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EMPOWERING IVD

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NOTICE of CHANGE dated 23/09/2022

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«HIV1 ELITe MGB Kit»
Ref. RTK600ING

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

- *Extension of the use of the product in association with «ELITe BeGenius®» instrument (REF INT040).*

Composition, use and performance of the product remain unchanged.

PLEASE NOTE

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

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HIV1 ELITe MGB® Kit

reagents for RNA reverse transcription and
cDNA Real Time amplification

REF **RTK600ING**

CE 0344 **IVD**

-20°C

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INTENDED USE

The **«HIV1 ELITe MGB® Kit»** product is quantitative nucleic acids reverse transcription and amplification assay for the **detection and quantification of the RNA** of Human immunodeficiency virus type 1 **(HIV1)** in RNA samples extracted from clinical specimens.

The assay is able to detect the RNA of HIV1 belonging to group M (subtypes A, B, C, D, F, G, H, J, K, L), group O, group N and major CRF subtypes CRF01-AE, CRF02-AG and CRF03-AB.

The assay is validated in association with **«ELITe InGenius®»** and **«ELITe BeGenius®»** system starting from human plasma samples collected in EDTA or in ACD.

The product is intended for use as an aid in the management of HIV1-infected individuals undergoing antiviral therapy, together with patient's clinical data and other laboratory test results.

This product is not intended for use as a screening test for the presence of HIV1 in blood or blood products or as a diagnostic test to confirm the presence of HIV1 infection.

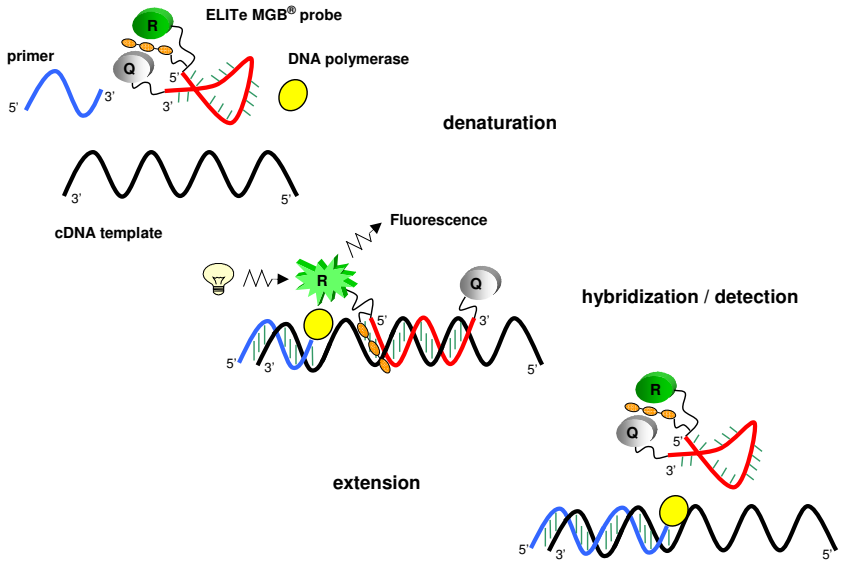
ASSAY PRINCIPLE

The assay consists of a reverse transcription and a real-time amplification reaction (one-step method) performed by **ELITe InGenius** and **ELITe BeGenius**, an automated and integrated system for extraction, reverse transcription, amplification and detection of nucleic acids and result interpretation.

Starting from RNA extracted by **ELITe InGenius** and **ELITe BeGenius** from sample to be tested, the complete HIV1 PCR Mix carries out a reaction of reverse transcription and amplification specific for the polymerase gene (integrase region) of HIV1 and for a region of the genomic RNA of MS2 phage (exogenous Internal Control of extraction and inhibition).

The HIV1 specific probes with ELITe MGB® and TaqMan™ MGB® technology, labelled by FAM fluorophore, are activated when hybridized with the specific product of the HIV1 amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled by AP525 fluorophore, is activated when hybridized with the specific product of Internal Control amplification reaction. As the specific product of the amplification reaction increases, the fluorescence emission increases and is measured and recorded by the instrument. The processing of the data determines the presence and the titre of HIV1 RNA in the sample.

In the following picture is synthetically shown the mechanism of activation and fluorescence emission of ELITe MGB® technology probe.



PRODUCT DESCRIPTION

The **HIV1 ELITe MGB Kit** product provides the following components:

- **HIV1 ELITe MGB® Mix**

This component provides the following two sub-components:

- "**HIV1 PCR Mix**", an optimized and stabilized mixture of oligonucleotides and reagents for reverse transcription and real-time amplification, pre-aliquoted into **four test tubes** (WHITE cap). Each tube contains **600 µL** of solution, enough for **24 tests** (processing at least 5 samples per session) in association with **ELITe InGenius** and **ELITe BeGenius**.
  Primers and probes for HIV1 (stabilized by MGB® group, labelled by FAM fluorophore and quenched by Eclipse non-fluorescent moiety) are specific for the polymerase gene (integrase region) of HIV1. The HIV1 signal is detected by Channel 1 (HIV1) of the **ELITe InGenius** and **ELITe BeGenius.**
  Primers and the probe for Internal Control (stabilized by MGB® group, labelled by AP525 fluorophore and quenched by Eclipse non-fluorescent moiety) are specific for a region of the phage **MS2** genomic RNA. The Internal Control signal is detected by Channel 2 (IC) of the **ELITe InGenius** and **ELITe BeGenius**.
  The reaction mixture provides also the buffer, magnesium chloride, the nucleotide triphosphates and the DNA Polymerase enzyme with hot start capability.
- "**RT EnzymeMix**", an optimized and stabilized mixture of enzymes for reverse transcription, pre-aliquoted into **two test tubes** (cap with BLACK insert). Each tube contains **20 µL** of solution, sufficient for **48 tests** (processing at least 5 samples per session) in association with **ELITe InGenius** and **ELITe BeGenius**.

The two sub-components are sufficient for **96 tests in association with ELITe InGenius** and **ELITe BeGenius,** by using respectively 20 µL and 0.3 µL for reaction.

- **HIV1 ELITe Standard**

This component provides the sub-components "**HIV1 Q-PCR Standard**", four stabilized solutions of plasmid DNA at **known titre**, each aliquoted into **one ready to use test tube**. Each tube contains **160 µL** of solution, sufficient for **2 sessions**. The plasmid DNA contains a region of polymerase gene of HIV1. The detection and quantification of HIV1 DNA as result of the analysis by **HIV1 ELITe MGB Mix** component in association with **ELITe InGenius** and **ELITe BeGenius** instrument allows to calculate the standard curve of the system (product batch and instrument) for HIV1 quantification.

The plasmid DNA concentration in copies / mL was determined through absorbance measurement by spectrophotometer. This plasmid DNA concentration was related to the "WHO International Standard 4th HIV-1 International Standard" (NIBSC, UK, code 16/194) by a conversion factor to allow calculation of concentration in International Unit / mL (IU / mL).

The component is sufficient for **2 separate analytic sessions in association with ELITe InGenius** and **ELITe BeGenius**, by using 20 µL for reaction.

- **HIV1 - ELITe Positive Control**

This component provides the sub-component "**HIV1 Positive Control**", a stabilized solution of plasmid DNA at **known titre**, aliquoted into **two ready-to-use test tubes**. Each test tube contains **160 µL** of solution, sufficient for **4 sessions**. The plasmid DNA contains a region of polymerase gene of HIV1. The detection and quantification of target DNA as result of the analysis with **HIV1 ELITe MGB Mix** component in association with **ELITe InGenius** instruments allows to validate the system (product batch and instrument) for HIV1 detection and quantification.

The component is sufficient for **8 separate analytic sessions in association with ELITe InGenius** and **ELITe BeGenius**, by using 20 µL for reaction.

- **HIV1 Internal Control**

This component provides the sub-component "**HIV1 CPE**", a stabilized solution of MS2 genomic RNA aliquoted into **eight ready-to-use test tubes**. Each tube contains **160 µL** of solution, sufficient for **12 tests** (processing at least 2 samples per session). The MS2 genomic RNA is used as exogenous Internal Control template. The detection of MS2 cDNA as result of the analysis with **HIV1 ELITe MGB Mix** component in association with **ELITe InGenius** and **ELITe BeGenius** instruments allows to validate the results of HIV1 negative samples.

The component is sufficient for **96 tests in association with ELITe InGenius** and **ELITe BeGenius,** by using 10 µL for extraction.

MATERIALS PROVIDED IN THE PRODUCT

Component	Sub-Component	Description	Quantity	Classification of hazards
HIV1 ELITe MGB Mix ref. RTS600ING	HIV1 PCR Mix ref. RTS600ING	mixture of reagents for reverse transcription and real-time amplification in tube with **WHITE cap**	**4 x 600 µL**	-
	RT EnzymeMix ref. RTS003-RT	Reverse transcription enzymes in tube with **cap with BLACK insert**	**2 x 20 µL**	-
HIV1 ELITe Standard ref. STD600ING	HIV1 Q-PCR Standard 10^5 ref. STD600ING-5	plasmid solution in tube with **RED cap**	**1 x 160 µL**	-
	HIV1 Q-PCR Standard 10^4 ref. STD600ING-4	plasmid solution in tube with **BLUE cap**	**1 x 160 µL**	
	HIV1 Q-PCR Standard 10^3 ref. STD600ING-3	plasmid solution in tube with **GREEN cap**	**1 x 160 µL**	
	HIV1 Q-PCR Standard 10^2 ref. STD600ING-2	plasmid solution in tube with **YELLOW cap**	**1 x 160 µL**	
HIV1 - ELITe Positive Control ref. CTR600ING	HIV1 Positive Control ref. CTR600ING	Plasmid solution in tube with **BLACK cap**	**2 x 160 µL**	-
HIV1 Internal Control ref. CPE600ING	HIV1 CPE ref. CPE600ING	Solution of plasmid DNAs and MS2 genomic RNA in tube with **NEUTRAL** cap	**8 x 160 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 (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- Sarstedt 2.0 mL tube skirted with screw-cap (Sarstedt Ref. 72.694.005).
- Molecular biology grade water.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of RNA from the samples to be analyzed and the consumables are **not** included in this product.

For automatic nucleic acids extraction, reverse transcription, real-time amplification and result interpretation of samples to be analysed, the instrument **ELITe InGenius** (ELITechGroup S.p.A., EG SpA, ref. INT030) and the following specific Assay Protocols (EG SpA) are required:

- parameters for calibrators amplification **«HIV1 ELITe_STD»**,
- parameters for positive control amplification **«HIV1 ELITe_PC»**,
- parameters for negative control amplification **«HIV1 ELITe_NC»**,
- parameters for Plasma samples to be analyzed **«HIV1 ELITe_PL_600_50»**.

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

- extraction cartridges **«ELITe InGenius® SP 1000»** (EG SpA, ref. INT033SP1000),
- consumables for extraction **«ELITe InGenius® SP 200 Consumable Set»** (EG SpA, ref. INT032CS),
- amplification cartridges **«ELITe InGenius® PCR Cassette»** (EG SpA, ref. INT035PCR),
- tips **«300 µL Filter Tips Axygen»** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S),
- boxes **«ELITe InGenius® Waste Box»** (EG SpA, ref. F2102-000).

For automatic nucleic acids extraction, Real Time amplification and result interpretation of samples to be analysed, the instrument **ELITe BeGenius** (ELITechGroup S.p.A., EG SpA, ref. INT040) and the following specific Assay Protocols (EG SpA) are required:

- parameters for calibrators amplification **«HIV1 ELITe_Be_STD»**,
- parameters for Positive Control amplification **«HIV1 ELITe_Be_PC»**,
- parameters for Negative Control amplification **«HIV1 ELITe_Be_NC»**,
- parameters for Plasma samples to be analyzed **«HIV1 ELITe_Be_PL_600_50»**,

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

- extraction cartridges **«ELITe InGenius® SP 1000»** (EG SpA, ref. INT033SP1000),
- consumables for extraction **«ELITe InGenius® SP 200 Consumable Set»** (EG SpA, ref. INT032CS),
- amplification cartridges **«ELITe InGenius® PCR Cassette»** (EG SpA, ref. INT035PCR),
- tips **«1000 µL Filter Tips Tecan»** (Tecan, Switzerland, ref. 30180118)
- boxes **«ELITe InGenius® Waste Box»** (EG SpA, ref. F2102-000).

WARNINGS AND PRECAUTIONS

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

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

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

The PCR Cassettes must be handled in order to avoid amplification product diffusion into the environment and sample and reagent contamination.

Warnings and precautions specific for the components

- **HIV1 ELITe MGB Mix**

The **HIV1 PCR Mix** must be stored at temperature lower than -20 °C in the dark.
The **HIV1 PCR Mix** must be used within one month from the first tube opening.
The **HIV1 PCR Mix** can be frozen and thawed for no more than **five times**: further freezing / thawing cycles may cause a loss of product performances.

The **RT EnzymeMix** must be stored at temperature lower than -20 °C.
The **RT EnzymeMix** must be used within one month from the first tube opening.
The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than 10 minutes during each use.
The **RT EnzymeMix** must not be exposed to temperatures higher than -20 °C for more than **ten times**: further uses may cause a loss of product performances.

- **HIV1 ELITe Standard**

The **HIV1 Q-PCR Standard** must be stored at temperature lower than -20°C.
The **HIV1 Q-PCR Standard** must be used within one month from the first tube opening.
The **HIV1 Q-PCR Standard** can be frozen and thawed for no more than **two times**: further freezing / thawing cycles may cause a loss in titre.
The **HIV1 Q-PCR Standard** can be kept on board in the **ELITe InGenius** or in the **ELITe BeGenius** instruments up to **two independent work sessions of two hours each** ("PCR Only" run mode).

- **HIV1 - ELITe Positive Control**

The **HIV1 Positive Control** must be stored at temperature lower than -20 °C.
The **HIV1 Positive Control** must be used within one month from the first tube opening.
The **HIV1 Positive Control** can be frozen and thawed for no more than **four times**: further freezing / thawing cycles may cause a loss of product performances.
The **HIV1 Positive Control** can be kept on board in the **ELITe InGenius** or in the **ELITe BeGenius** instruments up to **four independent work sessions of three hours each** ("Extraction+PCR Only" run mode).

- **HIV1 Internal Control**

The **HIV1 CPE** must be stored at temperature lower than -20 °C.
The **HIV1 CPE** must be used within one month from the first tube opening.
The **HIV1 CPE** can be frozen and thawed for no more than **twelve times**: further freezing / thawing cycles may cause a loss of product performances.
The **HIV1 CPE** can be kept on board in the **ELITe InGenius** or in the **ELITe BeGenius** instruments up to **six independent work sessions of three hours each** ("Extraction+PCR" run mode).

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Plasma collected in EDTA or ACD

Plasma Samples for nucleic acid extraction must be collected in EDTA or ACD, identified according to laboratory guidelines, transported and stored at room temperature (+18 / +25 °C) for a maximum of 24 hours or at +2 / +8 °C for a maximum of 3 days. Otherwise, they must be frozen and stored at -20 °C for a maximum of 1 month or at -70 °C for 6 months.

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

Note: The RNA extraction from plasma collected in EDTA or ACD is carried out with the **ELITe InGenius** system and with **ELITe InGenius Software** version 1.3 (or later equivalent versions) using the Assay protocol **HIV1 ELITe_PL_600_50**. This protocol processes 600 µL of sample, starting from secondary tube, adds 10 µL per extraction of the **HIV1 CPE** (Internal Control) and elutes the nucleic acids in 50 µL. Primary tube cannot be used in association with the Assay Protocol.

Purified nucleic acids can be stored at -20 °C for one month.

Other samples

At the moment there are no data available concerning product performance with other clinical samples such as whole blood, serum or CSF.

Interfering substances

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

Do not use Plasma collected in heparin in order to prevent inhibition of amplification reaction and frequent invalid results.

Amplification controls

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

- as calibrator set, use the four concentration levels of the **HIV1 ELITe Standard** component provided with this kit, in association with Assay Protocol **HIV1 ELITe_STD**,
- as amplification Positive Control, use the **HIV1 - ELITe Positive Control** component provided with this kit, in association with Assay Protocol **HIV1 ELITe_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit) in association with Assay Protocol **HIV1 ELITe_NC**.

Note: ELITe InGenius system requires approved and valid results of calibration curve and amplification controls for each lot of amplification reagent stored in its database.
The calibration curves, approved and stored in the database, will expire after **60 days**. At expiration date it is necessary to re-run the Q-PCR Standards in association with the amplification reagent lot.
The amplification control results, approved and stored in the database, will expire after **15 days**. At the expiration date it is necessary to re-run the Positive and Negative Controls in association with the amplification reagent lot.
Furthermore, the calibrators and amplification controls must be re-run when:

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

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used. External controls shall be used in accordance with local, state, federal accrediting organizations, as applicable.

PROCEDURE

The procedure to use the **HIV1 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 Calibrators (**HIV1 Q-PCR Standard**) were run, approved and not expired (Status) in association with the amplification reagent lot to be used. If there are not Calibrators approved or valid, run them as described in the following paragraphs,
- verify that the amplification Controls (**HIV1 Positive Control**, **HIV1 Negative Control**) were run, approved and not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification Controls approved or valid, run them as described in the following paragraphs,
- choose the type of run, following the instructions 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 ELITe MGB Kits and the ELITe InGenius instrument and the cited matrix.

The Assay Protocols available for sample testing with the product **HIV1 ELITe MGB Kit** are described in the table below:

Assay Protocol for HIV1 ELITe MGB Kit and ELITe InGenius			
Name	**Matrix**	**Report**	**Characteristics**
HIV1 ELITe_PL_600_50	Plasma	Positive / copies/mL / IU/mL / Negative	Extraction Input Volume: 600 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1.7 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 Customer Service.

Setup of the session

The product **HIV1 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. Calibration run (PCR only),
D. 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 “Laboratory Information Server” (LIS) through which it is possible to load the work session information. Refer to the instrument user’s manual for more details.

Before starting the session, it is mandatory to do the following:

1. Thaw for 30 minutes at room temperature (~+25 °C) the **HIV1 PCR Mix** (WHITE cap) test tubes needed for the session, remembering that the content of each test tube is enough for **24 tests**. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep in ice,

Note: Thaw **HIV1 PCR Mix** in the dark because this reagent is sensitive to the light.

2. Take the **RT EnzymeMix** (cap with BLACK insert) tubes necessary for the session remembering that the content of each tube is sufficient to set up **48 tests**. Gently shake the tubes, centrifuge for 5 seconds to bring the contents to the bottom and keep in ice,

Note: The **RT EnzymeMix** should not be exposed to temperatures above -20 °C for more than 10 minutes.

3. Prepare one 2 mL tube with screwed cap (Sarstedt Ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and mark it in a recognizable manner with a permanent marker,
4. Calculate the volumes of the two sub-components that are needed for preparing the **complete reaction mixture** on the basis of the number of samples to be analyzed, as described in the following table.

Note: In order to calculate the volumes of the two sub-components to be used for the **complete reaction mixture** preparation, it is necessary to define the number of samples (N) to be tested in the session and follow the table below.

Sample Number (N)	HIV1 PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

5. Prepare the **complete reaction mixture** by adding into the dedicated 2 mL tube the calculated volumes of the two components.
6. Mix by **vortexing at low speed** for 10 seconds three times, centrifuge the tube for 5 seconds to bring the content to the bottom and keep in ice.

Note: The **complete reaction mixture** should be used within **7** hours if kept on board in the refrigerated block. The complete reaction mixture **cannot** be stored for re-use.

Note: The **complete reaction mixture** is sensitive to the light, do not expose it to direct light.

The main steps for the setup of the three types of runs are described in the following paragraphs.

A. Integrated run

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

1. Thaw at room temperature (~+25 °C) the test tubes containing the samples to be analysed and handle according to laboratory guidelines and according to paragraph “Samples and Controls”. Remember that 600 µL of sample are needed for the analysis.
2. Thaw the **HIV1 CPE** tubes for the session at room temperature (~+25 °C) for 30 minutes. 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 1000 µL (even if 600 µL of sample will be used) and the “Extracted Elute Volume” is 50 µL.
5. For each Track of interest fill in the “SampleID” (SID) by typing or by scanning the sample barcode.
6. Select the Assay Protocol to be used in the “Assay” column (e.g. HIV1 ELITe_PL_600_50).
7. Ensure that the “Protocol” displayed is: “Extract + PCR”.
8. Select the sample loading position “Extraction Tube” in the “Sample Position” column. Click “Next” to continue the setup.
   For the analysis 600 µL of sample must be transferred in the “Extraction Tube”. Any exceeding volume will be left in the “Extraction Tube” by the **ELITe InGenius**.
9. Load **complete reaction mixture** and **HIV1 CPE** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **HIV1 PCR Mix** and **HIV1 CPE.** 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 1000** extraction cartridges, all the required consumables and the samples to be extracted, following the GUI instruction. Click “Next” to continue.
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 amplification products, the extraction cartridges and the consumables must be removed from the instrument and disposed without producing environmental contaminations. Avoid spilling the amplification products.

Note: The **complete reaction mixture** can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time needed to start a third work session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

B. Amplification run

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

1. Thaw at room temperature (~+25 °C) the test tubes containing the extracted nucleic acid samples to be analysed. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home” screen.
3. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 1000 µL (even if 600 µL of sample has been used) and the “Extracted Elute Volume” is 50 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 (e. g. HIV1 ELITe_PL_600_50).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure that the sample loading position in the “Sample Position” column is “Elution Tube (bottom row)”. Click “Next” to continue the setup.
8. Load the **complete reaction mixture** on the “Inventory Block” selected by following the GUI instruction and fill in the lot number and expiry date of **HIV1 PCR Mix**. 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.
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 in the “Elution tube” 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 amplification products and the consumables must be removed from the instrument and disposed without producing environmental contaminations. Avoid the spilling of the amplification products.

Note: The **complete reaction mixture** can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time needed to start a third work session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

C. Calibration run

To set up the Calibration run with Q-PCR Standards, carry out the following steps as per GUI:

1. Thaw **HIV1 Q-PCR Standard** tubes (Cal1: HIV1 Q-PCR Standards 10^2, Cal2: HIV1 Q-PCR Standards 10^3, Cal3: HIV1 Q-PCR Standards 10^4, Cal4: HIV1 Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for preparing 2 reactions. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home” screen.
3. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 1000 µL (even if 600 µL of sample will be used) and the “Extracted Elute Volume” is 50 µL.
4. In the Track of interest, select the Assay Protocol “HIV1 ELITe_STD” in the “Assay” column and fill in the lot number and expiry date of **HIV1 Q-PCR Standard**. Click “Next” to continue the setup.

5. Load the **complete reaction mixture** on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of **HIV1 PCR Mix**. Click "Next" to continue the setup.
6. Load and check the Tip Racks in the "Inventory Area" selected by following the GUI instruction. Click "Next" to continue the setup.
7. Load the **PCR Cassettes** and the **HIV1 Q-PCR Standard** tubes following the GUI instruction. Click "Next" to continue.
8. Close the instrument door.
9. 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 **HIV1 Q – PCR Standards** must be removed from the instrument, capped and stored at -20 °C.

Note: The **HIV1 Q-PCR Standards** can be used for 2 independent work sessions of 2 hours each.

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 without producing environmental contaminations. Avoid the spilling of the amplification products.

Note: The **complete reaction mixture** can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time needed to start a third work session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

D. Amplification run for Positive Control and Negative Control

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

1. Thaw **HIV1 Positive Control** tube at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for preparing 4 reactions. Mix gently, spin down the content for 5 seconds.
2. As **Negative Control**, transfer at least 50 µL of molecular biology grade water to an "Elution tube", provided with the **ELITe InGenius SP 200 Consumable Set**.
3. Select "Perform Run" from the "Home screen".
4. Even if no extraction will be carried out, ensure that the "Extraction Input Volume" is 1000 µL (even if 600 µL of sample will be used) and the "Extracted Elute Volume" is 50 µL.
5. In the Track of interest, select the Assay Protocol to be used in the "Assay" column.
6. For the Positive Control, select the Assay Protocol "HIV1 ELITe_PC" in the "Assay" column and fill in the lot number and expiry date of **HIV1 Positive Control**.
7. For the Negative Control, select the Assay Protocol "HIV1 ELITe_NC" in the "Assay" column and fill in the lot number and expiry date of the molecular biology grade water. Click "Next" to continue the setup.
8. Load the **complete reaction mixture** on the "Inventory Block" selected by following the GUI instruction and fill in the lot number and expiry date of **HIV1 PCR Mix**. 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**, the **HIV1 Positive Control** tube and the **Negative Control** tube following the GUI instruction. Click "Next" to continue.
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 **HIV1 Positive Control** must be removed from the instrument, capped and stored at -20 °C. The remaining **Negative Control** must be disposed.

Note: The **HIV1 Positive Control** can be used for 4 independent work sessions of 3 hours each.

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

Note: The **complete reaction mixture** can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time needed to start a third work session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

Review and approval of results

At the end of the run, the "Results Display" screen is automatically shown. In this screen the sample / Standard / 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 "Laboratory Information Server" (LIS) through which it is possible to 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 **HIV1 ELITe MGB Kit** through the following procedure:

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

A. Validation of Calibration curve

The fluorescence signals emitted by the probe for HIV1 (Channel "HIV1") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the Assay Protocol "**HIV1 ELITe_STD**".

The Calibration curve, specific for the amplification reagent lot, is recorded in the database (Calibration). It can be viewed and approved by personnel qualified as "Administrator" or "Analyst", following the GUI instructions.

The Calibration curve, specific for the amplification reagent lot, will expire **after 60 days**.

Note: if the Calibration curve does not meet the acceptance criteria, the "Failed" message is shown on the "Calibration" screen and it is not possible to approve the curve. The Calibrator amplification reactions have to be repeated.

Note: if the Calibration curve is run together with samples 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 amplification Positive Control and Negative Control results

The fluorescence signals emitted by the probe for HIV1 (Channel "HIV1") 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 "**HIV1 ELITe_PC**" and "**HIV1 ELITe_NC**".

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

Note: if the result of Positive Control or Negative Control amplification 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 reaction has to be repeated.

Note: if the Positive Control or Negative Control is run together with samples to be tested and its result is invalid, the samples can be approved but the results are not validated. In this case, the amplification of all samples must be repeated too.

C. Validation of Sample results

The fluorescence signals emitted by the probes for HIV1 (Channel "HIV1") 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 "HIV1 ELITe_PL_600_50".

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

1) Calibration Curve	**Status**
HIV1 Q-PCR Standards	APPROVED
2) Positive Control	**Status**
HIV1 Positive Control	APPROVED
3) Negative Control	**Status**
HIV1 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.

Result of sample run	Interpretation
HIV1: RNA Detected, quantity equal to "XXX" copies / mL or IU / mL.	**HIV1 RNA was detected** in the sample within the measurement range of the assay, quantity as shown.
HIV1: RNA Detected, quantity below "LLoQ" copies / mL or IU / mL	**HIV1 RNA was detected** in the sample below the lower limit of quantification (LLoQ) of the assay.
HIV1: RNA Detected, quantity beyond "ULoQ" copies / mL or IU / mL.	**HIV1 RNA was detected** in the sample beyond the upper limit of quantification (ULoQ) of the assay.
HIV1: RNA Not Detected or below the "LoD" copies / mL or IU / mL.	**HIV1 RNA was not detected** in the sample. The sample is negative for HIV1 RNA or its concentration is below the Limit of Detection (LoD) of the assay.
Invalid - Retest Sample.	**Not valid assay result** caused by Internal Control failure (incorrect extraction, inhibitors carry-over). The test should be repeated.

Samples reported as "Invalid - Retest Sample" by the **ELITe InGenius software** are not suitable for result interpretation. In this case, the Internal Control RNA was not efficiently detected due to problems in the reverse-transcription, amplification or extraction step (degradation or loss of RNA 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, by 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 "HIV1 RNA Not Detected or below LoD" are suitable for analysis but it was not possible to detect HIV1 RNA. In this case it cannot be excluded that the HIV1 RNA is present at a concentration below the limit of detection of the assay (see "Performance characteristics").

HIV1 RNA positive samples at a concentration below the LoD, when are detected by the assay, are reported as "HIV1: RNA Detected, quantity below LLoQ" (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".

D. Sample result reporting

The sample results are stored in the database and can be viewed or 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.

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Plasma collected in EDTA or ACD

Plasma samples for nucleic acid extraction, must be collected in EDTA or ACD, identified according to laboratory guidelines, transported and stored at room temperature (+18 / ~+25 °C) for a maximum of three days or at +2 / +8 °C for a maximum of five days. Otherwise, they must be frozen and stored at ~-20 °C for a maximum of one month or at ~-70 °C for 6 months.

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

Note: The RNA extraction from plasma collected in EDTA or ACD is carried out with the **ELITe BeGenius** system and with **ELITe BeGenius Software** version 2.1.0 (or later equivalent versions) using the Assay Protocol **HIV1 ELITe_Be_PL_600_50**. This protocol processes 600 µL of sample, adds 10 µL per extraction of the **HIV1 CPE** (Internal Control) and elutes the nucleic acids in 50 µL.

Purified nucleic acids can be stored at ~-20 °C for one month.

Other samples

At the moment there are no data available concerning product performance with other clinical samples such as whole blood, serum or CSF.

Interfering substances

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

Do not use Plasma collected in heparin in order to prevent inhibition of amplification reaction and frequent invalid results.

Amplification controls

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

- as calibrator set, use the four concentration levels of the **HIV1 ELITe Standard** product provided with this kit, in association with Assay Protocol **HIV1 ELITe_Be_STD**,
- as amplification Positive Control, use the **HIV1 - ELITe Positive Control** product provided with this kit, in association with Assay Protocol **HIV1 ELITe_Be_PC**,
- as amplification Negative Control, use molecular biology grade water (not provided with this kit) in association with Assay Protocol **HIV1 ELITe_Be_NC**.

Note: ELITe BeGenius system requires approved and valid results of calibration curve and amplification controls for each lot of amplification reagent stored in its database.
The calibration curves, approved and stored in the database, will expire after **60 days**. At expiration date it is necessary to re-run the Q-PCR Standards in association with the amplification reagent lot.
The amplification control results, approved and stored in the database, will expire after **15 days**. At the expiration date it is necessary to re-run the Positive and Negative Controls in association with the amplification reagent lot.
Furthermore, the calibrators and amplification controls must be re-run when:

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

Quality controls

The planned validation of the extraction and amplification procedure is recommended. Tested samples or certified reference material can be used. External controls shall be used in accordance with local, state, federal accrediting organizations, as applicable.

PROCEDURE

The procedure to use the **HIV1 ELITe MGB Kit** with the system **ELITe BeGenius** 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 BeGenius and select the login mode "**CLOSED**",
- verify that the Calibrators (**HIV1 Q-PCR Standard**) were run, approved and not expired (Status) in association with the amplification reagent lot to be used. If there are not Calibrators approved or valid, run them as described in the following paragraphs,
- verify that the amplification Controls (**HIV1 Positive Control**, **HIV1 Negative Control**) were run, approved and not expired (Status) in association with the amplification reagent lot to be used. If there are not amplification Controls approved or valid, run them as described in the following paragraphs,
- choose the type of run, following the instructions 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 ELITe MGB Kits and the ELITe BeGenius instrument and the cited matrix.
- The Assay Protocols available for sample testing with the product **HIV1 ELITe MGB Kit** are described in the table below:

Assay Protocol for HIV1 ELITe MGB Kit and ELITe BeGenius			
Name	**Matrix**	**Report**	**Characteristics**
HIV1 ELITe_Be_PL_600_50	Plasma	Positive / IU/mL / copies/mL / Negative	Extraction Input Volume: 600 µL Extraction Elution Volume: 50 µL Internal Control: 10 µL Dilution Factor: 1.7 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 Customer Service.
- **Setup of the session**
- The product **HIV1 ELITe MGB Kit** can be used with the **ELITe BeGenius** system in order to perform:
  A. Integrated run (Extract + PCR),
  B. Amplification run (PCR only),
  C. Calibration run (PCR only),
  D. 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 BeGenius** 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.

Before starting the session, it is mandatory to do the following:

1. Thaw for 30 minutes at room temperature (~+25 °C) the **HIV1 PCR Mix** (WHITE cap) test tubes needed for the session, remembering that the content of each test tube is enough for **24 tests**. Mix by vortexing for 10 seconds three times and centrifuge the tubes for 5 seconds to bring the content to the bottom and keep in ice,

Note: Thaw **HIV1 PCR Mix** in the dark because this reagent is sensitive to the light.

2. Take the **RT EnzymeMix** (cap with BLACK insert) tubes necessary for the session remembering that the content of each tube is sufficient to set up **48 tests**. Gently shake the tubes, centrifuge for 5 seconds to bring the contents to the bottom and keep in ice,

Note: The **RT EnzymeMix** should not be exposed to temperatures above -20 °C for more than 10 minutes.

3. Prepare one 2 mL tube with screwed cap (Sarstedt Ref. 72.694.005, not included in the kit) for the **complete reaction mixture** and mark it in a recognizable manner with a permanent marker,
4. Calculate the volumes of the two sub-components that are needed for preparing the **complete reaction mixture** on the basis of the number of samples to be analyzed, as described in the following table.

Note: In order to calculate the volumes of the two sub-components to be used for the **complete reaction mixture** preparation, it is necessary to define the number of samples (N) to be tested in the session and follow the table below.

Sample Number (N)	HIV1 PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

5. Prepare the **complete reaction mixture** by adding into the dedicated 2 mL tube the calculated volumes of the two components.
6. Mix by **vortexing at low speed** for 10 seconds three times, centrifuge the tube for 5 seconds to bring the content to the bottom and keep in ice.

Note: The **complete reaction mixture** should be used within **7** hours if kept on board in the refrigerated block. The complete reaction mixture **cannot** be stored for re-use.

Note: The **complete reaction mixture** is sensitive to the light, do not expose it to direct light.

The main steps for the setup of the three types of runs are described in the following paragraphs.

A. Integrated run

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

1. Thaw at room temperature (+18 / 25 °C) the test tubes containing the samples to be analysed and handle according to laboratory guidelines and according to paragraph "Samples and Controls". Remember that 600 µL of sample are needed for the analysis.
2. Thaw the **HIV1 CPE** tubes at room temperature (~+25°C) for 30 minutes 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. Remove the Racks from the "Cooler Unit" and place them on the preparation table.
5. Select the "run mode": "Extract + PCR".
6. Load the samples into the Racks 5 and 4 (start always from Rack 5).
7. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.

Note: If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the sample ID.

8. Check the Extraction Input Volume (600 µL) and the Extracted Elute Volume (50 µL).
9. Select the assay protocol to be used in the "Assay" column (i.e. HIV1 ELITe_Be_PL_600_50). Click "Next" to continue the setup.
10. If used, repeat step 7 to 9 for Rack 4.
11. Load the Elution tubes into the Racks 3 and 2 (start always from Rack 3).

Note: Elution tubes can be labelled to improve traceability.

12. Insert the Rack into the "Cooler Unit". Click "Next" to continue the setup.
13. If used, repeat steps 11 and 12 for Rack 2.
14. Load **CPE** and **complete reaction mixture** into the Rack 1.
15. Insert the Rack 1 into the "Cooler Unit". Click "Next" to continue the setup.
16. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click "Next" to continue the setup.

17. Load the Basket with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
18. Load the Basket with the “ELITe InGenius SP 1000” extraction cartridges and the required extraction consumables by following the GUI instruction. Click “Next” to continue the setup.
19. Close the instrument door.
20. Press “Start” to start the run.

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

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

Note: At the end of the run the “PCR Cassette” with the reaction products and the consumables must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

Note: The **complete reaction mixture** can be kept on board in the “Cooler Unit” up to 2 work sessions of 3 hours each and for the time needed to start a third work session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

B. Amplification run

To set up the amplification run starting from extracted RNA, carry out the steps below following the GUI:

1. Thaw at room temperature (~+25 °C) the test tubes containing the extracted nucleic acid samples to be analysed. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home screen”.
3. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
4. Select the “run mode”: “PCR Only”.
5. Load the samples into the Racks 3 and 2 (start always from Rack 3).
6. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
7. Even if extraction is not performed, check the Extraction Input Volume (600 µL) and the Extracted Elute Volume (50 µL).
8. Select the assay protocol to be used in the “Assay” column (e.g. HIV1 ELITe_Be_PL_600_50). Click “Next” to continue the setup.
9. If used, repeat step from 5 to 8 for Rack 2.
10. Load the **complete reaction mixture** into the Rack 1.
11. Insert the Rack 1 into the “Cooler Unit”. Click “Next” to continue the setup.
12. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
13. Load the Basket with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
14. Close the instrument door.
15. Press “Start” to start the run.

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

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

Note: At the end of the run the “PCR Cassette” with the reaction products must be removed from the instrument and eliminated without producing environmental contaminations. Avoid the spilling of the reaction products.

Note: **Note**: The **complete reaction mixture** can be kept on board in the “Cooler Unit” up to 2 work sessions of 3 hours each and for the time needed to start a third work session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

C. Calibration run

To set up the Calibration run with the Q-PCR Standards, carry out the steps below following the GUI:

1. Thaw **HIV1 Q-PCR Standard** tubes (Cal1: HIV1 Q-PCR Standards 10^2, Cal2: HIV1 Q-PCR Standards 10^3, Cal3: HIV1 Q-PCR Standards 10^4, Cal4: HIV1 Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for preparing 2 reactions. Mix gently, spin down the content for 5 seconds.
2. Select “Perform Run” from the “Home screen”.
3. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
4. Select the “run mode”: “PCR Only”.
5. Load the Calibrator tubes into the Rack 3.
6. Select the assay protocol to be used in the “Assay” column (HIV1 ELITe_Be_STD). Click “Next” button to continue the setup.
7. Load the **complete reaction mixture** into the Rack 2.
8. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
10. Load the Basket with “PCR Cassette” in the Inventory Area by 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 BeGenius** allows the user to view, approve, store the results and to print and save the report.

Note: At the end of the run the remaining Calibrators can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Q-PCR Standards.

Note: The **HIV1 Q-PCR Standards** can be used for 2 independent work sessions of 2 hours each.

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

Note: The **complete reaction mixture** can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time needed to start a third work session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

D. Positive Control and Negative Control run

To setup the amplification run with Positive Control and Negative Control, carry out the steps below following the GUI:

1. Thaw **HIV1 Positive Control** tubes at room temperature (~+25°C) for 30 minutes for the session. Each tube is sufficient for preparing 4 reactions. Mix gently, spin down the content for 5 seconds.
2. Transfer at least 50 µL of the molecular biology grade water (as Negative Control) for the sessions in one Elution tube, provided with the ELITe InGenius SP Consumable Set.
3. Select “Perform Run” from the “Home screen”.
4. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
5. Select the “run mode”: “PCR Only”.
6. Load the Positive Control and Negative Control tubes into the Rack 3.
7. Select the assay protocol to be used in the “Assay” column (HIV1 ELITe_Be_PC and HIV1 ELITe_Be_NC). Click “Next” button to continue the setup.
8. Load the **complete reaction mixture** into the Rack 2.

9. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
11. Load the Basket with “PCR Cassette” in the Inventory Area by 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 BeGenius** allows the user to view, approve, store the results and to print and save the report.

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

Note: The **HIV1 Positive Control** can be used for 4 independent work sessions of 3 hours each.

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

Note: The **complete reaction mixture** can be kept on board in the refrigerated block up to 2 work sessions of 3 hours each and for the time needed to start a third work session (7 hours in total). Mix gently and spin down the content for 5 seconds before starting the next session.

Review and approval of results

At the end of the run, the “Results Display” screen is automatically shown. In this screen the sample / Calibrator / 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.

The **ELITe BeGenius** system generates the results using the HIV1 ELITe MGB Kit through the following procedure:

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

Note: please, refer to the same **ELITe InGenius** chapters for the details.

PERFORMANCE CHARACTERISTICS
ELITe InGenius and ELITe BeGenius

Limit of Detection (LoD)

The Limit of Detection (LoD) of HIV1 ELITe MGB Kit was defined in association with Plasma samples and ELITe InGenius system.

The LoD was defined by testing a panel of HIV1 negative Plasma collected in ACD spiked by HIV1 certified reference material (4th WHO International Standard, NIBSC) at known titre. Six levels of dilutions were prepared starting from 100 IU / mL to 6 IU / mL. Each dilution level was processed in 24 replicates on ELITe InGenius system in “Extract + PCR” mode. The LoD was estimated by Probit regression analysis of the data as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following table.

Limit of Detection (IU / mL) for Plasma collected in ACD samples and ELITe InGenius			
Target	LoD	95% confidence interval	
		Lower bound	Upper bound
HIV1	60	40	122

The LoD as copies / mL for Plasma collected in ACD was calculated by applying the specific conversion factor (2.3 IU / copy). The analytical sensitivity as copies / mL is reported below.

Limit of Detection (copies / mL) for Plasma collected in ACD samples and ELITe InGenius			
Target	LoD	95% confidence interval	
		Lower bound	Upper bound
HIV1	26	17	53

The calculated LoD value was verified by testing 30 replicates of Plasma collected in ACD and 30 replicates of Plasma collected in EDTA spiked by HIV1 certified reference material (4th WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 27 out of 30 replicates give a positive result as per CLSI standard EP17-A.

The results are reported in the following table.

Verification of Limit of Detection for Plasma and ELITe InGenius					
Sample	Titer	Target	N	Positive	Negative
Plasma collected in ACD	60 IU / mL	HIV1	30	29	1
Plasma collected in EDTA	60 IU / mL	HIV1	30	28	2

The LoD value for HIV1 target was confirmed at 60 IU / mL for Plasma collected in ACD and Plasma collected in EDTA.

The calculated LoD value was verified in association with **ELITe BeGenius** by testing 30 replicates of Plasma collected in ACD and 30 replicates of Plasma collected in EDTA spiked by HIV1 certified reference material (4th WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 27 out of 30 replicates give a positive result as per CLSI standard EP17-A.

The results are reported in the following table.

Verification of Limit of Detection for Plasma and ELITe BeGenius					
Sample	Titer	Target	N	Positive	Negative
Plasma collected in ACD	60 IU / mL	HIV1	30	30	0
Plasma collected in EDTA	60 IU / mL	HIV1	30	27	3

The LoD value for HIV1 target was confirmed on **ELITe BeGenius** at 60 IU / mL for Plasma collected in ACD and Plasma collected in EDTA.

Matrix equivalence: Plasma EDTA versus Plasma ACD

The Equivalence of performances of HIV1 ELITe MGB Kit was verified using samples of Plasma collected in ACD and Plasma collected in EDTA in association with ELITe InGenius system.

A test was carried out on 30 samples of Plasma collected in EDTA and 30 samples of Plasma collected in ACD from the same 30 different donors (paired samples), tested negative for HIV1 by a CE IVD marked immunoassay. The samples were tested on ELITe InGenius system in “Extract + PCR” mode. The percentage negative agreement was evaluated. The percentage Coefficient of Variability (%CV) of Ct values of the Internal Control was calculated in order to evaluate the equivalence of the two matrices.

The results are reported in the following table.

Sample	N	Positive	Negative	% Negative agreement	IC Ct %CV	Whole IC Ct %CV
Plasma collected in EDTA	30	1	29	96.7%	1.80	1.81
Plasma collected in ACD	30	0	30		1.81	

The Plasma sample resulted positive showed a very low titre (lower than 60 IU / mL) that is compatible with a missing identification by the immunologic CE IVD assay used to certify the negativity of the sample.

A test was carried out on 30 paired samples of Plasma collected in EDTA and of Plasma collected in ACD, tested negative for HIV1 by a CE IVD marked immunoassay and spiked with certified reference material (4th WHO HIV1 International Standard, NIBSC) at a concentration of 3 x LoD (about 180 IU / mL). The samples were tested on ELITe InGenius system in “Extract + PCR” mode. The percentage positive agreement was evaluated. The percentage Coefficient of Variability (%CV) of Ct values of the HIV1 target was calculated the in order to evaluate the equivalence of the two matrices.

The results are reported in the following table.

Sample	N	Positive	Negative	% Positive agreement	HIV1 Ct %CV	Whole HIV1 Ct %CV	Bias (Log IU/mL)
Plasma collected in EDTA	30	30	0	100%	1.81	1.53	0.0744
Plasma collected in ACD	30	30	0		1.18		

In these tests, the 30 paired samples of Plasma collected in EDTA and of Plasma collected in ACD showed equivalent performances when analysed by HIV1 ELITe MGB Kit in association with ELITe InGenius system.

Further tests about matrices equivalence were performed during the Linear Measuring Range study.

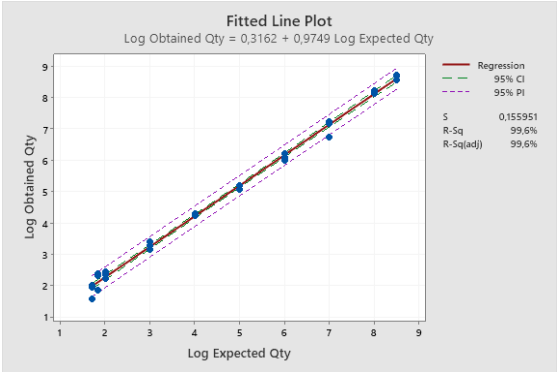
Linear measuring range

The Linear measuring range of HIV1 ELITe MGB Kit was determined in association with Plasma samples and ELITe InGenius and ELITe BeGenius systems.

The Linear measuring range was determined using a panel of dilutions of HIV1 Group M subtype B reference material (ZeptoMetrix) in HIV1 negative samples of Plasma collected in EDTA and **ELITe InGenius** and **ELITe BeGenius** systems. The panel consisted of ten dilution points from about 3.2 x10^8 IU / mL a 50 IU / mL. Each sample of the panel was tested in 3 replicates on ELITe InGenius and ELITE BeGenius systems in “Extract + PCR” mode.

The analysis of the obtained data, performed by polynomial regression and linear regression, demonstrated that the assay shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.996 for **ELITe InGenius**.

The results are reported in the following figure.



The Lower Limit of Quantification (LLoQ) was set at the LoD concentration that gives quantitative results precise (Standard Deviation = 0.3049 Log IU / mL) and accurate (Bias = 0.0759 Log IU / mL) within ±0.5 Log IU / mL: 60 IU / mL.

The Upper Limit of Quantification (ULoQ) was set at the highest concentration that gives quantitative results precise (Standard Deviation = 0.0816 Log IU / mL) and accurate (Bias = 0.1459 Log IU / mL) within ±0.5 Log IU / mL: 319,290,322 IU / mL.

The linear measuring range as copy / mL for Plasma EDTA is calculated by applying the specific conversion factor (2.3 IU / copy).

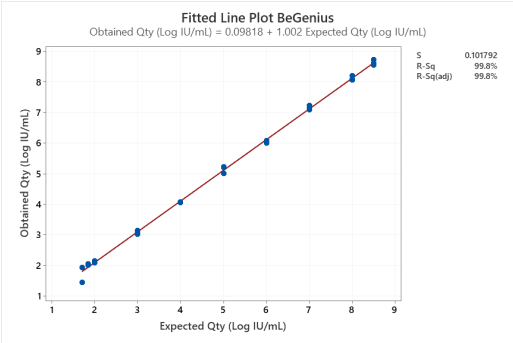
The final results are summarized in the following table.

Linear measuring range for Plasma EDTA samples and ELITe InGenius	
Lower Limit	Upper Limit
60 IU / mL	319,290,322 IU / mL
26 copies / mL	138,821,879 copies / mL

The Linear measuring range of HIV1 ELITe MGB® Kit was verifiedzs in association with Plasma samples and **ELITe BeGenius** system using a panel of dilutions of HIV1 Group M subtype B reference material (ZeptoMetrix) in HIV1 negative samples of Plasma collected in ACD. The panel consisted of ten dilution points from about 3.2 x 10^8 IU / mL to 50 IU / mL. Each sample of the panel was tested in 3 replicates on ELITe BeGenius® system in “Extract + PCR” mode.

The analysis of the obtained data, performed by polynomial regression and linear regression, demonstrated that the assay shows a linear response for all the dilutions with a Square Correlation Coefficient (R2) equal to 0.998 for **ELITe BeGenius**.

The results are reported in the following figure.



The Lower Limit of Quantification (LLoQ) was set at the LoD concentration that gives quantitative results precise (Standard Deviation = 0.3353 Log IU / mL) and accurate (Bias = 0.3004 Log IU / mL) within ±0.5 Log IU / mL: 60 IU / mL.

The Upper Limit of Quantification (ULoQ) was set at the highest concentration that gives quantitative results precise (Standard Deviation = 0.0891 Log IU / mL) and accurate (Bias = 0.1258 Log IU / mL) within ±0.5 Log IU / mL: 319,290,322 IU / mL.

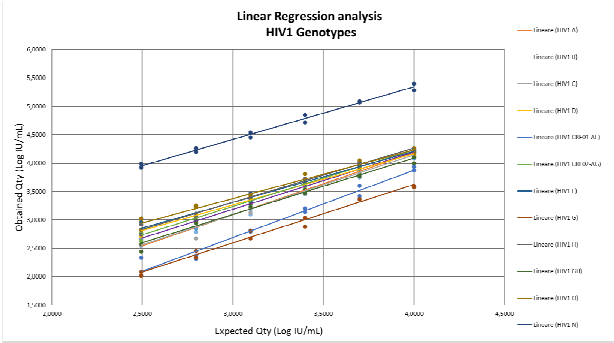
The linear measuring range as copy / mL for Plasma EDTA is calculated by applying the specific conversion factor (2.3 IU / copy).

The final results are summarized in the following table.

Linear measuring range for Plasma EDTA samples and ELITe BeGenius	
Lower Limit	Upper Limit
60 IU / mL	319,290,322 IU / mL
26 copies / mL	138,821,879 copies / mL

The linearity of quantification was verified by analysis of negative Plasma collected in EDTA spiked by HIV1 reference material (HIV-1 Subtype Panel, Institute of Virology, Erlangen University Hospital) for main HIV1 genotypes (Group M Subtype A, C, D, CRF01-AE, F, G, GH, H, CRF02-AG, Group O and Group N). Each HIV1 genotype was tested in a panel of 6 dilution levels. Each dilution level was tested in 2 replicates on ELITe InGenius system in “Extract + PCR” mode.

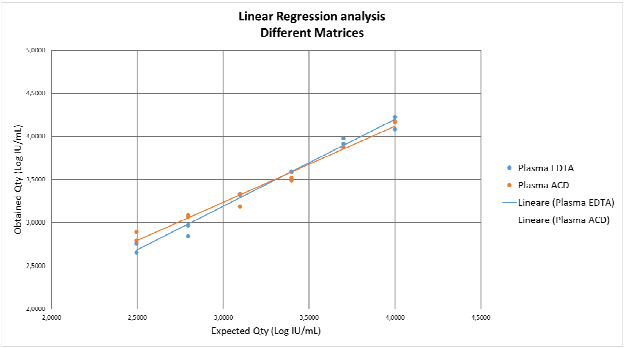
The results are reported in the following figure.



The linearity of quantification of the assay was confirmed for the main HIV1 genotypes (Group M Subtype A, C, D, CRF01-AE, F, G, GH, H, CRF02-AG, Group O and Group N): the R2 value ranged from 0.972 to 0.999 and the quantitative results fall within ±0.5 Log IU / mL with the exception of HIV1 Group N that was over-estimated of about 1.5 Log IU / mL in comparison with the theoretical value. However, this sample was also overestimated by "cobas® HIV-1 for use on the 6800 Systems" (Roche Diagnostics).

The linearity of quantification was verified by analysis of negative Plasma collected in EDTA and negative Plasma collected in ACD spiked by HIV1 Group M subtype B reference material (Zeptometrix). Each matrix was tested in a panel of 6 dilution levels. Each dilution level was tested in 2 replicates on **ELITe InGenius** system in "Extract + PCR" mode. Corresponding results of test with Plasma collected in EDTA were reported as reference.

The results are reported in the following figure.



The linearity of the assay was confirmed for Plasma collected in EDTA and Plasma collected in ACD giving quantitative results within ±0.5 Log IU / mL and an R2 respectively of 0.984 and 0.980.

Inclusivity: Efficiency of detection and quantification efficiency on different genotypes

The efficiency of detection for different genotypes of HIV1 was evaluated by *in silico* comparison of the sequences available in the nucleotide databases.

The analysis of the regions chosen for the hybridization of the primers and of the probe in the alignment of the sequences of the polymerase gene (integrase region) available in the database showed sufficient sequence conservation in the HIV1 genotypes of Group M (subtype A, B, C, D, F, G, H, J, K, L), Group O, Group N, Group P and CRF subtypes CRF01-AE, CRF02-AG, CRF03-AB. So, an efficient detection and quantification for the different HIV1 genotypes is expected.

The Inclusivity of the assay, as detection and quantification efficiency on different genotypes, was verified by testing two reference material panels:

- "1st WHO International Reference Preparation for HIV-1 CRF's", (NIBSC, code: 13/214) including Group O, CRF 11 GJ, CRF02-AG, CRF01-AE, CRF01 A, G, J, U, CRF BG 24, subtype J, subtype C, subtype G and CRF ADG,
- "HIV-1 Subtype Panel", (Institute of Virology, Erlangen University Hospital) a 20-member panel of diverse collection of HIV1 of Group M subtype A, B, C, D, CRF01-AE, F, G, H and GH, CRF02-AG, Group O and Group N.

Each sample of the panel was diluted at the concentration of 3 x LoD (about 180 IU / mL) in negative samples of Plasma collected in ACD and tested on **ELITe InGenius** system in "Extract + PCR" mode.

The results are reported in the following table.

1st WHO International Reference Preparation for HIV-1 CRF's					
Subtype/Group	**Strain**	**Theoretical IU/mL**	**Pos. / Rep.**	**Mean Ct**	**Mean IU/mL**
Group O	BCF01	180	3 / 3	31.92	2,808
CRF11 GJ	MP1307	180	3 / 3	34.60	478
CRF02-AG	P1261	180	3 / 3	35.34	299
CRF01-AE	CM244	180	3 / 3	36.48	142
CRF01 A,G,J,U	96CM1849	180	3 / 3	37.15	90
CRF BG 24	X2456	180	3 / 3	36.69	123
Subtype J	SE9173	180	3 / 3	35.91	210
Subtype C	X1936	180	3 / 3	36.01	199
Subtype G	P962	180	3 / 3	35.08	344
CRF ADG	24203	180	3 / 3	34.67	461

Erlangen HIV-1 Subtype Panel					
Sample ID	**Strain**	**Theoretical IU/mL**	**Pos. / Rep.**	**Mean Ct**	**Mean IU/mL**
HIV1 A1	92UG029	180	3/3	35.27	345
HIV1 A2	00KE_KER2018	180	3/3	34.62	506
HIV1 B1	92TH026	180	3/3	35.27	311
HIV1 B2	90TH_BK132	180	3/3	34.77	452
HIV1 C1	92BR025	180	3/3	35.01	372
HIV1 C2	99ET_14	180	3/3	34.74	466
HIV1 D1	92UG021	180	3/3	35.53	265
HIV1 D2	92UG035	180	3/3	34.12	683
HIV1 D3	92UG024	180	3/3	34.50	531
HIV1 E1	92TH022	180	3/3	36.28	166
HIV1 F1	93BR029	180	3/3	35.44	282
HIV1 F2	93BR020	180	3/3	35.58	251
HIV1 G	RU570	180	3/3	36.96	109
HIV1 H	VI525	180	3/3	35.11	361
HIV1 GH	VI557	180	3/3	35.17	336
HIV1 AG1	01CM.0005BBY	180	3/3	36.23	176
HIV1 AG2	01CM.0008BBY	180	3/3	34.74	451
HIV1 N	YBF30	180	3/3	30.60	7,694
HIV1 O1	MVP5180	180	3/3	34.47	555
HIV1 O2	CA-9	180	3/3	31.34	4,615

All the samples were correctly detected and quantified within the theoretical titer ±0.5 Log IU / mL (57 – 569 IU / mL) by the HIV1 ELITe MGB Kit in association with the **ELITe InGenius** instrument except for four samples that were overestimated. In particular, HIV1 Group N and Group O were overestimated more than 1 Log IU / mL in comparison with the theoretical value. However, these samples were also overestimated by "cobas HIV-1 for use on the 6800 Systems" (Roche Diagnostics).

Potential interfering markers: cross-reactivity

The Potential cross-reactivity with other unintended organisms of the assay was evaluated by *in silico* comparison of sequences available in the nucleotide databases.

The regions chosen for the hybridization of the primers and the probe were checked on the alignment of the sequences of other organisms available in the databases. The analysis of the hybridization regions showed absence of significant homologies with the unintended organisms, except for HIV2, the most related organism with HIV1, that has a homology close to 80%.

The absence of cross-reactivity with other organisms that can be found in clinical samples of Plasma, was also verified by testing a panel of certified reference materials.

Samples of genomic DNA or RNA from different potentially interfering markers (ATCC, NIBSC, ZeptoMetrix) were analyzed at high concentration (at least 10^5 copies / reaction) in three replicates in association with **ELITe InGenius** system in "PCR Only" mode. The genomic DNA or RNA of each organism were added with 80,000 Internal Control copies per reaction in order to mimic the extracted clinical sample.

The results are reported in the following table.

Sample ID	**HIV1 Pos. / Rep.**	**Outcome**
HIV2	3 / 3	Cross-reactivity
HTLV1	0 / 3	No cross-reactivity
HTLV2	0 / 3	No cross-reactivity
CMV	0 / 3	No cross-reactivity
EBV	0 / 3	No cross-reactivity
HAV	0 / 3	No cross-reactivity
HIV1	0 / 3	No cross-reactivity
HCV	0 / 3	No cross-reactivity
HEV	0 / 3	No cross-reactivity
HSV1	0 / 3	No cross-reactivity
HSV2	0 / 3	No cross-reactivity
HHV6	0 / 3	No cross-reactivity
VZV	0 / 3	No cross-reactivity

Sample ID	HIV1 Pos. / Rep.	Outcome
Flu A	0 / 3	No cross-reactivity
Flu B	0 / 3	No cross-reactivity
RSV	0 / 3	No cross-reactivity
ADV	0 / 3	No cross-reactivity
WNV	0 / 3	No cross-reactivity
DV3	0 / 3	No cross-reactivity
EV	0 / 3	No cross-reactivity
PVB19	0 / 3	No cross-reactivity
Staphylococcus aureus	0 / 3	No cross-reactivity
Candida albicans	0 / 3	No cross-reactivity

All the tested potential interfering markers showed no cross-reactivity for the HIV1 target using HIV1 ELITe MGB Kit, but HIV2 that can give positive results with a quantification 2,000 times lower than the theoretical HIV2 titre.

Potential interfering markers: Interference

The Absence of interference by other organisms that can be found in clinical samples of Plasma was verified by testing a panel of certified reference materials.

Samples of genomic DNA or RNA from different potentially interfering markers (ATCC, NIBSC, ZeptoMetrix) at high concentration (at least 10^5 copies / reaction) were spiked by HIV1 genomic RNA (PEI) at low concentration (about 20 copies / reaction). The samples were analyzed in three replicates in association with **ELITe InGenius** system in "PCR Only" mode. Each sample were added with 80,000 Internal Control copies per reaction in order to mimic the extracted clinical sample.

The results are reported in the following table.

Sample ID	HIV1 Pos. / Rep.	Outcome
HIV2	3 / 3	No interference
HTLV1	3 / 3	No interference
HTLV2	3 / 3	No interference
CMV	3 / 3	No interference
EBV	3 / 3	No interference
HAV	3 / 3	No interference
HIV1	3 / 3	No interference
HCV	3 / 3	No interference
HEV	3 / 3	No interference
HSV1	3 / 3	No interference
HSV2	3 / 3	No interference
HHV6	3 / 3	No interference
VZV	3 / 3	No interference
Flu A	3 / 3	No interference
Flu B	3 / 3	No interference
RSV	3 / 3	No interference
ADV	3 / 3	No interference
WNV	3 / 3	No interference
DV3	3 / 3	No interference
EV	3 / 3	No interference
PVB19	3 / 3	No interference
Staphylococcus aureus	3 / 3	No interference
Candida albicans	3 / 3	No interference

The presence of the tested potential interfering organisms showed no inhibition of the amplification of the HIV1 target using HIV1 ELITe MGB Kit.

Note: Even if HIV2 does not inhibit HIV1 detection, it can cause HIV1 over-quantification in samples from dually infected individuals due to HIV2 cross-reactivity.

Potential interfering substances

The effect of Potential interfering substances was evaluated by analyzing the panel "AcroMetrix® Inhibition Panel" (Thermo Fisher Scientific Inc.) containing endogenous substances, resulting from haemolysis, icterus and lipemia, and exogenous substances, EDTA and Heparin.

The samples of the inhibition panel were spiked with HIV1 certified reference material (PEI) at a concentration of 3 x LoD (about 180 IU / mL).

In addition, other 14 potential interfering substances were tested at relevant concentration: Ganciclovir, Azithromycin, Abacavir, Emtricitabine, Lamivudine, Tenofovir, Doravirine, Efavirenz, Rilpivirine, Atazanavir, Darunavir, Bictegravir, Elvitegravir, Maraviroc.

The substances were individually added to HIV1negative plasma collected in ACD spiked with HIV1 certified reference material (PEI) at a concentration of 3 x LoD (about 180 IU / mL).

The samples were processed in three replicates on **ELITe InGenius** system in "Extract + PCR" mode. The Ct values (reference and test samples) of the HIV1 target and the Internal Control were used to calculate the percentage Coefficient of Variability (%CV) in order to evaluate the possible interference.

The results are reported in the following table.

Sample	HIV1 Pos. / Rep.	HIV1 Ct %CV	IC Ct %CV	Outcome
EDTA	3 / 3	1.91	0.96	No interference
Heparin	0 / 3	N.A.	10.82	Interference
Haemolitic Blood high	3 / 3	0.99	1.35	No interference
Lipemic Plasma	3 / 3	0.94	0.44	No interference
Icteric Plasma	3 / 3	1.10	2.44	No interference
Ganciclovir	3 / 3	0.80	0.32	No interference
Azithromycin	3 / 3	0.92	0.41	No interference
Abacavir	3 / 3	1.61	0.53	No interference
Emtricitabine	3 / 3	1.06	0.65	No interference
Lamivudine	3 / 3	1.61	0.47	No interference
Tenofovir	3 / 3	0.84	0.92	No interference
Doravirine	3 / 3	1.03	0.59	No interference
Efavirenz	3 / 3	1.70	1.12	No interference
Rilpivirine	3 / 3	1.11	0.39	No interference
Atazanavir	3 / 3	0.87	0.73	No interference
Darunavir	3 / 3	1.53	0.91	No interference
Bictegravir	3 / 3	1.09	0.42	No interference
Elvitegravir	3 / 3	1.23	0.63	No interference
Maraviroc	3 / 3	1.68	0.78	No interference

Most of the tested substances do not interfere with the HIV1 or Internal Control amplification. The percentage %CV of Ct values were lower than 2.5%.

Heparin was confirmed to be capable of inhibiting the amplification of HIV1 but, thanks to the Internal Control Ct cut-off (IC Ct < 33), the samples result "invalid" and not "false negative".

Absence of cross-contamination

The Absence of cross-contamination was tested analyzing the results of five sessions in which HIV1 RNA negative plasma samples were alternated with plasma samples spiked by HIV1 certified reference material (ZeptoMetrix) at a concentration of $1x10^6$ IU/mL.

Five series of samples, alternating six positive samples with six negative samples, were tested on **ELITe InGenius** system in "Extract + PCR" mode.

The results are reported in the following table.

Samples	N	Negative	Positive
Plasma collected in ACD spiked at $1x10^6$ HIV1 IU/mL	30	0	30
Plasma collected in ACD negative for HIV1	30	30	0

None of the tested HIV1 negative samples gave false positive results. In this test no cross-contamination was detected intra-session and inter sessions.

Whole system failure rate

The Whole system failure rate, leading to false negative results, was verified in association with **ELITe InGenius**, by analysing a panel of samples spiked for HIV1 RNA at low titre.

100 different samples of plasma collected in EDTA and 30 different samples of Plasma collected in ACD, tested negative for HIV1 RNA were spiked with certified reference material (PEI) at a concentration of 3 x LoD (about 180 IU / mL). The samples were tested on ELITe InGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	N	Negative	Positive	Mean HIV1 IU/mL
Plasma collected in EDTA spiked by HIV1	100	0	100	247
Plasma collected in ACD spiked by HIV1	30	0	30	196

None of the tested HIV1 positive samples gave false negative results. In this test the whole system failure rate was equal to 0%.

The Whole system failure rate, leading to false negative results, was verified in association with **ELITe BeGenius** by analysing a panel of samples spiked for HIV1 RNA at low titre.

100 different samples of plasma collected in EDTA, tested negative for HIV1 RNA were spiked with certified reference material (PEI) at a concentration of 3 x LoD (about 180 IU / mL). The samples were tested on ELITe BeGenius system in "Extract + PCR" mode.

The results are summarized in the following table.

Samples	N	Negative	Positive	Mean HIV1 IU/mL
Plasma collected in EDTA spiked by HIV1	100	0	100	294

None of the tested HIV1 positive samples gave false negative results. In this test the whole system failure rate was equal to 0%.

Repeatability

The Repeatability of results obtained by the product HIV1 ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked by HIV1 certified reference material (PEI) at concentration of 3x LoD (about 180 IU / mL) and of 10x LoD (about 600 IU / mL).

The Repeatability was obtained through the analysis of panel samples in four replicates, in two runs per day, with the same lot of product, in two different days. Three lots of products were used on the same instrument by the same operator. Samples were processed in randomized positions on **ELITe InGenius** system in "Extract + PCR" mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability on ELITe InGenius

Sample	HIV1				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	Undet.	-	-	24 / 24	29.38	0.30	1.01
3x LoD	8 / 8	34.91	0.34	0.99				
10x LoD	8 / 8	33.08	0.24	0.73				

Inter – Session Repeatability on ELITe InGenius

Sample	HIV1				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	Undet.	-	-	48 / 48	29.40	0.27	0.91
3x LoD	16 / 16	35.00	0.37	1.07				
10x LoD	16 / 16	33.13	0.21	0.63				

In the Repeatability test on the **ELITe InGenius**, the assay detected the HIV1 target as expected and showed Ct values with %CV below 5 % for HIV1 and Internal Control.

The Repeatability of results obtained by the product HIV1 ELITe MGB Kit in association with the **ELITe BeGenius** system was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked by HIV1 certified reference material (4th WHO International Standard, NIBSC) at concentration of 3x LoD (about 180 IU / mL) and of 10x LoD (about 600 IU / mL).

The Repeatability was obtained through the analysis of panel samples in eight replicates, in one run per day, with the same lot of product, in two different days. Three lots of products were used on the same instrument by the same operator. Samples were processed in randomized positions on **ELITe BeGenius** system in "Extract + PCR" mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Repeatability as imprecision.

A summary of results is shown in the tables below.

Intra – Session Repeatability on ELITe BeGenius

Sample	HIV1				Internal Control			
	Pos./Rep.	Mean Ct	SD	% CV	Pos./Rep.	Mean Ct	SD	% CV
Negative	0 / 8	Undet.	-	-	24 / 24	29.71	0.40	1.34
3x LoD	8 / 8	35.90	0.72	2.02				
10x LoD	8 / 8	33.94	0.62	1.83				

Inter – Session Repeatability on ELITe BeGenius

Sample	HIV1				Internal Control			
	Pos./Rep.	Mean Ct	SD	% CV	Pos./Rep.	Mean Ct	SD	% CV
Negative	0 / 16	Undet.	-	-	48 / 48	29.78	0.32	1.06
3x LoD	16 / 16	35.99	0.59	1.64				
10x LoD	16 / 16	34.11	0.51	1.49				

In the Repeatability test on **ELITe BeGenius**, the assay detected the HIV1 target as expected and showed Ct values with %CV below 5 % for HIV1 and Internal Control.

Reproducibility

The Reproducibility of results obtained by the product HIV1 ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with HIV1 certified reference material (PEI) at concentration of 3 x LoD (about 180 IU / mL) and of 10 x LoD (about 600 IU / mL).

The Reproducibility was obtained through the analysis of panel samples in four replicates, in one run per day, in two days per site. Three different lots of product were used in three different sites with three different instruments by three different operators. Samples were processed in randomized positions on ELITe InGenius system in "Extract + PCR" mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Site Reproducibility on ELITe InGenius

Sample	HIV1				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 24	Undet.	-	-	72 / 72	29.51	0.40	1.36
3x LoD	24 / 24	35.01	0.66	1.88				
10x LoD	24 / 24	33.34	0.23	0.68				

Inter – Batch Reproducibility on ELITe InGenius

Sample	HIV1				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 48	Undet.	-	-	144 / 144	29.64	0.38	1.29
3x LoD	48 / 48	35.06	0.39	1.10				
10x LoD	48 / 48	33.31	0.32	0.97				

In the Reproducibility test on **ELITe InGenius**, the assay detected the HIV1 target as expected and showed Ct values with %CV below 5 % for HIV1 and Internal Control.

The Reproducibility of results obtained by the product HIV1 ELITe MGB Kit in association with the **ELITe BeGenius** system was tested by analysing a panel of Plasma samples. The panel included one negative sample and two samples spiked with HIV1 certified reference material (4th WHO International Standard, NIBSC) at concentration of 3 x LoD (about 180 IU / mL) and of 10 x LoD (about 600 IU / mL).

The Reproducibility was obtained through the analysis of panel samples in four replicates, in one run per day, in two days per instrument. Three different lots of product were used with three different instruments by three different operators. Samples were processed in randomized positions on **ELITe BeGenius** system in "Extract + PCR" mode.

The Ct values of the target and of Internal Control were used to calculate the %CV in order to evaluate the Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Instrument Reproducibility on ELITe BeGenius								
Sample	HIV1				Internal Control			
	Pos./Rep.	Mean Ct	SD	% CV	Pos./Rep.	Mean Ct	SD	% CV
Negative	0 / 24	Undet.	-	-	72 / 72	29.97	0.74	2.47
3 x LoD	24 / 24	35.86	0.59	1.65				
10 x LoD	24 / 24	34.12	0.41	1.19				

Inter – Batch Reproducibility on ELITe BeGenius								
Sample	HIV1				Internal Control			
	Pos./Rep.	Mean Ct	SD	% CV	Pos./Rep.	Mean Ct	SD	% CV
Negative	0 / 48	Undet.	-	-	144 / 144	29.92	0.56	1.87
3x LoD	48 / 48	35.79	0.67	1.87				
10x LoD	48 / 48	34.06	0.33	0.98				

In the Reproducibility test on **ELITe BeGenius**, the assay detected the HIV1 target as expected and showed Ct values with %CV below 5 % for HIV1 and Internal Control.

Conversion factor to International Units

The Conversion factor, to express the quantitative results in International Units / mL starting from copies / mL, was calculated using a panel of four dilutions (0.5 Log between dilutions) of the certified calibrated reference material "4th WHO HIV1 International Standard" (NIBSC) in Plasma collected in EDTA tested negative for HIV1 RNA.

Each point of the panel was tested in 27 replicates using three different lots of product on three different instruments in three different days. Samples were processed in randomized positions on ELITe InGenius system in "Extract + PCR" mode.

The Conversion factor was calculated by the analysis of the logarithmic concentration difference between the reference titre in IU / mL and the obtained results in copies / mL and it is equal to 2.3 IU / copy.

A summary of results is shown in the table below.

Conversion factor to International Units, Fc = 2.3 IU / copy						
Reference Sample			Result			Log difference (ref. - test)
IU / mL	Log IU / mL	N	Mean c. / mL	Mean IU / mL	Mean Log IU / mL	
10,000	4.0000	27	4,052	9,320	3.9623	+0.0377
3,162	3.5000	26	1,379	3,172	3.4913	+0.0087
1,000	3.0000	27	500	1,149	3.0494	-0.0494
316	2.5000	27	156	359	2.5366	-0.0366

As the equivalence between Plasma collected in EDTA and Plasma ACD was demonstrated (see Matrix Equivalence and Linear Measuring Range), the Conversion factor can be applied to the two matrices.

The Conversion factor, to express the quantitative results in International Units / mL starting from copies / mL, was verified on **ELITe BeGenius** and **ELITe InGenius** systems using a panel of dilutions (0.5 Log between dilutions) of the certified calibrated reference material (4th WHO International Standard, NIBSC) in Plasma collected in EDTA tested negative for HIV1 RNA. The panel consisted of five dilution points from about 4,0 Log IU/mL to 1.9 Log IU/mL. Each point of the panel was tested in 4 replicates.

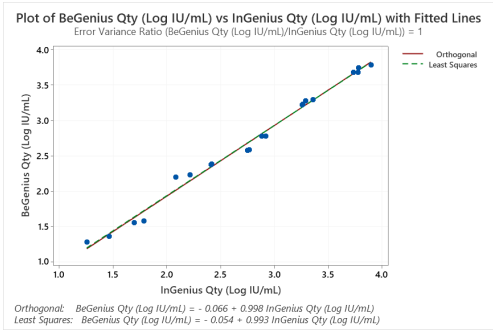
The target quantification precision, as standard deviation of Log IU/mL, was lower than 0.5 log.

The target quantification accuracy, as difference between Theoretical and Measured concentrations in Log IU/mL, was lower than 0.5 Log.

These results confirmed the Conversion factor calculated for Plasma samples with **ELITe InGenius.**

The results obtained by **ELITe InGenius** and **ELITe BeGenius** were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

The results are summed up in the following figure.



The Orthogonal Regression analysis generated an intercept equal to - 0.066 (95% CI: - 0.194; 0.061) and a slope equal to 0.998 (95% CI: 0.953; 1.042). The linear regression analysis generated a R2 of 0.991.

Reproducibility with Reference Material

The Reproducibility of the assay results compared with results obtained using other methods in different laboratories has been verified by testing the proficiency study panel "QCMD 2018 Human Immunodeficiency Virus RNA EQA Programme" (Qnostics).

Each point of the panel was tested on **ELITe InGenius** system in "Extract + PCR" mode.

The quantity values of the consensus of commercial real time amplification systems were compared with the results of the assay in order to evaluate the accuracy as bias.

The results are reported in the following table.

QCMD 2018 HIV1 panel		Consensus	Test results	Log difference (ref. - test)
Sample ID	Sample Content	Log c/mL	Log c/mL	
HIV1RNA18S-01	HIV1 Negative	n.a.	n.a.	n.a.
HIV1RNA18S-02	HIV1 Type B	3.777	3.920	-0.143
HIV1RNA18S-03	HIV1 Type B	3.767	3.908	-0.141
HIV1RNA18S-04	HIV1 Type B	2.315	2.449	-0.134
HIV1RNA18S-05	HIV1 Type AG	2.987	3.466	-0.479
HIV1RNA18S-06	HIV1 Type C	2.345	2.316	+0.029
HIV1RNA18S-07	HIV1 Type C	3.357	3.307	+0.050
HIV1RNA18S-08	HIV1 Type C	2.330	2.140	+0.190

In this test, the assay correctly detected all the panel members. The seven positive samples were quantified within the range of technology consensus ± 0.5 Log IU / mL.

The Reproducibility of the assay results has been also verified by testing the panel "HIV-1 RNA EDTA AccuSet™ Performance Panel" (SeraCare).

Each point of the panel was tested on ELITe InGenius system in "Extract + PCR" mode.

The reference quantification value of the samples obtained using the "COBAS AmpliPrep/COBAS TaqMan HIV-1 Test" (Roche Diagnostics) and the "Abbott m2000 RealTime HIV-1 Assay" (Abbott), provided by SeraCare, were compared with the results of the assay in order to evaluate the accuracy as bias.

The results are reported in the following tables.

SeraCare HIV1 panel		Roche COBAS results	Test results	Difference
Sample ID	Batch	Log c/mL	Log c/mL	(ref. - test)
SeraCare_01	10047508	2.7482	3.1209	-0.3727
SeraCare_02	10056461	n.a.	n.a.	n.a.
SeraCare_03	10047514	2.2405	2.2068	+0.0337
SeraCare_04	10047519	4.3546	4.2142	+0.1405
SeraCare_05	10044233	3.0799	3.0881	-0.0082
SeraCare_06	10056463	3.2601	3.5190	-0.2590
SeraCare_07	10047518	4.5548	4.8794	-0.3246
SeraCare_08	10047515	4.1651	4.0840	+0.0810
SeraCare_09	10047517	4.5124	4.6529	-0.1405
SeraCare_10	10047520	4.8556	4.8175	+0.0382
SeraCare_11	10057462	4.1700	4.1761	-0.0061
SeraCare_12	10057427	n.a.	n.a.	n.a.

SeraCare HIV1 panel		Abbott m2000 results	Test results	Difference
Sample ID	Batch	Log c/mL	Log c/mL	(ref. - test)
SeraCare_01	10047508	3.0934	3.1209	-0.0275
SeraCare_02	10056461	n.a.	n.a.	n.a.
SeraCare_03	10047514	2.1038	2.2068	-0.1030
SeraCare_04	10047519	4.3077	4.2142	+0.0935
SeraCare_05	10044233	3.1232	3.0881	+0.0351
SeraCare_06	10056463	3.5190	3.5190	+0.0000
SeraCare_07	10047518	4.5339	4.8794	-0.3456
SeraCare_08	10047515	4.2273	4.0840	+0.1433
SeraCare_09	10047517	4.7333	4.6529	+0.0804
SeraCare_10	10047520	4.7928	4.8175	-0.0247
SeraCare_11	10057462	4.0934	4.1761	-0.0826
SeraCare_12	10057427	n.a.	n.a.	n.a.

In this test, the assay correctly detected all the panel members. The ten positive samples were quantified within the range of reference result (commercial real time amplification systems) ± 0.5 Log IU / mL.

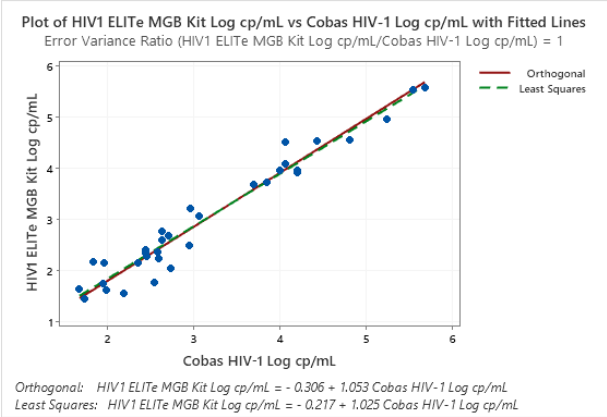
Diagnostic Sensitivity: method correlation

The diagnostic sensitivity of the assay, as correlation of results obtained with different methods, was evaluated in association with **ELITe InGenius** by analysing HIV1 RNA positive clinical patients undergoing antiviral therapy and within the measuring range of the HIV1 ELITe MBG Kit and of a CE IVD marked molecular diagnostic reference methods (“cobas HIV-1 for use on the 4800 System” and “cobas HIV-1 for use on the 6800 System”, Roche Diagnostics, Cobas HIV-1). As **ELITe BeGenius** has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic sensitivity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

The correlation study was performed at the site 1 on 33 HIV1 RNA positive clinical samples of Plasma collected in EDTA using the “cobas HIV-1 for use on the 6800 System” as comparator.

Each sample was tested carrying out the whole analysis procedure, extraction, reverse transcription, amplification, detection and result interpretation by the ELITechGroup S.p.A. products and by the reference methods. The results obtained by the HIV1 ELITe MBG Kit and the reference methods were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

The results are summed up in the following figure.



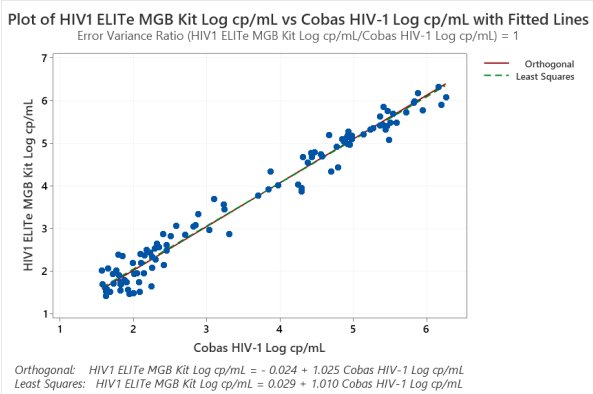
In this test, the orthogonal regression analysis generated a slope equal to 1.053 (95% IC: 0.967; 1.138) and an intercept equal to -0.306 (95% CI: - 0.591; - 0.020). The linear regression analysis generated an R2 of 0.950.

The correlation study was performed at site 2 and site 3 on the following 107 samples of Plasma collected in EDTA using the “cobas HIV-1 for use on the 4800 System” as comparator:

- site 2: 29 HIV1 RNA positive clinical samples of Plasma collected in EDTA,
- site 3: 78 HIV1 RNA positive clinical samples of Plasma collected in EDTA.

Each sample was tested carrying out the whole analysis procedure, extraction, reverse transcription, amplification, detection and result interpretation by the ELITechGroup S.p.A. products and by the reference methods. The results obtained by the HIV1 ELITe MBG Kit and the reference methods were analysed by orthogonal and linear regression in order to calculate the correlation between the methods.

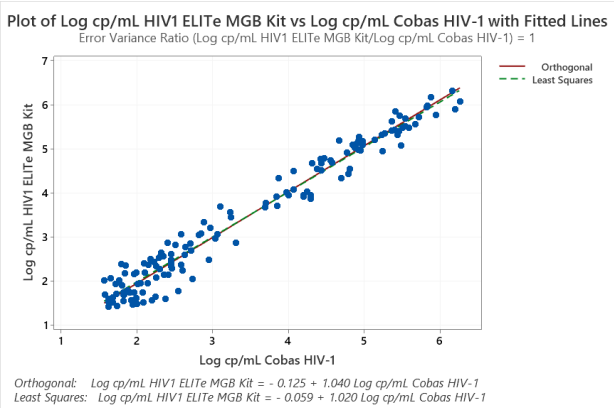
The results are summed up in the following figure.



In this test, the orthogonal regression analysis generated a slope equal to 1.025 (95% IC: 0.992; 1.059) and an intercept equal to -0.024 (95% CI: -0.152; 0.104). The linear regression analysis generated an R2 of 0.971.

As the two reference methods (“cobas HIV-1 for use on the 4800 System” and “cobas HIV-1 for use on the 6800 System”, Roche Diagnostics, Cobas HIV-1) have equivalent performances, the correlation study was also performed on the merged results of the three different sites.

The results are summed up in the following figure.



In this test, the orthogonal regression analysis generated a slope equal to 1.040 (95% CI: 1.006 - 1.073) and an intercept equal to -0.125 (95% CI: - 0.248 – 0.002). The linear regression analysis generated an R2 of 0.964.

Diagnostic Specificity: confirmation of negative samples

The Diagnostic Specificity of the assay, as percentage negative agreement of results obtained with different methods, was evaluated in association with **ELITe InGenius** by analysing HIV1 RNA negative clinical samples tested by a CE IVD marked molecular diagnostic reference methods (“cobas HIV-1 for use on the 4800 System” and “cobas HIV-1 for use on the 6800 System”, Roche Diagnostics, Cobas HIV-1). As **ELITe BeGenius** has equivalent analytical performances to ELITe InGenius, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic Specificity of the assay obtained in association with ELITe InGenius is also applicable to ELITe BeGenius.

As the two reference methods (“cobas HIV-1 for use on the 4800 System” and “cobas HIV-1 for use on the 6800 System”, Roche Diagnostics, Cobas HIV-1) have equivalent performances, the Diagnostic Specificity study was performed on the merged results of the three different sites on the following 196 samples of Plasma collected in EDTA:

- site 1: 90 HIV1 RNA negative clinical samples of Plasma collected in EDTA,
- site 2: 77 HIV1 RNA negative clinical samples of Plasma collected in EDTA,
- site 3: 29 HIV1 RNA negative clinical samples of Plasma collected in EDTA.

Each sample was tested carrying out the whole analysis procedure, extraction, reverse transcription, amplification, detection and result interpretation by the ELITechGroup S.p.A. products. The results obtained by the HIV1 ELITe MBG Kit were analysed in order to calculate the percentage negative agreement with the reference methods.

The results, after discrepant analysis, are summed up in the following table.

Samples	N	Positive	Negative	Invalid	Diagnostic Specificity
HIV1 RNA negative Plasma collected in EDTA	196	1	195	0	99.5%

In this test, 195 sample were confirmed negative. one sample gave a discordant positive result with titre lower than the LoD of the HIV1 ELITe MBG Kit and of the reference methods. This sample has a very low titre that could randomly generate positive calls. The Diagnostic Specificity of the HIV1 ELITe MBG Kit was equal to 99.5%.

Note: The complete data and results from the tests carried out to evaluate the product's performance characteristics with matrices and instrument are recorded in Section 7 of the Product Technical File for the "HIV1 ELITe MGB Kit", FTP 600ING.

REFERENCES

J. Müller et al. (2007) *J. Virol. Methods* 142: 127 - 135.
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30.

PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: Plasma collected in EDTA or ACD.

Plasma collected in EDTA or in ACD may be obtained from whole blood stored at +2 / +25 °C for no longer than 24 hours.

Do not use Plasma collected in heparin with this product: heparin inhibits the amplification reaction of nucleic acids and causes invalid results.

At the moment there are no data available concerning product performances with other clinical samples such as whole blood, serum or CSF.

This product is not intended for use as a screening test for the presence of HIV1 in blood or blood products or as a diagnostic test to confirm the presence of HIV1 infection.

This product shows cross-reactivity with HIV2 that can give positive results with a quantification 2,000 times lower than the theoretical HIV2 titre. Nevertheless, given product intended use, HIV2 epidemiology and implementation of clear diagnostic algorithms (e.g. CDC) aimed to distinguish HIV1 from HIV2 infection, cross-reactivity of HIV2 does not represent a real issue. However, HIV1 ELITe MGB Kit is not ideal for aid in the management of HIV1 and HIV2 dually infected individuals. In this case, HIV2 does not inhibit HIV1 detection, but it can cause HIV1 over-quantification due to HIV2 cross-reactivity.

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 product.

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.

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

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

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

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

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 delay in obtaining final results.

Possible polymorphisms, insertion or deletions within the region of the target RNA covered by the product primers and probes may impair detection and quantification of target RNA.

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 Q-PCR Standard reaction or Positive Control reaction **Invalid Standard curve**	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture, Q-PCR Standards and Positive Control. Check the volumes of complete reaction mixture, Q-PCR Standards and Positive Control.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Complete reaction mixture degradation or of its sub-components	Do not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Use a new aliquot of sub-components.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 2 independent sessions (2 hours each in the Extraction Area). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area). Use new aliquots of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

Invalid Negative Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of complete reaction mixture and Negative Control. Check the volumes of complete reaction mixture and Negative Control.
Contamination of the complete reaction mixture or of its sub-components	Prepare again the complete reaction mixture. Use a new aliquot of components
Contamination of the Negative Control.	Do not use the Negative Control for more than 1 session. Use a new aliquot of molecular biology grade water.
Contamination of the 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**
Instrument setting error.	Check the position of complete reaction mixture and sample. Check the volumes of complete reaction mixture and sample.
Complete reaction mixture preparation error.	Check the volumes of reagents used during the preparation of the complete reaction mixture.
Complete reaction mixture degradation or of its sub-components.	Do not use the complete reaction mixture for more than three sessions (7 hours in the Inventory Area). Do not leave the complete reaction mixture at room temperature for more than 30 minutes. Do not leave the RT EnzymeMix at temperatures higher than -20 °C for more than 10 minutes. Prepare again the complete reaction mixture. Use a new aliquot of sub-components.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to sample interfering substances.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in a “Extract + PCR” session.
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 1:10 dilution in molecular biology grade water of the primary sample in a “Extract + PCR” session.

TH Error, SDM Error, Ct Error	
Possible Causes	**Solutions**
Sample with anomalous plot shape.	If significant amplification is observed in PCR plot generating error: - 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 1:10 dilution in molecular biology grade water of the primary sample in a “Extract + PCR” session.

SYMBOLS

Catalogue Number.


Upper limit of temperature.

Batch code.


Use by (last day of month).

in vitro diagnostic medical device.

Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device. Certification released by DEKRA Certification B.V., the Netherland.

Contains sufficient for "N" tests.


Attention, consult instructions for use.

Contents.


Keep away from sunlight.


Manufacturer.

NOTICE TO PURCHASER: LIMITED LICENSE

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

ELITe MGB® detection reagents are covered by one or more of U.S. Patent numbers 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.

ELITe InGenius® and ELITe BeGenius® technology is covered by patents and requests for patents.

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

HIV1 ELITe MGB® Kit used in association with Genius series®
Ref: RTK600ING

Caution, this document is a simplified version of the official instruction for use. This document is available only in English.
Please refer to the complete document before use: www.elitechgroup.com

Intended use

The "HIV1 ELITe MGB® Kit" product is a quantitative nucleic acids reverse transcription and amplification assay for the detection and the quantification of the RNA of Human Immunodeficiency Virus type 1 (HIV1) in RNA samples extracted from clinical specimens.
The assay is able to detect the RNA of HIV1 belonging to group M (subtypes A, B, C, D, F, G, H, J, K, L), group O, group N and major CRF subtypes CRF01-AE, CRF02-AG and CRF03-AB.
The assay is validated in association with "ELITe InGenius®" and "ELITe BeGenius®" system starting from human plasma collected in EDTA or in ACD samples.
The product is intended for use as an aid in the management of HIV1-infected individuals undergoing antiviral therapy, together with patient's clinical data and other laboratory test results.

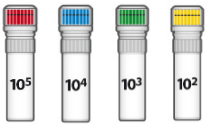
Amplified sequence

Sequence	Gene	Fluorophore	Channel
Target	HIV1 polymerase gene (integrase region)	FAM	HIV1
Internal Control	MS2	AP525	IC

Validated matrix

› **Plasma EDTA**
› **Plasma ACD**

Kit content

HIV1 ELITe MGB Mix		HIV1 ELITe Standard	HIV1 - ELITe Positive Control	HIV1 Internal Control
 X 4	 X 2	 X 1	 X 2	 X 8
HIV PCR Mix 4 tubes of 600 µL 96 reactions per kit 5 freeze-thaw cycles	RT EnzymeMix 2 tubes of 20 µL 96 reactions per kit 10 freeze-thaw cycles	Ready-to-use Calibrators: 10^5, 10^4, 10^3, 10^2 1 set of 4 tubes of 160 µL 2 freeze-thaw cycles	Ready-to-use PC 2 tubes of 160 µL 8 reactions per kit 4 freeze-thaw cycles	Ready-to-use IC 8 tubes of 160 µL 96 extractions per kit 12 freeze-thaw cycles

Maximum shelf-life: **18 months**
Storage Temperature: **-20 °C**

Material required not provided in the kit

› ELITe InGenius instrument: INT030
› ELITe BeGenius instrument: INT040
› ELITe InGenius SP 1000 Extraction Cartridge: INT033SP1000
› ELITe InGenius PCR Cassette: INT035PCR
› ELITe InGenius SP200 Consumable Set: INT032CS
› ELITe InGenius Waste Box: F2102-000
› Filter Tips 300 µL: TF-350-L-R-S
› 1000 µL Filter Tips Tecan: 30180118

ELITe InGenius and ELITe BeGenius protocol

› Sample volume	600 µL	› Unit of quantitative result	International Unit: IU/mL Copies: Copies/mL
› HIV1 CPE volume	10 µL		
› Total elution volume	50 µL	› Conversion factor to IU	2.3 IU/copy
› PCR elution input volume	20 µL	› Frequency of controls	15 days
› Complete PCR Mix volume	20 µL	› Frequency of calibration	60 days

ELITe InGenius and ELITe BeGenius Performance

Matrix	Limit of Detection	Method Correlation	Diagnostic Specificity
Plasma	**60 IU / mL** 26 copies / mL	**R^2 = 0.964** *141 quantified samples*	**99.5%** 195 *confirmed samples / 196 tested samples*

reference methods:
"cobas® HIV1 for use on the 4800 Systems" and
"cobas® HIV1 for use on the 6800 Systems", Roche Diagnostics.

Sample preparation

Plasma samples collected in EDTA or ACD samples must be identified according to laboratory guidelines, transported and stored at room temperature (~+25 °C) for a maximum of one day or at +2 / +8 °C for a maximum of three days. Otherwise, they must be frozen and stored at ~-20 °C for a maximum of one month or at ~-70 °C for six months. Do not use Plasma collected in heparin in order to prevent inhibition of amplification reaction and frequent invalid results.

ELITe InGenius Procedures

The user is guided step-by-step by the ELITe InGenius software to prepare the run. All the steps: extraction, reverse transcription, amplification and result interpretation are automatically performed. Three operational modes are available: complete run, or extraction only, or PCR only.

Before analysis

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

2. Verify calibrators: **HIV1 Q-PCR Standard** in the "Calibration" menu
Verify controls: **HIV1 Positive Control** and **HIV1 Negative Control** in the "Controls" menu
Note: Both must have been run, approved and not expired

3. Thaw the **HIV1 PCR Mix** and the **HIV1 CPE** tubes
Vortex gently
Spin down 5 sec
Keep the **RT EnzymeMix** in ice

4. Prepare the complete reaction mixture

Sample Number (N)	HIV1 PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

5. Vortex gently
Spin down 5 sec
Keep the complete reaction mixture in ice. Do not expose to direct light.

Procedure 1 - Complete run: Extraction + PCR

1. Select "Perform Run" on the touch screen

2. Verify the extraction volumes: Input: "1000 µL", elution: "50 µL"

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

4. Select the "Assay protocol" of interest: HIV1 ELITe_PL_600_50

5. Select the method "Extract + PCR" and the sample position: Extraction Tube

6. Load the complete reaction mixture and the Internal Control in the inventory block

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

8. Close the door
Start the run

9. View, approve and store the results

Procedure 2 - PCR only

1 to 4: Follow the Complete Run procedure described above

5. Select the method "PCR only" and set the sample position "Elution Tube"

6. Load the complete reaction mixture in the inventory block

7. Load: PCR cassette rack and the Elution tube rack with the extracted nucleic acid

8. Close the door
Start the run

9. View, approve and store the results

Procedure 3 - Extraction only

1 to 4: Follow the Complete Run procedure described above

5. Select the method "Extraction Only" and set the sample position: Extraction Tube

6. Load the Internal Control in the inventory block

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

8. Close the door
Start the run

9. Archive the eluate sample

ELITe BeGenius Procedures

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

Before analysis

1. Switch on ELITe BeGenius.
Log in with username and password
Select the mode "Closed"

2. Verify calibrators: **HIV1 Q-PCR Standard** in the "Calibration" menu
Verify controls: **HIV1 Positive Control** and **HIV1 Negative Control** in the "Controls" menu
Note: Both must have been run, approved and not expired

3. Thaw the **HIV1 PCR Mix** and the **HIV1 CPE** tubes
Vortex gently
Spin down 5 sec
Keep the **RT EnzymeMix** in ice

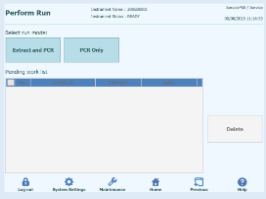
4. Prepare the complete reaction mixture

Sample Number (N)	HIV1 PCR Mix	RT EnzymeMix
1 ≤ N ≤ 5	(N + 1) x 20 µL	(N + 1) x 0.3 µL
6 ≤ N ≤ 11	(N + 2) x 20 µL	(N + 2) x 0.3 µL
N = 12	290 µL	4.4 µL

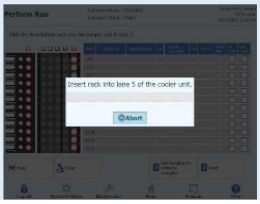
5. Vortex gently
Spin down 5 sec
Keep the complete reaction mixture in ice. Do not expose to direct light.

Procedure 1 - Complete run: Extraction + PCR

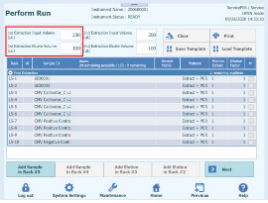
1. Select "Perform Run" on the touch screen and then click on the run mode «Extraction and PCR»



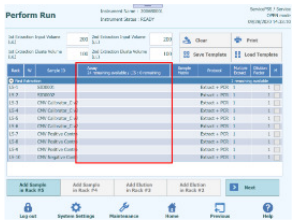
2. Insert the Sample Rack with the barcoded samples in the cooling area. The barcode scan is already active



3. Verify the extraction volumes: Input: "600 µL", Eluate: "50 µL"

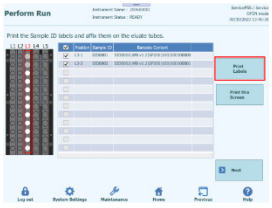


4. Select the "Assay protocol" of interest



Note: if a second extraction is performed repeat steps from 2 to 4

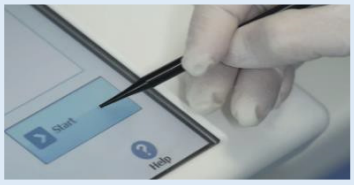
5. Print the labels to barcode the empty elution tubes. Load the tubes in the Elution Rack and insert it in the cooling area



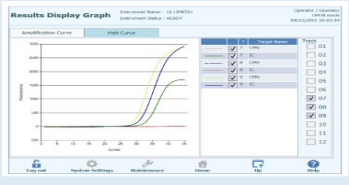
6. Load the complete reaction mixture and the CPE Internal Control in Reagent Rack and insert it in the cooling area

7. Load: Filter Tips, Extraction rack, and PCR rack

8. Close the door. Start the run



9. View, approve and store the results



Procedure 2 - PCR only

1. Select "Perform Run" on the touch screen and the click on the run mode «PCR Only»	**2.** Load the extracted nucleic acid barcoded tubes in the Elution Rack and insert it in the cooling area	**3.** Select the "Assay protocol" of interest
4. Load the complete reaction mixture in Reagent Rack and insert it in the cooling area Load filter tips and the PCR rack	**5.** Close the door. Start the run	**6.** View, approve and store the results

Procedure 3 - Extraction only

1 to 4 : Follow the Complete Run procedure described above	**5.** Select the protocol "Extraction Only" in the Assay Protocol selection screen.	**6.** Load the CPE Internal Control in the Elution Rack and insert it in the cooling area
7. Load : Filter Tips and the Extraction Rack	**8.** Close the door Start the run	**9.** Archive the eluate sample