Enterovirus R-gene™ - ref.: 69-005

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EXTRACTION

- IN EXTRACTION ROOM -

- Schedule a pre-treatment for stools.
- Homogenize **IC1** and samples to be analysed:

CSF, throat, nasopharyngeal secretions, stool, cell culture.

- Add 10 µL **IC1** to the appropriate volume of each sample to be extracted in 1 extraction tube.
- Add 10 µL **IC1** to the appropriate volume of **W0** in 1 extraction tube.

- Perform extraction as below:

INSTRUMENT	KIT	Sample + IC1 Volumes	SAMPLE TYPE	PROTOCOL	ELUTION VOLUME
QIAcube	QIAamp® Viral RNA Mini Kit	140 µL of sample + 10 µL IC1	CSF	Purification of viral RNA from cell-free body fluids.	40 µL
			Respiratory samples. Stool.		60 µL
			CSF		50 µL
			Respiratory samples. Stool.		60 µL
MagNAPure Compact® Instrument	MagNA Pure Compact Nucleic Acid Isolation Kit I	200 µL of sample + 10 µL IC1	CSF. Respiratory samples.	Total_NA_Plasma_100_400	50 µL
	MagNA Pure Compact RNA Isolation Kit		CSF. Respiratory samples.	RNA_Blood_V3_2	50 µL
NucliSENS® easyMAG™	NucliSens® Magnetic extraction reagents		CSF. Respiratory samples.	Generic	50 µL
				Specific A + matrix	70 µL
			Stool.	Specific A + 100 µL silica	50 µL
Versant™ kPCR Molecular System SP	Versant™ Sample Preparation 1.0	400 µL of sample + 10 µL IC1 (extract 250 µL)	CSF	Sample Preparation Protocol 5	65 µL (eluat 50 µL)

Store extracted samples and reagents at -18°C/-22°C

ANCILLARY REAGENTS:

- **CELL Control r-gene™ - ref.: 71-106**
- **Colour Compensation r-gene™ - ref.: 71-103**

for LightCycler® Instruments use only.

GLOSSARY:

IC1	Internal Control 1
W0	Water for extraction
R9	Enterovirus premix
RT	Reverse Transcriptase
CT	Crossing Threshold
IC1sample	Sample Extraction + Inhibition Control
IC1W0	Reference Extraction + Inhibition Control

AMPLIFICATION

- IN AMPLIFICATION ROOM -

Validated on:

LightCycler®	SmartCycler®	ABI®	Rotor-Gene™	Stratagene® Versant™ kPCR AD
530 & 560	FCTC / FAM & Cy3	FAM & VIC	GREEN & YELLOW	FAM & HEX
including LC480		including ABI StepOne™ and ABI Fast		

AMPLIFICATION PROGRAM

- Enter the following program:

STEPS		TIME	TEMPERATURE	CYCLES	FLUORESCENCE ACQUISITION
Reverse Transcription		30 min.	50°C	1	None
Activation Hot Start Taq Polymerase		15 min.	95°C	1	None
Amplification	Denaturation	10 sec.	95°C	45	None
	Annealing	40 sec.	60°C		530 + 560 nm
	Elongation	25 sec.	72°C		None

Note 1: for LightCycler® instruments, set **SEEK TEMPERATURE** to the temperature corresponding to the fluorescence acquisition.
Note 2: for LightCycler® instruments, add a cooling step: 30 sec. / 40°C / 1 cycle at the end of the PCR.
Note 3: for ABI® instruments, select **NONE** in **PASSIVE REFERENCE**.
Note 4: for Rotor-Gene™, calibrate the signal by clicking on **GAIN OPTIMISATION**.
Note 5: for Stratagene® and Versant™ kPCR Molecular System AD instruments, select **NONE** in **REFERENCE DYE**.

AMPLIFICATION PREPARATION

Preparation of the premix

- Plan "**n**" number of amplification tubes taking into account:
  - ⇨ number of sample to be amplified;
  - ⇨ positive control (PC9);
  - ⇨ negative/ inhibition control (**W0**);
- Pipet 15 µL x (n + 1) Amplification Premix (R9).
- Add 0.1 µL x (n + 1) Reverse Transcriptase (RT).

Preparation of PCR assay

- Distribute 15 µL of the previous premix (R9 + RT) in amplification tubes.
- Add 10 µL extracted sample, positive control (PC9) and extracted **W0**.
- Centrifuge for 15 sec, depending on the instruments.
- Place the amplification tubes in the instrument and run the program above according to the protocol of the instrument used.

RESULTS

INTERPRETATION

Conditions for test validation:

- Negative Inhibition Control (530 nm): NO SIGNAL.
- IC1W0 (560 nm): CT ≤ 36 CYCLES.
- PC9 (530 nm): 18 ≤ CT ≤ 25 CYCLES.

Sample result interpretation

EXTRACTION and INHIBITION CONTROL	CT [IC1sample] ≤ CT [IC1W0] + 3 cycles		CT [IC1sample] > CT [IC1W0] + 3 cycles	
	NON INHIBITED and correctly extracted sample		INHIBITED or badly extracted sample	
SAMPLE	Calculated CT	Non calculated CT	Calculated CT	Non calculated CT
ENTEROVIRUS detection	Sample validated as positive	Sample validated as negative	Sample validated as positive	NOT VALID

This outlined procedure does not replace the original protocol.

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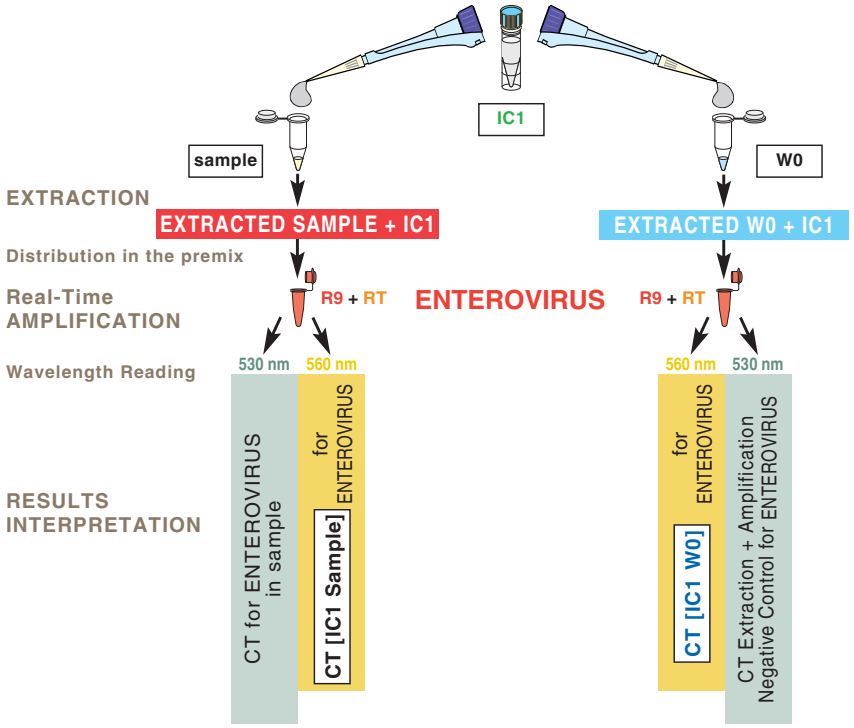
PRINCIPLE for ENTEROVIRUS DETECTION



Sample interpretation

CT [IC1sample] ≤ CT [IC1W0] + 3 cycles

The sample is correctly extracted and doesn't contain inhibitory agents of amplification.

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