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NOTICE of CHANGE dated 06/12/2022

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«Adenovirus ELITe MGB® Kit»
Ref. RTS078PLD

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

- *Update of LoD and LloQ for whole blood and plasma*

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
	DIE REVIEW VON DIESER IFU IST KOMPATIBLE MIT DER VORIGE VERSION VON DEM TEST-KIT

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EMPOWERING IVD

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ADENOVIRUS ELITe MGB® Kit

reagent for DNA Real Time amplification

REF RTS078PLD

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INTENDED USE

The **«ADENOVIRUS ELITe MGB® Kit»** product is part of a qualitative and quantitative nucleic acids amplification assay for the **detection and quantification of the DNA of human Adenovirus (ADV)**, genotypes A, B, C, D, E, F and G (including 57 serotypes), in DNA samples extracted from whole blood collected in EDTA, plasma collected in EDTA, nasal washes and nasal swabs.

The product is intended for use in the diagnosis and monitoring of Adenovirus infections alongside clinical data of the patient and other laboratory tests outcomes.

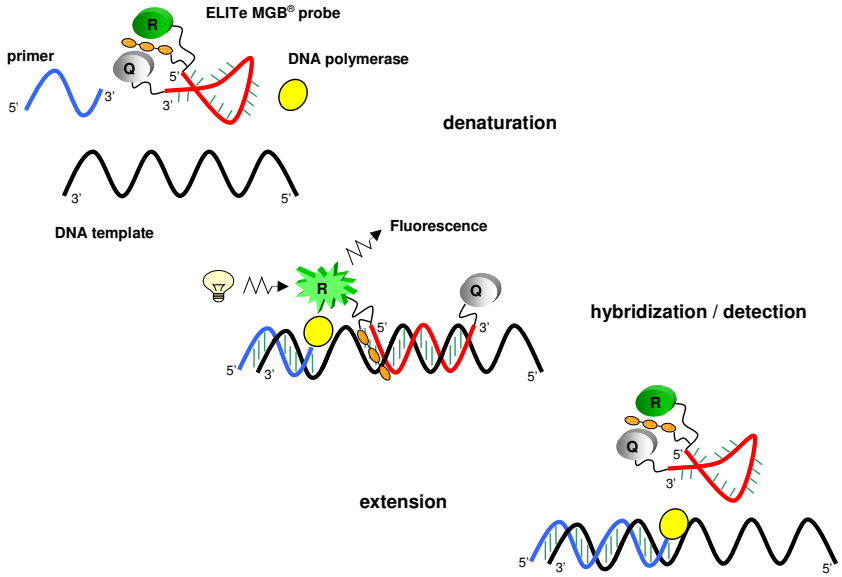
ASSAY PRINCIPLES

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

In each well, two amplification reactions are performed starting from DNA extracted from the samples being tested: a specific reaction for a region of the **Hexon** protein gene of ADV and a specific reaction for a region of the human **beta Globin** gene (Internal Control of inhibition). The ADV specific probe with ELITe MGB® technology, labelled with FAM fluorophore, is activated when hybridizes with the specific product of the ADV amplification reaction. The Internal Control specific probe with ELITe MGB® technology, labelled with AP525 fluorophore (analogous to VIC), is activated when hybridizes with the specific product of the 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 allows detecting the presence and the titre of ADV DNA in the starting sample.

The assay is validated with the systems described in this user manual.

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



PRODUCT DESCRIPTION

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

The primers and the ADV specific probe (stabilized by MGB® group, labelled with FAM fluorophore and quenched by a non-fluorescent molecule) are specific for a region of the **Hexon** protein gene of ADV.

The primers and the probe for the Internal Control (stabilized by MGB® group, labelled with AP525 fluorophore, analogous to VIC, and quenched by a non-fluorescent molecule) are specific for the **promoter and 5' UTR** region of the human **beta Globin** gene.

The reaction mixture provides buffer, magnesium chloride, triphosphate nucleotides, AP593 fluorophore (used instead of ROX or CY5) as passive reference for fluorescence normalisation, the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by the amplification product, the "hot start" DNA polymerase enzyme.

The product is sufficient for **96 tests in association with «ELITe InGenius®»** and **«ELITe BeGenius®»** systems, including standards and controls.

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

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
ADV Q - PCR Mix	complete reaction mixture	**4 x 540 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).
- Molecular biology grade water.
- Programmable thermostat with optical fluorescence detection system 7300 Real Time PCR System or 7500 Fast Dx Real-Time PCR Instrument calibrated following manufacturer's instructions.
- Programmable thermostat with optical fluorescence detection system cobas z 480 analyzer, calibrated following manufacturer's instructions.

OTHER PRODUCTS REQUIRED

The reagents for the extraction of DNA from the samples, the positive control of extraction, the positive control of the amplification, the known quantity DNA standards and the consumables **are not** included in this product.

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For automatic DNA extraction, amplification and interpretation of sample analysis with the instrument **«ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) the following generic products are required: the extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200), the consumables for extraction and amplification of nucleic acids from biological samples **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS), **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000), **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR) and **«300 µL Universal Filter Tips »** (Axygen BioScience Inc., CA, USA, ref. TF-350-L-R-S).

For automatic DNA extraction, amplification and interpretation of sample analysis, the instrument **« ELITe InGenius»** (ELITechGroup S.p.A., ref. INT030) and the following specific Assay protocols (ELITechGroup S.p.A.), are required:

for the calibrators **«ADV ELITe_STD»**,
for the positive control of amplification **«ADV ELITe_PC»**,
for negative control of amplification **«ADV ELITe_NC»**,
for samples analysis **«ADV ELITe_WB_200_100»** and **«ADV ELITe_PL_200_100»**

For automatic sample analysis with the instrument **«ELITe BeGenius»** (ELITechGroup S.p.A., ref. INT040) it is validated the use of the following generic product: the extraction cartridges **«ELITe InGenius® SP 200»** (ELITechGroup S.p.A., ref. INT032SP200), the consumables for extraction and amplification of nucleic acids from biological samples **«ELITe InGenius® SP 200 Consumable Set»** (ELITechGroup S.p.A, ref. INT032CS), **«ELITe InGenius® Waste Box»** (ELITechGroup S.p.A, ref. F2102-000), **«ELITe InGenius® PCR Cassette»** (ELITechGroup S.p.A, ref. INT035PCR) and **«1000 µL Filter Tips Tecan»** (Tecan, Switzerland, ref. 30180118).

For automatic DNA extraction, amplification and interpretation of sample analysis, the instrument **«ELITe BeGenius®»** (ELITechGroup S.p.A., ref. INT040) and the following specific Assay protocols (ELITechGroup S.p.A.), are required:

for the calibrators **«ADV ELITe_Be_STD»**,
for the positive control of amplification **«ADV ELITe_Be_PC»**,
for negative control of amplification **«ADV ELITe_Be_NC»**,
for samples analysis **«ADV ELITe_Be_WB_200_100»** and **«ADV ELITe_Be_PL_200_100».**

For automatic DNA extraction from samples to be analyzed, it is validated the use of generic product **«ELITe STAR 200 Extraction kit»** (ELITechGroup S.p.A., ref. INT011EX), kit for extraction of DNA and RNA from non-cellular and cellular samples with the **«ELITe STAR»** instrument (ELITechGroup S.p.A., ref. INT010).

For automatic DNA extraction and preparation of microplates for amplification of samples to be analyzed, it is validated the use of generic product **«ELITe GALAXY 300 Extraction Kit»** (ELITechGroup S.p.A., ref. INT021EX), kit for extraction of DNA and RNA from non-cellular and cellular samples with the instrument **«ELITe GALAXY»** (ELITechGroup S.p.A., ref. INT020). The instrument «ELITe GALAXY» can also carry out the PCR Setup.

For automatic DNA extraction from samples to be analyzed, the generic products **«NucliSENS® easyMAG® Reagents»** (bioMérieux SA, ref. 280130, 280131, 280132, 280133, 280134, 280135), kits for extraction of nucleic acid from biological samples, with the instrument **«NucliSENS® easyMAG®»** (bioMérieux SA, ref. 200111) are also validated.

For automatic DNA extraction from samples to be analyzed, the products **«QIAsymphony® DNA Mini Kit»** (QIAGEN GmbH, ref. 931236) and **«QIAsymphony® DSP Virus / Pathogen Midi kit»** (QIAGEN GmbH, ref. 937055), kits for extraction of nucleic acid from biological samples, with the instrument **«QIAsymphony® SP/AS»** (QIAGEN GmbH, ref. 9001297, 9001301) and related generic products are also validated.

For automatic DNA extraction from samples to be analyzed, the product **«MagNA Pure 24 Total NA Isolation Kit»** (Roche, ref. 07658036001), kit for extraction of nucleic acid from biological samples, with the instrument **«MagNA Pure 24 System»** (Roche, ref. 07290519001) is also validated.

When a 7300 Real-Time PCR System is used, it is required the use of generic product **«MicroAmp™ Optical 96-Well Reaction Plate»** (Life Technologies, ref. N8010560), microplates with 0.2 mL wells and adhesive sealing sheets for real time amplification.

When a 7500 Fast Dx Real-Time PCR Instrument is used, it is required the use of generic product: **«MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL»** (Life Technologies, ref. 4346906), microplates with 0.1 mL wells and adhesive sealing sheets for real time amplification.

When a cobas z 480 analyzer is used, it is required the use of generic product **«AD-plate 0.3ml»** (Roche, ref. 05232724001), microplates with 0.3 mL wells and adhesive sealing sheets for real time amplification.

If detection of ADV DNA is required for qualitative analysis, use the product **«ADENOVIRUS - ELITe Positive Control»** (ELITechGroup S.p.A., ref. CTR078PLD) or the product **«ADENOVIRUS - ELITe Positive Control RF»** (ELITechGroup S.p.A., ref. CTR078PLD-R), positive control of plasmid DNA.

If detection and quantification of ADV DNA is required for quantitative analysis, use the product **«ADENOVIRUS ELITe Standard»** (ELITechGroup S.p.A., ref. STD078PLD), four dilutions of known quantity plasmid DNA to obtain the standard curve.

As positive control of nucleic acids extraction from non-cellular samples and inhibition control, it is required the use of generic product **«CPE - Internal Control»** (ELITechGroup S.p.A., ref. CTRCPE), a stabilised plasmid solution containing two plasmid DNAs and genomic RNA of MS2 phage.

WARNINGS AND PRECAUTIONS

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

General warnings and precautions

Handle and dispose of all biological samples as if they were able to transmit infective agents. Avoid direct contact with 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 in the product before running the assay.
While running the assay, follow the instructions provided in the product.
Do not use the product after the indicated expiry date.
Only use the reagents provided in the product and those recommended by the manufacturer.
Do not use reagents from different batches.
Do not use reagents from other manufacturers.

Warnings and precautions for molecular biology

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

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

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

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The samples must be exclusively used for this type of analysis. Samples must be handled under a laminar airflow hood. Tubes containing different samples must never be opened at the same time. 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, free from DNA and RNA.

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

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

Warnings and precautions specific for the components

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

The **ADV Q - 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 **ADV Q - PCR Mix** can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each.

ELITe InGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with clinical samples:

Whole blood collected in EDTA

The whole blood samples for DNA extraction must be collected in EDTA and identified according to laboratory guidelines, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of three days, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods.

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

N.B.: when the DNA extraction from whole blood is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the extraction protocol **ADV ELITe_WB_200_100**. These protocols process 200 µL of sample, add the **CPE** Internal Control at 10 µL per extraction and elute the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of tube loaded. Refer to the instruction for use of the extraction kit for more information.

Plasma collected in EDTA

The plasma samples for nucleic acid extraction must be collected in EDTA and identified according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of three days, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

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

Note: when the DNA extraction from 200 µL of plasma is carried out with the **ELITe InGenius** and with **ELITe InGenius Software** version 1.3 (or later equivalent versions), use the extraction protocol **ADV ELITe_PL_200_100**. These protocols process 200 µL of sample, add the **CPE** Internal Control at 10 µL per extraction and elute the nucleic acids in 100 µL

When the primary tube is used, the volume of the sample varies according to the type of tube loaded. Refer to the instruction for use of the extraction kit for more information.

Other samples

There are no data available concerning product performance with DNA extracted from the following clinical samples: nasal washes, nasal swabs, fecal supernatant and cerebrospinal fluid.

Interfering substances

The DNA extracted from the sample must not contain heparin, in order to prevent inhibition problems and the possibility of frequent invalid results.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

Amplification calibrators and 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 **ADENOVIRUS ELITe Standard**, in association with protocol **«ADV ELITe_STD»**,
- as amplification Positive Control use the **ADENOVIRUS - ELITe Positive Control**, in association with protocol **«ADV ELITe_PC»,**
- as amplification Negative Control, use molecular grade water (not provided with this kit) in association with protocol **«ADV ELITe_NC»,**

Note: ELITe InGenius with **ELITe InGenius Software** require 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.
The Calibrators and amplification Controls must be retested if any of the following events occurs:
- a new lot of amplification reagents is started,
- the results of Quality Control analysis (see following paragraph) are out of specification,
- any major maintenance is performed on the **ELITe InGenius** instrument.

ELITe InGenius® PROCEDURE

The procedure to use the **ADENOVIRUS ELITe MGB® Kit** with the system **ELITe InGenius** consists of three steps:

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

Verification of the system readiness

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

- switch on the **ELITe InGenius** and select the mode "**CLOSED**";
- verify that the Calibrators (**ADV Q-PCR Standard**) have been run, approved and are not expired (status). This can be checked under the "Calibration" menu in the Home page;
- verify (Controls) that the amplification Controls (**ADV Positive Control, ADV Negative Control**) have been run, approved and are not expired (status). This can be checked under the "Control" menu in the Home page;
- choose the type of run and set up the run, following the instructions of Graphical User Interface (GUI) for the session set up and using the Assay Protocols provided by ELITechGroup. These IVD protocols were specifically validated with ELITe MGB® kits matrices and ELITe InGenius instrument.

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The Assay protocols available for **«ADENOVIRUS ELITe MGB® Kit»** are described in the table below.

Assay protocol for ADENOVIRUS ELITe MGB kit and ELITe InGenius			
Name	**Matrix**	**Report unitage**	**Characteristics**
ADV ELITe_WB_200_100	Whole Blood	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
ADV ELITe_PL_200_100	Plasma	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Protocols for qualitative analysis are available on request.

Setup of the session

The **ADENOVIRUS ELITe MGB® Kit** in association to the **ELITe InGenius** can be used in order to perform:

A. Integrated run (Extract + PCR)
B. Amplification run (PCR only)
C. Calibration run (PCR only)
D. Amplification Positive and/ or Negative Control run (PCR only)

The amplification thermal profile is included in the assay protocols available with the instrument and is automatically recalled when the assay protocol is selected.

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

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

A. Integrated run

To set up the integrated run carry out the steps below following the **SW Graphical User Interface (GUI)**:

1. Thaw ADV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in a sufficient number for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

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

2. Thaw a sufficient number of CPE tubes for the session. Each tube is sufficient for 12 extractions. Mix gently, spin down the content for 5 seconds.
3. Select "Perform Run" from the "Home".
4. Select the Extraction Input Volume: 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and ensure that the Extracted Elute Volume is 100 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 (i.e. ADV ELITe_WB_200_100).
7. Ensure that the "Protocol" displayed is: "Extract + PCR".

8. Select the sample loading position in the “Sample Position” column:
   - if a primary tube is used select “Primary Tube”, the Primary tube can be use only starting from 200 µL of samples.
   - if a secondary tube is used select “Extraction Tube”.
   - Click “Next” to continue the setup.
9. Load CPE and ADV Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the “PCR Cassette”, the “ELITe InGenius SP 200” or “ELITe InGenius SP1000” extraction cartridges, all the required consumables and the samples to be extracted following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

After process completion, the **ELITe InGenius** allows 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 any spilling of the extracted sample.

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

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

B. Amplification run

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

1. Thaw ADV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in a sufficient number for the session. Each tube is sufficient for 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

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

2. Select “Perform Run” from the “Home”.
3. Even if no extraction will be carried out , ensure that the Extraction Input Volume is 200 µL to process 200 µL of sample or 1000 µL to process 1000 µL of sample and that the Extracted Elute Volume is 100 µL.
4. For each Track of interest type the “SampleID” (SID) by typing or by scanning the sample barcode.
5. Select the assay protocol to be used in the “Assay” column (i.e. ADV ELITe_WB_200_100).
6. Select “PCR Only” in the “Protocol” column.
7. Ensure the Eluted sample loading position in the “Sample Position” column is “Elution tube (bottom raw)”. Click “Next” to continue the setup.
8. Load ADV Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
9. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load the “PCR Cassette”, and the extracted Nucleic Acid samples following the GUI instruction. Click “Next” to continue the setup.
11. Close the instrument door.

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12. Press “Start” to start the run.

After process completion, the **ELITe InGenius** 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 and stored at -20 °C. Avoid any 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 disposed of without producing environmental contaminations. Avoid any spilling of the reaction products.

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

C. Calibration run

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

1. Thaw ADV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in a sufficient number for the sessio.. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

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

2. Thaw ADV Q - PCR Standard tubes (Cal1: ADV Q-PCR Standards 10^2, Cal2: ADV Q-PCR Standards 10^3, Cal3: ADV Q-PCR Standards 10^4, Cal4: ADV Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Select “Perform Run” from the “Home screen”.
4. Even if no extraction will be carried out, ensure that the “Extraction Input Volume” is 200 µL to process 200 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
5. Starting from the Track of interest, select the assay protocol to be used in the “Assay” column (ADV ELITe_STD) and fill with the lot number and expiry date for the ADV Q - PCR Standard. Click “Next” button to continue the setup.
6. Load the ADV Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
7. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
8. Load the **ADV Q-PCR Standard** tubes and “PCR Cassette” on board, following the GUI instruction. Click “Next” to continue the setup.
9. Close the instrument door.
10. Press “Start” to start the run.

After process completion, the **ELITe InGenius** 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.

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

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. 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 set up the amplification Positive Control and Negative Control run carry on the steps below following the GUI:

1. Thaw ADV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in a sufficient number for the session. Each tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

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

2. Thaw ADV - ELITe Positive Control tubes at room temperature (~+25°C) for 30 minutes, for Positive Control amplification. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. Transfer at least 50 µL of molecular biology grade water for the sessions in one Elution tube, provided with the ELITe InGenius® SP Consumable Set.
4. Select “Perform Run” from the “Home screen”.
5. Even if no extraction will be carried out, ensure that the Extraction Input Volume: 200 µL to process 200 µL of sample and ensure that the Extracted Elute Volume is 100 µL.
6. For the positive control, select ADV ELITe_PC and fill in the lot number and expiry date for the ADV Positive Control.
7. For the negative control, select ADV ELITe_NC and fill in the lot number and expiry date for the molecular biology grade water.
8. Click “Next” to continue the setup.
9. Load ADV Q-PCR Mix on the “Inventory Block” selected by following the GUI instruction. Click “Next” to continue the setup.
10. Load and check the Tip Racks in the “Inventory Area” selected by following the GUI instruction. Click “Next” to continue the setup.
11. Load the amplification “PCR cassette”, the ADV Positive Control tube and/or the Negative Control tube, following the GUI instruction. Click “Next” to continue the setup.
12. Close the instrument door.
13. Press “Start” to start the run.

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

Note: The Positive Control and the Negative Control must be run as amplification control, to set up the “Control Charts”. Four Positive Control and Negative Control results, from 4 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: At the end of the run the remaining Positive Control can be removed from the instrument, capped and stored at -20 °C. The remaining Negative Control must be disposed.

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

Note: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

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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”).

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

The **ELITe InGenius** generates the results using the **«ADENOVIRUS 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.

A. Validation of Calibration curve

The fluorescence signals emitted by the specific ADV probe ("ADV") in the Calibrator amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol “ADV ELITe_STD”.

The Calibration curve, specific for the amplification reagent lot, is stored in the database after the approval of the “Administrator” or “Analyst” personnel by following the GUI instruction.
The Calibration curve, specific for the amplification reagent lot, will expire after 60 days.

Before analysing any sample, it is absolutely mandatory to generate and to approve the Calibration curve for the lot of amplification reagent used. The availability of Calibration curve results with “Approved” (Status) is shown in the “Calibration” window of the ELITe InGenius software.

Note: When the Calibration curve does not meet the acceptance criteria, the “not passed” message is shown on the “Calibration” menu and it is not possible to approve the curve. The Calibrator amplification reactions have to be repeated.

Note: When the Calibration Curve is run together with samples and its result is invalid, the entire session is invalid and the amplification of all samples must be repeated.

B. Validation of amplification Positive Control and Negative Control result

The fluorescence signals emitted by the specific ADV probe ("ADV ") in the Positive Control and Negative Control amplification reactions are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocols “ADV ELITe_PC” and “ADV ELITe_NC”.

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

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

Before analysing any sample and after approval of the Calibration curve, it is absolutely mandatory to generate and to approve an amplification Positive Control and Negative Control results for the lot of amplification reagent used. The availability of an amplification Positive Control and Negative Control results with “Approved” (Status) is shown in the “Controls” window of the ELITe InGenius software. If the amplification Positive Control and Negative Control results are missing, generate them as described above.

Note: When the Positive Control or Negative Control result does not meet the acceptance criteria, the “not passed” message is shown on the “Controls” menu and it is not possible to approve the result. The Positive Control or Negative Control amplification reaction has to be repeated.

Note: When the Positive Control or Negative Control are run as an amplification control together with samples and its result is invalid, the entire session is invalid and the amplification of all samples must be repeated.

C. Validation of Samples results

The fluorescence signals emitted by the specific ADV probe ("ADV") and by the specific Internal Control probe ("IC") in each sample amplification reaction are analysed automatically and interpreted by the instrument software with the parameters included in the assay protocol.

Note: Before analysing any sample, it is mandatory to generate and to approve the Calibration curve and the amplification Controls for the lot of reagent used. It is recommended, but optional, to run Positive and Negative Control together with the Calibrators. The availability of a Calibration curve and amplification Positive and Negative Control results with "Approved" (Status) is shown in the "Calibration" and "Controls" windows of the GUI.

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

The Sample run is valid when the three conditions reported in the table below are met.

1) Calibration curve	**Status**
ADV Q-PCR Standard	APPROVED
2) Positive Control	**Status**
ADV Positive Control	APPROVED
3) Negative Control	**Status**
ADV Negative Control	APPROVED

For each Sample the calculation of the viral load is automatically performed by **ELITe InGenius software** as established by the algorithm and the assay protocol parameters.

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

Result of Sample run	Interpretation
ADV: DNA Detected, quantity equal to XXX copies / mL	**ADV DNA detected** within the measurement range of the assay, quantity as shown.
ADV: DNA Detected, quantity below LLoQ copies / mL	**ADV DNA detected** below the lower limit of quantification of the assay
ADV: DNA Detected, quantity beyond ULoQ copies / mL	**ADV DNA detected** beyond the upper limit of quantification of the assay
ADV: DNA Not Detected or below LoD copies / mL	**ADV DNA not detected or below the Limit of Detection** of the assay.
Invalid - Retest Sample	**Not valid assay result** due to Internal Control failure (extraction failure or presence of inhibitor).

Samples not suitable for analysis are reported as "Invalid - Retest Sample" by the ELITe InGenius software. In this case, the Internal Control DNA was not detected due to potential problems in the amplification or extraction step (degradation of DNA, loss of DNA during the extraction or inhibitors carry-over in the eluate) that may lead to false negative call.

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

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

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 "Administrator" or "Analyst" personnel by 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".

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D. Samples result reporting

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

The "Sample Report" shows the details of a sample run sorted by Sample ID (SID).

The "Track Report" shows the details of a sample run track by selected track.

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

ELITe BeGenius®

SAMPLES AND CONTROLS

Samples

This product must be used with the following clinical samples:

Whole blood collected in EDTA

The whole blood samples for nucleic acid extraction must be collected in EDTA and identified according to laboratory guidelines, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of three days, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods.

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

Note: when the DNA extraction from whole blood is carried out with the **ELITe BeGenius®** and with **ELITe BeGenius® Software** version **2.1.0** (or later equivalent versions), use the extraction protocol **ADV ELITe_Be_WB_200_100** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

When the primary tube is used, the volume of the sample varies according to the type of the tube loaded. Refer to the instruction for use of the extraction kit for more information on how to set up and perform the extraction procedure.

Plasma collected in EDTA

The plasma samples for nucleic acid extraction must be collected in EDTA and identified according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of three days, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

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

Note: when the DNA extraction from 200 µL of plasma is carried out with the **ELITe BeGenius®** and with **ELITe BeGenius® Software** version **2.1.0** (or later equivalent versions), use the extraction protocol **ADV ELITe_Be_PL_200_100** This protocol processes 200 µL of sample, adds the **CPE** Internal Control at 10 µL / extraction and elutes the nucleic acids in 100 µL.

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: nasal washes, nasal swabs, fecal supernatant and cerebrospinal fluid.

Interfering substances

The DNA extracted from the sample must not contain heparin, haemoglobin, dextran, Ficoll®, ethanol or 2-propanol in order to prevent inhibition problems and the possibility of frequent invalid results.

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

Amplification calibrators and amplification controls

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

as calibrator set, use the four concentration levels of the **ADENOVIRUS ELITe Standard**, in association with the protocol **«ADV ELITe_Be_STD»**,
as amplification Positive Control use the **ADENOVIRUS - ELITe Positive Control**, in association with the protocol **«ADV ELITe_Be_PC»**,
as amplification Negative Control, use Molecular biology grade water (not provided with this kit), in association with protocol **«ADV ELITe_Be_NC»**.

Note: ELITe BeGenius with **ELITe BeGenius Software** require 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.

The Calibrators and amplification Controls must be retested if any of the following events occurs:
- a new lot of amplification reagents is started,
- the results of Quality Control analysis (see following paragraph) are out of specification,
- any major maintenance is performed on the instrument.

Quality controls

External quality controls shall be used in accordance with local, state, federal accrediting organizations, as applicable. External quality controls are available on the market.

ELITe BeGenius® PROCEDURE

The procedure to use the **«ADENOVIRUS ELITe MGB Kit»** with the system **ELITe BeGenius** consists of three steps:

- System readiness verification
- Set up of the session
- Review and approval of results

System readiness verification

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

- switch on the **ELITe BeGenius** and select the mode "**CLOSED**";
- verify that the Calibrators (**ADV Q-PCR Standard**) have been run, approved and not expired (status). This can be checked under the "Calibration" menu in the Home page;
- verify that the amplification Controls (**ADV - Positive Control**, **ADV Negative Control**) have been run, approved and not expired (status). This can be checked under the "Control" menu in the Home page;
- choose the type of run and set up the run, following the instructions Graphical User Interface (GUI) for the session set up and using the Assay Protocols provided by ELITechGroup. These IVD protocols were specifically validated with ELITe MGB Kits, matrices and ELITe BeGenius instrument.

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The Assay protocols available for **«ADENOVIRUS ELITe MGB® Kit»** are described in the table below.

Assay protocols for «ADENOVIRUS ELITe MGB Kit» and ELITe BeGenius

Name	Matrix	Report unitage	Characteristics
ADV ELITe_Be_WB_200_100	Whole Blood	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
ADV ELITe_Be_PL_200_100	Plasma	copies/mL or IU/mL	Extraction Input Volume: 200 µL Extracted Elute Volume: 100 µL Internal Control: 10 µL PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Protocols for qualitative analysis are available on request.

Setup of the session

The **ADENOVIRUS ELITe MGB Kit** in association to the **ELITe BeGenius** can be used in order to perform:

A. Sample run, (EXTR + PCR),
B. Amplification run (PCR only),
C. Calibration run (PCR only),
D. Positive and Negative Control run (PCR only).

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

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

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

A. Sample run

To set up the integrated run, carry out the steps below following the **GUI**:

1. Thaw ADV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

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

2. Thaw the CPE tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session. Each new 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 (200 µL) and the Extracted Elute Volume (100 µL).
9. Select the assay protocol to be used in the “Assay” column (i.e. ADV ELITe_Be_WB_200_100). 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 step 12 for Rack 2..
14. Load CPE and ADV Q-PCR Mix 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 200” 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 PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

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B. Amplification run

To set up the amplification run, with eluted samples, carry out the steps below following the GUI:

Thaw ADV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

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

1. Select “Perform Run” from the “Home screen”.
2. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
3. Select the “run mode”: “PCR Only”.
4. Load the samples into the Racks 3 and 2 (start always from Rack 3).
5. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
6. Even if extraction is not performed, check the Extraction Input Volume (200 µL) and the Extracted Elute Volume (100 µL).
7. Select the assay protocol to be used in the “Assay” column (e.g. ADV ELITe_Be_WB_200_100). Click “Next” to continue the setup.
8. Repeat step from 7 to 9 for Rack 2
9. Load ADV Q-PCR Mix into Rack1.
10. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
11. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
12. Load the Basket with “PCR Cassette in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
13. Close the instrument door.
14. Press “Start” to start the run.

After process completion, the **ELITe 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: The PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. 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 ADV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

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

2. Thaw the ADV Q - PCR Standard tubes (Cal1: ADV Q-PCR Standards 10^2, Cal2: ADV Q-PCR Standards 10^3, Cal3: ADV Q-PCR Standards 10^4, Cal4: ADV Q-PCR Standards 10^5) at room temperature (~+25°C) for 30 minutes. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
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 Calibrator tubes into the Rack 3.
7. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
8. Select the assay protocol to be used in the “Assay” column (ADV ELITe_Be_STD). Click “Next” button to continue the setup.
9. Load ADV Q-PCR Mix into Rack 2.
10. Insert the Rack 2 into the “Cooler Unit”. Click “Next” to continue the setup.
11. Load and check the Tip Racks in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
12. Load the basket with the with “PCR Cassette” in the Inventory Area by following the GUI instruction. Click “Next” to continue the setup.
13. Close the instrument door.
14. Press “Start” to start the run.

After process completion, the **ELITe 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: 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 PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. Mix gently and spin down the content for 5 seconds before starting the next session.

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D. Positive Control and Negative Control run

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

1. Thaw ADV Q - PCR Mix tubes at room temperature (~+25°C) for 30 minutes in sufficient number for the session for the session. Each new tube is sufficient for preparing 24 reactions in optimal reagent consumption conditions. Mix gently, spin down the content for 5 seconds.

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

2. Thaw the ADV - ELITe Positive Control tubes at room temperature (~+25°C) for 30 minutes, for Positive Control amplification. Each tube is sufficient for 4 sessions. Mix gently, spin down the content for 5 seconds.
3. 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.
4. Select “Perform Run” from the “Home screen”.
5. Remove Racks 1, 2 and 3 from the “Cooler Unit” and place them on the preparation table.
6. Select the “run mode”: “PCR Only”.
7. Load the Positive Control and Negative Control tubes into the Rack 3.
8. Insert the Rack into the “Cooler Unit”. Click “Next” to continue the setup.
9. Select the assay protocol to be used “ADV ELITe_Be_PC” and “ADV ELITe_Be_NC” in the “Assay” column. Click “Next” button to continue the setup.
10. Load ADV Q-PCR Mix into the Rack 2.
11. Insert the Rack 2 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 Positive Control can be removed from the instrument, capped and stored at -20 °C. Avoid the spilling of the Positive Controls.

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 PCR Mix can be used for 5 independent work sessions of 3 hours each or can be kept on board in the refrigerated block up to 3 consecutive work sessions of 3 hours each. 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").

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

The **ELITe BeGenius** generates the results using the ADV 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

Analytical sensitivity: Limit of Detection

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

The LoD of this assay was tested using plasmid DNA containing the amplification product whose initial concentration was measured by spectrophotometer. The plasmid DNA was diluted to a titre of 10 copies / 20 µL in human genomic DNA at a titre of 500 ng / 20 µL. This sample was tested in PCR Only in 24 replicates carrying out the amplification by ELITechGroup S.p.A. products on two different instruments. The results are reported in the following table.

Samples	N	positive	negative
10 copies plasmid DNA + 500 ng of human genomic DNA	24	24	0

Whole blood

The analytical sensitivity of this assay used in association to **Whole Blood** and **ELITe InGenius** was verified with a panel of ADV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for human adenovirus DNA for nucleic acid amplification techniques" (NIBSC code: 16/324, United Kingdom) in ADV DNA - negative matrix. The panel consisted of six points around the limit concentration. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, run set up, extraction of nucleic acids, real time amplification and data interpretation with **ELITe InGenius** and ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The analytical sensitivity as copies / mL is calculated by applying the specific conversion factor reported at page 29

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The final results are reported in the following tables.

Limit of Detection with ELITe InGenius			
Matrix	95% positivity	95% confidence range	
		lower limit	upper limit
Whole Bood	**117 IU / mL**	76 IU / mL	296 IU / mL
	45 copies / mL	29 copies / mL	114 copies / mL

The calculated LoD value for **Whole Blood** was verified in association with **ELITe InGenius** and **ELITe BeGenius** by testing 20 replicates of whole blood collected in EDTA spiked by ADV certified reference material ("1st WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 18 out of 20 replicates give a positive result as per CLSI standard EP17-A.

The results are reported in the following tables.

Limit of Detection for Whole Blood and ELITe InGenius					
Sample	**Titer**	**Target**	**N**	**Positive**	**Negative**
Whole blood collected in EDTA	117 IU / mL	ADV	20	20	0

Limit of Detection for Whole Blood and ELITe BeGenius					
Sample	**Titer**	**Target**	**N**	**Positive**	**Negative**
Whole blood collected in EDTA	117 IU / mL	ADV	20	19	1

The LoD value for ADV target was confirmed at 117 IU / mL in association with Whole Blood samples.

Plasma:

The analytical sensitivity of this assay used in association with **Plasma** and **ELITe BeGenius** was verified with a panel of ADV dilutions within the limiting concentration. The panel was prepared by diluting the "1st WHO International Standard for human adenovirus DNA for nucleic acid amplification techniques" (NIBSC code: 16/324, United Kingdom) in ADV DNA - negative matrix. The panel consisted of at least six points around the limit concentration. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, run set up, extraction of nucleic acids, real time amplification and data interpretation with **ELITe BeGenius** and ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The analytical sensitivity as copies / mL is calculated by applying the specific conversion factor reported at page 29.

The final results are reported in the following tables.

Limit of Detection with ELITe BeGenius			
Matrix	95% positivity	95% confidence range	
		lower limit	upper limit
Plasma	**89 IU / mL**	60 IU / mL	204 IU / mL
	47 copies / mL	32 copies / mL	107 copies / mL

The calculated LoD value for **Plasma** was verified in association with **ELITe BeGenius** and **ELITe InGenius** by testing 20 replicates of plasma collected in EDTA spiked by ADV certified reference material ("1st WHO International Standard, NIBSC) at the claimed concentration. The LoD is confirmed if at least 18 out of 20 replicates give a positive result as per CLSI standard EP17-A.

The results are reported in the following tables.

Limit of Detection for Plasma and ELITe BeGenius					
Sample	**Titer**	**Target**	**N**	**Positive**	**Negative**
Plasma collected in EDTA	89 IU / mL	ADV	20	20	0

Limit of Detection for Plasma and ELITe InGenius					
Sample	**Titer**	**Target**	**N**	**Positive**	**Negative**
Plasma collected in EDTA	89 IU / mL	ADV	20	20	0

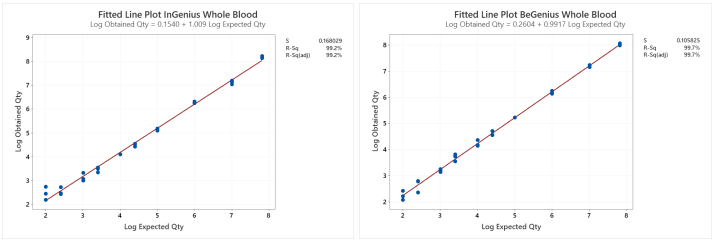
The LoD value for ADV target was confirmed at 89 IU / mL in association with Plasma collected in EDTA.

Linear measuring range

Whole Blood:

The linear measuring range of ADENOVIRUS ELITe MGB® Kit used in association with **Whole Blood** and **ELITe InGenius** and **ELITe BeGenius** was tested using a panel prepared by diluting a ADV reference material ("1st WHO International Standard, NIBSC) in ADV DNA - negative matrix. The panel consisted of ten dilution points from $6.5x10^7$ to 10^2 IU / mL. Each sample of the panel was tested in 3 replicates.

The analysis of the obtained data, performed by linear regression, demonstrated that the assay in association with Whole Blood samples shows a linear response for all the dilution levels with a Square Correlation Coefficient (R2) equal to 0.992 for **ELITe InGenius** and 0.997 for **ELITe BeGenius**.



The Lower Limit of Quantification (LLoQ) was set at the concentration, that gives quantitative results precise (Standard Deviation equal to 0.1978 Log IU / mL for ELITe InGenius and 0.2865 Log IU / mL for ELITe BeGenius) and accurate (Bias equal to 0.1627 Log IU / mL for ELITe InGenius and 0.0287 Log IU / mL for ELITe BeGenius): 117 IU / mL.

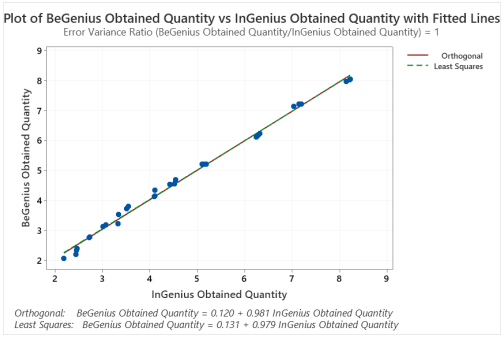
The Upper Limit of Quantification (ULoQ) was set at the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.0458 Log IU / mL for ELITe InGenius and 0.0358 Log IU / mL for ELITe BeGenius) and accurate (Bias equal to -0.3943 Log IU / mL for ELITe InGenius and -0.2099 Log IU / mL for ELITe BeGenius): 65,000,000 IU / mL.

The linear measuring range as copies / mL for Whole blood is calculated by applying the specific conversion factor reported at page 29

The final results are summarized in the following table.

Linear measuring range for whole blood samples and ELITe InGenius and ELITeBeGenius		
Unit of measure	lower limit	upper limit
IU / mL	**117**	**65,000,000**
copies / mL	**45**	**25,000,000**

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.

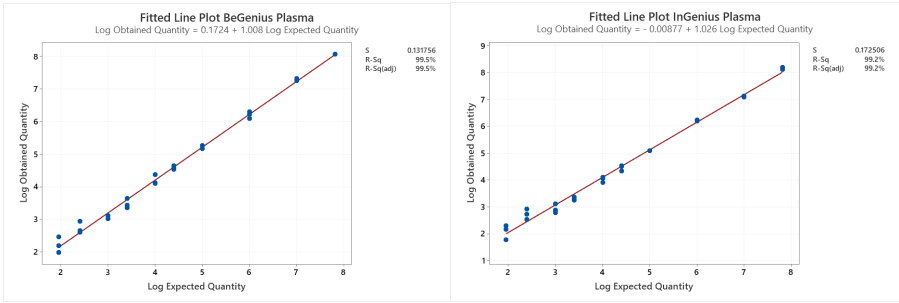


The orthogonal regression analysis generated a slope equal to 0.981 (95% CI: 0.955; 1.006) and an intercept equal to 0.120 (95% CI: - 0.010; 0.249). The linear regression analysis generated a R2 of 0.995.

Plasma:

The linear measuring range of ADENOVIRUS ELITe MGB® Kit used in association with **Plasma** and **ELITe InGenius** and **ELITe BeGenius** was tested using a panel prepared by diluting a ADV reference material ("1st WHO International Standard, NIBSC) in ADV DNA - negative matrix. The panel consisted of ten dilution points from $6.5x10^7$ to 89 IU / mL. Each sample of the panel was tested in 3 replicates.

The analysis of the obtained data, performed by linear regression, demonstrated that the assay in association with Plasma samples shows a linear response for all the dilution levels with a Square Correlation Coefficient (R2) equal to 0.995 for **ELITe BeGenius** and 0.992 for **ELITe InGenius**.



The Lower Limit of Quantification (LLoQ) was set at the LoD concentration, that gives quantitative results precise (Standard Deviation equal to 0.2738 Log IU / mL for ELITe InGenius and 0.2535 Log IU / mL for ELITe BeGenius) and accurate (Bias equal to -0.0397 Log IU / mL for ELITe InGenius and -0.0521 Log IU / mL for ELITe BeGenius): 89 IU / mL.

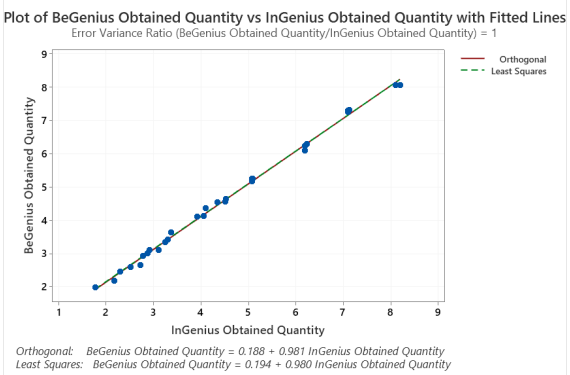
The Upper Limit of Quantification (ULoQ) was set at the highest concentration tested, that gives quantitative results precise (Standard Deviation equal to 0.0515 Log IU / mL for ELITe InGenius and 0.0015 Log IU / mL for ELITe BeGenius) and accurate (Bias equal to 0.3222 Log IU / mL for ELITe InGenius and 0.2488 Log IU / mL for ELITe BeGenius): 65,000,001 IU / mL.

The linear measuring range as copies / mL for Plasma is calculated by applying the specific conversion factor reported at page 29.

The final results are summarized in the following table.

Linear measuring range for plasma samples and ELITe InGenius and ELITeBeGenius		
Unit of measure	lower limit	upper limit
IU / mL	**89**	**65,000,001**
copies / mL	**47**	**34,210,527**

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.



In this test, the orthogonal regression analysis generated a slope equal to 0.981 (95% CI: 0.962; 1.000) and an intercept equal 0.188 (95% CI: 0.094; 0.282). The linear regression analysis generated a R2 of 0.994

Repeatability

The Repeatability of results obtained by the product ADENOVIRUS ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of Whole blood samples collected in EDTA. The panel included one negative sample and two samples spiked by ADV certified reference material "1st WHO International Standard for human adenovirus DNA for nucleic acid amplification techniques" (NIBSC code 16/324, United Kingdom) at concentration of 3 x LoD (about 354 IU / mL) and of 10 x LoD (about 1180 IU / mL).

The Intra – Session Repeatability on **ELITe InGenius** was obtained through the analysis of panel samples in eight replicates, in two runs per day, with the same lot of product, with the same instrument, by the same operator, on the same day. Samples were processed in randomized positions on **ELITe InGenius** system in "Extract + PCR" mode.

The Inter – Session Repeatability on **ELITe InGenius** was obtained through the analysis of panel samples in eight replicates, in two runs per day, with the same lot of product, with the same instrument, by the same operator, on two different days. 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 ELITe InGenius								
Sample	**ADV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	25.20	0.44	1.73
3 x LoD	8 / 8	35.58	0.51	1.43				
10 x LoD	8 / 8	34.09	0.49	1.44				

Inter – Session Repeatability ELITe InGenius								
Sample	**ADV**				**Internal Control**			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	N.A.	N.A.	N.A.	48 / 48	25.14	0.45	1.80
3 x LoD	16 / 16	35.67	0.55	1.53				
10 x LoD	16 / 16	34.05	0.42	1.23				

In the Repeatability test on **ELITe InGenius**, the assay detected the ADV target as expected and showed low %CV of Ct values that did not exceed 1.5% for ADV and 1.8% for Internal Control.

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

The Inter – Session Repeatability on **ELITe BeGenius** was obtained through the analysis of panel samples in eight replicates, in one run per day, with the same lot of product, with the same instrument, by the same operator, on two different days. 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 ELITe BeGenius								
Sample	**ADV**				**Internal Control**			
	Pos. / Rep.	**Mean Ct**	**SD**	**% CV**	**Pos. / Rep.**	**Mean Ct**	**SD**	**% CV**
Negative	0 / 8	N.A.	N.A.	N.A.	24/24	27.33	0.44	1.62
3 x LoD	8 / 8	36.72	0.38	1.03				
10 x LoD	8 / 8	34.99	0.28	0.80				

Inter – Session Repeatability ELITe BeGenius								
Sample	**ADV**				**Internal Control**			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 16	N.A.	N.A.	N.A.	47 / 47	27.13	0.48	1.77
3 x LoD	15 / 15	36.61	0.40	1.10				
10 x LoD	16 / 16	34.90	0.31	0.88				

In the Repeatability test on **ELITe BeGenius**, the assay detected the ADV target as expected and showed low %CV of Ct values that did not exceed 1.1% for ADV and 1.8% for Internal Control.

Reproducibility

The Reproducibility of results obtained by the product ADENOVIRUS ELITe MGB Kit in association with the **ELITe InGenius** and **ELITe BeGenius** systems was tested by analysing a panel of whole blood samples. The panel included one negative sample and two samples spiked with ADV certified reference material "1st WHO International Standard for human adenovirus DNA for nucleic acid amplification techniques" (NIBSC code 16/324, United Kingdom) at concentration of 3 x LoD (about 354 IU / mL) and of 10 x LoD (about 1180 IU / mL).

The Inter – Instrument Reproducibility on **ELITe InGenius** was obtained through the analysis of panel samples in eight replicates, in one run per day, in two days, using the same lot and two different instruments by two different operators. Samples were processed in randomized positions on **ELITe InGenius** system in "Extract + PCR" mode.

The Inter – Batch Reproducibility on **ELITe InGenius** was obtained through the analysis of panel samples in eight replicates, in two runs per day, using two different lots and 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 Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe InGenius

Sample	ADV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	24.48	0.59	2.43
3 x LoD	8 / 8	35.91	0.67	1.87				
10 x LoD	8 / 8	34.42	0.39	1.13				

Inter – Batch Reproducibility ELITe InGenius

Sample	ADV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	24.44	0.82	3.35
3 x LoD	8 / 8	35.85	0.45	1.25				
10 x LoD	8 / 8	34.34	0.50	1.45				

In the Reproducibility test on **ELITe InGenius**, the assay detected the ADV target as expected and showed low %CV of Ct values that did not exceed 1.9% for ADV and 3.4%for Internal Control.

The Inter – Instrument Reproducibility on **ELITe BeGenius** was obtained through the analysis of panel samples in eight replicates, in one run per day, in two days, using the same lot and two different instruments by two different operators. Samples were processed in randomized positions on **ELITe BeGenius** system in "Extract + PCR" mode.

The Inter – Batch Reproducibility on **ELITe BeGenius** was obtained through the analysis of panel samples in eight replicates, in two runs per day, using two different lots and 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 Reproducibility as imprecision.

A summary of results is shown in the table below.

Inter – Instrument Reproducibility ELITe BeGenius

Sample	ADV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	27.76	0.83	2.98
3 x LoD	8 / 8	37.72	0.97	2.58				
10 x LoD	8 / 8	36.17	0.91	2.51				

Inter – Batch Reproducibility ELITe BeGenius

Sample	ADV				Internal Control			
	Pos. / Rep.	Mean Ct	SD	% CV	Pos. / Rep.	Mean Ct	SD	% CV
Negative	0 / 8	N.A.	N.A.	N.A.	24 / 24	26.81	0.57	2.12
3 x LoD	8 / 8	37.09	0.60	1.62				
10 x LoD	8 / 8	35.38	0.32	0.92				

In the Reproducibility test on **ELITe BeGenius**, the assay detected the ADV target as expected and showed low %CV of Ct values that did not exceed 2.6% for ADV and 3 % for Internal Control.

ADENOVIRUS ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS078PLD

Reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of a calibrated reference material, was evaluated using as reference material the calibrated panel QCMD 2013 Adenovirus EQA Panel (Qnostics Ltd, UK) a panel of Adenovirus dilutions. Each sample of the panel was tested in 4 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation, using **ELITe InGenius** and ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with reference materials and ELITeInGenius

Sample	Sample Status	Positive / Replicates
ADV13-01	Frequently detected	4/4
ADV13-02	Detected	2/4
ADV13-03	Frequently detected	4/4
ADV13-04	Negative	0/4
ADV13-05	Frequently detected	4/4
ADV13-06	Detected	4/4
ADV13-07	Frequently detected	4/4
ADV13-08	Detected	4/4
ADV13-09	Frequently detected	4/4
ADV13-10	Detected	4/4

All samples were correctly detected. The sample ADV13-02, positive at low titre, below the theoretical limit of detection of the assay, it was detected in 2 replicates out of 4.

Further tests were carried out using as reference material the calibrated panel «AcroMetrix™ Adenovirus Plasma Panel» (Acrometrix, Life Technologies, US). Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation with **ELITe InGenius** and ELITechGroup S.p.A. products. The results are reported in the following table.

Tests with calibrated reference materials and ELITeInGenius

Sample	Nominal titre copies / mL	Nominal titre Log_{10} copies / mL	Positive / Replicates	Mean results Log_{10} copies / mL
Adenovirus 1E3	1000	3.000	2/2	3.030
Adenovirus 1E4	10000	4.000	2/2	3.926
Adenovirus 1E5	100000	5.000	2/2	5.052
Adenovirus 1E6	1000000	6.000	2/2	5.893
Adenovirus 1E7	10000000	7.000	2/2	6.873

All samples were detected as positive, with a titre within the expected value ± 0.5 Log.

Conversion factor to International Units

Whole blood

The conversion factor, to convert a quantitative result from copies / mL to International Units / mL, was calculated using a panel of six dilutions of calibrated reference material approved by WHO ("1st WHO International Standard for human adenovirus DNA for nucleic acid amplification techniques" (NIBSC code 16/324, United Kingdom)) in whole blood matrix tested negative for ADV DNA. The panel had 6 dilution steps of 0.5 Log. Each point of the panel was tested in 20 replicates carrying out the whole analysis, extraction, amplification, detection and result interpretation with **ELITe InGenius** and ELITechGroup S.p.A. products.

The result is reported in the following table.

Conversion factor to International Units with ELITe InGenius	
Matrix	Fc (IU / copies)
Whole Blood	**2.6**

The Conversion Factor of ADENOVIRUS ELITe MGB® Kit used in association with **Whole Blood** collected in EDTA and **ELITe InGenius** and **ELITe BeGenius** was verified analysing the results obtained during the Linear measuring range study.

The target quantification precision, as Standard Deviation of Log IU/mL, was lower than 0.5 Log for both **ELITe InGenius** and **ELITe BeGenius**.

The target quantification accuracy, as difference between the Theoretical and Measured concentrations in Log IU / mL, was lower than 0.5 Log for both **ELITe InGenius** and **ELITe BeGenius.**

These results confirmed the Conversion factor calculated for whole blood with **ELITe InGenius**.

Plasma

The conversion factor, to convert a quantitative result from copies / mL to International Units / mL, was calculated using a panel of six dilutions of calibrated reference material approved by WHO ("1st WHO International Standard for human adenovirus DNA for nucleic acid amplification techniques" (NIBSC code 16/324, United Kingdom)) in plasma matrix tested negative for ADV DNA. The panel had 6 dilution steps of 0.5 Log. Each point of the panel was tested in 18 replicates carrying out the whole analysis, extraction, amplification, detection and result interpretation with **ELITe BeGenius** and ELITechGroup S.p.A. products.

The results is reporte in the following table.

Conversion factor to International Units with ELITe BeGenius	
Matrix	Fc (IU / copies)
plasma	**1.9**

The Conversion Factor of ADENOVIRUS ELITe MGB® Kit used in association with **Plasma** collected in EDTA and **ELITe InGenius** and **ELITe BeGenius** was verified analysing the results obtained during the Linear measuring range study.

The target quantification precision, as Standard Deviation of Log IU/mL, was lower than 0.5 Log for both **ELITe InGenius** and **ELITe BeGenius**.

The target quantification accuracy, as difference between the Theoretical and Measured concentrations in Log IU / mL, was lower than 0.5 Log for both **ELITe InGenius** and **ELITe BeGenius.**

These results confirmed the Conversion factor calculated for plasma with **ELITe BeGenius**.

Diagnostic sensitivity: confirmation of positive samples

Whole Blood

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing some clinical samples of Whole Blood collected in EDTA positive for Adenovirus DNA in association to **ELITe InGenius**. 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 test was performed on 30 whole blood samples, collected in EDTA negative for Adenovirus DNA, that were spiked for ADV DNA adding ADV12-01 sample, from QCMD 2012 Adenovirus EQA Panel (Qnostics Ltd, UK) Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **ELITe InGenius** and with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
whole blood collected in EDTA and positive for ADV DNA	30	30	0

All samples were confirmed as positives.
The diagnostic sensitivity of the assay in this test was equal to 100%.

Plasma

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was evaluated by analyzing some clinical samples of Plasma collected in EDTA positive for Adenovirus DNA in association to **ELITe BeGenius**. As **ELITe InGenius** has equivalent analytical performances to **ELITe BeGenius**, 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 BeGenius** is also applicable to **ELITe InGenius**.

The test was performed on 75 Plasma samples collected in EDTA positive for Adenovirus DNA. Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **ELITe BeGenius** and with ELITechGroup S.p.A. products. The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA and positive for ADV DNA	75	75	0

All samples were confirmed as positives.
The diagnostic sensitivity of the assay in this test was equal to 100%.

Diagnostic specificity: confirmation of negative samples

Whole Blood

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analyzing some clinical samples of Whole Blood collected in EDTA negative for Adenovirus DNA in association with **ELITe InGenius**. 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 test was performed on 30 whole blood samples collected in EDTA that were presumably negative for Adenovirus DNA. Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **ELITe InGenius** and with ELITechGroup S.p.A. products. The results are summed up in the following table.

Samples	N	positive	negative
whole blood collected in EDTA and negative for ADV DNA	30	0	30

All samples were confirmed to be negative for ADV - DNA.
The diagnostic specificity of the assay in this test was equal to 100%.

Plasma

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated by analyzing some clinical samples of Plasma collected in EDTA negative for Adenovirus DNA in association with **ELITe BeGenius**. As **ELITe InGenius** has equivalent analytical performances to **ELITe BeGenius**, 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 BeGenius** is also applicable to **ELITe InGenius**.

The test was performed on 38 plasma samples collected in EDTA that were negative for Adenovirus DNA. Each sample was tested carrying out the whole analysis procedure, extraction, amplification, detection and result interpretation with **ELITe BeGenius** and with ELITechGroup S.p.A. products. The results are summed up in the following table.

Samples	N	positive	Negative
Plasma collected in EDTA and negative for ADV DNA	38	1	37

37 out of 38 samples were confirmed to be negative for ADV - DNA. One sample resulted discrepant positive at low titer.

The diagnostic specificity of the assay in this test was equal to 97.4%.

ADENOVIRUS ELITe MGB® Kit
reagent for DNA Real Time amplification

REF RTS078PLD

ABI 7500 Fast Dx Real-Time PCR Instrument
ABI 7300 Real-Time System

SAMPLES AND CONTROLS

Samples

This product must be used with **DNA extracted** from the following clinical samples: whole blood collected in EDTA, plasma collected in EDTA, nasal washes, nasal swabs.

Whole blood collected in EDTA

The whole blood samples for nucleic acids extraction must be collected in EDTA according to laboratory guidelines, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of three days, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing.

When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when you carry out the DNA extraction from whole blood (cellular sample) using **«EXTRAblood»** kit, please, follow the instructions for use manual: start from **200 µL** of sample (2 million of cells maximum), elute the DNA in **100 µL** of elution buffer.

Note: when you carry out the DNA extraction from whole blood with **ELITe STAR** and with **software version 3.4.13** (or later equivalent versions), use the extraction protocol **UUNI_E100_S200_ELI**, that uses 200 µL of sample and elutes the extract in 100 µL. Samples in primary tubes can be directly loaded on **«ELITe STAR»**. A minimum volume of 700 µL is always required for each sample. Add **200 µL** of **CPE** into Proteinase-Carrier tube as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from whole blood with the **ELITe GALAXY** with **software version 1.3.1** (or later equivalent versions) use the extraction protocol **xNA Extraction (Universal)**, that uses 300 µL of sample and elutes the extract in 200 µL. Samples in primary tubes can be directly loaded on **«ELITe GALAXY»**. A minimum volume 400-650 µL, dependent on the tube class used, is always required for each sample. Add **10 µL** / **sample** of **CPE.** The CPE must be added to **IC + Carrier solution** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from whole blood with the instrument **«NucliSENS® easyMAG®»**, please follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer

100 µL of sample in the 8 well strip, load the strip on the instrument and run the extraction without lysis incubation. After the instrument added **EasyMAG® Lysis Buffer**, without removing the strip, mix three times the strip content by the supplied multichannel pipet using the program number 3. Incubate for 10 minutes, then add the **NucliSENS® easyMAG® Magnetic Silica** and proceed with the extraction. Elute the nucleic acids in **50 µL** of elution buffer.

Note: when you carry out the DNA extraction from whole blood with the instrument **«QIAsymphony® SP/AS»** and the kit **«QIAsymphony® DNA Mini Kit»** with **software version 3.5**, use the extraction protocol **"Virus Blood_200_V4_default IC"** and follow these directions: the instrument is able to use a primary tube, sample volume required for the extraction is **200 µL**, it's always requested a minimum dead volume of **100 µL**. Load on the instrument, in the "internal control" slot, the tubes containing buffer ATE, as indicated in the instruction for use manual of the kit; indicate the position where eluates will be dispensed and specify the elution volume of **60 µL**. For details on the extraction procedure follow indications in the instruction for use manual of the kit.

Plasma collected in EDTA

The plasma samples for nucleic acid extraction must be collected in EDTA according to laboratory guidelines, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of three days, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing.

Note: when you carry out the DNA extraction from plasma with the **ELITe STAR** and with **software version 3.4.13** (or later equivalent versions), use the extraction protocol **UUNI_E100_S200_ELI**, that uses 200 µL of sample and elutes the extract in 100 µL. Samples in primary tubes can be directly loaded on **«ELITe STAR»**. A minimum volume of 600 µL is always required for each sample. Add **200 µL** of **CPE** into Proteinase-Carrier tube as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Note: when you carry out the DNA extraction from plasma with the **ELITe GALAXY** with **software version 1.3.1** (or later equivalent versions) use the extraction protocol **xNA Extraction (Universal)**, that uses 300 µL of sample and elutes the extract in 200 µL. Samples in primary tubes can be directly loaded on **«ELITe GALAXY»**. A minimum volume 400-650 µL, dependent on the tube class used, is always required for each sample. Add **10 µL** / **sample** of **CPE.** The CPE must be added to **IC + Carrier solution** as indicated in the manual of the extraction kit. For the extraction procedure refer to the instruction for use manual of the extraction kit.

Nasal washes

The nasal washes, intended for DNA extraction, must be collected according to laboratory guidelines, diluted in sterile physiological solution or sterile PBS, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of four hours, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing.

Note: when you carry out the DNA extraction from nasal washes (non cellular sample) using **«EXTRAblood»** kit, please, follow the instructions for use manual: start from **200 µL** of sample, add **5 µL** of **CPE** for the internal control at the beginning of the extraction, recover the DNA in **60 µL** of elution buffer.

Note: when you carry out the DNA extraction from nasal washes with the instrument **«NucliSENS® easyMAG®»**, please follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **500 µL** of sample in the 8 well strip, add **5 µL** of **CPE** for the internal control before adding the **NucliSENS® easyMAG® Magnetic Silica**. Elute the nucleic acids in **100 µL** of elution buffer.

Nasal swabs

The nasal swabs, intended for DNA extraction, must be collected according to laboratory guidelines, diluted in transport medium for cell cultures or sterile physiological solution or sterile PBS, transported at +2 / +8 °C and stored at +2 / +8 °C for a maximum of four hours, otherwise they must be frozen and stored at -20 °C for a maximum of thirty days or at -70 °C for longer periods.

It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing.

Note: when you carry out the DNA extraction from nasal swabs (non cellular sample) using **«EXTRAblood»** kit, please, follow the instructions for use manual: start from **200 µL** of sample, add **5 µL** of **CPE** for the internal control at the beginning of the extraction, recover the DNA in **60 µL** of elution buffer.

Note: when you carry out the DNA extraction from nasal swabs with the instrument **«NucliSENS® easyMAG®»**, please follow the extraction protocol **Generic 2.0.1** and follow these directions: transfer **500 µL** of sample in the 8 well strip, add **5 µL** of **CPE** for the internal control before adding the **NucliSENS® easyMAG® Magnetic Silica**. Elute the nucleic acids in **100 µL** of elution buffer.

Other samples

There are no data available concerning product performance with DNA extracted from the following clinical samples: fecal supernatant, cerebrospinal fluid.

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Interfering substances

The DNA extracted from the sample must not contain heparin, haemoglobin, dextran, Ficoll®, ethanol or 2-propanol in order to prevent the problem of inhibition and the possibility of frequent invalid results.

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

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

Amplification controls

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

For the negative control, use molecular biology grade water (not provided with this product) added to the reaction in place of the DNA extracted from the sample.

For the positive control, use the **«ADENOVIRUS - ELITe Positive Control»** product or the **«ADENOVIRUS ELITe Standard»** product.

Quality controls

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

PROCEDURE

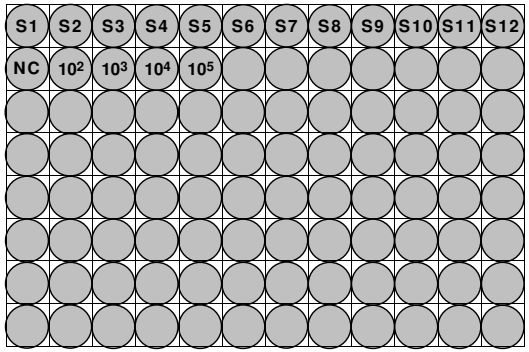
Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

When **7300 Real-Time PCR System** instrument is used.

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session,
- set (Detector Manager) the "detector" for the ADV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “ADV”,
- set (Detector Manager) the "detector" for the Internal Control probe with the "reporter" = "VIC" (AP525 is analogous to VIC) and the "quencher" = "none" (non fluorescent) and call it “IC”,
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "ROX" (AP593 is used instead of ROX, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

Note: In order to determine the DNA titre in the starting sample, set up a series of reactions with the **Q - PCR Standards** (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the **Standard curve**.

See below, by way of example, how you can organise the quantitative analysis of 12 samples.



Legend: S1 - **S12**: Samples to be analysed; **NC**: Negative Control of amplification,
10^2: 10^2 standard copies; **10^3**: 10^3 standard copies; **10^4**: 10^4 standard copies; **10^5**: 10^5 standard copies.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:
- add to amplification stage the step (Add Step) of **extension at 72°C**;

Note: the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60°C.

- modify timing as indicated in the following table,
- set the number cycles to **45**,
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;

Thermal cycle		
Stage	**Temperatures**	**Timing**
Decontamination	50° C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60° C (fluorescence acquisition)	30 sec.
	72° C	20 sec.

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

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the real time thermal cycler, switch on the computer, run the dedicated software and open an "absolute quantification" session and set “Run mode: Fast 7500”,
- set (Detector Manager) the "detector" for the ADV probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “ADV”,
- set (Detector Manager) the "detector" for the internal control probe with the "reporter" = "VIC" (AP525 is similar to VIC) and the "quencher" = "none" (non fluorescent) and call it “IC”,
- for each well in use in the microplate, set (Well Inspector) the "detector" (type of fluorescence that is to be measured), the "passive reference" = "CY5" (AP593 is used instead of CY5, normalisation of the measured fluorescence) and the type of reaction (sample, negative amplification control, positive amplification control or known quantity standard). Add this information to the **Work Sheet** enclosed at the end of this manual or print the microplate set up. The **Work Sheet** must be followed carefully during the transfer of the reaction mixture and samples into the wells.

Note: In order to determine the DNA titre in the starting sample, set up a series of reactions with the **Q - PCR Standards** (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the **Standard curve**.

The setup of the quantitative analysis of some samples is shown, by way of example, in the previous paragraph describing the procedure for the **7300 Real Time PCR System** instrument.

Referring to the instrument documentation, set on the dedicated software (Instrument > Thermal Cycler Protocol > Thermal Profile) the parameters of the **thermal cycle**:
- add to amplification stage the step (Add Step) of **extension at 72 °C**;

Note: the fluorescence acquisition (Instrument > Thermal Cycler Protocol > Settings > Data Collection) must be set during the step of hybridization at 60 °C.

- modify timing as indicated in the following table,
- set the number cycles to **45**,
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**,

Thermal cycle		
Stage	**Temperatures**	**Timing**
Decontamination	50 °C	2 min.
Initial denaturation	94 °C	2 min.
Amplification and detection (45 cycles)	94 °C	10 sec.
	60 °C (data collection)	30 sec.
	72 °C	20 sec.

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

Before starting the session, it is important to do the following:
- take and thaw the tubes containing the samples to be analysed. Mix gently, spin down the content for 5 seconds and keep them on ice;
- take and thaw the **ADV Q - PCR Mix** tubes required for the session, remembering that each tube is sufficient for preparing **25 reactions**. Mix gently, spin down the contents for 5 seconds and keep them on ice;
- take and thaw the **ADV - Positive Control** or the **ADV Q - PCR Standard** tubes. Mix them gently, centifuge them for 5 seconds spinning down the contents and keep them on ice;
- take the **Amplification microplate** that will be used during the session, being careful to handle it with powder-free gloves and not to damage the wells.

1. Accurately pipet **20 µL** of **ADV Q - PCR Mix** on the bottom of the **Amplification microplate** wells, as previously established in the **Work Sheet**. Avoid creating bubbles.

Note: If not all the reaction mixture is used, store the remaining volume in the dark at -20°C for no longer than one month. Freeze and thaw the reaction mixture from a maximum of **5 TIMES**.

2. Accurately pipet, by placing into the reaction mixture, **20 µL** of **extracted DNA** from the first sample in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the sample by pipetting the **extracted DNA** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other samples of **extracted DNA**.
3. Accurately pipet, by placing into the reaction mixture, **20 µL** of **molecular biology grade water** (not provided with this product) in the well of **Amplification microplate** of the negative control of amplification, as previously established in the **Work Sheet**. Mix well the negative control by pipetting the **molecular biology grade water** three times into the reaction mixture. Avoid creating bubbles.
4. On the basis of the result required (qualitative or quantitative), one of these two options must be followed:

   - When a **qualitative** result is required (detection of ADV DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **ADV - Positive Control** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the **ADV - Positive Control** three times into the reaction mixture. Avoid creating bubbles.

   - When a **quantitative** result is required (quantification of ADV DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **ADV Q - PCR Standard 10^2** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **ADV Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other **ADV Q - PCR Standards** (**10^3, 10^4, 10^5**).
5. Accurately seal the **Amplification microplate** with the **Amplification Sealing Sheet**.
6. Transfer the **Amplification microplate** into the real time thermal cycler in the amplification / detection of amplification products area and start the thermal cycle for the amplification saving the session setting with an univocal and recognizable file name (e.g. "year-month-day-ADV-EGSpA").

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

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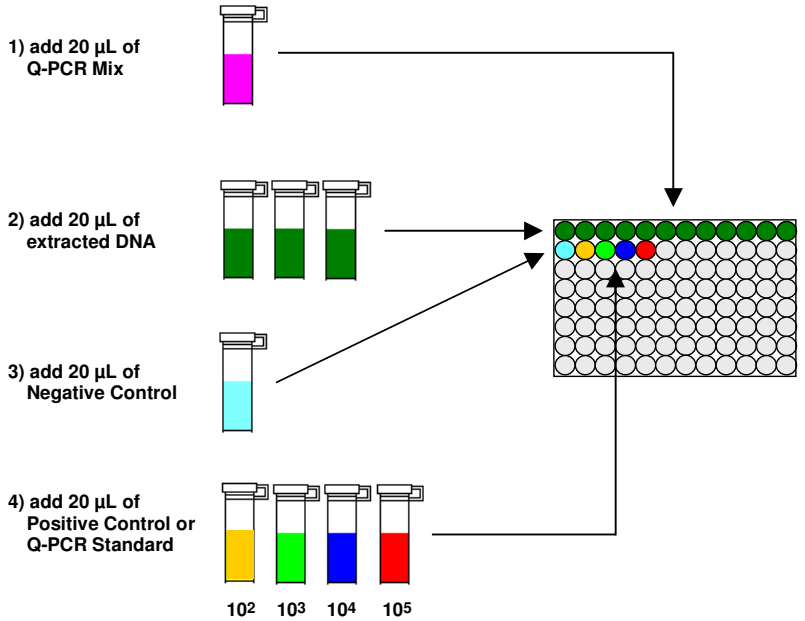
The following figure shows synthetically the preparation of the amplification reaction.



Note: if the preparation of the amplification is performed with the instrument **«QIAsymphony® SP/AS»**, insert the microplate containing the exctracts, the regents and the amplification microplate in the dedicated slots, using the special adaptors, then follow indications in the instruction for use manual of the setup module and the steps required by the software.

Note: if the preparation of the amplification reaction is performed with the **«ELITe GALAXY»** instrument, load the elution microplate, the complete reaction mixture and the amplification microplate as indicated in the instrument user manual and following the steps required by the GUI.

Qualitative analysis of the results

The recorded values of the fluorescence emitted by the specific ADV probe (FAM detector "ADV") and by the specific Internal Control probe (VIC detector "IC") in the amplification reactions must be analysed by the instrument software.

Before starting the analysis, referring to the instrument documentation, it is necessary to:
- set manually (Results > Amplification plot > delta Rn vs Cycle) the calculation range for the **Baseline** (fluorescence background level) from cycle 6 to cycle 15;

Note: In the case of a positive sample with a high titre of ADV DNA, the FAM fluorescence of the ADV specific probe may begin to increase before the cycle 15. In this case the calculation range for the **Baseline** must be adapted from cycle 6 to the cycle in which the FAM fluorescence of the sample begins to increase, as detected by the instrument software (Results > Component).

When a **7300 Real-Time PCR System** instrument is used:

- set manually the **Threshold** for the FAM detector “ADV” to **0.1**;
- set manually the **Threshold** for the VIC detector "IC" to **0.05**.

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

- set manually the **Threshold** for the FAM detector "ADV" to **0.2**;
- set manually the **Threshold** for the VIC detector "IC" to **0.1**.

The values of fluorescence emitted by the specific probes in the amplification reaction and the **Threshold** value of fluorescence allow determining the **Threshold cycle (Ct)**, the cycle in which the fluorescence reached the **Threshold** value.

In the **Positive Control*** amplification reaction, the **Ct** value of ADV (Results > Report) is used to validate the amplification and the detection as described in the following table:

Positive Control reaction detector FAM "ADV"	Assay result	Amplification / Detection
Ct ≤ 25	**POSITIVE**	**CORRECT**

If the result of the **Positive control** amplification reaction is **Ct > 25** or **Ct Undetermined** for ADV, the target DNA was not correctly detected. This means that problems occurred during the amplification or detection step (incorrect dispensation of the reaction mix or of the positive control, degradation of the reaction mix or of the positive control, incorrect setting of the position of the positive control, incorrect setting of the thermal cycle) which may lead to incorrect results. The session is not valid and needs to be repeated starting from the amplification step.

*** Note:** When this product is used for the quantification of ADV DNA, the **Q - PCR Standard** reactions were set up instead of the **Positive Control** reaction. In this case, validate the amplification and the detection by referring to the amplification reaction of **Q - PCR Standard 10^5** (**Ct ≤ 25**).

In the **Negative control** amplification reaction, the **Ct** value of ADV (Results > Report) is used to validate the amplification and the detection as described in the following table:

Negative control reaction detector FAM "ADV"	Assay result	Amplification / Detection
Ct Undetermined	**NEGATIVE**	**CORRECT**

If the result of the **Negative control** amplification reaction is different from **Ct Undetermined** for ADV, the target DNA was detected. This means that problems occurred during the amplification step (contamination) which may lead to incorrect results and false positives. The session is not valid and needs to be repeated starting from the amplification step.

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

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

This product is able to detect a minimal quantity of about 10 copies of DNA of the Hexon protein gene of ADV in the amplification reaction (limit of detection, see Performance Characteristics paragraph).

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

Sample reaction		Sample suitability	Assay result	ADV DNA
detector FAM "ADV"	**detector VIC "IC"**			
Ct Undetermined	**Ct > 35 or Ct Undetermined**	**unsuitable**	**invalid**	**-**
	Ct ≤ 35	**suitable**	**valid, negative**	**NOT DETECTED**
Ct Determined	**Ct > 35 or Ct Undetermined**	**suitable**	**valid, positive**	**DETECTED**
	Ct ≤ 35	**suitable**	**valid, positive**	**DETECTED**

If the result of the amplification reaction of a sample is **Ct Undetermined** for ADV and **Ct > 35** or **Ct Undetermined** for the Internal Control, it means that it was impossible to detect efficiently the DNA for the Internal Control. In this case problems occurred during the amplification step (inefficient or absent amplification) or during the extraction step (degradation of the sample DNA, sample with insufficient cells number, loss of DNA during the extraction or presence of inhibitors) which may lead to incorrect results and false negatives. The sample is not suitable, the assay is invalid and it needs to be repeated starting from the extraction of a new sample.

If the result of the amplification reaction of a sample is **Ct Undetermined** for ADV and **Ct ≤ 35** for the Internal Control, it means that the ADV DNA is not detected in the DNA extracted from the sample; but it can not be excluded that the ADV DNA has a lower titre than the detection limit of the product (see the paragraph about Performance Characteristics). In this case the result could be a false negative.

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

Note: When in the amplification reaction of a sample the ADV DNA is detected, the Internal Control may result as Ct > 35 or Ct Undetermined. In fact, the low efficiency amplification reaction for the Internal Control may be displaced by competition with the high efficiency amplification reaction for ADV DNA. In this case the sample is nevertheless suitable and the positive result of the assay is valid.

Quantitative analysis of the results

After carrying out the procedure for qualitative analysis of the results it is possible to perform the quantitative analysis of the results of the positive samples.

In the amplification reactions of the four **Q - PCR standards**, the **Ct** values of ADV are used to calculate the **Standard Curve** (Results > Standard Curve) for the amplification session and to validate the amplification and the detection as described in the following table:

Standard Curve detector FAM "ADV"	Acceptability range	Amplification / Detection
Correlation coefficient (R2)	**0.990 ≤ R2 ≤ 1.000**	**CORRECT**

If the **Correlation coefficient (R2)** value does not fall within the limits, this means that problems occurred during the amplification or detection step (incorrect dispensation of the reaction mixture or of the standards, degradation of the reaction mixture or of the standards, incorrect setting of the position of the standards, incorrect setting of the thermal cycle) which may lead to incorrect results. The session is not valid and needs to be repeated starting from the amplification step.

The **Ct** values of ADV in the amplification reaction of each **sample** and the **Standard Curve** of the amplification session are used to calculate the **Quantity** of target DNA present in the amplification reactions of the samples.

This product is able to quantify from 1,000,000 to 10 copies of DNA of the Hexon protein gene of ADV in the amplification reaction corresponding to the genome Equivalents per reaction (linear measuring range, see Performance Characteristics), as described in the following table:

Sample result detector FAM "ADV"	ADV genome Equivalents per reaction
Quantity > 1 x 10^6	MORE THAN 1,000,000
1 x 10^1 ≤ Quantity ≤ 1 x 10^6	= Quantity
Quantity < 1 x 10^1	LESS THAN 10

The results (**Quantity**) of each **sample** (Results > Report) are used to calculate the genome Equivalents (**gEq**) of ADV present in the sample used in the extraction (**Nc**) according to this formula:

$$\text{Nc (gEq / mL)} = \frac{\text{Ve x Quantity}}{\text{Vc x Va x Ep}}$$

Where:
Vc is the quantity of the sample used in the extraction in rate to the required unit of measurement;
Ep is the efficiency of the procedure, extraction and amplification, **expressed in decimal**,
Ve is the total volume of the extraction product **expressed in µL**;
Va is the volume of the extraction product used in the amplification reaction **expressed in µL**;
Quantity is the result of the amplification reaction of the sample **expressed in gEq per reaction**.

When «**EXTRAblood**» extraction kit is used with whole blood samples collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for whole blood and «EXTRAblood»
Nc (gEq / mL) = 25 x Quantity

When «**EXTRAblood**» extraction kit is used with nasal washes and nasal swabs samples and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for nasal washes, nasal swabs and «EXTRAblood»
Nc (gEq / mL) = 15 x Quantity

When **«ELITe STAR»** is used with whole blood samples collected in EDTA or plasma samples collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for whole blood, plasma and «ELITe STAR»
Nc (gEq / mL) = 28 x Quantity

When **«ELITe GALAXY»** is used with whole blood samples collected in EDTA or plasma samples collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for whole blood, plasma and «ELITe GALAXY»
Nc (gEq / mL) = 35 x Quantity

When «**NucliSENS® easyMAG®**» extraction system is used with whole blood samples collected in EDTA and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for whole blood and «NucliSENS® easyMAG®»
Nc (gEq / mL) = 50 x Quantity

When «**NucliSENS® easyMAG®**» extraction system is used with nasal washes and nasal swabs samples and the result **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for nasal washes, nasal swabs and «NucliSENS® easyMAG®»
Nc (gEq / mL) = 10 x Quantity

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When «**QIAsymphony® SP/AS**» extraction system is used with whole blood samples collected in EDTA and the result is **expressed in gEq / mL** is required, the formula becomes:

Simplified formula for whole blood and «QIAsymphony® SP/AS»
Nc (gEq / mL) = 23 x Quantity

Calculation of the linear measuring range limits

When a particular extraction method is used, the linear measuring range limits as gEq / mL of the sample may be calculated from the linear measuring range of the amplification reaction according to this formula:

$$\text{Lower limit (gEq / mL)} = \frac{\text{Ve x 10 gEq}}{\text{Vc x Va x Ep}}$$

$$\text{Upper limit (gEq / mL)} = \frac{\text{Ve x 1,000,000 gEq}}{\text{Vc x Va x Ep}}$$

When «**EXTRAblood**» extraction kit is used with cellular samples, the formula becomes:

Linear measuring range limits (gEq / mL) with «EXTRAblood»
Lower limit (gEq / mL) = 25 x 10 gEq Upper limit (gEq / mL) = 25 x 1,000,000 gEq
from 250 to 25,000,000 gEq / mL

When «**EXTRAblood**» extraction kit is used with non cellular samples, the formula becomes:

Measuring range limits (gEq / mL) with «EXTRAblood»
Lower limit (gEq / mL) = 15 x 10 gEq Upper limit (gEq / mL) = 15 x 1,000,000 gEq
from 150 to 15,000,000 gEq / mL

When **«ELITe STAR»** is used with cellular samples or non-cellular samples, the formula becomes:

Measuring range limits (gEq / mL) with «ELITe STAR»
Lower limit (gEq / mL) = 28 x 10 gEq Upper limit (gEq / mL) = 28 x 1,000,000 gEq
from 280 to 28,000,000 gEq / mL

When **«ELITe GALAXY»** is used with cellular samples or non-cellular samples, the formula becomes:

Measuring range limits (gEq / mL) with «ELITe GALAXY»
Lower limit (gEq / mL) = 35 x 10 gEq Upper limit (gEq / mL) = 35 x 1,000,000 gEq
from 350 to 35,000,000 gEq / mL

When «**NucliSENS® easyMAG®**» extraction system is used with cellular samples, the formula becomes:

Measuring range limits (gEq / mL) with «NucliSENS® easyMAG®»
Lower limit (gEq / mL) = 50 x 10 gEq **Upper limit (gEq / mL) = 50 x 1,000,000 gEq**
from 500 to 50,000,000 gEq / mL

When «**NucliSENS® easyMAG®**» extraction system is used with non cellular samples, the formula becomes:

Measuring range limits (gEq / mL) with «NucliSENS® easyMAG®»
Lower limit (gEq / mL) = 10 x 10 gEq **Upper limit (gEq / mL) = 10 x 1,000,000 gEq**
from 100 to 10,000,000 gEq / mL

When «**QIAsymphony® SP/AS**» extraction system is used with cellular samples, the formula becomes:

Measuring range limits (gEq / mL) with «QIAsymphony® SP/AS»
Lower limit (gEq / mL) = 23 x 10 gEq **Upper limit (gEq / mL) = 23 x 1,000,000 gEq**
from 230 to 23,000,000 gEq / mL

PERFORMANCE CHARACTERISTICS

Analytical sensitivity: limit of detection

The analytical sensitivity of this assay allows detecting the presence of about 10 target DNA molecules in the 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of the assay, as detection limit, was tested using a plasmid DNA containing the amplification product whose initial concentration was measured by spectrophotometer. The plasmid DNA was diluted to a titre of 10 copies / 20 µL in human genomic DNA at a titre of 500 ng / 20 µL. This sample was tested in 50 replicates carrying out the amplification by ELITechGroup S.p.A. products.

The final results are summed up in the following table.

Samples	No.	positive	negative
10 copies of plasmid DNA + 500 ng of human genomic DNA	50	50	0

The analytical sensitivity of this assay used in association to whole blood samples collected in EDTA and **ELITe GALAXY** was verified with a panel of Adenovirus dilutions within the limiting concentration. The panel was prepared by diluting the ADV12-01 sample of the "QCMD 2012 Adenovirus EQA Panel" (Qnostics, Ltd, UK) in Adenovirus DNA - negative EDTA whole blood. The viral concentrations ranged from 10 gEq / mL to 560 gEq / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The analytical sensitivity as gEq/mL is reported below

Limit of Detection for whole blood samples and ELITe GALAXY (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**169 gEq / mL**	105 gEq / mL	430 gEq / mL

The analytical sensitivity of this assay used in association to plasma samples collected in EDTA and **ELITe GALAXY** was verified with a panel of Adenovirus dilutions within the limiting concentration. The panel

was prepared by diluting the ADV12-01 sample of the "QCMD 2012 Adenovirus EQA Panel" (Qnostics, Ltd, UK) in Adenovirus DNA - negative EDTA plasma. The viral concentrations ranged from 10 gEq / mL to 560 gEq / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products. The statistical analysis was performed by the Probit regression. The limit of detection was calculated for the concentrations at which the probability of a positive result is the 95%.

The analytical sensitivity as gEq/mL is reported below

Limit of Detection for plasma samples and ELITe GALAXY (gEq / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**80 gEq / mL**	59 gEq / mL	196 gEq / mL

Analytical sensitivity: linear measuring range

The analytical sensitivity of this assay allows the quantification from 1,000,000 to 10 molecules of target DNA in the 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of the assay, as linear measuring range, was determined using a panel of dilutions (1 $\log_{10}$ between one dilution and the next) of a plasmid DNA containing the amplification product whose initial concentration was measured by a spectrophotometer. The dilutions from 10^7 molecules per reaction to 10^1 molecules per reaction were tested in 9 replicates carrying out the amplification by ELITechGroup S.p.A. products.

The analysis of the obtained data, performed by linear regression, demonstrated that the assay displays a linear response for all the dilutions (linear correlation coefficient greater than 0.99).

The upper limit of the linear measuring range was set at 10^6 molecules per reaction, corresponding to the genome Equivalents per reaction, within one logarithm from the highest concentration Q - PCR Standard amplification standard (10^5 molecules / 20 µL).

The lower limit of the linear measuring range was set at 10 molecules per reaction, corresponding to the genome Equivalents per reaction, within one logarithm from the lowest concentration Q - PCR Standard amplification standard (10^2 molecules / 20 µL).

The final results are summed up in the following table.

Linear measuring range (gEq / reaction)	
Upper limit	1,000,000 gEq / reaction
Lower limit	10 gEq / reaction

The linear measuring range limits as gEq / mL referring to the used extraction kit are calculated at page 25.

Analytical sensitivity: Precision and Accuracy

The precision of the assay, as the variability of results obtained with several replicates of a sample tested within the same amplification session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 28.6% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The accuracy of the assay, as the difference between the mean of results obtained with several replicates of a sample within the same amplification session and the theoretical concentration value of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 13.1% of measured quantities, within the range from 10^6 molecules to 10^1 molecules in the 20 µL of DNA added to the amplification reaction.

The precision and the accuracy were determined using data obtained for the study of the linear measuring range.

Analytical sensitivity: reproducibility with calibrated reference material

The analytical sensitivity of the assay, as reproducibility of results, was checked testing a sample of certified reference material.

The tests were carried out using as certified reference material the sample CE Marked Material Human Adenovirus serotype 2 for Nucleic Acid Amplification Techniques (NIBSC, UK). The sample of

certified reference material was tested in 2 replicates carrying out the extraction procedure and each replicate was amplified in duplicate, by ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with certified reference material				
Sample	Virus	Ct Expected Result	Positive / Replicates	Ct Mean
08/114-001	Human Adenovirus type 2	~ 30	4 / 4	29.10

The sample was correctly detected in all the replicates. The quantification of the sample, expressed as Ct value, is similar to that declared from the supplier.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, as confirmation of positive clinical samples, was tested using some clinical samples spiked with ADV DNA.

The diagnostic sensitivity was evaluated using as reference material 24 whole blood samples collected in EDTA (Biological Sample Library Europe S.A.S., France) tested negative for the ADV DNA with a CE IVD nested amplification product and spiked with certified reference material (CE Marked Material Human Adenovirus serotype 2 for Nucleic Acid Amplification Techniques, NIBSC) to obtain a Ct value of about 36 (equal to about 500 gEq /mL). Each sample was tested carrying out the whole analysis, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Whole blood collected in EDTA spiked for ADV DNA	24	24	0

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

The diagnostic sensitivity was evaluated using 30 samples of plasma collected in EDTA and negative for ADV DNA, that were spiked for ADV DNA adding ADV12-01 sample, from QCMD 2012 Adenovirus EQA Panel (Qnostics Ltd, UK) and 30 whole blood samples collected in EDTA negative for ADV DNA, that were spiked for Adenovirus DNA adding ADV12-05 sample, from QCMD 2012 Adenovirus EQA Panel (Qnostics Ltd, UK). Each sample was used to carry out the whole analysis procedure: extraction with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA spiked for ADV DNA	30	30	0
Whole blood collected in EDTA spiked for Adenovirus DNA	30	29	1

29/30 whole blood samples are reported as positive for ADV DNA. One samples gave a negative result. This result was confirmed with a second amplification and is probably due to due to a likely presence of an inhibitor.

The diagnostic sensitivity of the assay in this test was equal to 98%.

The diagnostic sensitivity was evaluated using 30 samples of plasma collected in EDTA negative for Adenovirus DNA, that were spiked for Adenovirus DNA adding ADV12-01 sample, from QCMD 2012 Adenovirus EQA Panel (Qnostics Ltd, UK) and 30 whole blood samples collected in EDTA negative for Adenovirus DNA, that were spiked for Adenovirus DNA adding ADV12-01 sample, from QCMD 2012 Adenovirus EQA Panel (Qnostics Ltd, UK). Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA spiked for Adenovirus DNA	30	30	0
Whole blood collected in EDTA spiked for Adenovirus DNA	30	30	0

All spiked samples were correctly detected as positive for ADV DNA.

The diagnostic sensitivity of the assay in this test was equal to100%.

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The diagnostic sensitivity was evaluated using as reference material 23 nasal washes samples spiked positive for the ADV DNA with a CE IVD nested amplification product. Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Nasal washes spiked for ADV DNA	23	23	0

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

Diagnostic sensitivity: detection and quantification efficiency with different genotypes

The diagnostic sensitivity of the assay, as detection and quantification efficiency with different genotypes, was evaluated by comparison of sequences with nucleotide databases.

The analysis of the regions chosen for the hybridisation of the primers and of the fluorescent probe in the alignment of the sequences available in the database for the region of the Hexon protein gene of ADV, including genotypes A, B, C, D, E, F and G (corresponding to 57 serotypes), showed their conservation and absence of significant mutations.

The diagnostic sensitivity of the assay, as detection and quantification efficiency with different genotypes, was checked using some plasmid constructs corresponding to genotypes showing nucleotide variations in the sequence of the amplified region.

The diagnostic sensitivity of the assay was checked using plasmids containing the sequence of the amplified region of the following genotypes: A serotype 12, A serotype 18, A serotype 31, B serotype 3, B serotype 16, B serotype 34, B serotype 35, C serotype 1, D serotype 19, E serotype 4, F serotype 40, F serotype 41 and G serotype 52. The initial concentration of the plasmids was measured by a spectrophotometer. Plasmids were diluted to a titre of 50,000, 5,000 and 500 copies per reaction. These samples were tested in 3 replicates carrying out the amplification by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Tests with plasmids corresponding to different genotypes						
	Expected Qty log_{10} gEq / reac.	-	Expected Qty log_{10} gEq / reac.	-	Expected Qty log_{10} gEq / reac.	-
	4.699	-	3.699	-	2.699	-
Genotype, serotype	Mean Qty detected log_{10} gEq / reac.	Standard Deviation	Mean Qty detected log_{10} gEq / reac.	Standard Deviation	Mean Qty detected log_{10} gEq / reac.	Standard Deviation
A, serotype 12	4.774	0.010	3.930	0.007	2.996	0.071
A, serotype 18	4.626	0.016	3.670	0.006	2.641	0.070
A, serotype 31	4.855	0.015	3.943	0.011	2.936	0.083
B, serotype 3	4.744	0.019	3.814	0.007	2.789	0.064
B, serotype 16	4.738	0.022	3.750	0.053	2.786	0.034
B, serotype 34	4.635	0.020	3.661	0.035	2.589	0.019
B, serotype 35	4.971	0.009	3.988	0.022	2.941	0.017
C, serotype 1	4.766	0.017	3.790	0.033	2.782	0.022
D, serotype 19	4.765	0.018	3.805	0.050	2.811	0.032
E, serotype 4	4.995	0.011	4.059	0.008	3.079	0.024
F, serotype 40	4.871	0.039	3.966	0.008	2.920	0.064
F, serotype 41	4.814	0.004	3.889	0.005	2.832	0.025
G, serotype 52	4.848	0.039	3.944	0.017	2.905	0.064

All samples were correctly detected in all the replicates. The obtained quantification is within the range defined by the expected value ± 0.4 log_{10}.

Analytical specificity: absence of cross-reactivity potential interfering markers

The analytical specificity of the assay, as absence of cross-reactivity with other potential interfering markers, was evaluated by comparison of sequences with nucleotide databases.

The analysis of the alignment of the sequences of the primers and of the fluorescent probe with the sequences available in databases for organisms other than ADV, including CMV and EBV complete genomes, showed their specificity and the absence of significant homology.

The analytical specificity of the assay, as absence of cross-reactivity with other potential interfering markers, was checked using some clinical samples negative for ADV DNA and positive for DNA of other pathogens.

The analytical specificity was checked using as reference material 20 whole blood samples collected in EDTA, that were negative for ADV DNA but positive for DNA of other pathogens including CMV and EBV, tested with CE IVD real time amplification products. Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Whole blood collected in EDTA positive for CMV DNA	13	0	13
Whole blood collected in EDTA positive for EBV DNA	5	0	4
Whole blood collected in EDTA positive for HHV6 DNA	2	0	1

An EBV positive sample and a HHV6 positive sample resulted invalid.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative clinical samples, was tested using some clinical samples negative for ADV DNA.

The diagnostic specificity was evaluated using as reference material 24 whole blood samples collected in EDTA, that were negative for ADV DNA, tested with a CE IVD nested amplification product. Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Whole blood collected in EDTA negative for ADV DNA	24	0	24

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

The diagnostic specificity was evaluated using 30 plasma samples collected in EDTA that were presumably negative for ADV DNA (tested with a real time amplification CE IVD product) and 30 whole blood samples collected in EDTA that were presumably negative for ADV DNA (tested with a real time amplification CE IVD product). Each sample was used to carry out the whole analysis procedure: extraction with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA negative for ADV DNA	30	0	30
Whole blood collected in EDTA presumably negative for ADV DNA	30	0	28

Two samples of whole blood gave invalid result due to a likely presence of an inhibitor. 28 samples resulted valid for analysis and were confirmed negative.

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

The diagnostic specificity was evaluated using 34 plasma samples collected in EDTA that were presumably negative for Adenovirus DNA and 34 whole blood samples collected in EDTA that were presumably negative for Adenovirus DNA (tested with a real time amplification CE IVD product). Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	N	positive	negative
Plasma collected in EDTA presumably negative for Adenovirus DNA	34	0	34
Whole blood collected in EDTA presumably negative for Adenovirus DNA	34	1	33

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All negative plasma samples were correctly detected as negative for Adenovirus DNA.

33/34 negative whole blood samples were confirmed negative, 1/34 sample resulted discrepant positive (45 gEq/mL). This sample at low titre is below the limit of detection of the method in test and probably also of the reference method for Adenovirus – DNA, this sample can randomly test either negative or positive.

The diagnostic specificity of the assay in this test was equal to 98.5%.

The diagnostic specificity was evaluated using as reference material 20 nasal washes samples that were negative for ADV DNA, tested with a CE IVD nested amplification product. Each sample was tested carrying out the whole analysis procedure, extraction and amplification, by ELITechGroup S.p.A. products.

The results are summed up in the following table.

Samples	No.	positive	negative
Nasal washes negative for ADV DNA	20	2	18

Two samples reported a positive result with a viral titre equal to 150 and 46 gEq/mL. Due to the low viral titre, the samples couldn't have been detected during the analysis with the reference method.

The diagnostic specificity of the assay in this test was equal to 90%.

Note: The complete data and results of the tests carried out to evaluate the product performance characteristics with matrices and instruments are recorded in the Product Technical File "ADENOVIRUS ELITe MGB® Kit", FTP RTS078PLD.

Roche cobas z 480 analyzer

SAMPLES AND CONTROLS

Samples

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

Whole blood collected in EDTA

The whole blood samples for DNA extraction must be collected in EDTA and identified according to laboratory guidelines, transported at +2° / +8°C and stored at +2° / +8°C for a maximum of three days, otherwise they must be frozen and stored at -20°C for a maximum of thirty days or at -70°C for longer periods. It is recommended to split the samples to be frozen into aliquots in order to prevent repeated cycles of freezing and thawing. When using frozen samples, thaw the samples just immediately before the extraction in order to avoid possible nucleic acid degradation.

Note: when you carry out DNA extraction from whole blood samples with the **"MagNA Pure 24 System"** instrument with **software version 1.0** (or equivalent later versions), use the **"Pathogen200"** extraction protocol and follow these instructions: dispense **350 µL** of sample into the MagNA Pure Tube 2.0 mL, load the tube into the instrument and begin the extraction. This protocol processes 200 µL of sample, adds **CPE** 20 µL / extraction and elutes the nucleic acids into 100 µL. The **CPE** must be diluted 1:2 in ultra-pure molecular biology grade water. For details of the extraction procedure, follow the instructions contained in the kit's User Manual carefully.

Other samples

There are no data available concerning product performance with DNA extracted from the following clinical samples: plasma, nasal washes, nasal swabs, fecal supernatant and cerebrospinal fluid.

Interfering substances

The DNA extracted from the sample must not contain heparin, haemoglobin, dextran, Ficoll®, ethanol or 2-propanol in order to prevent inhibition problems and the possibility of frequent invalid results.

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

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

Amplification controls

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

For the negative control, add ultra-pure molecular biology grade water (not included in the kit) to the reaction instead of the DNA extracted from the sample.

For the positive control, use the **«ADENOVIRUS - ELITe Positive Control»** product or alternatively **«ADENOVIRUS - ELITe Positive Control RF»** product or the **«ADENOVIRUS ELITe Standard»** product.

Quality controls

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

PROCEDURE

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

When **cobas z 480 analyzer (Roche)** instrument is used:

Before starting the session, referring to the instrument documentation, it is necessary to:
- switch on the control computer and the real time thermal cycler. Open the dedicated software and in the main window, open an "New Experiment" session;
- set the reaction volume ("Reaction volume") to 40 µL;
- assign an identifier to each sample ("Sample editor");
- define the reaction's Thermal Cycle according to the following table:

Thermal Cycle		
Stage	**Temperatures**	**Periods**
Decontamination	50°C	2 mins.
Initial denaturation	94°C	2 mins.
Amplification and detection (45 cycles)	94°C	10 sec.
	60°C (fluorescence acquisition)	30 sec.
	72°C	20 sec.

Note: fluorescence acquisition occurs individually, set Ramp Rate (°C/sec) to 4.4 °C/sec.

- select the signal detection channels: "detector" for the ADV sensor with "channel FAM 465-510" and "detector" for the IC internal control sensor with "channel VIC 540-580";

Fill in the **Work Plan** attached at the end of this User Manual, transcribing this information or printing the microplate's layout. This **Work Plan** must be followed carefully when transferring the reaction mixture and samples into the wells.

Note: to determine the concentration of DNA in the source sample, you must perform a series of reactions with **Q - PCR Standard** (10^5 copies, 10^4 copies, 10^3 copies, 10^2 copies) to obtain the **Standard Curve**.

See below, by way of example, how you can organise the quantitative analysis of 12 samples.

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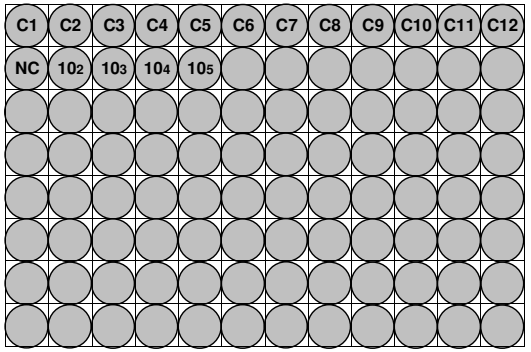


Legend: C1 - **C12**: Samples to be analyzed; **NC**: Negative amplification control;
10^2: Standard 10^2 copies; **10^3**: Standard 10^3 copies; **10^4**: Standard 10^4 copies; **10^5**: Standard 10^5 copies.

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

Before starting the session, it is necessary to:
- retrieve and thaw the test tubes containing the samples to be analyzed. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve and thaw the test tubes containing **ADV Q - PCR Mix** required for the session, remembering that the contents of each tube is enough to perform **25 reactions**. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve and thaw the test tubes containing **ADV – Positive Control** or alternatively **ADV - ELITe Positive Control RF** or **ADV Q - PCR Standard**. Shake the tubes gently and then place them in the centrifuge for 5 seconds to send the contents to the bottom and then keep them on ice;
- retrieve the **AD-plate** to be used in the session, making sure you handle it wearing dust-free gloves and do not damage the wells.

1. Without creating any bubbles and depositing it precisely on the bottom, transfer **20 µL** of reaction mixture **ADV Q - PCR Mix** into the wells on the **AD-plate** as previously established in the **Work Plan**.

Note: If not using all the reaction mixture, store any remaining mixture at -20°C for a maximum of one month. Freeze and thaw the reaction mixture a maximum of **5 TIMES**.

2. Depositing it precisely into the reaction mixture, transfer **20 µL** of **extracted DNA** from the first sample in the corresponding well on the **AD-plate** as previously established in the **Work Plan**. Mix the sample well by pipetting the **extracted DNA** three times into the reaction mixture. Be sure not to create any bubbles. Proceed in the same manner with all the other **extracted DNA**.
3. Depositing it precisely into the reaction mixture, transfer **20 µL** of **ultra-pure molecular biology grade water** (not supplied with the product) into the well on the **AD-plate** containing the negative amplification control as previously established in the **Work Plan**. Mix the negative control well by pipetting the **ultra-pure molecular biology grade water** three times into the reaction mixture. Be sure not to create any bubbles.
4. On the basis of the result required (qualitative or quantitative), one of these two options must be followed:

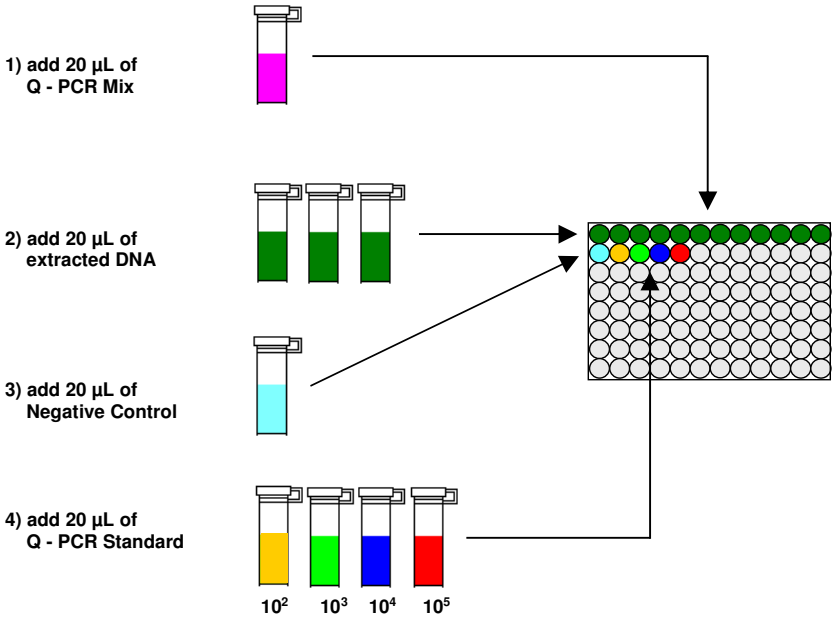
   - When a **qualitative** result is required (detection of Adenovirus DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **ADV - Positive Control** or alternatively **ADV - ELITe Positive Control RF** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the **ADV - Positive Control** three times into the reaction mixture. Avoid creating bubbles.
   - When a **quantitative** result is required (quantification of Adenovirus DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **ADV Q - PCR Standard 10^2** in the corresponding well of

Amplification microplate, as previously established in the **Work Sheet**. Mix well the standard by pipetting the **ADV Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other **ADV Q - PCR Standards** (**10^3, 10^4, 10^5**).

5. Carefully seal the **AD-plate** using the **Sealing Film.**
6. Transfer the **AD-plate** into the real-time Thermal Cycler in the amplification/detection of amplification products area and start the amplification thermal cycle, saving the session settings under a unique and recognizable identifier (e.g. "year-month-day-ADV-EGSpA").

Note: At the end of the thermal cycle, the **AD-plate** and reaction products must be removed from the instrument and disposed of in a way that does not cause environmental pollution. **Never remove** the **Sealing Film from the Amplification microplate** to avoid any leakage of the reaction products.

The following figure shows synthetically the preparation of the amplification reaction..



Qualitative results analysis

The emitted fluorescence values recorded by the ADV detector and Internal Control (IC) detector during the amplification reactions must be analyzed by the instrument's software.

Select the menu " Analysis" and choose "Absolute Quant/Fit Points" (2 points)

Select the group of samples to be analyzed

In accordance with the instrument's documentation, before starting the analysis you must:
- manually enter the calculation range (Background button) for the **Background Fluorescence Level** from cycle 2 to cycle 6.

- manually set the **Threshold** and **Noiseband** for the FAM "ADV" detector to **0.80**;
- manually set the **Threshold** and **Noiseband** for the VIC "IC" detector to **1.5**

The fluorescence values emitted by the specific detectors in the amplification reaction and the **Threshold** and **Noiseband** fluorescence values are used to determine the **Threshold Cycle** (**Ct**), i.e. the cycle in which the fluorescence **Threshold** is reached.

The **Ct** values for ADV in the amplification reactions of the four **Q - PCR Standard** are used to calculate the **Standard Curve** (Results > Standard Curve) of that amplification session and to validate the

amplification and detection as shown in the following table:

Reaction Q - PCR Standard 10^5 "ADV" detector	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive control** amplification reaction is **Ct > 25** or **Ct Undetermined** for ADV, the target DNA was not correctly detected. This means that problems occurred during the amplification or detection step (incorrect dispensation of the reaction mix or of the positive control, degradation of the reaction mix or of the positive control, incorrect setting of the position of the positive control, incorrect setting of the thermal cycle) which may lead to incorrect results. The session is not valid and needs to be repeated starting from the amplification step.

*** Note:** When this product is used for the quantification of ADV DNA, the **Q - PCR Standard** reactions were set up instead of the **Positive Control** reaction. In this case, validate the amplification and the detection by referring to the amplification reaction of **Q - PCR Standard 10^5** (**Ct ≤ 25**).

During the **Negative Control** amplification reaction, the value of **Ct** for ADV (Analysis window) is used to validate amplification and detection as shown in the following table:

Negative Control Reaction "ADV" detector	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative Control** amplification reaction is other than **Ct Undetermined** for ADV, the presence of the DNA target has been detected. Problems occurred during the amplification stage (contamination) which can lead to incorrect results and false positives. The session is invalid and must be repeated from the amplification stage.

During the amplification reactions for each **sample**, the value of **Ct** for ADV is used to detect the presence of the DNA target, whilst the value of **Ct** for the Internal Control is used to validate the extraction, amplification and detection.

Note: Check using the instrument's software (Analysis window) that the **Ct** is determined by a rapid and regular increase in fluorescence values and not by peaks or an increase of the background signal (irregular or noisy background).

Results like **Ct** from each **sample**'s amplification reactions (Analysis window) are used as shown in the following table:

Sample reaction		Sample suitability	Assay result	ADV DNA
"ADV" detector	"IC" detector			
Ct Undetermined	Ct > 35 or Ct Undetermined	not suitable	invalid	-
	Ct ≤ 35	suitable	valid, negative	NOT DETECTED
Ct Determined	Ct > 35 or Ct Undetermined	suitable	valid, positive	DETECTED
	Ct ≤ 35	suitable	valid, positive	DETECTED

If the result of a sample's amplification reaction is **Ct Undetermined** for ADV and **Ct > 35** or **Ct Undetermined** for the Internal Control, it was not possible to detect the Internal Control DNA efficiently. In this case, problems occurred during the amplification stage (inefficient or null amplification) or in the extraction stage (degraded sample DNA, sample with insufficient number of cells, loss of DNA during extraction or presence of inhibitors in the extracted DNA) which can cause incorrect results and false negatives. The sample is not suitable, the assay is not valid and must be repeated starting from the extraction of a new sample.

If the result of a sample's amplification reaction is **Ct Undetermined** for ADV and

Ct ≤ 35 for the Internal Control, the ADV DNA was not detected in the DNA extracted from the sample but it cannot be excluded that the ADV DNA is present at a concentration lower than the product's limit of detection (see Performance Characteristics). In this case, the result would constitute a false negative.

The results obtained with this assay must be interpreted by considering all the clinical data and the results of other laboratory tests connected to the patient.

Note: When ADV DNA is detected during the amplification reaction of a sample, amplification of the Internal Control can produce a result of Ct > 35 or Ct Undetermined. In fact, the low-efficiency Internal Control amplification reaction can be eliminated from the competition with the high-efficiency ADV reaction. In this case, the sample is then suitable and the positive assay result is valid.

Quantitative results analysis

After having performed the qualitative analysis procedure, you can carry out the quantitative analysis of the results relating to the positive sample.

If the result of the amplification reaction for the **Q - PCR Standard 10^5** is **Ct > 25** or **Ct Undetermined** or if the Ct values of the four Q - PCR standards don't fit regularly the standard curve the DNA target was not correctly detected. Problems occurred during the amplification or detection stage (incorrect dispensing of the reaction mixture or standards, degradation of the reaction mixture or standards, incorrect setting of the standard positions, incorrect setting of the thermal cycle) which can cause incorrect results. The session is invalid and must be repeated from the amplification stage.

The **Ct** values for ADV in the amplification reactions of each **sample** and the **Standard Curve** (**Standard Curve** button) from the amplification session are used to calculate the **Quantity** of DNA target present in the amplification reactions relating to the samples.

This product is able to quantify from 1,000,000 down to around 10 copies per reaction, from 25,000,000 to 250 copies per mL of whole blood using the **MagNA Pure 24** extraction system (see Performance Characteristics), as shown in the following table:

Sample result FAM "ADV" detector	ADV copies per reaction
Quantity > 1 x 10^6	GREATER THAN 1,000,000
1.0 x 10^1 ≤ Quantity ≤ 1 x 10^6	= Quantity
Quantity < 1.0 x 10^1	LESS THAN 10

The results (**Quantity**) relating to each **sample** (Analysis window) are used to calculate the **copies** of ADV present in the source sample (**Nc**) according to this formula:

$$Nc = \frac{Ve \times Quantity}{Vc \times Va \times Ep}$$

Where:
Vc is the quantity of sample used in the extraction in relation to the required unit of measure;
Ep is the efficiency of the procedure, extraction and amplification, **expressed in decimals**,
Ve is the total volume obtained from the extraction **expressed in µL**;
Va is the volume of extraction product used in the amplification reaction **expressed in µL**;
Quantity is the result of the amplification reaction relating to the sample **expressed in copies per reaction**.

When using samples of whole blood collected in EDTA and the **MagNA Pure 24** extraction system and the result is to be **expressed in copies / mL**, the formula becomes:

Simplified formula for whole blood and MagNA Pure 24
Nc (copies / mL) = 25 x Quantity

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PERFORMANCE CHARACTERISTICS

Analytical sensitivity: limit of detection

The analytical sensitivity of this assay, as limit of detection, allows for the detection of around 10 copies in 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of this assay, as limit of detection, has been tested using a plasmid DNA containing the amplification product whose initial concentration was measured using a spectrophotometer. The plasmid DNA was diluted to a concentration of 10 copies / 20 µL in 150,000 copies of pBETAGLOBIN / 20 µL. This sample was used in 36 replicates to carry out amplification using ELITechGroup S.p.A. products. The final results are summarized in the following table.

Samples	N	positives	negatives
10 copies of plasmid DNA + 150,000 copies of Beta-globin	36	36	0

Analytical sensitivity: linear measuring range

The analytical sensitivity of this assay, as linear measuring range, allows for the quantification from around 1,000,000 to 10 copies in 20 µL of DNA added to the amplification reaction.

The analytical sensitivity of this assay was evaluated using a panel of dilutions (1 Log_{10} between one dilution and the next) of plasmid DNA containing the amplification product, whose initial concentration was measured using a spectrophotometer. The points of the panel from 10^7 molecules per reaction to 10^1 molecules per reaction were used in 9 replicates to carry out amplification using ELITechGroup S.p.A. products. Analysis of the obtained data, performed using linear regression, showed that the assay has a linear response for all panel points (linear correlation coefficient greater than 0.99).

The lower limit of the linear measuring range was set at around 10 copies / reaction within one logarithm from the lowest concentration of Q - PCR Standard amplification standard (10^2 copies / 20 µL).

The upper limit of the linear measuring range was set at 10^6 copies / reaction within one logarithm from the highest concentration of Q - PCR Standard amplification standard (10^5 copies / 20 µL).

The results are shown in the following table.

Linear measuring range using MagNA Pure 24		
	Lower limit	Upper limit
copies / mL	**250**	**25,000,000**
copies / reaction	**10**	**1,000,000**

Conversions from copies / mL to copies / reaction and vice versa were calculated as shown on page 39.

Analytical sensitivity: Precision and Accuracy

The precision of this assay, in terms of the variability of the results obtained in the same amplification session using different replicates of a sample, allowed to obtain a mean Variation Coefficient percentage (VC%) of the values of Ct lower than 2% in the range from 10^6 molecules to 10^1 molecules in 20 µL of DNA added to the amplification reaction.

The precision of this assay, in terms of the variability of the results obtained in the same amplification session using different replicates of a sample, allowed to obtain a mean Variation Coefficient percentage (VC%) of the measured quantities of around 9% in the range from 10^6 molecules to 10^1 molecules in 20 µL of DNA added to the amplification reaction.

The accuracy of this assay, in terms of the difference between the mean of the results obtained in the same amplification session using different replicates of a sample and the sample's theoretical concentration value, allowed to obtain a mean Inaccuracy percentage of the measured Log quantity of around 1% in the range from 10^6 molecules to 10^1 molecules in 20 µL of DNA added to the amplification reaction.

Precision and accuracy were determined using the data obtained during the experiments assessing the linear measuring range.

Analytical sensitivity: reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of value of a calibrated reference material, was evaluated using as reference material the «ADENOVIRUS Molecular 'Q' Panel» (Qnostics Ltd, UK). Each sample of the panel was tested in 4 replicates carrying out the whole analysis procedure: extraction using the **MagNA Pure 24** automatic extraction system and amplification using ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials and «MagNA Pure 24»	
Sample	Positives / Replicates
ADVMQP01-High	4/4
ADVMQP01-Medium	4/4
ADVMQP01-Low	4/4
ADVMQP01-Negative	0/4

All samples were correctly detected.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity was evaluated using as reference material 29 samples of whole blood collected in EDTA negative for ADV DNA samples which were spiked for ADV DNA adding ADVMQP01-High sample (Qnostics Ltd, UK).

Each sample was used carrying out the whole analysis procedure: extraction using the **MagNA Pure 24** automatic extraction system and amplification using ELITechGroup S.p.A. products. The results are summarized in the following table.

Samples	N	positives	negatives
Whole blood collected in EDTA spiked for Adenovirus DNA	29	29	0

All whole blood samples were valid at first test and confirmed positive for ADV DNA.

The total diagnostic sensitivity of the assay was 100%.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity was evaluated using as reference material 41 samples of whole blood collected in EDTA presumably negative for ADV DNA.

Each sample was used carrying out the whole analysis procedure: extraction using the **MagNA Pure 24** automatic extraction system and amplification using ELITechGroup S.p.A. products. The results are summarized in the following table.

Samples	N	positives	negatives
Whole blood collected in EDTA presumably negative for ADV DNA	41	0	41

All whole blood samples were valid at first test and confirmed negative for ADV DNA.

The total diagnostic specificity of the assay was 100%.

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Robustness: invalid results using clinical samples

The robustness of this assay, in terms of the evaluation of invalid results using clinical samples in first analysis, was verified by analyzing clinical samples.

The number of invalid samples was verified using the results of clinical samples which were negative and positive for Adenovirus DNA having been analyzed using the **MagNA Pure 24** automatic extraction system and through amplification using ELITechGroup S.p.A. products. The results are shown in the following table.

Samples	N	Invalid	%
Whole blood collected in EDTA	71	1	1.5

Note: The complete data and results from the tests carried out to evaluate the product's performance characteristics with matrices and instruments are recorded in Section 7 of the Product Technical File for the " Adenovirus ELITe MGB® Kit", FTP RTS078PLD.

REFERENCES

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Wong S. et al. (2008) J. Med. Virol. 80: 856 - 865
E. A. Lukhtanov et al. (2007) Nucleic Acids Res. 35: e30

PROCEDURE LIMITATIONS

Use this product only with DNA extracted from the following clinical samples: whole blood collected in EDTA, plasma collected in EDTA, nasal washes and nasal swabs.

Do not use DNA extracted from heparinized samples with this product: heparin inhibits the amplification reaction of nucleic acids and causes invalid results.

Do not use extracted DNA that is contaminated with haemoglobin, dextran, Ficoll®, ethanol or 2-propanol with this product: these substances inhibit the amplification reaction of nucleic acids and may cause invalid results.

There are no data available concerning product performance with DNA extracted from the following clinical samples: fecal supernatant, cerebrospinal fluid.

Use this product only with the validated instruments and associated clinical samples indicated in the section "Samples and Controls".

There are no data available concerning inhibition caused by antiviral, antibiotic, chemotherapeutic or immunosuppressant drugs.

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

Owing to its high analytical sensitivity, the real time amplification method used in this product is sensitive to cross-contaminations from the ADV positive clinical 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, the cross-contaminations can be avoided only by good laboratory practices and following carefully these instructions for use manual.

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 qualified personnel trained in molecular biology techniques, such as extraction, amplification and detection of nucleic acids, to avoid incorrect results.

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

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

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

A negative result obtained with this product means that the ADV DNA is not detected in the DNA extracted from the sample; but it cannot be excluded that the ADV DNA 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 failed internal control and require retesting, starting from extraction, that can lead to a delay in obtaining final results.

Possible polymorphisms within the region of the viral genome covered by the product primers and probes may impair detection and quantification of ADV DNA.

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, as the prenatal diagnosis, this residual risk could contribute to wrong decisions with potentially dangerous effects for the patient.

TROUBLESHOOTING

Target DNA not detected in the Positive Control or Q - PCR Standard reactions or invalid correlation coefficient of the Standard curve	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Take care when dispensing reactions into the microplate wells and comply with the work sheet. Check the volumes of reaction mixture dispensed. Check the volumes of positive control or standard dispensed.
Incorrect session setup on ELITe InGenius and ELITe BeGenius.	Check the position of reaction mixture, positive control or standards. Check the volumes of reaction mixture, positive control or standards.
Probe degradation.	Use a new aliquot of reaction mixture.
Positive control or standard degradation.	Use a new aliquot of positive control or standard.
Instrument setting error.	Check the position settings for the positive control or standard reactions on the instrument. Check the thermal cycle settings on the instrument.

Target DNA detected in the Negative control reaction	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples, negative controls, positive controls or standards into the microplate wells and comply with the work sheet.
Incorrect session setup on ELITe InGenius and ELITe BeGenius.	Check the position of reaction mixture, positive control or standards. Check the volumes of reaction mixture, positive control or standards.
Error while setting the instrument	Check the position settings of the samples, negative controls, positive controls or standards on the instrument
Microplate badly sealed.	Take care when sealing the microplate.
Contamination of the molecular biology grade water.	Use a new aliquot of water.
Contamination of the reaction mixture.	Use a new aliquot of reaction mixture.

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Contamination of the extraction / preparation area for amplification reactions.	Clean surfaces and instruments with aqueous detergents, wash lab coats, replace test tubes and tips in use.

Target and Internal Control DNA not detected in the sample reactions	
Possible Causes	**Solutions**
Incorrect dispensing into the microplate wells.	Avoid spilling the contents of the sample test tube. Always change tips between one sample and another. Take care when dispensing samples into the microplate wells and comply with the work sheet.
Incorrect session setup on ELITe InGenius and ELITe BeGenius	Check the position of reaction mixture or samples. Check the volumes of reaction mixture or samples.
Internal Control degradation.	Use new aliquots 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 and amplification of sample.
Incorrect reagent storage.	Verify that reaction mix was not exposed to room temperature for more than 30 minutes.
Problems during extraction	Verify quality and concentration of extracted DNA.
Instrument error.	Contact ELITechGroup Technical Service.

Irregular or high background fluorescence in the reactions	
Possible causes	**Solutions**
Incorrect dispensing of sample.	Take care, by pipetting three times, when mixing samples, negative controls and positive controls or standards into the reaction mixture. Avoid creating bubbles.
Baseline setting error.	Set the baseline calculation range within cycles where the background fluorescence has already stabilized (check the "Results", "Component" data) and the signal fluorescence has not yet started to increase, e.g. from cycle 6 to cycle 15. Use the automatic baseline calculation by setting the "Auto Baseline" option.
With ELITeInGenius: Error 30103	
Possible Causes	**Solutions**
Too high concentration of target in the sample.	If significant amplification is observed in PCR plot: - repeat the amplification of eluted sample in molecular biology grade water, in a “PCR only” session or - repeat the extraction with a dilution of the primary sample in molecular biology grade water, in a “Extract + PCR” session.

SYMBOLS

REF	Catalogue Number.
	Upper limit of temperature.
LOT	Batch code.
	Use by (last day of month).
IVD	*in vitro* diagnostic medical device.
CE	Fulfilling the requirements of the European Directive 98\79\EC for *in vitro* diagnostic medical device.
	Contains sufficient for "N" tests.
	Attention, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

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

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