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EMPOWERING IVD

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NOTICE of CHANGE dated 03/06/2024

IMPORTANT COMMUNICATION FOR THE USERS OF PRODUCT:

«PVB19 ELITe MGB® Kit»
Ref. RTS070PLD

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

- *Update for the use of the product in association with «ELITe BeGenius®» instrument (REF INT040) and amniotic fluid matrix.*

  Update of PERFORMANCE CHARACTERISTICS (pag.16):

  - *Linear measuring range*
  - *Conversion factor to International Units*

- *Addition of UDI information.*

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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Parvovirus B19 ELITe MGB® Kit

reagents for DNA Real-Time PCR

REF RTS070PLD

CE IVD

-20°C

UDI 08033891483623

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INTENDED USE

The product **Parvovirus B19 ELITe MGB® Kit** is an *in vitro* diagnostic medical device intended to be used by healthcare professionals as a qualitative and quantitative nucleic acids Real-Time PCR assay for the **detection and quantification of the DNA of Parvovirus B19 human virus (PVB19)**, genotypes 1, 2, 3a and 3b, in DNA samples extracted from clinical specimens.

The assay is validated in association with the **ELITe InGenius®** and **ELITe BeGenius®** instruments, automated and integrated systems for extraction, Real-Time PCR and results interpretation, using human specimens of whole blood (peripheral and from bone marrow) collected in EDTA and amniotic fluid.

The assay is also validated in association with the **7300 Real-Time PCR System** and **7500 Real-Time PCR Instrument**, using human specimens of whole blood (peripheral and from bone marrow) collected in EDTA and plasma collected in EDTA.

This product is intended for use as an aid in the diagnosis and monitoring of Parvovirus B19 infections. The results must be interpreted in combination with all relevant clinical observation and laboratory outcomes.

ASSAY PRINCIPLE

The assay is a quantitative Real-Time PCR detecting the DNA of Parvovirus, isolated from specimens and amplified using the assay reagent **PVB19 Q PCR Mix,** that contains primers and probes with ELITe MGB technology.

The ELITe MGB probes are activated when hybridize with the related PCR products. **ELITe InGenius** and **ELITe BeGenius** monitor fluorescence increase and calculate the threshold cycle (Ct) and the melting temperatures (Tm). The **PVB19** DNA quantity is calculated based on a stored calibration curve.

In the ELITe MGB probes the fluorophores are quenched in the random-coiled, single-stranded state of probe. The fluorophores are active in the probe / amplicon duplex as the quencher is spatially separated from the fluorophore. Note the fluorophore is not cleaved during PCR and can be utilized for dissociation analysis and melting temperature calculation.

PRODUCT DESCRIPTION

The **Parvovirus B19 ELITe MGB Kit** provides the assay reagent **PVB19 Q PCR Mix**, an optimized and stabilized PCR mixture that contains the specific primers and probes for:

- PVB19 **VP1** region detected in Channel **PVB19;** the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled by FAM dye.
- Internal Control (IC), specific for the **promoter and 5' UTR** region of the **human beta Globin** gene, detected in Channel **IC**; the probe is stabilized by MGB, quenched by the Eclipse Dark Quencher®, and labelled with AquaPhluor® 525 (AP525) dye.

The **PVB19 Q PCR Mix** also contains buffer, magnesium chloride, nucleotide triphosphates, AP593 fluorophore (analogue of ROX or Cy5) as passive reference for fluorescence normalisation, the enzyme Uracil N-glycosidase (UNG) to inactivate contamination by the amplification product, the "hot start" DNA Polymerase.

The product **Parvovirus B19 ELITe MGB Kit** contains sufficient reagents for **96 tests** on **ELITe InGenius** and **ELITe BeGenius (24 tests each tube)** and for **100 tests** on **other systems (25 tests each tube),** with 20 µL used per reaction.

The **Parvovirus B19 ELITe MGB Kit** can be also used in association with other equivalent instruments.

MATERIALS PROVIDED IN THE PRODUCT

Component	Description	Quantity	Classification of hazards
PVB19 Q - PCR Mix **ref. RTS070PLD**	Mixture of reagents for Real-Time PCR in tube with **transparent cap**	**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 (~13,000 RPM).
- Micropipettes and sterile tips with aerosol filter or sterile positive displacement tips (0.5-10 µL, 2-20 µL, 5-50 µL, 50-200 µL, 200-1000 µL).
- 2.0 mL sterile screw capped tubes (Sarstedt, Germany, ref. 72.694.005).
- Molecular biology grade water.
- 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.

OTHER PRODUCTS REQUIRED

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

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

Instruments and Softwares	Products and Reagents
ELITe InGenius (ELITechGroup S.p.A., EG SpA ref. INT030) **ELITe InGenius Software** version 1.3.0.17 (or later) **PVB19 ELITe_STD**, Assay Protocol with parameters for Calibrators analysis **PVB19 ELITe_PC**, Assay Protocol with parameters for Positive Control analysis **PVB19 ELITe_NC**, Assay Protocol with parameters for Negative Control analysis **PVB19 ELITe_WB_200_100**, Assay Protocol with parameters for whole blood specimen analysis **PVB19 ELITe_AF_200_100**, Assay Protocol with parameters for amniotic fluid specimen analysis	**ELITe InGenius SP200** (EG SpA, ref. INT032SP200) **ELITe InGenius SP 200 Consumable Set** (EG SpA, ref. INT032CS) **ELITe InGenius PCR Cassette** (EG SpA, ref. INT035PCR), **ELITe InGenius Waste Box** (EG SpA, ref. F2102-000) **300 µL Filter Tips Axygen** (Corning Life Sciences Inc., ref. TF-350-L-R-S) with ELITe InGenius only **1000 µL Filter Tips Tecan** (Tecan, Switzerland, ref. 30180118) with ELITe BeGenius only **CPE – Internal Control** (EG SpA, ref. CTRCPE) **Parvovirus B19 ELITe Standard** (EG SpA, ref. STD070PLD) **Parvovirus B19 – ELITe Positive Control** (EG SpA, ref. CTR070PLD)
ELITe BeGenius (EG SpA ref. INT040) **ELITe BeGenius Software** version 2.1.0. (or later) **PVB19 ELITe_Be_STD**, Assay Protocol with parameters for Calibrators analysis **PVB19 ELITe_Be_PC**, Assay Protocol with parameters for Positive Control analysis **PVB19 ELITe_Be_NC**, Assay Protocol with parameters for Negative Control analysis **PVB19 ELITe_Be_WB_200_100**, Assay Protocol with parameters for whole blood specimen analysis **PVB19 ELITe_Be_AF_200_100**, Assay Protocol with parameters for amniotic fluid specimen analysis	
7300 Real-Time PCR System (ThermoFisher Scientific, ref. 4351101) **QIAsymphony® SP/AS** (QIAGEN GmbH, Ref. 9001297, 9001301) **NucliSENS® easyMAG®** (bioMérieux SA, Ref. 200111) **ELITe GALAXY** (EG S.p.A., ref. INT020) **ELITe STAR** (EG SpA ref. INT010)	**MicroAmp™ Optical 96-Well Reaction Plate** (LifeTechnologies, ref. N8010560) **CPE – Internal Control** (ELITechGroup S.p.A., ref. CTRCPE) **Parvovirus B19 ELITe Standard** (EG SpA, ref. STD070PLD) **Parvovirus B19 – ELITe Positive Control** (EG SpA, ref. CTR070PLD) **QIAsymphony® Midi kit** (QIAGEN GmbH, Ref. 931236) **NucliSENS® easyMAG® Reagents** (bioMérieux SA, Ref. 280130, 280131, 280132, 280133, 280134, 280135), **ELITe GALAXY 300 Extraction Kit** (EG S.p.A., ref. INT021EX) **InviMag Universal Kit / IG** (INVITEK, ref. 2450120100).

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Instruments and Softwares	Products and Reagents
7500 Fast Dx Real-Time PCR Instrument (ThermoFisher Scientific, ref. 4406985) **QIAsymphony® SP/AS** (QIAGEN GmbH, Ref. 9001297, 9001301) **NucliSENS® easyMAG®** (bioMérieux SA, Ref. 200111) **ELITe GALAXY** (EG S.p.A., ref. INT020) **ELITe STAR** (EG SpA ref. INT010)	**«MicroAmp™ Fast Optical 96-Well Reaction Plate» with Barcode, 0.1 mL** (Life Technologies, ref. 4346906) **CPE – Internal Control** (ELITechGroup S.p.A., ref. CTRCPE) **Parvovirus B19 ELITe Standard** (EG SpA, ref. STD070PLD) **Parvovirus B19 – ELITe Positive Control** (EG SpA, ref. CTR070PLD) **QIAsymphony® Midi kit** (QIAGEN GmbH, Ref. 931236) **NucliSENS® easyMAG® Reagents** (bioMérieux SA, Ref. 280130, 280131, 280132, 280133, 280134, 280135), **ELITe GALAXY 300 Extraction Kit** (EG S.p.A., ref. INT021EX) **InviMag Universal Kit / IG** (INVITEK, ref. 2450120100).

A conversion factor (Fc) allows to express the results of the quantitative analysis in International Units (IU) of PVB19 of "3rd WHO International Standard for Parvovirus B19 DNA for Nucleic Acid Amplification (NAT) Assay " (NIBSC, ref. 12/208, United Kingdom).

WARNINGS AND PRECAUTIONS

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

General warnings and precautions Handle and dispose of all biological samples as if they were infectious. Avoid direct contact with biological samples. Avoid splashing or spraying. Tubes, tips and other materials that come into contact with the biological samples must be treated for at least 30 minutes with 3% sodium hypochlorite (bleach) or autoclaved for one hour at 121°C before disposal.

Handle and dispose of all reagents and all materials used to carry out the assay as if they were infectious. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be handled and disposed of in compliance with adequate safety standards. Disposable combustible material must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal. Do not allow extraction reagents to contact sodium hypochlorite (bleach).

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

Warnings and precautions for molecular biology

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

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

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

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

The samples must be suitable and, if possible, dedicated for this type of analysis. Samples must be handled under a laminar airflow hood. Pipettes used to handle samples must be exclusively used for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

The reagents must be handled under a laminar airflow hood. The pipettes used to handle the reagents must be exclusively used for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips used must be sterile, free from DNases and RNases, and free from DNA and RNA.

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

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

Warnings and precautions specific for the components

Component	Storage temperature	Use from first opening	Freeze / Thaw cycles	On board stability (ELITe InGenius and ELITe BeGenius)
PVB19 Q PCR Mix	-20 °C or below (protected from light)	one month	up to five	up to five separate* sessions of three hours each or up to 7 consecutive hours (2 sessions of 3 hours each and the time needed to start a third session)

* with intermediate freezing

SPECIMENS AND CONTROLS for ELITe InGenius and ELITe BeGenius

Specimens

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

Specimen	Collection requirements	Transport/Storage conditions			
		+16 / +26 °C (room temperature)	+2 / +8 °C	-20 ± 10 °C	-70 ± 15 °C
Whole Blood	EDTA	≤ 24 hours	≤ 3 days	≤ 30 days	≤ 30 days
Amniotic fluid	collected without preservatives	≤ 4 hours	≤ 4 hours	≤ 30 days	≤ 30 days

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

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

Assay Protocols for Parvovirus B19 ELITe MGB Kit					
Specimen	**Instrument**	**Sample transfer**	**Assay Protocol Name**	**Report**	**Characteristics**
Whole blood	**ELITe InGenius**	Not Required	**PVB19 ELITe_WB_200_100**	copies / mL or IU / mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	Not Required	**PVB19 ELITe_Be_WB_200_100**	copies / mL or IU / mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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Amniotic fluid	**ELITe InGenius**	required, in Extraction tube	**PVB19 ELITe_AF_200_100**	copies / mL or IU / mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL
	ELITe BeGenius	required, in 2 mL Sarstedt Tube	**PVB19 ELITe_Be_AF_200_100**	copies / mL or IU / mL	Extraction Input Volume: 200 µL Extraction Elution Volume: 100 µL Internal Control: 10 µL Sonication: NO Dilution Factor: 1 PCR Mix volume: 20 µL Sample PCR input volume: 20 µL

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

Note: Pipetting samples to the **Extraction tube** or to the **2 mL Sarstedt Tube** might **generate contamination.** Use the appropriate pipettes and follow all recommendations reported in the "Warnings and Precautions" section.

Note: The volume of the sample in a primary tube varies according to the type of the tube loaded. Refer to the extraction kit instructions for use or **ELITe InGenius** and **ELITe BeGenius** manual for more information on how to set up and perform the extraction procedure.

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

Refer to "Potentially Interfering Substances" in the Performance Characteristics section to check data concerning interfering substances.

PCR calibrators and controls

The Calibration curve must be generated and approved for each lot of PCR reagent.

- For the calibration curve, use the four levels of the product **Parvovirus B19 ELITe Standard** (not provided with this kit) with the **PVB19 ELITe_STD** or **PVB19 ELITe_Be_STD** Assay Protocols.

Note: the concentrations of Q – PCR Standard are expressed in copies / reaction (10^5 copies / rxn, 10^4 copies / rxn, 10^3 copies / rxn, 10^2 copies /rxn).

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

- For the Positive Control, use the product **Parvovirus B19 - ELITe Positive Control** (not provided with this kit) with the **PVB19 ELITe_PC** or **PVB19 ELITe_Be_PC** Assay Protocols,
- For the Negative Control, use molecular biology grade water (not provided with this kit) with the **PVB19 ELITe_NC** or **PVB19 ELITe_Be_NC** Assay Protocols.

Note: The **ELITe InGenius** and **ELITe BeGenius** allow generation and storage of the calibration curve and PCR control validation for each lot of PCR reagent.
Calibration curves expire after **60 days**, at which time it is necessary to re-run the calibration.
PCR control results expire after **15 days**, at which time it is necessary to re-run the positive and negative controls.
The Calibrators and PCR controls must be re-run if any of the following events occur:

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

Quality controls

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

ELITe InGenius PROCEDURE

The procedure to use the **Parvovirus B19 ELITe MGB Kit** with the **ELITe InGenius** consists of three steps:

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

STEP 1 – Verification of the system readiness

Before starting the session:

- switch on the **ELITe InGenius** and login in "**CLOSED**" mode,
- in the "Calibration" menu on the Home page, verify the Calibrators (**PVB19 Q - PCR Standard**) are approved and valid (Status) for the **PVB19 PCR Mix** lot to be used. If no valid Calibrators are available for the **PVB19 PCR Mix** lot, perform calibration as described in the following sections,
- in the "Controls" menu on the Home page, verify the PCR Controls (**PVB19 - Positive Control**, **PVB19 Negative Control**) are approved and valid (Status) for the PCR Mix lot to be used. If no valid PCR Controls are available for the **PVB19 PCR Mix** lot, run the PCR Controls as described in the following sections,
- choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see "Specimens and Controls").

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

Protocols for qualitative analysis are available on request.

STEP 2 – Session Setup

The **Parvovirus B19 ELITe MGB Kit** can be used on **ELITe InGenius** to perform:

A. Sample run (Extract + PCR),
B. Eluted sample run, (PCR Only),
C. Calibration run (PCR Only),
D. Positive Control and Negative Control run (PCR Only).

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

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

Before to setup a run:

Thaw the needed **PVB19 Q PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session). Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.

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

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

	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. If required, transfer 200 µL of sample in an Extraction tube previously labelled. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	**Thaw** the **Elution tube** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select "**Perform Run**" from the "Home" screen.	Select "**Perform Run**" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.
4	For each sample, assign a Track and enter the "SampleID" (SID) by typing or by scanning the sample barcode.	For each sample, assign a Track and enter the "SampleID" (SID) by typing or by scanning the sample barcode.
5	**Select** the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").
6	Ensure the "Protocol" displayed is: "Extract + PCR".	Select "PCR Only" in the "Protocol" column.
7	Select the sample loading position as "Primary tube" or "Extraction Tube" in the "Sample Position" column. Ensure the "**Dilution factor**" is "**1**".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)". Ensure the "**Dilution factor**" is "**1**".
8	Click "Next" to continue.	Click "Next" to continue.
9	**Load CPE** and the **PCR Mix** on the "Inventory Block" referring to the "Load List" and enter CPE and PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **PCR Mix** on the "Inventory Block" referring to the "Load List" and enter PCR Mix lot number, expiry date and number of reactions for each tube.
10	Click "Next" to continue.	Click "Next" to continue.
11	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Racks if necessary.
12	Click "Next" to continue.	Click "Next" to continue.
13	**Load** PCR Cassette, ELITe InGenius SP 200 extraction cartridges, and all required consumables and samples to be extracted.	**Load** PCR Cassette and Elution tubes with samples extracted.
14	Click "Next" to continue.	Click "Next" to continue.
15	Close the instrument door.	Close the instrument door.
16	Press "Start".	Press "Start".

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw Positive Control tubes** at room temperature for 30 minutes. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block. **Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.	Ensure "Extraction Input Volume" is 200 µL and "Extracted Elute Volume" is 100 µL.
4	For the Q-PCR Standard, assign the "Track", **select** the **Assay Protocol** (see "Specimen and Controls") in the "Assay" column and enter the reagent lot number and expiry date.	**Select** the **Assay Protocol** in the "Assay" column (see "Specimens and Controls"). Enter the lot number and expiry date of the Positive Control and of the molecular biology grade water.
5	Ensure "PCR Only" is selected in the "Protocol" column.	Ensure "PCR Only" is selected in the "Protocol" column.
6	Ensure the sample loading position in "Sample Position" column is "Elution Tube (bottom row)".	Ensure the sample loading position in the "Sample Position" column is "Elution Tube (bottom row)".
7	**Load** the **PCR Mix** on the "Inventory Block" referring to the Load List and enter the PCR Mix lot number, expiry date and number of reactions for each tube.	**Load** the **PCR Mix** on the "Inventory Block" referring to the "Load List" and enter the PCR Mix lot number, expiry date and number of reactions for each tube.
8	Click "Next" to continue.	Click "Next" to continue.
9	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.
10	Click "Next" to continue.	Click "Next" to continue.
11	**Load** the PCR Cassette and the Q-PCR Standard tubes.	**Load** PCR Cassette, Positive Control and Negative Control.
12	Click "Next" to continue.	Click "Next" to continue.
13	Close the instrument door.	Close the instrument door.
14	Press "Start"	Press "Start".

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

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

Note: At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session); mix gently and spin down the content for 5 seconds before starting the next session.

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

Note: The **PVB19 Q-PCR Standard** can be used for 4 separate sessions of 2 hours each.

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

Note: The **PVB19 Positive Control** can be used for 4 separate sessions of 3 hours each.

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

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STEP 3 - Review and approval of results

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

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

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

The **ELITe InGenius** generates results with the **PVB19 ELITe MGB Kit** through the following procedure:

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

A. Validation of Calibration curve

The **ELITe InGenius software** interprets the PCR results for the target of the Calibrator reactions with the **PVB19 ELITe STD** Assay Protocol parameters. The resulting Ct versus concentration produces the Calibration curve.

The Calibration curves, specific for the PCR reagent lot, are recorded in the database (Calibration). They can be viewed and approved by "Administrator" or "Analyst" users, following the GUI instructions.

The Calibration curve expires **after 60 days**.

Note: If the Calibration curve does not meet the acceptance criteria, the "Failed" message is shown on the "Calibration" screen. In this case, the results cannot be approved and the Calibrator amplification reactions must be repeated. In addition, if samples were included in the run, these are not quantified and must also be repeated to generate quantitative results.

B. Validation of amplification Positive Control and Negative Control results

The **ELITe InGenius software** interprets the PCR results for the target of the Positive Control and Negative Control reactions with the **PVB19 ELITe_PC** and **PVB19 ELITe_NC** Assay Protocols parameters. The resulting Ct values are converted to concentration and used to verify the system (reagents lot and instrument).

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

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

The **ELITe InGenius software** processes the Positive Control and Negative Control results and generates Control Charts. Four approved Positive Control and Negative Control results are used to set up the initial Control Chart. For subsequent controls, the results are analyzed by the software to ensure the system performances are within the acceptance criteria, shown in the Control Chart plots. Refer to the instrument manual for more details.

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

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

C. Validation of Sample results

The **ELITe InGenius software** interprets the PCR results for the target (Channel **PVB19**) and the Internal Control (Channel **IC**) with the **PVB19 ELITe_WB_200_100** and **PVB19 ELITe_AF_200_100** Assay Protocol parameters. The resulting target Ct values are converted to concentration.

Results are shown in "Results Display" screen.

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

1) Calibration Curve	Status
PVB19 Q-PCR Standard	APPROVED
2) Positive Control	**Status**
PVB19 Positive Control	APPROVED
3) Negative Control	**Status**
PVB19 Negative Control	APPROVED

The sample results are automatically interpreted by the **ELITe InGenius software** using Assay Protocol parameters.

The possible result messages are listed in the table below.

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

Result of sample run	Interpretation
PVB19: DNA Detected, quantity equal to XXX copies / mL or IU / mL	**PVB19 DNA was detected** in the sample within the assay measurement range, its concentration is shown.
PVB19: DNA Detected, quantity below LLoQ copies / mL or IU / mL	**PVB19 DNA was detected** in the sample, its concentration is below the assay -Lower Limit of Quantification
PVB19: DNA Detected, quantity beyond ULoQ copies / mL or IU / mL	**PVB19 DNA was detected** in the sample, its concentration is above the assay Upper Limit of Quantification
PVB19: DNA Not detected or below LoD copies / mL or IU / mL	**PVB19 DNA was not detected** in the sample. The sample is negative for the target DNA, or its concentration is below the assay **Limit of Detection.**
Invalid - Retest Sample	**Not valid assay result** caused by Internal Control failure (due to e.g. incorrect extraction, inhibitors carry-over). The test should be repeated.

Samples reported as “Invalid-Retest Sample”: in this case, the Internal Control DNA was not efficiently detected, which could be due to problems in sample collection, extraction or PCR steps (e.g. incorrect sampling, degradation or loss of DNA during the extraction or inhibitors in the eluate), which may cause incorrect results.

If sufficient eluate volume remains, the eluate can be retested (as is or diluted) by an amplification run in “PCR Only” mode. If the second result is invalid, the sample must be retested starting from extraction of a new sample using “Extract + PCR” mode (see “Troubleshooting”)

Samples reported as “PVB19: DNA Not detected or below “LoD" copies / mL” are suitable for analysis but PVB19 was not detected. In this case the sample may be either negative for PVB19 DNA or the PVB19 DNA is present at a concentration below the Limit of Detection of the assay (see “Performance Characteristics”).

PVB19 DNA positive samples at a concentration below the Limit of Detection (and Lower Limit of Quantification) of the assay, if detected, are reported as “PVB19: DNA Detected, quantity below “LLoQ” copies / mL” (see “Performance Characteristics”).

PVB19 DNA positive samples within the Linear Measuring Range are detected and are reported as “PVB19: DNA Detected, quantity equal to “XXX” copies / mL”. (see “Performance Characteristics”)

PVB19 DNA positive samples that are above the Upper Limit of Quantification are reported as “PVB19: DNA Detected, quantity beyond “ULoQ” copies / mL” and they are not suitable for quantification. If needed the sample may be diluted before extraction or PCR and retested to yield results within the Linear Measuring Range of the assay (see “Performance Characteristics”).

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

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

D. Sample result reporting

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

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

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

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

ELITe BeGenius PROCEDURE

The procedure to use the **Parvovirus B19 ELITe MGB Kit** with the **ELITe BeGenius** consists of three steps:

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

STEP 1 - Verification of the system readiness

Before starting the session:

- switch on the **ELITe BeGenius** and login in “**CLOSED**” mode,

- in the “Calibrations” menu on the Home page, verify the Calibrators (**PVB19 Q - PCR Standard**) are approved and valid (Status) for the **PVB19 PCR Mix** lot to be used. If no valid Calibrators are available for the **PVB19 PCR Mix** lot, perform calibration as described in the following sections,

- - in the “Controls” menu on the Home page, verify that the PCR Controls (**PVB19 Positive Control, PVB19 Negative Control**) are approved and valid (Status) for the **PVB19 PCR Mix** lot to be used. If no valid PCR Controls are available for the **PVB19 PCR Mix** lot, run the PCR Controls as described in the following sections,

choose the type of run, following the instructions on the Graphical User Interface (GUI) for the session setup and using the Assay Protocols provided by EG SpA (see “Specimens and Controls”).

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

Protocols for qualitative analysis are available on request.

STEP 2 – Session Setup

The **PVB19 ELITe MGB Kit** can be used on the **ELITe BeGenius** to perform:

A. Sample run (Extract + PCR),
B. Eluted sample run (PCR Only),
C. Calibration run (PCR Only),
D. Positive Control and Negative Control run (PCR Only).

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

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

Before to setup a run:

Thaw the needed **PVB19 PCR Mix** tubes at room temperature for 30 minutes. Each tube is sufficient for **24 tests** in optimized conditions (2 or more tests per session). Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.

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

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

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	A. Sample run (Extract + PCR)	B. Eluted sample run (PCR Only)
1	**Identify samples** and, if needed, thaw at room temperature, mix gently, spin down the contents for 5 seconds and keep on ice or cool block. If required, transfer **200 µL of sample** in a 2 mL Sarstedt tube (not provided) previously labelled. **Thaw** the needed **CPE tubes** at room temperature for 30 minutes. Mix gently, spin down the contents for 5 seconds and keep on ice or cool block. Each tube is sufficient for 12 extractions.	If needed, **thaw the Elution tube** containing the extracted nucleic acids at room temperature. Mix gently, then spin down the contents for 5 seconds and keep on ice or cool block.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen.
3	Remove all the Racks from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) of the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode": "Extract + PCR".	Select the "Run mode": "PCR Only".
5	Load the samples into the "Sample Rack". (Note: when secondary tubes "2 mL Tubes" are loaded, use the blue adaptors for the "Sample Rack").	Load the samples into the "Elution Rack".
6	**Insert the "Sample Rack"** into the "Cooler Unit" starting from the "Lane 5" (L5). If needed, insert the "Sample ID" (SID) for each "Position" used. (If secondary tubes are loaded, flag "2 mL Tube". If secondary tubes are not barcoded, type manually the "Sample ID").	**Insert the "Elution Rack"** into the "Cooler Unit" starting from "Lane 3" (L3) If needed, for each "Position" enter the "Sample ID", the "Sample matrix", the "Extraction kit" and the "Extracted eluate vol." (eluate volume).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.	Ensure the "Extraction Input Volume" is 200 µL and the "Extracted Elute Volume" is 100 µL.
9	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").	Select the Assay Protocol in the "Assay" column (see "Specimens and Controls").
10	Click "Next" to continue.	Click "Next" to continue.
11	When more than 12 samples are processed, repeat the procedure from point 6.	When more than 12 samples are processed, repeat the procedure from point 6.
12	Load the "**Elution tubes**" into the "**Elution Rack**" (Elution tubes can be labelled with barcode to improve traceability).	Not applicable
13	Insert the "**Elution Rack**" into the "Cooler Unit" starting from "Lane 3" (L3). When more than 12 samples are processed, repeat using "Lane 2" (L2).	Not applicable
14	Click "Next" to continue.	Not applicable
15	Load CPE and the PCR Mix into the "Reagent/Elution Rack".	Load the PCR Mix into "Reagent/Elution Rack".
16	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix and / or CPE enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2) if available or in "Lane 1" (L1). If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
17	Click "Next" to continue	Click "Next" to continue.
18	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Rack(s)" in the "Inventory Area" and replace Tip Rack(s) if necessary.
19	Click "Next" to continue.	Click "Next" to continue.
20	Load the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area.	Load the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area.
21	Click "Next" to continue.	Click "Next" to continue.
22	Load the "**Extraction Rack**" with the "ELITe InGenius SP 200" extraction cartridges and required extraction consumables.	Not applicable
23	Close the instrument door.	Close the instrument door.
24	Press "Start".	Press "Start".

	C. Calibration run (PCR Only)	D. Positive Control and Negative Control run (PCR Only)
1	**Thaw** the needed **Q-PCR Standard tubes** (Cal1: Q-PCR Standard 10^2, Cal2: Q-PCR Standard 10^3, Cal3: Q-PCR Standard 10^4, Cal4: Q-PCR Standard 10^5) for 30 minutes at room temperature. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block.	**Thaw the Positive Control tubes** at room temperature for 30 minutes. Mix gently then spin down the contents for 5 seconds and keep on ice or cool block. **Prepare** the **Negative Control** by transferring at least 50 µL of molecular biology grade water to an "Elution tube", provided with the ELITe InGenius SP 200 Consumable Set.
2	Select "Perform Run" from the "Home" screen.	Select "Perform Run" from the "Home" screen
3	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.	Remove the "Racks" from "Lane 1, 2 and 3" (L1, L2, L3) from the "Cooler Unit" and place them on the preparation table.
4	Select the "Run mode: PCR Only".	Select the "Run mode": "PCR Only".
5	**Load the Q-PCR** Standard tubes into the "Elution Rack".	**Load** the **Positive Control and Negative Control tubes** into the "Elution Rack".
6	**Insert** the **"Elution Rack"** into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	**Insert** the **"Elution Rack"** into the "Cooler Unit" starting from the "Lane 3" (L3). If needed, for each "Position" enter the "Reagent name" and the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
7	Click "Next" to continue.	Click "Next" to continue.
8	Ensure the "Extraction Input Volume" (200 µL) and the "Extracted Elute Volume" (100 µL).	Ensure the "Extraction Input Volume" (200 µL) and the "Extracted Elute Volume" (100 µL).
9	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").	Select the **Assay Protocol** in the "Assay" column (see "Specimens and Controls").
10	Click "Next" to continue.	Click "Next" to continue.
11	**Load** the **PCR Mix** into "Reagent/Elution Rack".	**Load** the **PCR Mix** into "Reagent/Elution Rack".
12	**Insert** the "**Reagent/Elution Rack**" into the "Cooler Unit" in "Lane 2" (L2) If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).	Insert the "Reagent/Elution Rack" into the "Cooler Unit" in "Lane 2" (L2). If needed, for each PCR Mix enter the "S/N" (serial number), the "Lot No." (lot number), the "Exp. Date" (expiry date) and the "T/R" (number of reactions).
13	Click "Next" to continue.	Click "Next" to continue.
14	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.	Verify the tips in the "Tip Racks" in the "Inventory Area" and replace Tip Rack(s) if necessary.
15	Click "Next" to continue.	Click "Next" to continue.
16	**Load** the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area.	**Load** the "**PCR Rack**" with "**PCR Cassette**" in the Inventory Area.
17	Click "Next" to continue.	Click "Next" to continue.
18	Close the instrument door.	Close the instrument door.
19	Press "Start".	Press "Start".

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

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

Note: At the end of the run the **PCR Mix** can be removed from the instrument, capped and stored at -20 °C or below or can be kept on board in the refrigerated block for up to 7 hours (2 sessions of 3 hours each and the time needed to start a third session); mix gently and spin down the content for 5 seconds before starting the next session.

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

Note: The **PVB19 Q-PCR Standard** can be used for 4 separate sessions of 2 hours each.

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

Note: The **PVB19 Positive Control** can be used for 4 separate sessions of 3 hours each.

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

STEP 3 -Review and approval of results

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

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

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

The **ELITe BeGenius** generates the results with the **Parvovirus B19 ELITe MGB Kit** through the following procedure:

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

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

PERFORMANCE CHARACTERISTICS WITH ELITe InGenius and ELITe BeGenius

Analytical sensitivity: Limit of Detection (LoD)

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

The LoD of this assay was determined testing on ELITe InGenius, 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 presence of human genomic DNA at a titre of 500 ng / 20 µL. The results summed up in the following table.

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

Whole Blood collected in EDTA

The theoretical LoD value was verified by testing on ELITe InGenius and on ELITe BeGenius a pool of whole blood samples collected in EDTA spiked with reference material of Parvovirus B19 (3rd WHO International Standard, NIBSC) at claimed concentration (125 IU / mL).
The results are reported in the following tables.

Limit of Detection for whole blood samples on ELITe InGenius and on ELITe BeGenius

Sample	LoD	N	Valid	Positive	Negative
Whole Blood collected in EDTA	125 IU / mL	20	20	20	0
	250 copies / mL				

The results obtained confirmed the claimed concentration for the target of Parvovirus B19 ELITe MGB Kit on both ELITe InGenius and ELITe BeGenius. The LoD value for PVB19 Target in association to whole blood samples was confirmed at 125 IU / mL, corresponding to 250 copies / mL.

The value in copies / mL is calculated by applying the specific conversion factor reported in the paragraph "Analytical sensitivity".

Amniotic Fluid

The LoD of the assay used in association to amniotic fluid was determined on ELITe InGenius instrument, by testing a panel of Parvovirus B19 (PVB19) negative amniotic fluid spiked with reference material of PVB19 (3rd WHO International Standard, NIBSC). Probit regression analysis was performed on the results, and the LoD estimated as the concentration corresponding to 95% probability of a positive call.

The results are reported in the following tables.

Limit of Detection for amniotic fluid samples and ELITe InGenius			
Target	LoD	95% confidence interval	
		Lower bound	Upper bound
PVB19	40 IU / mL	35 IU / mL	50 IU / mL
	40 copies / mL	35 copies / mL	50 copies / mL

The LoD as copies / mL for amniotic fluid was calculated by applying the specific Conversion factor (1 IU / copy) reported in the paragraph "Analytical sensitivity".

The calculated LoD value was verified by testing on ELITe InGenius and ELITe BeGenius a pool of amniotic fluid spiked with PVB19 certified reference material (3rd WHO International Standard, NIBSC) at the claimed concentration.

The results obtained confirmed the claimed concentration for the target of Parvovirus B19 ELITe MGB Kit on both ELITe InGenius and ELITe BeGenius.

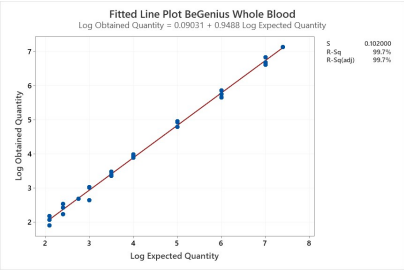
Analytical sensitivity: linear measuring range

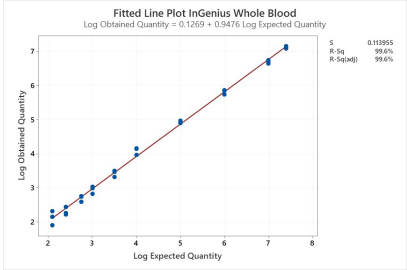
The Linear measuring range of the assay was verified with Whole Blood and Amniotic Fluid samples on ELITe InGenius and ELITe BeGenius.

For Whole blood collected in EDTA

The linear measuring range was verified using a panel of dilutions of PVB19 reference material (3rd WHO International Standard, NIBSC) in negative EDTA Whole Blood samples.

The results are reported in the following figures.





The final results are summarized in the following table.

Linear measuring range for whole blood samples and ELITe InGenius and ELITe BeGenius		
Unit	Lower limit	Upper limit
IU / mL	**125**	**25,000,000**
copies / mL	**250**	**50,000,000**

The linear measuring range as copies / mL for EDTA Whole Blood is calculated by applying the specific conversion factor reported in the following section.

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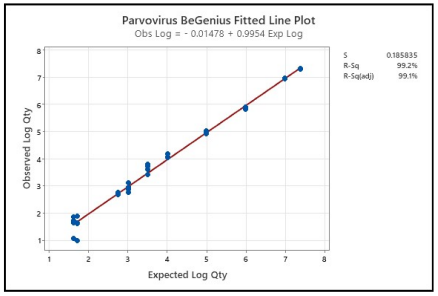
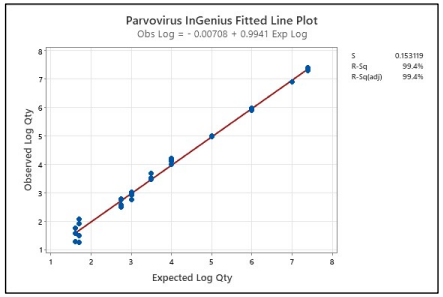
For Amniotic Fluid

The linear measuring range was verified using a panel of dilutions of PVB19 reference material (NIBSC) in negative amniotic fluid samples.

The results are reported in the following figures.





The final results are summarized in the following table.

Linear measuring range for amniotic fluid samples and ELITe InGenius and ELITe BeGenius		
Unit	Lower limit	Upper limit
IU / mL	**40**	**25,000,000**
copies / mL	**40**	**25,000,000**

The linear measuring range as copies / mL for Amniotic Fluid is calculated by applying the specific conversion factor reported in the following section.

Repeatability

The Intra-Session and Inter-Session Repeatability of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Whole Blood samples collected in EDTA, including one negative sample and two samples spiked by PVB19 certified reference material (3rd WHO International Standard, NIBSC).

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

Intra – Session Repeatability ELITe InGenius					
Sample	PVB19				
	N.	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A:	N.A.	100%
3 x LoD	8	35.81	0.52	1.46	100%
10 x LoD	8	34.31	0.28	0.83	100%

Intra – Session Repeatability ELITe BeGenius					
Sample	PVB19				
	N.	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	36.53	0.53	1.46	100%
10 x LoD	8	34.79	0.21	0.61	100%

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

Inter – Session Repeatability ELITe InGenius					
Sample	PVB19				
	N.	Mean Ct	SD	% CV	% Agreement
Negative	16	N.A.	N.A.	N.A.	100%
3 x LoD	16	35.87	0.44	1.23	100%
10 x LoD	16	34.19	0.28	0.83	100%

Inter – Session Repeatability ELITe BeGenius					
Sample	PVB19				
	N.	Mean Ct	SD	% CV	% Agreement
Negative	16	N.A.	N.A.	N.A.	100%
3 x LoD	16	36.40	0.45	1.23	100%
10 x LoD	16	34.68	0.27	0.79	100%

In the Repeatability test, the Parvovirus B19 ELITe MGB Kit detected the target and showed a maximum variability of target Ct values as %CV equal to 1.46%.

Reproducibility

The Inter-Instruments and the Inter -Batch Reproducibility of the assay was evaluated on ELITe InGenius and ELITe BeGenius by analysis of a panel of Whole Blood samples collected in EDTA, including one negative sample and two samples spiked by PVB19 certified reference material (3rd WHO International Standard, NIBSC).

A summary of Inter-Instrument Reproducibility (on two different instruments) is shown in the tables below.

	Inter – Instrument Reproducibility ELITe InGenius				
Sample	PVB19				
	N.	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	36.89	0.62	1.68	100%
10 x LoD	8	34.85	0.26	0.74	100%

	Inter – Instrument Reproducibility ELITe BeGenius				
Sample	PVB19				
	N.	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	37.00	0.37	1.00	100%
10 x LoD	8	35.14	0.51	1.46	100%

A summary of Inter-batch Reproducibility (on two lots) is shown in the tables below:

	Inter – Batch Reproducibility ELITe InGenius				
Sample	PVB19				
	N.	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	36.80	0.67	1.82	100%
10 x LoD	8	35.18	0.71	2.02	100%

	Inter – Batch Reproducibility ELITe BeGenius				
Sample	PVB19				
	N.	Mean Ct	SD	% CV	% Agreement
Negative	8	N.A.	N.A.	N.A.	100%
3 x LoD	8	37.41	0.27	0.72	100%
10 x LoD	8	35.31	0.32	0.90	100%

In the Inter - Instrument and Inter - batch Reproducibility test, the Parvovirus B19 ELITe MGB Kit correctly detected all the samples as expected and showed a maximum variability of target Ct values as %CV equal to 2.02%.

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Analytical sensitivity: reproducibility with certified reference material

The analytical sensitivity of the assay, as reproducibility of values of a calibrated reference material, was evaluated using as reference material the calibrated panel QCMD 2014 B19 Virus DNA EQA Panel (Qnostics Ltd, UK) a panel of PVB19 dilutions. Each sample of the panel was tested in 2 replicates carrying out the whole procedure of analysis, extraction, amplification, detection and result interpretation, using **ELITe InGenius** and ELITechGroup S.p.A. products.

The results in IU / mL were calculated applying the conversion factor (Fc = 0.3 IU/copy) for **ELITe InGenius** and plasma and are reported in the following table.

Tests with calibrated reference materials and ELITe InGenius®				
Sample	Consensus virus conc. Log_{10} IU / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
B19DNA14-01	4.788	0.507	2/2	4.948
B19DNA14-02	2.878	0.437	2/2	2.902
B19DNA14-03	4.848	0.400	2/2	4.850
B19DNA14-04	Negative	NA	0/2	NA
B19DNA14-05	5.802	0.465	2/2	5.847
B19DNA14-06	1.936	0.672	2/2	1.779
B19DNA14-07	3.913	0.371	2/2	3.955
B19DNA14-08	3.844	0.507	2/2	4.063

All samples were correctly detected. All positive samples were quantified within the range defined by the Consensus ± 1 Standard Deviation (SD).

Conversion factor to International Units

The Conversion factor (Fc) to report the quantitative results in International Units / mL starting from copies / mL, was calculated on ELITe InGenius using the certified calibrated reference material of PVB19 (NIBSC).

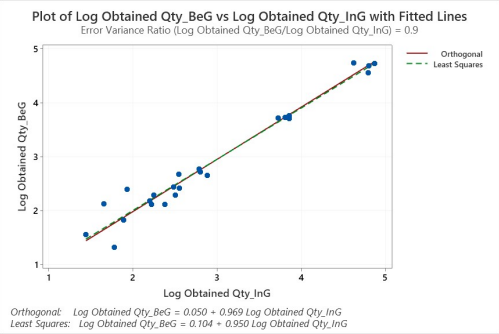
Whole Blood collected in EDTA

The Conversion factor was determined as 0.5 IU / copy on ELITe InGenius using a panel of dilutions of PVB19 reference material (2th WHO International Standard, NIBSC) in negative EDTA Whole Blood samples.

A summary of results is shown in the table below.

Conversion factor to International Units, Fc = 0.5 IU / copy						
Sample			Result			Log difference (ref. - test)
IU / mL	Log IU / mL	N	Mean copies / mL	Mean IU / mL	Mean Log IU / mL	
100,000	5.0000	10	21,4661	107,331	5.0210	-0.0210
10,000	4.0000	10	21,433	10,716	4.0080	-0.0080
1,000	3.0000	10	2,136	1,068	2.9850	+0.0150

The Conversion Factor value was verified on **ELITe InGenius** and **ELITe BeGenius** using the certified calibrated reference material (3th WHO International Standard, NIBSC), verified from 5.0000 Log IU / mL to 2.0970 Log IU / mL. The results obtained were analysed by orthogonal and linear regression in order to calculate their correlation.



The Orthogonal Regression analysis generated an intercept equal 0.050 (95% CI: - 0.197; 0.296) and a slope equal to 0.969 (95% CI: 0.889; 1.048). The linear regression analysis generated a R^2 of 0.963.

Amniotic Fluid

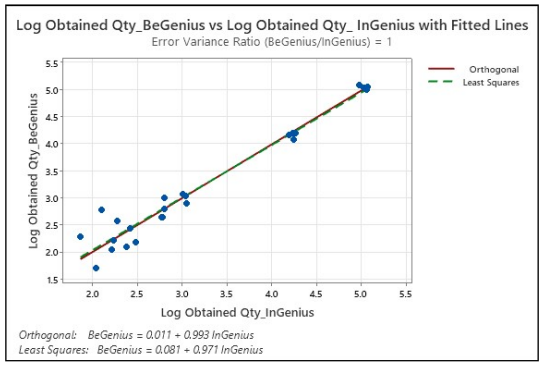
The Conversion factor was determined as 1 IU / copy on ELITe InGenius using a panel of dilutions of PVB19 reference material (3rd WHO International Standard, NIBSC) in negative amniotic fluid samples.

A summary of results is shown in the table below.

Conversion factor to International Units, Fc = 1 IU / copy						
Sample			Result			Log difference (ref. - test)
IU / mL	Log IU / mL	N	Mean copies / mL	Mean IU / mL	Mean Log IU / mL	
316,228	5.5000	16	273,604	273,604	5.4360	0.0640
100,000	5.0000	16	88,997	88,997	4.9470	0.0530
31,623	4.5000	16	34,559	34,559	4.5340	-0.0340
10,000	4.0000	16	11,089	11,089	4.0390	-0.0390
3,162	3.5000	15*	3,704	3,704	3.5580	-0.0580
1,000	3.0000	16	932	932	2.9520	0.0480

**One same resulted as an outlier was excluded by analysis.*

The Conversion Factor value was verified on **ELITe InGenius** and **ELITe BeGenius** using the certified calibrated reference material (3th WHO International Standard, NIBSC), verified from 5.0000 Log IU / mL to 2.0970 Log IU / mL. The results obtained were analysed by orthogonal and linear regression in order to calculate their correlation.



The Orthogonal Regression analysis generated an intercept equal 0.0112 (95% CI: 0.2879; 0.3103) and a slope equal 0.9928 (95% CI: 0.9046; 1.0810). The linear regression analysis generated a R^2 of 0.957.

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The results for each matrix are summarized in the following table.

Conversion factor to International Units with ELITe InGenius and ELITe BeGenius	
Matrix	Fc (IU / copies)
Whole Bood	**0.5**
Amniotic fluid	**1.0**

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity of the assay, assessed by positive clinical samples, was evaluated in association with **ELITe InGenius** by analyzing clinical samples of Whole Blood collected in EDTA and amniotic fluid certified positive for the target or spiked with reference material. As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic sensitivity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius.**

The results are summed up in the following table.

Samples	N	Positive	Negative	% Diagnostic Sensitivity
Whole blood collected in EDTA and spiked with PVB19 DNA	30	30	0	100
Amniotic fluid spiked with PVB19 DNA	30	30	0	100

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was evaluated in association with **ELITe InGenius** by analyzing clinical samples of Whole Blood collected in EDTA and amniotic fluid certified negative for the target. As **ELITe BeGenius** has equivalent analytical performances to **ELITe InGenius**, the diagnostic performances of the assay performed on the two instruments are also considered equivalent. Therefore, the Diagnostic specificity of the assay obtained in association with **ELITe InGenius** is also applicable to **ELITe BeGenius.**

The results are summed up in the following table.

Samples	N	Positive	Negative	% Diagnostic Specificity
whole blood collected in EDTA and negative for PVB19 DNA	30	0	30	100
Amniotic fluid negative for PVB19 DNA	30	0	30	100

The IC Ct cut-off value is set at 35 for whole blood samples collected in EDTA and amniotic fluid samples when tested with ELITe InGenius and ELITe BeGenius.

SPECIMENS AND CONTROLS FOR OTHER SYSTEMS

Samples

This product must be used with **DNA extracted** from the following clinical samples: whole blood (peripheral and from bone marrow) collected in EDTA, plasma collected in EDTA.

Whole Blood collected in EDTA

The whole blood samples (peripheral and from bone marrow) 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 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.

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 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 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.

NOTE: when you carry out the DNA extraction from plasma 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.

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NOTE: when you carry out the DNA extraction from plasma with the instrument **«QIAsymphony® SP/AS»** and the kit **«QIAsymphony® DSP Virus / Pathogen Midi kit»** with **software version 3.5**, use the extraction protocol **"Virus Cell free 500_V3_DSP_default IC"** and follow these directions: the instrument is able to use a primary tube, sample volume required for the extraction is **500 µL**, it's always requested a minimum dead volume of 100 µL. Prepare the solution containing AVE buffer and RNA carrier, according to the instruction manual of the extraction kit. Add **6 µL / sample** of **CPE** to the solution for each requested sample. Load on the instrument, in the "internal control" slot, the tubes containing the solution, 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 **85 µL**. For details on the extraction procedure follow indications in the instruction for use manual of the kit.

Other samples:

There are no data available concerning product performance with DNA extracted from the following clinical samples: amniotic fluid, suspensions of leucocytes and suspensions of granulocytes.

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 **«Parvovirus B19 - ELITe Positive Control»** product or the **«Parvovirus B19 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.

OTHER SYSTEMS PROCEDURE

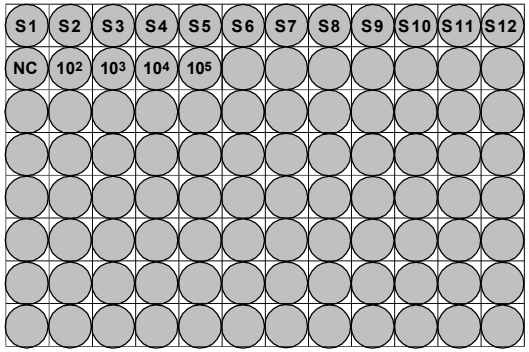
Setting of the real time amplification session
(To perform in the amplification / detection of amplification products area)

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 PVB19 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it "PVB19";
- 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**;
- optional: add dissociation stage (Add Dissociation Stage) and set the temperature from **40°C** to **80°C**.

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.
Dissociation (optional)	95 °C	15 sec.
	40 °C	30 sec.
	80 °C	15 sec.

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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 PVB19 probe with the "reporter" = "FAM" and the "quencher" = "none" (non fluorescent) and call it “PVB19”;
- 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 set up 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 table **"Thermal cycle"**;
- set the number cycles to **45**;
- set the volume for the software emulation of thermal transfer to reaction ("Sample volume") to **30 µL**;
- optional: add dissociation stage (Add Dissociation Stage) and set the temperature from **40 °C** to **80 °C**.

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.
Dissociation (optional)	95 °C	15 sec.
	40 °C	1 min.
	80 °C	15 sec.
Dissociation (optional)	60 °C	15 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 **PVB19 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 **PVB19 - Positive Control** or the **PVB19 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 **PVB19 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 PVB19 DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **PVB19 - Positive Control** in the corresponding well of **Amplification microplate**, as previously established in the **Work Sheet**. Mix well the positive control by pipetting the **PVB19 - Positive Control** three times into the reaction mixture. Avoid creating bubbles.

When a **quantitative** result is required (quantification of PVB19 DNA): accurately pipet, by placing into the reaction mixture, **20 µL** of **PVB19 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 **PVB19 Q - PCR Standard 10^2** three times into the reaction mixture. Avoid creating bubbles. Proceed in the same way with the other **PVB19 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-PVB19-EGSpA").

N. B.: 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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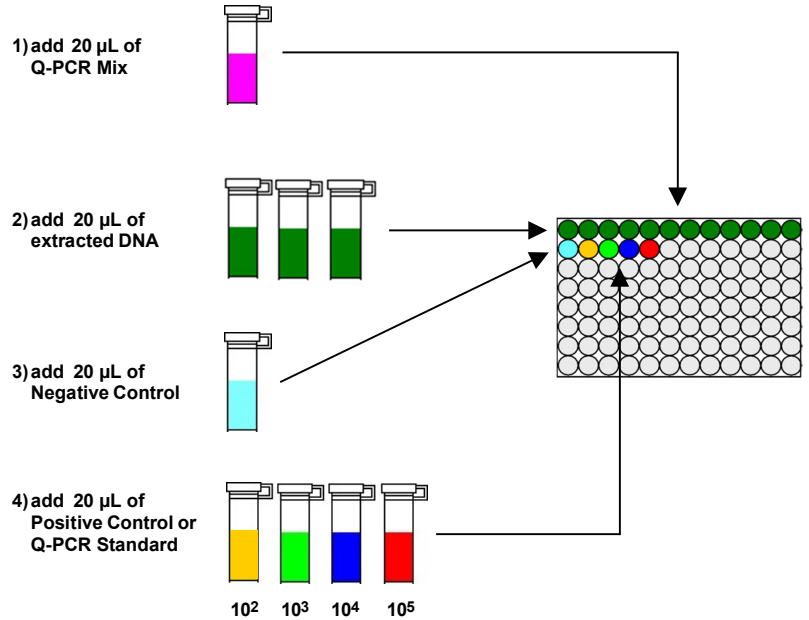
The following figure shows synthetically the preparation of the amplification reaction.



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 PVB19 probe (FAM detector "PVB19") 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 PVB19 DNA, the FAM fluorescence of the PVB19 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 “PVB19” 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 “PVB19” 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 PVB19 (Results > Report) is used to validate the amplification and the detection as described in the following table:

Positive Control reaction detector FAM "PVB19"	Assay result	Amplification / Detection
Ct ≤ 25	POSITIVE	CORRECT

If the result of the **Positive control** amplification reaction is **Ct > 25** or **Ct Undetermined** for PVB19, 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 PVB19 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 PVB19 (Results > Report) is used to validate the amplification and the detection as described in the following table:

Negative control reaction detector FAM "PVB19"	Assay result	Amplification / Detection
Ct Undetermined	NEGATIVE	CORRECT

If the result of the **Negative control** amplification reaction is different from **Ct Undetermined** for PVB19, 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 PVB19 is used to detect the target DNA, while the **Ct** value of Internal Control is used to validate extraction, amplification and detection.

N.B: 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 VP1 region of PVB19 in the amplification reaction (limit of detection, see Performance Characteristics paragraph).

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	PVB19 DNA
detector FAM "PVB19"	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

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If the result of the amplification reaction of a sample is **Ct Undetermined** for PVB19 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 PVB19 and **Ct ≤ 35** for the Internal Control, it means that the PVB19 DNA is not detected in the DNA extracted from the sample; but it can not be excluded that the PVB19 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 PVB19 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 PVB19 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 PVB19 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 "PVB19"	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 PVB19 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 VP1 region of PVB19 in the amplification reaction (linear measuring range, see Performance Characteristics), as described in the following table:

Sample result detector FAM "PVB19"	PVB19 copies per reaction
Quantity > 1×10^6	MORE THAN 1,000,000
1×10^1 ≤ Quantity ≤ 1×10^6	= Quantity
Quantity < 1×10^1	LESS THAN 10

The results (**Quantity**) of each **samples** (Results > Report) are used to calculate the copies of PVB19 present in the extracted sample (**Nc**) according to this formula:

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

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 copies per reaction**,

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

Simplified formula for whole blood, plasma and «ELITe STAR»
Nc (copies / 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 copies / mL** is required, the formula becomes:

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

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

Simplified formula for plasma and «NucliSENS® easyMAG®»
Nc (copies / mL) = 10 x Quantity

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

Simplified formula for plasma and «QIAsymphony® SP/AS»
Nc (copies / mL) = 12 x Quantity

Calculation of the linear measuring range limits

When a particular extraction method is used, the linear measuring range limits may be calculated from the linear measuring range of the amplification reaction according to the following formula:

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

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

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When **«ELITe STAR»** extraction system is used with whole blood samples collected in EDTA or plasma samples collected in EDTA, the formula becomes:

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

When **«ELITe GALAXY»** extraction system is used with whole blood samples collected in EDTA or plasma samples collected in EDTA, the formula becomes:

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

When «**NucliSENS® easyMAG®**» extraction system is used with plasma samples collected in EDTA, the formula becomes:

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

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

Measuring range limits (copies / mL) with «QIAsymphony® SP/AS»
Lower limit (copies / mL) = 12 x 10 copies **Upper limit (copies / mL) = 12 x 1,000,000 copies**
from 120 to 12,000,000 copies / mL

Convertion of results to International Units (IU)

Fc is the conversion factor established using the reference calibrated material approved by WHO "2nd WHO International Standard for Parvovirus B19 DNA for Nucleic Acid Amplification (NAT) Assay", NIBSC ref. 99/802, United Kingdom (see Performance Characteristics paragraph).

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

Simplified formula for whole blood and ELITe STAR
Fc = 0.98 IU / copies **Nc (IU / mL) = Nc (copies / mL) x Fc**
Nc (IU / mL) = 27.4 x Quantity

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

Simplified formula for plasma and ELITe STAR
Fc = 0.69 IU / copies **Nc (IU / mL) = Nc (copies / mL) x Fc**
Nc (IU / mL) = 19.3 x Quantity

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

Simplified formula for whole blood and «ELITe GALAXY»
Fc = 0.82 IU / copies **Nc (IU / mL) = Nc (copies / mL) x Fc**
Nc (IU / mL) = 28.7 x Quantity

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

Simplified formula for plasma and «ELITe GALAXY»
Fc = 0.87 IU / copies **Nc (IU / mL) = Nc (copies / mL) x Fc**
Nc (IU / mL) = 30.5 x Quantity

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

Simplified formula for plasma and «NucliSENS® easyMAG®»
Fc = 1 IU / copies **Nc (IU / mL) = Nc (copies / mL) x Fc**
Nc (IU / mL) = 10 x Quantity

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

Simplified formula for plasma and «QIAsymphony® SP/AS»
Fc = 1 IU / copies **Nc (IU / mL) = Nc (copies / mL) x Fc**
Nc (IU / mL) = 12 x Quantity

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PERFORMANCE CHARACTERISTICS WITH OTHER SYSTEMS

Analytical sensitivity: limit of detection

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

The analytical sensitivity of this assay, as detection limit, was tested using plasmidic DNA containing the amplification product whose initial concentration was measured by spectrophotometer. The plasmidic 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 plasmidic DNA + 500 ng of human genomic DNA	50	50	0

The analytical sensitivity of this assay used in association to whole blood samples and **ELITe STAR** was verified with a panel of Parvovirus B19 dilutions within the limiting concentration. The panel was prepared by diluting the “2nd WHO International Standard for Parvovirus B19 DNA for Nucleic Acid Amplification (NAT) Assay” (NIBSC code 99/802, United Kingdom) in Parvovirus B19 DNA - negative EDTA whole blood. The viral concentrations ranged from 3.161 IU / mL to 1000 IU / mL. Each sample of the panel was tested in 12 replicates carrying out the whole analysis procedure, extraction with **ELITe STAR** 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 final results are reported in the following tables.

Limit of Detection for whole blood samples and ELITe STAR (IU / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**293 IU / mL**	159 IU / mL	1053 IU / mL

Limit of Detection for whole blood samples and ELITe STAR (copies / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**299 copie / mL**	162 copie / mL	1074 copie / mL

The analytical sensitivity of this assay used in association to plasma samples and **ELITe STAR** was verified with a panel of Parvovirus B19 dilutions within the limiting concentration. The panel was prepared by diluting the “2nd WHO International Standard for Parvovirus B19 DNA for Nucleic Acid Amplification (NAT) Assay” (NIBSC code 99/802, United Kingdom) in Parvovirus B19 DNA - negative EDTA plasma. The viral concentrations ranged from 3.161 IU / mL to 1000 IU / mL. Each sample of the panel was tested in 8 replicates carrying out the whole analysis procedure, extraction with **ELITe STAR** 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 final results are reported in the following tables.

Limit of Detection for plasma samples and ELITe STAR (IU / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**100 IU / mL**	45 IU / mL	987 IU / mL

Limit of Detection for plasma samples and ELITe STAR (copies / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**145 copie / mL**	65 copie / mL	1430 copie / mL

The analytical sensitivity of this assay used in association to whole blood samples and **ELITe GALAXY** was verified with a panel of Parvovirus B19 dilutions within the limiting concentration. The panel was prepared by diluting the "2nd WHO International Standard for Parvovirus B19 DNA for Nucleic Acid Amplification (NAT) Assay" (NIBSC code 99/802, United Kingdom) in Parvovirus B19 DNA - negative EDTA whole blood. The viral concentrations ranged from 10 IU / mL to 560 IU / 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 final results are reported in the following tables.

Limit of Detection for whole blood samples and ELITe GALAXY (IU / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**145 IU / mL**	80 IU / mL	562 IU / mL

Limit of Detection for whole blood samples and ELITe GALAXY (copies / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**177 copie / mL**	98 copie / mL	685 copie / mL

The analytical sensitivity of this assay used in association to plasma samples and **ELITe GALAXY** was verified with a panel of Parvovirus B19 dilutions within the limiting concentration. The panel was prepared by diluting the "2nd WHO International Standard for Parvovirus B19 DNA for Nucleic Acid Amplification (NAT) Assay" (NIBSC ref. 99/802, United Kingdom) in Parvovirus B19 DNA - negative EDTA plasma. The viral concentrations ranged from 10 IU / mL to 560 IU / 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 final results are reported in the following tables.

Limit of Detection for plasma samples and ELITe GALAXY (IU / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**79 IU / mL**	54 IU / mL	174 IU / mL

Limit of Detection for plasma samples and ELITe GALAXY (copies / mL)			
		95% confidence range	
		lower limit	upper limit
95% positivity	**91 copie / mL**	62 copie / mL	200 copie / 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 this assay, as linear measuring range, was determined using a panel of dilutions (1 $\log_{10}$ between one dilution and the next) of a plasmidic 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 panel points (linear correlation coefficient greater than 0.99).

The upper limit of the linear measuring range was set at 10^6 molecules 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, within one logarithm from the lowest concentration Q - PCR Standard amplification standard (10^2 molecules / 20 µL).

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The final results are summed up in the following table.

Linear measuring range (IU / reaction)	
Upper limit	1,000,000 DNA copies / reaction
Lower limit	10 DNA copies / reaction

The linear measuring range limits as IU / mL referring to the used extraction kit are calculated at page 33.

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 session, allowed to obtain a mean percentage Coefficient of Variation (% CV) of about 20.4% 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 session and the theoretical concentration of the sample, allowed to obtain a mean percentage Inaccuracy (% Inacc.) of about 12.4% 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 compared with results obtained using other assays in different laboratories, was checked testing a calibrated reference material.

The tests were carried out using as calibrated reference material a panel of dilutions of PVB19 within the limit concentration (QCMD 2008 B19 Virus EQA Panel, Qnostics Ltd, UK). Each sample of the panel was tested in duplicates carrying out the whole analysis, extraction and amplification with ELITechGroup S.p.A. products.

The results are reported in the following table.

Tests with calibrated reference materials				
Sample	Commercial assay consensus virus conc. Log_{10} IU / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
B1908-01	PVB19, 2.396	0.534	2/2	2.636
B1908-02	PVB19, 3.966	0.596	2/2	4.182
B1908-03	PVB19, 2.822	0.574	2/2	2.750
B1908-04	Negative, NA	NA	0/2	Not detected
B1908-05	PVB19, 2.894	0.607	2/2	2.928
B1908-06	PVB19, 2.061	0.577	2/2	1.969
B1908-07	PVB19, 2.926	0.648	2/2	3.026
B1908-08	PVB19, 3.575	0.595	2/2	3.627

All samples were correctly detected. All the quantitative results obtained are within the range defined by the commercial assay Consensus ± 1 Standard Deviation (SD).

Further tests were carried out using as calibrated reference material a panel of dilutions of PVB19 within the concentration limit (QCMD 2012 B19 Virus EQA Panel, Qnostics Ltd, UK). Each sample was tested in duplicates carrying out the whole analysis procedure: extraction with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

The results in IU / mL were calculated applying the conversion factor for **ELITe STAR** and plasma and are reported in the following table.

Tests with calibrated reference materials and ELITe STAR				
Sample	Commercial assay consensus virus conc. Log_{10} IU /mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
B1912-01	PVB19, 1.684	0.488	2/2	2.242
B1912-02	PVB19, 3.716	0.522	2/2	4.078
B1912-03	Negative, NA	-	0/2	-
B1912-04	PVB19, 6.378	0.686	2/2	6.675
B1912-05	PVB19, 4.486	0.641	2/2	4.849
B1912-06	PVB19, 2.687	0.577	1/2	2.837
B1912-07	PVB19, 5.565	0.487	2/2	5.639
B1912-08	PVB19, 2.704	0.386	2/2	2.839

All samples were correctly detected. Six (6) out of seven positive samples were quantified within the range defined by the Consensus ± 1 SD and one sample (B1912-01) was quantified within ± 2 SD. This result can be explained because the sample titer is below the limit of detection of the system.

Further tests were carried out using as calibrated reference material a panel of dilutions of PVB19 within the concentration limit (QCMD 2014 B19 Virus EQA Panel, Qnostics Ltd, UK). Each sample of the panel was tested in duplicates carrying out the whole analysis procedure: extraction ad PCR Setup with **ELITe GALAXY** System and amplification with ELITechGroup S.p.A. products.

The results in IU / mL were calculated applying the conversion factor for **ELITe GALAXY** and plasma and are reported in the following table.

Tests with calibrated reference materials and ELITe GALAXY				
Sample	Commercial assays consensus virus conc. Log_{10} IU / mL	Standard Deviation	Positive / Replicates	Mean results Log_{10} IU / mL
B19DNA14-01	PVB19, 4.788	0.507	2/2	4.977
B19DNA14-02	PVB19, 2.878	0.437	2/2	3.115
B19DNA14-03	PVB19, 4.848	0.400	2/2	4.848
B19DNA14-04	Negative, NA	-	0/2	-
B19DNA14-05	PVB19, 5.802	0.465	2/2	5.996
B19DNA14-06	PVB19, 1.936	0.672	2/2	1.653
B19DNA14-07	PVB19, 3.913	0.371	2/2	3.972
B19DNA14-08	PVB19, 3.844	0.507	2/2	4.555

All samples were correctly detected. Six (6) out of seven positive samples were quantified within the range defined by the Consensus ± 1 SD and one sample (B19DNA14-08) was quantified within ± 2 SD.

Analytical sensitivity: Conversion factor to International Units

Whole blood collected in EDTA

The conversion factor was determined using a panel of three dilutions (0.5 log_{10} dilution step) of the calibrated reference material approved by WHO ("2nd WHO International Standard for Parvovirus B19 DNA for Nucleic Acid Amplification (NAT) Assay", NIBSC ref. 99/802, United Kingdom) in whole blood collected in EDTA.

Each point of the panel was tested in 15 replicates carrying out the whole analysis, extraction with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

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The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 0.9 IU per copy of PVB19 detected with whole blood samples.The results are reported in the following table.

Conversion to International Units with whole blood and ELITe STAR Fc = 0.98 IU / copy				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity copies / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
31,623	4.500	29,023	28,434	4.443
10,000	4.000	9,631	9,435	3.947
3,162	3.500	4,346	4,258	3.607

Each point of the panel was tested in 15 replicates carrying out the whole analysis, extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 0.8 IU per copy of PVB19 detected with whole blood samples. The final results are summed up in the following table.

Conversion to International Units with whole blood and ELITe GALAXY Fc = 0.82 IU / copy				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity copies / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
31,623	4.500	48,688	39,924	4.471
10,000	4.000	13,885	11,386	4.029
3,162	3.500	6,085	4,990	3.506

Plasma collected in EDTA

The conversion factor was determined using a panel of three dilutions (0.5 log_{10} dilution step) of the calibrated reference material approved by WHO ("2nd WHO International Standard for Parvovirus B19 DNA for Nucleic Acid Amplification (NAT) Assay", NIBSC ref. 99/802, United Kingdom) in plasma collected in EDTA.

Each point of the panel was tested in 15 replicates carrying out the whole analysis, extraction with **ELITe STAR** and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 0.6 IU per copy of PVB19 detected with plasma samples.The results are reported in the following table.

Conversion to International Units with plasma and ELITe STAR Fc = 0.69 IU / copy				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity copies / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
31,623	4.500	39,888	27,403	4.425
10,000	4.000	14,901	10,237	3.987
3,162	3.500	5,862	4,027	3.588

Each point of the panel was tested in 15 replicates carrying out the whole analysis, extraction and PCR Setup with **ELITe GALAXY** and amplification with ELITechGroup S.p.A. products.

The analysis of the data obtained allows to calculate a mean conversion factor (Fc) equal to 0.8 IU per copy of PVB19 detected with plasma samples. The final results are summed up in the following table.

Conversion to International Units with plasma and ELITe GALAXY Fc = 0.87 IU / copy				
Expected conc. IU / mL	Expected conc. Log_{10} IU / mL	Mean Quantity copies / mL	Mean Quantity IU / mL	Mean Quantity Log_{10} IU / mL
31,623	4.500	30,768	26,768	4.423
10,000	4.000	15,154	13,184	4.119
3,162	3.500	3,378	2,939	3.458

The mean conversion factor to be used with this assay in association with **«NucliSENS® easyMAG®» or «QIAsymphony® SP/AS»** extraction systems to convert quantitative result from copies / mL to International Units / mL was defined equal to 1 IU per target DNA copy.

Diagnostic sensitivity: detection and quantification efficiency with different genotypes / subtypes

The diagnostic sensitivity of the assay, as detection and quantification efficiency on different genotypes / subtypes, 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 VP1 region of PVB19, including genotypes 1, 2, 3a and 3b, showed conservation and absence of significant mutations.

The diagnostic sensitivity of the assay, as detection and quantification efficiency on different genotypes / subtypes, was checked using some plasmidic constructs corresponding to genotypes 1, 2 and 3a or 3b.

The diagnostic sensitivity of the assay was checked using three plasmids containing the sequence of the amplified region of genotypes 1, 2 and 3 (the same for subtypes 3a and 3b). The plasmids were diluted from 10^5 copies per reaction to 10^2 copies per reaction. These samples were tested in three 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 genotype 1, genotype 2, genotype 3				
Expected concentration copies / reaction	Expected concentration Log_{10} copies / reaction	Mean Quantity detected genotype 1 Log_{10} copies / reaction	Mean Quantity detected genotype 2 Log_{10} copies / reaction	Mean Quantity detected genotype 3 Log_{10} copies / reaction
100,000	5.000	5.013	4.882	4.849
10,000	4.000	4.009	3.910	3.862
1,000	3.000	3.024	2.911	2.848
100	2.000	2.037	2.026	1.921

The results are within the range defined by the expected value ± 0.2 Log_{10}.

Diagnostic sensitivity: confirmation of positive samples

The diagnostic sensitivity was evaluated using 30 samples of plasma collected in EDTA and negative for PVB19 DNA, that were spiked for PVB19 DNA adding B1912-05 sample, from QCMD 2012 B19 Virus EQA Panel (Qnostics Ltd, UK) and 30 whole blood samples collected in EDTA and negative for PVB19 DNA, that were spiked for PVB19 DNA adding B1912-05 sample, from QCMD 2012 B19 Virus EQA Panel (Qnostics Ltd, UK).Each sample was used to carry out the whole analysis procedure: extraction with **ELITe STAR** System and amplification with ELITechGroup S.p.A. products. The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA spiked for PVB19 DNA	30	30	0
Plasma collected in EDTA spiked for PVB19 DNA	30	30	0

All spiked samples were correctly detected as positive for PVB19 DNA.
The diagnostic sensitivity of the assay in this test was equal to100%.

The diagnostic sensitivity was evaluated using 30 samples of plasma negative for Parvovirus B19 DNA, that were spiked for Parvovirus B19 DNA adding B1912-05 sample, from QCMD 2012 B19 Virus EQA Panel (Qnostics Ltd, UK) and 30 whole blood samples negative for Parvovirus B19 DNA, that were spiked for Parvovirus B19 DNA adding B1912-05 sample, from QCMD 2012 B19 Virus EQA Panel (Qnostics Ltd, UK). Each sample was used to carry out the whole analysis procedure: extraction and PCR Setup with **ELITe GALAXY** System and amplification with ELITechGroup S.p.A. products. The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA spiked for Parvovirus B19 DNA	30	30	0
Plasma collected in EDTA spiked for Parvovirus B19 DNA	30	30	0

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All spiked samples were correctly detected as positive for PVB19 DNA.
The diagnostic sensitivity of the assay in this test was equal to100%.

Analytical specificity: absence of cross-reactivity potential interfering markers

The analytical specificity of the assay, as absence of cross-reactivity with other potential interference 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 PVB19, including Parvovirus 4, Bocavirus and Dependovirus complete genomes, the human virus that are most similar to PVB19, showed their specificity and the absence of significant homology.

The analytical specificity of the assay, as absence of cross-reactivity with other potential interference markers, was checked using some clinical samples negative for the PVB19 DNA and positive for DNA of other pathogens.

The analytical specificity was checked using as reference material 22 whole blood (peripheral) samples collected in EDTA, that were negative for PVB19 DNA but positive for DNA of other pathogens as PVB19, EBV, CMV, VZV, HSV1 and HHV8 (tested with CE IVD amplification products). Each sample was tested carrying out the whole analysis procedure: extraction and amplification with ELITechGroup S.p.A. products. The results are summed up in the following table.

Samples	N	positive	negative
Whole blood collected in EDTA negative for PVB19 DNA and positive for DNA of other pathogens	22	0	22

No cross-reactivity was detected with samples positive for DNA of other pathogens.

Diagnostic specificity: confirmation of negative samples

The diagnostic specificity of the assay, as confirmation of negative samples, was tested using some PVB19 DNA negative clinical samples of whole blood collected in EDTA, tested negative for PVB19 DNA.

The diagnostic specificity was evaluated using 30 plasma samples collected in EDTA that were negative for PVB19 DNA and 30 whole blood samples collected in EDTA that were negative for PVB19 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
Whole blood collected in EDTA negative for PVB19 DNA	30	0	30
Plasma collected in EDTA negative for PVB19 DNA	30	0	30

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 negative for Parvovirus B19 DNA and 34 whole blood samples collected in EDTA that were negative for Parvovirus B19 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 Parvovirus B19 DNA	34	0	34
Whole blood collected in EDTA presumably negative for Parvovirus B19 DNA	33	1	32

One whole blood sample resulted discrepant positive (31 copies / mL). This sample titre was below the limit of detection of the method. The diagnostic sensitivity of the assay in this test was equal to 98.5%.

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 "Parvovirus B19 ELITe MGB® Kit", FTP RTS070PLD.

REFERENCES

F. McOmish et al. (1993) *J. Clin. Microbiol.* 31: 323 - 328
E. A. Lukhtanov et al. (2007) *Nucleic Acids Res.* 35: e30
K. Linnet et al. (2004) *Clin. Chem.* 50: 732 - 740.

PROCEDURE LIMITATIONS

Use this product only with the following clinical samples: whole blood (peripheral and from bone marrow) collected in EDTA, plasma collected in EDTA and amniotic fluid.

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.

Do not use with this product extracted DNA containing high quantity of human genomic DNA that may inhibit the amplification reaction of nucleic acids.

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

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

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

This product must be handled by qualified personnel trained in the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of personal protective equipment and areas that are suitable for the processing of potentially infective biological samples and chemical preparations classified as dangerous to prevent accidents with potentially serious consequences for the user and other persons.

This product requires the use of personal protective equipment and instruments dedicated to work session setup to avoid false positive results.

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

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 indicates that the target DNA is not detected in the DNA extracted from the sample; however it cannot be excluded that the target DNA has a lower titer than the product detection limit (see “Performance Characteristics”). In this case the result could be a false negative.

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

Possible polymorphisms, insertions or deletions within the region of the DNA targeted by the product primers and probes may impair detection and quantification of target DNA.

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

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

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TROUBLESHOOTING

ELITe InGenius and ELITE BeGenius

Invalid Q-PCR Standard reaction, Standard curve or Positive Control reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Q-PCR Standards and Positive Control. Check the volumes of PCR Mix, Q-PCR Standards and Positive Control.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area, Cool Block or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit) Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Q-PCR Standards or Positive Control degradation.	Do not use the Q-PCR Standard for more than 4 independent sessions (2 hours each in the Extraction Area or in the Cooler Unit). Do not use the Positive Control for more than 4 independent sessions (3 hours each in the Extraction Area or in the Cooler Unit). Use new aliquots of Q-PCR Standards or Positive Control.
Instrument error.	Contact ELITechGroup Technical Service.

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

Invalid Sample reaction	
Possible Causes	**Solutions**
Instrument setting error.	Check the position of PCR Mix, Internal Control, and sample. Check the volumes of PCR Mix, Internal Control and sample.
PCR Mix degradation.	Do not use the PCR Mix for more than 7 independent sessions (3 hours each in the Inventory Area or in the Cooler Unit). Do not use the PCR Mix for more than 3 consecutive sessions (7 hours in the Inventory Area Cool Block or in the Cooler Unit). Do not leave the PCR Mix at room temperature for more than 30 minutes. Use a new aliquot of PCR Mix.
Internal Control template degradation.	Use a new aliquot of Internal Control.
Inhibition due to interfering substances in the sample.	Repeat the amplification with a 1:2 dilution in molecular biology grade water of eluted sample in a “PCR Only” session. Repeat the extraction with a 1:2 dilution in molecular biology grade water of the sample in an “Extract + PCR” session.
Instrument error.	Contact ELITechGroup Technical Service.

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

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

Abnormal high rate of positive results within the same session (reactions with similar late Ct values)	
Possible Causes	**Solutions**
Sample-to-sample contamination during preanalytical steps.	Clean the micropipette with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner after pipetting each sample. Do not use Pasteur pipettes. The pipettes must be of the positive displacement type or used with aerosol filter tips. Introduce samples in the last positions of the instruments, as indicated by the GUI. Follow the loading sequence indicated by the software.
Laboratory environmental contamination.	Clean all surfaces in contact with the operator and samples (including the pipettes) with fresh 3% sodium hypochlorite solution (bleach) or DNA/RNA cleaner. Perform an U.V. decontamination cycle. Use a new tube of PCR Mix and / or CPE.

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Open Platform:

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.
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.
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 molecular biology grade water.	Use a new aliquot of sterile water.
Contamination of the reaction mixture.	Use a new aliquot of reaction mixture.
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.

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.

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

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.
UDI	Unique Device Identification
	Caution, consult instructions for use.
CONT	Contents.
	Keep away from sunlight.
	Manufacturer.

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NOTICE TO PURCHASER: LIMITED LICENSE

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ELITe MGB® detection reagents are covered by one or more of U.S. Patent numbers, 7319022, 7348146, 7381818, 7541454, i 7671218, 7718374, 7723038, 7759126, 7767834, , 8008522, 8067177, 8163910, 8389745, 8969003, 9056887, 9085800, 9169256, 9328384, 10677728, 10738346, 10890529, and EP patent numbers 1687609, 1781675, 1789587, 2689031, 2714939, 2736916, 2997161 as well as applications that are currently pending.

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

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