INSTRUCTION FOR USE

GeneProof®
Molecular diagnostics for your routine

GeneProof Enterovirus PCR Kit

CE IVD

1. LIST OF PRODUCT VARIANTS

Product	Package	REF
GeneProof Enterovirus PCR Kit	25 reactions	EV/ISEX/025
GeneProof Enterovirus PCR Kit	100 reactions	EV/ISEX/100

2. INTENDED PURPOSE AND USE

Indication	*in vitro* diagnostic medical device
Regulatory Status	CE IVD / EC Directive 98/79/EC
Function	Diagnostics, aid to diagnosis or monitoring
What is Detected / Target	Enterovirus
Automated / Manual detection	Manual
Type of analysis	Qualitative and quantitative
Validated Specimen	CSF, stool*, swab
Testing Population	EU population
Intended User	For professional use in laboratories with trained staff
Test Principle	Real-time polymerase chain reaction (PCR) – amplification of the specific Target Sequence and detection using TaqMan probes with fluorophore-based detection

**NOTE: Only in combination with NucleoSpin® RNA Stool.*

3. TECHNICAL SPECIFICATION

Target Sequence	5' UTR RNA
Analytical Specificity	Enterovirus A – D, 100 %
Analytical Sensitivity (LoD with 95% probability)	**Sensitivity** / **Performed on** 158.34 cp/µl – Amplirun® Enterovirus 68 RNA control, Vircell 0.57 cp/µl – Amplirun® Enterovirus 71 RNA control, Vircell 0.59 cp/µl – Amplirun® Coxsackie B5 RNA control, Vircell
Diagnostic Specificity	93.75 % ($CI_{95\%}$: 77.78 % - 98.91 %)
Diagnostic Sensitivity	98.72 % ($CI_{95\%}$: 92.09 % - 99.93 %)
Positive predictive value	97.47 % ($CI_{95\%}$: 90.31 % - 99.56 %)
Negative predictive value	96.77 % ($CI_{95\%}$: 81.49 % - 99.83 %)
Linear range	10^9 – $10^{2.5}$ cp/ml with precision ± 0.5 log (on CSF)
Dynamic range	10^9 – 316.2 cp/ml with precision ± 0.5 log (on CSF)
Trueness (of expected concentration)	-0.13 log ($CI_{95\%}$: -0.23 – -0.02) using GeneProof PathogenFree DNA Isolation Kit -0.20 log ($CI_{95\%}$: -0.24 – -0.16) using croBEE 201A Nucleic Acid Extraction Kit
Precision – repeatability	• Intra-assay SD of log concentration = 0.091 ($CI_{95\%}$: 0.073 – 0.123)
Precision – reproducibility	• Inter-assay SD of log concentration = 0.084 ($CI_{95\%}$: 0.053 – 0.207) • Inter-lot SD of log concentration = 0.133 ($CI_{95\%}$: 0.083 – 0.326) • Total SD of log concentration = 0.117 ($CI_{95\%}$: 0.073 – 0.287)
Reporting Units	cp/ml
Extraction/Inhibition Control	PCR inhibition and RNA extraction efficiency control by Internal Control (IC)
Validated Extraction Methods	croBEE 201A Nucleic Acid Extraction Kit GeneProof PathogenFree RNA Isolation Kit NucleoSpin® RNA Stool
Applied Instruments	**Instrument Name** / **EV** / **Internal Control (IC)** croBEE Real-Time PCR System – FAM – HEX AMPLilab Real-Time PCR System – FAM – HEX Applied Biosystems 7300 / 7500 Real-Time PCR System – FAM – JOE AriaMx Real-Time PCR System – FAM – HEX BioQaunt-96 Real-Time PCR System – FAM – HEX CFX Connect™ / CFX96™/ Dx Real-Time PCR Detection System – FAM – HEX Gentier 96E/96R Real-Time PCR System – FAM – HEX LightCycler® 480 – FAM – HEX LineGene 9600 Plus – FAM – HEX Mic qPCR Cycler – FAM – HEX QuantStudio™ 3 / 5 Real-Time PCR System – FAM – VIC Rotor-Gene 3000 / 6000 / Q – FAM – HEX SLAN® Real-Time PCR System – FAM – HEX
Detection Channels	FAM (EV), HEX/JOE/VIC (IC)
External Quality Assessment	Regularly tested in QCMD and INSTAND e.V. External Quality Assessment Panels – results at *www.geneproof.com*

4. INTERFERENCES

The evaluation and settings of pathological values for interference testing was performed according to CLSI guidelines EP7-A2, guidelines and recommendations of Czech Society of Clinical Biochemistry.

Endogenous and Exogenous Interferences

Tested Substance	Tested Level(s)	Observed Interference	Tested Substance	Tested Level(s)	Observed Interference
CSF					
Albumin	60 g/l	None	Lactic acid	16.5 mmol/l	None
Glucose	55 mmol/l	None	-	-	-
NASOPHARYNGEAL SWAB					
Whole blood	2 % (v/v)	None	Oxymetazoline (Nasivin)	0.0625 mg/ml	None
Mucin	60 µg/ml	None	Phenylephrine (Vibrocil)	0.375 mg/ml	None
Xylometazoline (Olynth)	0.25 mg/ml	None	Budesonidum (Tafen)	100 µg	Partial
Sodium chloride (Quixx)	6.5 mg/ml	None	Neomycin sulfas, bacitracinum (Pamycon)	300 U.I. (neomycin) 25 U.I. (bacitracin)	None

NOTE: In the case of partial interference, inhibition may occur with the risk of a false negative result at a given concentration of interferent.

5. KIT CONTENT

Reagent	Content	Vial Title	Cap Colour	Guaranteed Volume [µl]	Number of Vials	
					EV/ISEX/025 – for 25 rxn	EV/ISEX/100 – for 100 rxn
Master Mix	Mixture of PCR enzymes, target specific primers and TaqMan probes in buffer	Master Mix EV	Blue	375	1	4
Calibrator	DNA oligonucleotide in buffer	Calibrator A EV 10^5 cp/µl	Black	200	1	1
		Calibrator B EV 10^4 cp/µl	Brown	200	1	1
		Calibrator C EV 10^3 cp/µl	White	200	1	1
		Calibrator D EV 10^2 cp/µl	Transparent	200	1	1
Internal control	Inactivated viral particle in buffer	Internal Control RNA IC	Red	250	1	4

6. CALIBRATOR INFORMATION

The use of all 4 calibrators is necessary for correct sample quantification. The automatic quantification based on the analysis of calibrators is generated automatically as a part of analytical process performed in the PCR instrument. Each calibrator consists of target specific DNA. Each calibrator must be designated as „standard“ in the PCR instrument. The concentration of each calibrator must be entered when samples are defined in the PCR plate set up in the data analysis software.

NOTE: In the case of qualitative detection, the Calibrator C 10^3 cp/µl serves as a positive control.

7. TRANSPORT AND STORAGE

Storage Conditions	(-20 ± 5) °C
Transport Conditions	-20 °C and below
In-use Stability	thaw a maximum of 5 times or use within 30 days after the first use of a particular vial, whichever comes first

8. ASSAY PROCEDURE

SPECIMEN COLLECTION, TRANSPORTATION AND HANDLING

1. Samples for RNA extraction must be collected and transported following professional guidelines, preferably at (5 ± 3) °C.
2. Samples for RNA extraction must be transported and processed by the laboratory in the shortest possible time (preferably within 4 hours).

NUCLEIC ACID PURIFICATION

3. Prepare specimens for the assay according to the corresponding extraction kit manual.
4. Thaw required amount of Internal Control (IC or UNIC) vials, mix and briefly centrifuge.
5. Add the Internal Control (IC or UNIC) directly into the sample at the beginning of the extraction process so that 1 µl of the resulting elution volume contains 0.1 µl of the IC:

Elution Volume	25 µl	50 µl	100 µl	200 µl
Internal Control (IC or UNIC)	2.5 µl	5 µl	10 µl	20 µl

6. Continue extraction according to the appropriate protocol.
   *NOTE: In case of using *UNIC = GeneProof Universal Internal Control (more information in chapter 12. Additional Products), see Instruction for Use of GeneProof Universal Internal Control.*

PCR SETUP PROTOCOL

7. Thaw required vials and reagents completely.
8. Gently vortex and briefly centrifuge all vials before setting up the PCR run.
   NOTE:
   Keep the reagents at (5 ± 3) °C for the shortest time possible until the PCR reaction is set up.
9. Add 15 µl of Master Mix into PCR tubes.
10. Add 10 µl of the extracted nucleic acid sample or 10 µl of Calibrator into the individual PCR tubes and mix by pipetting. The total reaction mix volume is 25 µl.
11. Close the tubes, centrifuge shortly, insert them into the real-time PCR device and amplify according to the following PCR profile.
    NOTE:
    It is recommended to perform at least 1 negative control and at least 1 full range of calibrators (for a quantitative kit) for each individual PCR run. Use your own negative control (not provided) in the form of nuclease-free water. For more information see chapter 10. Run Validity.

AMPLIFICATION PROFILE

Follow the thermocycler manufacturer's manual for setting the instrument and for analysis.

Universal PCR Profile

WARNING! For the Universal PCR Profile, the reaction volume must be set to 40 µl.

Step	Process	Temperature [°C]	Time	Cycles	Fluorescence Acquisition
1	UNG decontamination/ Reverse Transcription	42	15 min	1 cycle	
2	Initial denaturation	95	10 min	1 cycle	
3	Denaturation	95	5 s	45 cycles	
	Annealing	60	40 s		FAM, HEX/JOE/VIC
	Extension	72	20 s		

9. INTERPRETATION OF RESULTS

Channel FAM (EV)	Channel HEX/JOE/VIC (IC)	Result	Interpretation
+	+	Valid	Enterovirus positive
+	-	Valid	Enterovirus positive
-	+	Valid	Enterovirus negative
-	-	Invalid	-

NOTE: For interpretation of PCR run see chapter 10. Run Validity.

10. RUN VALIDITY

OVERALL VALIDITY OF DETECTION

	Signal	Channel	Run validity	Recommendation
Calibrator C10^3 (qualitative detection) or Calibrator Set (quantitative detection)	+	FAM	Valid	-
Calibrator C10^3 (qualitative detection) or Calibrator Set (quantitative detection)	-	FAM	Invalid	Repeat PCR run
Negative control	-	FAM	Valid	-
Negative control	+	FAM	Invalid	Repeat PCR run

NOTE: If the issue persists, please contact Customer Support, see Contact information.

11. QUANTITATIVE DETECTION EVALUATION

Use the following formula to calculate the viral load concentration in cp/ml for manual extraction (using GeneProof PathogenFree RNA Isolation Kit):

VLC – Viral load concentration [cp/ml]
SC - Sample concentration [cp/µl]
EV - Elution volume [µl]
IV - Isolation volume [µl]

$$VLC = \frac{SC \times EV \times 10^3}{IV}$$

To easily calculate pathogen concentrations using manual or automated extraction, you can use the calculator at *www.geneproof.com*

VALIDITY OF QUANTITATIVE DETECTION

Channel	Calibrators				Result	Recommendation
	A10^5	B10^4	C10^3	D10^2		
Target specific channel (FAM)	++++	+++	++	+	Valid exact quantification	-
Internal Control channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	≥0.98					
Target specific channel (FAM)	++++	+++	++	+	Reduced quantification accuracy	Repeat PCR run
Internal Control channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	<0.98					
Target specific channel (FAM)	No signal of one or more calibrators				Invalid quantification	Repeat PCR run
Internal Control channel (HEX/JOE/VIC)	+/-	+/-	+/-	+/-		
R^2	N/A					

R^2 – Determination coefficient – parameter evaluating the quality of standard curve

NOTE: If the problem persists, please contact Customer Support.

12. ADDITIONAL PRODUCTS

GeneProof Universal Internal Control

GeneProof Universal Internal Control (UNIC) is intended to be used as the Internal Control for microbiological GeneProof PCR kits and as an alternative product to Internal Controls included in the GeneProof microbiological PCR kits. The UNIC works only in combination with GeneProof PCR kits. It is intended to simplify the user's workflow in cases where multiple detection kits with single extract are used. For more details see the Instruction for Use for UNIC.

GeneProof Universal Control Plus

Product	REF
GeneProof Universal Internal Control	UNIC/GP/050

NOTE: IC is applied to the solution only once. Add UNIC instead of IC from the package of the PCR kit. Do not add IC and UNIC to the same sample at the same time.

13. MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

CONSUMABLE MATERIAL

96-well reaction plates or PCR tubes (0.2 ml volume), pipette tips with filters, powder-free gloves, biohazard waste bin, nuclease-free water

DEVICES

Real-time PCR instrument (see chapter 3. Technical Specification), nucleic acid extraction system or kit (see chapter 3. Technical Specification), desktop centrifuge (for 0.2 ml PCR tubes or 96-well plates), vortex mixer, freezer (-20 ± 5) °C, refrigerator (5 ± 3) °C, cooling rack

14. WARNINGS, PRECAUSIONS AND PROCEDURE LIMITATIONS

- Patient management decisions should never be made based solely on the results from this test. Other laboratory and clinical factors must also be considered in making clinical decisions.
- Any serious incident occurred in relation to the using of GeneProof PCR Kit shall be reported to the manufacturer and to the competent local authority.
- Use separate working places for sample preparation / nucleic acid extraction and amplification reactions. Never introduce an amplified product in reagent and/or nucleic acid extraction (sample preparation) area.
- Dispose of unused reagents and waste in accordance with national, federal, state or local regulations.
- Use all necessary protective equipment (protective disposable gloves, a laboratory coat and eye protection) when handling specimens and kit reagents.
- Avoid microbial and ribonuclease contamination of the reagents when removing aliquots from reagent vials.
- Use RNase- and DNase-free filter pipette tips only.
- Use new tips for each pipetting step.
- Close the kit components' vials immediately after use and never interchange lids.
- Do not pool reagents from different lots or from different vials within the same lot.
- Do not substitute the reagents between different lots.
- Do not use reagents from damaged or leaking vials.
- Be very careful when handling the calibrators or the clinical material; incorrect handling could result in contamination and the consequent impairment of the kit components! The manufacturer is not responsible for the kit impairment due to incorrect handling.

Procedure Limitations:

- Read the whole Instruction for Use properly before starting the manipulation. Not following these instructions can lead to an erroneous result which can cause misdiagnosis or inappropriate treatment!
- Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.
- Do not use kit after the expiry date.
- The presence of UNG decontamination step reduces the risk of lower levels of amplicon contamination. However, contamination from very high levels of amplicons can be avoided only by good laboratory practices and careful adherence to the procedures specified in this Instruction for Use. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

- This product is designed for use with the applied PCR instruments and validated extraction methods mentioned in chapter 3. Technical Specification.
- Specimens should be handled as if infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories.

Clinical Limitations:

- Detection of pathogen's RNA is dependent on the number of viruses present in the specimen and may be affected by specimen collection methods and patient factors.
- Use only with validated specimens (see chapter 2. Intended Purpose and Use) otherwise incorrect results could occur.

15. EXPLANATION OF SYMBOLS

Symbol	Explanation	Symbol	Explanation
CE	this product complies with the relevant EU requirements	LOT	Lot number
IVD	*in vitro* diagnostic medical device	Σ	Contains sufficient amount for n-tests
REF	Catalogue number		Temperature limitation
	Manufacturer		Expiry date
	Read Instruction for Use		

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