr-1 DNA detected
Ciprofloxacin	5 µg / mL	3 / 3	Mcr-1 DNA detected
Azythromycin	10 µg / mL	3 / 3	Mcr-1 DNA detected

All the samples resulted positive for the target. None of the tested substances at the tested concentrations were found to interfere with the target detection.

Repeatability

The Repeatability of results obtained by the assay in association with the ELITe InGenius system was tested by analysing a panel of rectal matrix with one negative sample and three samples spiked with the mcr-1 positive reference material (DSMZ) at concentrations of about 0.5x LoD, 1.5x LoD and 3x LoD.

The Repeatability was calculated through the analysis of panel samples in three replicates, in two runs per day, with the same lot of product. Three different lots of product were tested in three different days with the same instrument and by the same operator. Samples were processed on ELITe InGenius system in “Extract + PCR” mode.

The Ct values of each level of dilution were used to calculate the %CV in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the tables below.

Repeatability				
Sample	**Pos. / rep.**	**mcr-1 Mean Ct**	**mcr-1 Std Dev**	**mcr-1 %CV**
3x LoD (96 CFU/mL)	18/18	36.23	0.44	1.23
1.5x LoD (48 CFU/mL)	18/18	36.82	0.59	1.61
0.5x LoD (16 CFU/mL)	10/18	37.97	0.47	1.25
Negative rectal matrix	0/18	N.A.	N.A.	N.A.

In the Repeatability test, the assay detected the mcr-1 target as expected and showed low %CV of target Ct value that did not exceed 5 %.

Reproducibility

The Reproducibility of results obtained by the assay in association with the ELITe InGenius system was tested by analysing a panel of rectal matrix with one negative sample and three samples spiked with the mcr-1 positive reference materials (DSMZ) at concentrations of about 0.5x LoD, 1.5x LoD and 3x LoD.

The Reproducibility was calculated through the analysis of panel samples in three replicates, in two runs per day. Three different lots of products were tested in three different days, on three different instrument and by three different operators. Samples were processed on ELITe InGenius system in “Extract + PCR” mode.

The Ct values of each level of dilution were used to calculate the %CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the tables below.

Reproducibility				
Sample	**Pos. / rep.**	**mcr-1 Mean Ct**	**mcr-1 Std Dev**	**mcr-1 %CV**
3x LoD (96 CFU/mL)	18/18	36.07	0.60	1.66
1.5x LoD (48 CFU/mL)	18/18	36.86	0.53	1.44
0.5x LoD (16 CFU/mL)	9/18	37.91	0.71	1.88
Negative rectal matrix	0/18	N.A.	N.A.	N.A.

In the Reproducibility test, the assay detected the mcr-1 target as expected and showed a low %CV of target Ct value that did not exceed 5 %.

Diagnostic sensitivity (Positive Percent Agreement): confirmation of positive samples

Given the difficulty of finding mcr-1 and mcr-2 genes positive clinical samples, due to the low-incidence of transmissible Colistin-resistance, spiked samples were analyzed to assess the diagnostic sensitivity of the product.

The diagnostic sensitivity of the assay, as confirmation of positive samples, was evaluated by analyzing 77 rectal swab samples spiked with mcr-1 or mcr-2 positive *E. coli* isolates.

56 rectal swab samples were certified as negative by culture method and then were spiked with different mcr-1 positive *E. coli* isolates.

21 rectal swab samples presumably negative for *Enterobacteriaceae* harboring Colistin transmissible resistance were spiked with the mcr-2 positive *E. Coli* strain NCTC 14378.

All the samples were tested with COLISTIN-R ELITe MGB Kit and ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	N	mcr-1 positive	mcr-2 positive	negative	invalid
Rectal Swab mcr-1 spiked samples	56	55	0	0	1
Rectal Swab mcr-2 spiked samples	21	0	21	0	0

55 out of 56 mcr-1 spiked samples were confirmed mcr-1 positive; one sample gave an invalid result and was excluded from the analysis.

All the mcr-2 spiked samples (21 out of 21) were confirmed mcr-2 positive.

In this test, the diagnostic sensitivity for rectal swab samples was equal to 100 % both for mcr-1 and mcr-2 targets.

Diagnostic specificity (Negative Percent Agreement): confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analyzing 58 mcr-1 and mcr-2 negative rectal swab samples.

The 58 rectal swab samples were certified as negative by culture method and then were tested with COLISTIN-R ELITe MGB Kit and ELITe InGenius system in “Extract + PCR” mode.

The results are summarized in the following table.

Samples	N	mcr-1 positive	mcr-2 positive	negative	invalid
Rectal Swab mcr-1 and mcr-2 negative samples	58	1	0	54	3

In the test, 54 out of 58 samples were confirmed valid and negative; one sample resulted discordant mcr-1 low positive. Three samples gave an invalid result and were excluded from the analysis. In this test, the diagnostic specificity for rectal swab samples was equal to 98 % for mcr-1 target and 100 % for mcr-2 target.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instrument are recorded in the Product Technical File "COLISTIN-R ELITe MGB Kit", FTP 202ING.

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H. Giamarellou (2016) Int. J. Antimicrob. Agents. 48 (6): 614 - 621
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30

PROCEDURE LIMITATIONS

Use this product only with the following clinical sample: human rectal swabs.

Do not use this product with specimens of animal and food origin.

Do not use this product with samples containing too much faecal matrix: samples with high turbidity inhibit the amplification reaction of nucleic acids and can cause invalid results.

There are no data available concerning product performance with DNA extracted from the following human clinical samples: blood culture, faecal supernatant, urine.

The results obtained with this product depend on an adequate 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 products for nucleic acid extraction.

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the positive samples, the positive controls and the same amplification products. Cross-contaminations cause false positive results. The product format is able to limit cross-contaminations. However, cross-contaminations can be avoided only 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 work clothes 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 special clothing and instruments dedicated to work session setup to avoid false positive results.

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

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

In case of co-infections, the sensitivity for one target can be affected by the amplification of a second target.

Results obtained with this product may sometimes be invalid due to failure of internal control. In this case the sample shall be retested, starting from extraction, which can lead to a delay in obtaining final results.

Possible polymorphisms within the region of the target DNA covered by the product primers and probes may impair detection of target DNA.

This product is not able to detect mcr types from 3 to 5 and from 7 to 10. This product may detect mcr-6 type within the mcr-2 detector.

This product is able to detect the transmissible (plasmid-mediated) Colistin resistance through mcr-1 and mcr-2 genes, while is not able to detect chromosomal resistance.

As with any other diagnostic medical device, the results obtained with this product must be interpreted taking into consideration all the clinical data and other laboratory tests done on the patient.

As with any other diagnostic medical device, there is a residual risk of invalid, false positive and false negative results obtained 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.

TROUBLESHOOTING

Invalid Positive Control reaction	
Possible Causes	**Solutions**
Session setup error.	Check the position of PCR Mix and positive control. Check the volumes of PCR Mix and positive control.
Positive control degradation.	Use a new aliquot of positive control.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Session setup error.	Check the position of PCR Mix and Negative Control. Check the volumes of PCR Mix and Negative Control.
Contamination of the Negative Control.	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, of Racks or of Inventory Block.	Clean surfaces with aqueous detergents, wash lab coats, replace test tubes and tips in use.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Sample reaction	
Possible Causes	**Solutions**
Session setup error.	Check the position of PCR Mix and sample. Check the volumes of PCR Mix and sample.
Internal Control template degradation.	Use new aliquots of Internal Control.
Inhibition due to sample interfering substances (*e.g., turbidity*)	Repeat the amplification with a 1:2 or 1:5 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction with a further 1:2 dilution in eNAT® medium of the tested sample in a “Extract + PCR” session.
PCR Mix degradation.	Use a new aliquot of PCR Mix.
Instrument error.	Contact ELITechGroup Technical Service.

Error 30103	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - selected the track related to the sample and approve manually the result. If a Ct value is required: - repeat the amplification with a 1:10 dilution in molecular biology grade water of eluted sample in a “PCR only” session or - repeat the extraction with a further 1:10 dilution in eNAT® medium of the tested sample in a “Extract + PCR” session.

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SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*In vitro* diagnostic medical device
CE 0344	Fulfilling the requirements of the IVDR Regulation 2017/746/EC for *in vitro* diagnostic medical device. Certification released by DEKRA Certification B.V., the Netherland.
UDI	Unique Device Identification
	Contains sufficient for "N" tests.
	Attention, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

NOTICE TO THE USERS

Any serious incident the has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and /or the patient is established. At the moment of the current revision of the IFU, no serious incident or recall of the device has occurred.

A “Summary of Safety and Performance” will be made available to the public via the European database on medical devices (Eudamed) when this informatic system will be functional.

NOTICE TO PURCHASER: LIMITED LICENSE

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

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COLISTIN_R ELITe MGB® Kit used with ELITe InGenius®
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This document is a simplified version of the official instruction for use. Before use please refer to the complete instruction for use downloadable at: www.elitechgroup.com
This document is available only in English.

A. Intended use

The product **"COLISTIN-R ELITe MGB® Kit"** is an in vitro diagnostic medical device intended to be used by healthcare professionals as qualitative nucleic acids amplification assay for the detection and identification of the transmissible Colistin-resistance mcr-1 and mcr-2 gene DNA of *Enterobacteriaceae* in clinical samples.

The assay is validated in association with the **"ELITe InGenius®"** instrument, an automated integrated system for extraction, real time PCR and results interpretation, starting from rectal swabs.

The product is intended for use as an aid in the diagnosis of infections of *Enterobacteriaceae* positive for Colistin transmissible resistance genes, together with the patient's clinical data and other laboratory test results.

B. Amplified sequence

Target for Qualitative Application	Gene	Fluorophore
Target 1	mcr-1 gene	AP639 (CH 5)
Target 2	mcr-2 gene	FAM (CH 1)
Internal Control	IC2	AP525 (CH 2)

C. Validated Matrixes

› **Rectal Swabs**

D. Tube type collection

Copan Ref.	Description
480CE	eSwab

E. Kit content

COL PCR Mix (Neutral)
X **4 (RTS202ING-48)**
tubes of 280 µL 48 reactions per kit (ref. RTS202ING-48) 7 freeze-thaw cycles

Maximum Shelf-life: 24 Months

Storage temp.: - 20 °C

F. Material required not provided in the kit

› ELITe InGenius instrument: INT030
› ELITe InGenius SP200 Extraction Cartridge: INT032SP200
› ELITe InGenius PCR Cassette: INT035PCR
› ELITe InGenius Waste Box: F2102-000
› 300 µL Filter Tips Axygen: TF-350-L-R-S
› COLISTIN-R - ELITe Positive Control: CTR202ING
› CPE - Internal Control: CTRCPE
› eSWAB: 480CE
› eNAT 606CS01R

G. ELITe InGenius® Protocol

Protocol	Volume
Sample	200 µL
Total eluate	100 µL
PCR eluate input	20 µL
Complete PCR Mix	20 µL
Control Frequency	15 days
Calibration Frequency	60 days

H. Performance

Target	Matrix	Limit of Detection	Diagnostic Sensitivity	Diagnostic Specificity
mcr-1	Rectal swab	**32 CFU/mL**	**100%** 55/55*	**98%** 54/55*
mcr-2	Rectal swab	**50 CFU/mL**	**100%** 21/21*	**100%** 55/55*

*confirmed samples / tested samples

I. Procedures

The user is guided step-by-step by the ELITe InGenius software to setup the run. All the steps, extraction, amplification, melting curve analysis and result interpretation, are automatically performed. Three operational mode are available: complete run, extraction only or PCR only.

Before analysis

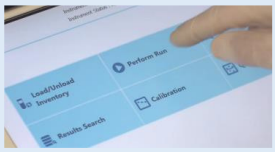
1. Switch on ELITe InGenius
Login with username and password
Select the mode "Closed"

2. Verify controls: COL pos. and neg. controls in the "Control menu"
NB: Both must have been run, approved and not expired

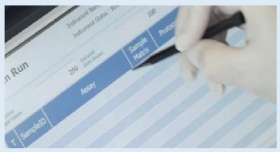
3. Thaw COL PCR Mixes and the Internal Control tubes
Vortex gently
Spin down 5 sec

Procedure - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen



2. Verify the extraction volumes:
Input: "200 µL", eluate: "100 µL"



3. Scan the sample barcodes with hand-barcode reader or type the sample ID

4. Select the "Assay protocol" of interest

5. Select the sample position: "Extraction tube"

6. Load the PCR Mixes and the Internal Control in the inventory block

7. Load: PCR cassette, Extraction cartridge, Elution tube, Tip, Extraction tube racks

8. Close the door Start the run

9. View, approve and store the results