quanty HEV
REF: RT-41
Detection and quantification of the Hepatitis E Virus genome with *Real Time PCR*

INTRODUCTION AND PURPOSE OF USE

The **quanty HEV** system is a quantitative test that allows the RNA amplification and quantification, by means of *Real Time PCR*, of ORF3 region of Hepatitis E virus RNA.
The Procedure allows the detection of the RNA target by means a retro-amplification reaction.
The analysis of the results is made using a Real Time PCR analyzer (thermal cycler integrated with a system for fluorescence detection and a dedicated software).

CONTENT

The kit contains reagents enough to perform 24 tests

	Quantity	Description
R1	2 x 8 µl	**Enzyme mMix** Reverse Transcriptase e Taq Polymerase enzymes (Blu cap)
R2	2 x 200 µl	**HEV probe Mix** Upstream primer, downstream primer, Target probes (FAM for HEV and VIC for internal Control), Nuclease-free water, ROX, dNTPs, Tris-HCl, KCl, $MgCl_2$ (Green Cap)
R3	2 x 35 µl	synthetic RNA corresponding to ORF3 gene 100.000 cps/µl
R4	2 x 35 µl	synthetic RNA corresponding to ORF3 gene 10.000 cps/µl
R5	2 x 35 µl	synthetic RNA corresponding to ORF3 gene 1.000 cps/µl
R6	2 x 35 µl	synthetic RNA corresponding to ORF3 gene 100 cps/µl
R7	2 x 160 µl	**Inhibition control** (murine RNA - GAPDH)
R8	1 x 50 µl	**Negative Control**

Instruction for use: **ST. RT41-ENG.4**

MATERIALS AND STRUMENTATION REQUIRED BUT NOT SUPPLIED

Disposable latex powder-free gloves or similar material;
Bench microcentrifuge (12,000 - 14,000 rpm);
Micropipettes and Sterile tips with aerosol filter;
Vortex;
Plastic materials (microplate and optical adhesive cover);
EZ1 Advanced XL DSP Virus Card. - Ref. 9018703 - QIAGEN.

Reagents

The **quanty HEV** kit was evaluated using the following extraction method:

Manual Extraction

Ref. 52906. QIAmp Viral RNA Mini Kit
The kit allows the manual RNA extraction from tested samples . The kit contains reagents for 250 samples. (QIAGEN)

Automatic extraction

Ref. 62724. EZ1 XL DSP Virus Kit.
The kit allows the automatic viral RNA from Human samples.
The kit contains reagents for 48 samples. (QIAGEN)

Alternative nucleic acid extraction systems and kits might also be appropriate following the manufacturer instruction. The suitability of the nucleic acid extraction procedure for use with *quanty HEV* has to be validated by the user.

Instruments

The **quanty HEV** kit was evaluated using the following instruments:

Extraction System

Ref. 9001492. EZ1 Advanced XL.
Robotic Workstation for the automatic purification of the nucleic acids until 14 samples simultaneously (QIAGEN)

Real Time PCR

The ***quanty HEV*** kit was developed and validated to be used with the following real time PCR instruments:

- *7500 Fast* from Lifetechnologies
- *Rotor-Gene Q* MDx from QIAGEN
- *Versant kPCR AD from Siemens or Stratagene MX3005P/MX3000P*
- LightCycler 480 from Roche

Please ensure that the instruments have been installed, calibrated, checked and maintained according to the manufacturer's instruction and raccommendations.

SAMPLES AND STORAGE

The ***quanty HEV*** system must be used with extracted RNA from the following biological samples: **Plasma** and **Serum**.
Collected material must be shipped and stored at +2 - +8°C. Store the samples at -20°C if not used within 3 days.

PRECAUTIONS USE

This kit is for in *vitro* diagnostic (IVD), for professional use only and not for in vivo use.
After reconstitution, the amplification master mix could be used in one time (12 reactions). Repeat thawing and freezing of reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.
At all times follow Good Laboratory Practice (GLP) guidelines.
Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
Avoid any contact between hands and eyes or nose during specimens collection and testing.
Handle and dispose all used materials into appropriate bio-hazard waste containers. It should be discarded according to local law.
Keep separated the extraction and the reagents preparation.
Never pipette solutions by mouth.
Avoid the air bubbles during the master mix dispensing. Eliminate them before starting amplification.
Wash hands carefully after handling samples and reagents.
Do not mix reagents from different lots.
It is not infectious and hazardous for the health (see Material Safety data Sheet – MSDS).
Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
Read carefully the instructions notice before using this test.
Do not use beyond the expiration date which appears on the package label.
Do not use a test from a damaged protective wrapper.

LIMIT OF THE METHOD

The extreme sensitivity of gene amplification may cause false positives due to cross-contamination between samples and controls. Therefore, you should:

- physically separate all the products and reagents used for amplification reactions from those used for other reactions, as well as from post-amplification products;
- use tips with filters to prevent cross-contamination between samples;
- use disposable gloves and change them frequently;
- carefully open test tubes to prevent aerosol formation;
- close every test tube before opening another one.

The proper functioning of the retro-amplification mix depends on the correct collection, correct transportation, correct storage and correct preparation of a biological sample.
As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and other laboratory tests done on the patient.
A negative result obtained with this product suggests that the RNA of HEV was not detected in RNA extracted from the sample, but it may also contain HEV-RNA at a lower titre than the detection limit for the product (detection limit for the product, see paragraph on Performance Characteristics); in this case the result would be a false negative.
As with any diagnostic device, with this product there is a residual risk of obtaining invalid, false positives or false negatives results.

STORAGE AND STABILITY

Store the product ***quanty HEV*** at **–20°C**.
The ***quanty HEV*** kit is shipped on dry ice. The kit components should be frozen.
If one or more components are not frozen upon receipt or if the tubes have been compromised during transport, contact Clonit srl for assistance.
An intact and well stored product has a stability of 6 months from the date of production. Do not use beyond the expiration date which appears on the package label.
Repeat thawing and freezing of reagents (more than twice) should be avoided, as this might affect the performance of the assay. The reagents should be frozen in aliquots, if they are to be used intermittently.

ANALYTICAL PROCEDURE

Manual Extraction

Ref. 52906. QIAmp Viral RNA Mini Kit

Procedure to plasma and Serum

Follow the instructions inside the kit *QIAmp Viral RNA Mini Kit*.
After the incubation period for 10 min at room temperature, add 5 µl of Inhibition Control
Follow the instructions inside the kit
Elute the sample in 50 µl of buffer AVE

Samples are now ready for amplification or storage at -80°C

Automatic extraction

Ref. 62724. EZ1 XL DSP Virus Kit

Procedure to plasma and Serum

Follow the instructions inside the kit *EZ1 XL DSP Virus Kit*.
Volume of sample to be used:

Plasma [µl]	*Serum [µl]*
200	*200*

Preparation of the Carrier and the Internal Control (Inhibition Control).
Solve completely the lyophilize RNA carrier in elution buffer (AVE), from 310 µl, split in aliquots and store to –20 ± 5°C. Not thawing and freezing the aliquots more than 2 times.
For each analyzed sample, dilute 3,6 µl of a original solution include the RNA Carrier and 10 µl of *Inhibition Control* in total volume of 60 µl using elution buffer (AVE)
Select the protocol starting from 200 µl of samples with the elution of 60 µl.

Follow the instructions inside the kit *EZ1 XL DSP Virus Kit*.

Samples are now ready for amplification or storage at -80°C

SOFTWARE SETTING

Lifetechnologies 7500 fast

Turn the instrument and the computer on and open the control software. Click on "**Advance Setup**": by default the software will shows the page "**experiment properties**". Write in the "**experiment name**" the file name, choose the type of instrument (**7500 o 7500fast),** the type of reaction (**quantitation standard curve**)**,** the type of used reagent (**Taqman®Reagents**) and the reaction time of analysis (**Standard ≈ 2 hours to complete a run**).
Open the page named "**page setup"** (sheet **Define Target and Samples**).
In the window **Define Targets** set:

Target	Reporter	Quencher
HEV probe:	FAM	TAMRA
Inhibition Control (IC) probe:	VIC	TAMRA

Set the samples' name in the window "**Define Samples**".
In the same page "**plate setup**" select the sheet "**Assign Target and Samples**". On the screen you will see the microplate draft.
Select an area of the plate where the controls will be placed: select wells of the plate and set both targets (HEV and IC). Select "**Assign target to selected wells**" in the blank, the "**task Standard (S)**" for HEV target and set the controls' concentration.
Choose an area in the plate where negative control will be placed: select "**Assign target to selected wells**" in the blank, the "**task Negative (N)**" for the HEV target.
Select an area of the plate where samples will be placed: select the wells and set both targets (HEV and IC). Link every well to a sample, through the window "**Assign samples to selected wells**".
For each sample, select in the blank "**Assign targets to selected wells**" the "**task UnKnown (U)**" for the HEV target.
Set ROX as passive reference, using it as normalizer of detecting fluorescence.
Open "**Run Method**" (sheet **Graphic View**) and set the right thermal cycling:

cycles	denaturation	annealing	extension
1	50° C 30 min		
1	95°C 2 min		
45	95° C 15 sec	55° C 45 sec	72° C 15 sec

In the window "**Reaction volume plate per well**" set a volume of 30 µl.
After preparing the plate, and correctly inserting it in the instrument, press the button "**Start Run**".

Rotor Gene Q MDx

The experiments can be **set using the Quick Start Wizard or the** Advanced Wizard, which appears when the software is started.
Select the wizard "**Advanced**". As a first step, select the model "**Two Step Reaction**" with a double click in the "**New Run**".
In the next window, select the type of rotor installed on the instrument from the list that appears. Check the "**Locking Ring Attached**", check the checkbox and then click "**Next**".
Enter the name of the operator and the reaction volume of 30 µl, and then click "**Next**".
In the next window click on "**edit profile**". Set the following thermal cycle:

cycles	denaturation	annealing	extension
1	50° C 30 min		
1	95°C 2 min		
45	95° C 15 sec	55° C 45 sec	72° C 15 sec

Select the annealing / extension from the thermal profile and click on "**Acquiring A to cycling**."
In the next window, select yellow from the available channels and add it to acquiring channel along with the green channel and click "**OK**". In the next window click on "**OK**" and then click "**Next**".
Click on "Edit Gain" button and set the following values for each channel:

Reporter	Gain
Green	5
Yellow	5

To begin the course, click on the button "**Start Run**". You can save the model before you begin your run by clicking on "**Save Template**".
After clicking on the button "**Start R**un" window appears "**Save As**". The stroke can be saved in the desired position by the user.
Once the run started, the window "**Edit Samples**" allows you to set the name of samples and controls in the positions in which they were loaded on the instrument.
Select the locations where they were positioned the controls of known concentration and designate them as HEV Standard. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**Standards**". Enter the concentrations of the controls.
Select the location where you placed the Negative Control and name it as Negative Control. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**Negative Controls**"
Select the location of each sample and enter the name or code of the patient. Clicking on the box next to "**Type**" correspondent, in the dropdown menu "**Samples**" you can select the type of sample being analyzed. Select "**UnKnown**"
At the end of the operation click "**OK**" in the "**edit samples**" and wait until the end of the race for the analysis (see "**Interpretation of Results**").

Versant kPCR AD or Stratagene MX3005P/MX3000P

Turn the instrument on and wait until both green lamps have fixed light, turn on the computer and start the control software. In the main screen will appear the window "**New Experiment Options**": select "**Experiment type": quantitative PCR (Multiple Standard).**
Turn the lamp on 20 minutes before doing a new experiment. For turning the lamp on, click on the icon of the lamp in the tool bar or select "**Lamp On**" from the menu **"Instruments".**
Verify the right setting of the gain of the fluorescent reporters: in the menu of settings, choose: **"Instrument"** and then **"Filter set gain setting"**.

Reporter	Gain
FAM	4
HEX	4
ROX	1

Click on button **"setup"** in the tool bar and choose "**Plate Setup".**
Sign the wells corresponding to calibrators. Define the calibrator's positions in right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
Standard	FAM/HEX/ROX	ROX	None

Clicking on every single well, the window "**well information**" will appear: choose the name of the calibrator.
In the window "**Select Quantity**", set the concentrations of the 4 calibrators, following the instructions indicated in the paragraph **Interpretation of the results**.
Sign the wells correspondent to Negative control. Define the NTC positions in right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
NTC	FAM/HEX/ROX	ROX	None

Clicking on every single well, the window "**well information**" will appear: set NTC as the name.
Sign the wells correspondent to the Samples. Define the samples positions in right menu, setting:

Well type:	Collect Fluorescent Data:	Reference Dye:	Replicate Symbol:
UnKnown	FAM/HEX/ROX	ROX	None

Clicking on every single well, the window "**well information**" will appear: set the name or the code of the sample.
It's possible, indeed, set near the name of fluorescent reporter the name of analyzed targets:

FAM	HEX
HEV	Internal Control

In the tool bar choose the sheet **"Thermal Profile Setup"**, set the correct thermal cycle and reading the fluorescence in the annealing step:

cycles	denaturation	annealing	extension
1	50° C 30 min		
1	95°C 2 min		
45	95° C 15 sec	55° C 45 sec	72° C 15 sec

After preparing the plate and inserting it in the instrument, press the button "**Run**", selecting the sheet Thermal profile status and check the correctness of thermal profile.
Select the box **Turn Lamp Off** at the end of execution. Push the button Start: the software will ask you to indicate the name of saved file. The analysis will start.

LightCycler 480

Turn the instrument and the computer on and start the control software. In the principal screen, on "**Experiment Creation**" select the "**Plate type**" and push the "**New experiment**" button. The window "experiment" appears. On the "Run protocol" sheet set: Thermal protocol, Reaction volume (30µl) and Detection format (**dual colour hydrolysis probe**).

cycles	denaturation	annealing	extension
1	50° C 30 min		
1	95°C 2 min		
45	95° C 15 sec	55° C 45 sec	72° C 15 sec

Push the "**Subset editor**" button and in this window, select an area of the plate where controls and samples will be placed.
Push the "**Sample editor**" button. Select the correct workflow (Step1: **Abs quantification**), choose the samples Subset created in the step before and insert the name for each well. Choose the correct type for each well: Positive CTR, Negative CTR or Unknown sample.

Push again the "**experiment button**", insert the plate in the instrument and push "**start run**".

PREPARATION OF THE REACTIONS:

Defrost a tube of **Enzyme mMix**;
Defrost a tube of **HEV probes Mix;**
Mix carefully by vortex **6,5µl** of **Enzyme mMix** and **190µl** of **HEV probes Mix** (the mix as produced is enough to prepare **12 reactions** of amplification: **4 positive controls, 1 negative control and 7 samples**).
Distribute, in the amplification plate, **15µl of just reconstituted mix** in chosen positions as already set on the instrument software.
Distribute, in the negative control position, **15 µl** of solution taken by the negative control vial.
Distribute, in chosen position for each sample, **15 µl** of corresponding sample.
Distribute, in chosen positions for the positive controls, **15µl** of 10^2 **copies/µl,** 10^3 **copies/µl,** 10^4 **copies/µl and** 10^5 **copies/µl**.
Seal up accurately the plate using an optical adhesive film and verify that there aren't air bubbles in the mix, to avoid the amplification interferences.
For the Rotor-Gene Q MDx, seal each tube with the appropriate cap. The air bubbles presence is not influently; the rotor centrifugal force will allow automatic deletion.
Transfer the plate in the instrument and push the button "**Start Run**".

QUANTITATIVE ANALYSIS

Lifetechnologies 7500 Fast, StepOne Plus.

At the end of the PCR run, the software automatically opens the "**Analysis**" window in the "**Amplification plot**" sheet on the menu on the left.
Select the wells corresponding to the positive control, negative control and samples for analysis.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **HEV target**. Check the correct setting of the threshold.
Select in the "**Option**" window inside the "**Target**" pop-up menu the **IC Control target**. Check the correct setting of the threshold.

The analysis of the results is made selecting from the menu in the left the page **"Analysis"**. From the page "**Standard Curve**", maintaining open the sheet "**view well plate**" in the right side of the software select the wells containing the points of the curve and verify the parameters described in the paragraph "**Interpretation of the Results**" (coefficient of correlation, slope ecc…).
From the page "**Amplification Plot**" verify the amplification plot for every single sample.
Opening the sheet "**view well table**" in the right side of the software it is possible to verify the data obtained from experiments: Threshold Cycles, emitted fluorescence and the target quantification expressed in copies/reaction or copies/ml, depending on the settings of the calibration curve.
Clicking from the menu file and selecting the box export, the window "**export properties**" will open. Indicate the file name, select the position to save it (**Browse**) and click on button
"Start export". In this way the software will permit to save a excel file with all the data corresponding to selected experiment.

Rotor Gene Q MDx

At the end of the PCR run open the "**Analysis**" window. Select the "**Quantification**" sheet and click on "**cycling A (green)".**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.
Open the "**Analysis**" window. Select the "**Quantification**" sheet and click on "**cycling A (yellow)".**
Select from the menu **"Dynamic Tube"** and subsequently **"Slope correct"**.
Check the correct setting of the threshold in the space provided "**CT calculation – Threshold"**.

Also in this case, you can print a report of the analysis by clicking on the **"Report"** window and selecting the file in the first **Quantification cycling A (green)** and then the file **cycling A (yellow).**

Versant kPCR AD or Stratagene MX3005P/MX3000P
Click on button "**Analysis**" in the toolbar. The software will open in default the sheet "**Analysis Term Setting**". Activate the buttons FAM and HEX in the lower part of the screen and select testing samples.
Click on sheet "**results**"; the software will open in default the page "**Amplification plot**". Check the correct setting of the threshold in the specific window "**Threshold fluorescence**", in the menu on the right of the screen.

Selecting the box "**Standard Curve**" from menu "**Area to Analyze**" it's possible to visualize the data related to the calibration curve and verify the parameters described in paragraph "**Interpretation of Results**" (coefficient of correlation, slope ecc…). Selecting the box **Text report**" from menu "**Area to Analyze**" in the right side of the screen it's possible to verify the data obtained from the experiments: Threshold Cycles, emitted Fluorescences and the target quantification expressed in copies/reaction o copies/ml depending on the settings of the calibration curve.
From the window **Text Report** it's possible to export the results obtained clicking **file, export** on main menu.

LightCycler 480
When the run is completed select analysis and choose the correct kind of analysis you want: "**Abs Quant/Fit Points**". Choose the samples subset you want to analyze. Select the "**NoiseBand**" sheet, under the plot you can choose "**NoiseBand (Fluoresc.)**"; and move the line of the NoisBand on the plot with the mouse of your PC. Repeat this action for each fluorophore using the "**Filter comb"** button.
Clicking the sheet "**Analysis**" you can set the threshold choosing the option "Threshold(manual).

After setting parameters push the "**Calculate**" button. Repeat this action for each fluorophore.

INTERPRETATION OF RESULTS

Through the Real Time PCR reaction it is possible to obtain the RNA quantification of HEV-RNA, by setting the values of the positive controls of the calibration curve. To calculate these values, all the dilutions steps that the sample has undergone during the extraction and amplification stages must be considered.
The Ct values obtained from the amplification of 4 controls of known titre are used by the software for the calculation of the calibration curve from which the unknown samples are interpolated.
A proper functioning of the amplification mix can be verified analyzing these parameters:

Parameters	**Reference**
RTS conc. 10^5 copie/µl (FAM)	Ct ≤ 22
Correlation Coefficients	0.990 ≤ r^2 ≤ 1
Slope	-3,6 ≤ Slope ≤-3.2
PCR Efficiency	90 ≤ Efficienza ≤ 100

If the RTS amplification reaction at a concentration of 10^5 copies produces a Ct > 22 or undetermined the session can't be considered valid and must be repeated.

Verify that the correlation coefficient value (r^2), the slope or the reaction efficiency fit to the limited indicated in the above table or do not deviate much from them, which represent the ideal range for a proper PCR reaction.

By correctly setting the standards concentration as a function of the extraction system you can get the quantization of the sample directly in copies / ml:

		Manual Extr. Ref. 52906 (QIAGEN)	**Automatic Extr. Ref. 62724 (QIAGEN)**	**Alternative extract.**
	RTS 1	35.700.000 copies	30.000.000 copies	1.500.000 copies
	RTS 2	3.570.000 copies	3.000.000 copies	150.000 copies
	RTS 3	357.000 copies	300.000 copies	15.000 copies
	RTS 4	35.700 copies	30.000 copies	1.500 copies

When alternative systems are used sample concentration expressed in copies/ml will be obtained using the formula:

$$copie/ml = \frac{1000}{Ve} \times \frac{Ev}{Ea} \times C_{reaz}$$

where:
- **Ve:** extracted sample Volume expressed in µl
- **Ev:** eluted sample Volume during extraction stage expressed in µl
- **Ea:** extracted sample volume used for amplification expressed in µl
- **C_{reaz}:** copies provided by the instrument.

As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and the other laboratory tests done on the patient.
As with any diagnostic device, with this product there is a residual risk of obtaining invalid, false positives or false negatives results.

The use of positive and negative controls in each amplification session allow to verify the correct functioning of the amplification mix and the absence of any contamination.
In the amplification reaction of each sample, the Ct values for the internal control specific probe are used to validate the analysis session, from extraction process until detection step.
In the amplification reaction of each sample, the Ct values for the internal control specific probe are used to validate the analysis session, from reverse-transcription process until detection step.
Be sure that emitted fluorescence from internal control amplification has not a Ct > 30 or undetermined. If a sample shows an undetermined HEV RNA and an internal control Ct >30, this means that there have been problems in the extraction stage or in the retro-amplification stage; therefore the sample could be a false negative.
Repeat the sample.
It can be considered valid the samples with a Ct > 30 for the internal control, and a high concentration of HEV RNA. In this case, the competitive nature of PCR reaction can hide or disadvantage the internal control amplification.

Detector FAM	Detector VIC/Hex	PCR Run	Sample
Ct undetermined	Ct > 30 or undetermined	Not Valid	repeat
Ct undetermined	Ct < 30	Valid	Negative
High Ct	Ct < 30	Valid	Positive
Low Ct	Ct > 30 or undetermined	Valid	HighPositive

PERFORMANCES

Analytical sensitivity:
It is considered as analytical sensitivity the highest dilution (title) to which a positive sample can be diluted without the system losing the ability to detect with a positivity rate of ≥ 95%. The analytical sensitivity of the system was assessed by analyzing synthetic RNA, quantified by spectrophotometric analysis, containing the regions of interest (ORF3) of the virus in serial dilutions.

Analytical sensitivity of ***quanty HEV*** determined by Probit analysis

Instrument	Copies/µl	95% confidence interval
7500 Fast	0.348 cps/ul	Inf. 0.153 cps/ul Sup.3.364 cps/ul
RotorGene Q MDx	0.39 cps/ul	Inf. 0.178 cps/ul Sup.2.149 cps/ul
Versant kPCR AD	0.3 cps/ul	Inf. 0.12 cps/ul Sup.1.84 cps/ul

Instrument	Copies/ml	95% confidence interval
7500 Fast	104 cps/ml	Inf. 45.9 cps/ml Sup.1000 cps/ml
RotorGene Q MDx	117 cps/ml	Inf. 53 cps/ml Sup. 645 cps/ml
Versant kPCR AD	90 cps/ml	Inf. 36 cps/ml Sup. 552 cps/ml

Clinical sensitivity:
It is considered as clinical sensitivity the ability to detect true positive samples in the totality of the samples screened as positive. The analysis was made on HEV positive samples and the test was performed following the method recommendations. Positive samples were confirmed with an other CE approved method available on the market.

Obtained results show a clinical sensitivity of 100%.

Diagnostic Specificity:
It is considered as diagnostic specificity the ability of the method to detect trues negative samples. The diagnostic specificity of the system was evaluated analyzing human samples tested and confirmed as HEV negative with an other CE marked method available on the market.

Diagnostic specificity is 100% for material extracted from plasma.

Analytical Specificity:
Test's specificity was guaranteed by the use of specific primers for HEV.
The alignment of the choose regions for specific primers' hybridization for HEV with available sequences of the ORF3 region present in database, demonstrated: their conservation, the absence of significative mutations and the complete specificity for the analysed target.
The Genotypes included in the WHO reference panel have been successfully detected by the kit "**quanty HEV"** showing that the kit is able to amplify:
Genotype 1
Genotype 2
Genotype 3
Genotype 4

Traceability versus WHO Control
The used standard (PEI code 8578/13, Version 2.0, dated November) was established as the 1st WHO International Reference Panel for Hepatitis E Virus RNA genotypes for Nucleic Acid Amplification technology (NAT) based assays.

Samples	Matrix	Genotype	Average Study Log (IU/ml)	Obtained Results IU/ml
8567/13	Plasma	1a	2.64	2.60
8568/13s	Stool	1a	4.25	4.64
8569/13	Plasma	1e	3.25	3.66
8570/13	Plasma	3b	4.20	3.38
8571/13	Plasma	3c	3.40	2.61
8572/13	Plasma	3e	3.50	2.75
8573/13	Plasma	3f	3.84	3.71
8574/13s	Stool	3	4.98	4.36
8575/13	Plasma	4c	4.07	3.57
8576/13	Plasma	4g	3.77	3.69
8577/13s	Stool	2a	5.42	5.63

Average conversion factor quanty HEV Kit:

1 copy/ml = 1 IU/ml

Baylis SA, Terao E, Hanschrmann KM, Collaborative Sudy to establish the 1st **WHO International Reference Panel for Hepatitis E Virus RNA genotypes for Nucleic Acid Amplification technology (NAT) based assays.** WHO Report 2015, WHO/BS/2015.2264

Cross-Reactivity:
The exam of alignment of the region chosen for hybridization of primers specific for HEV with the sequences available in the bank data of the ORF3 region showed the their conservation, the absence of significant mutations and the complete specificity for the target analyzed.
To check the cross-reactivity of the assay, samples tested as positive for other viruses were analysed following the method instructions.

Sample	Positive sample	Obtained Results cps/ml
1	HCV	Negative
2	HBV	Negative
3	HDV	Negative
4	Enterovirus	Negative
5	HIV	Negative

INTERFERENCES:

Verify that in the RNA extracted from the sample there is no contamination from mucoproteins and haemoglobin, to exclude possible inhibition of PCR reaction. The interference due to contaminants can be detected through a spectrophotometric analysis, verifying the ratio between the absorbance readings at 260 nm (maximum absorbtion of Nucleic Acids) and 280 nm (maximum absorbtion of Proteins). A pure RNA should have a ratio of approximately 2.

QUALITY CONTROL

It is recommended to include in each analytical run, as quality control of every extraction, amplification and detection step, an already tested negative and positive sample, or a reference material with known concentration.
In accordance with the Clonit srl ISO EN 13485 Certified quality Management System, each lot of **quanty HEV** is tested against predetermined specification to ensure consistent product quality.

BIBLIOGRAPHY

Sally A. Baylis, Saeko Mizusawa, Yoshiaki Okada, Kay-Martin O. Hanschmann. **Collaborative Study to Establish a World Health Organization International Standard for Hepatitis E Virus RNA for Nucleic Acid Amplification Technology (NAT)-Based Assays**. WHO/BS/2011.2175

Sally A. Baylis at al. **Standardization of HEV Nucleic Acid Amplification Technique based assays: an Initial study to evaluate a panel of HEV Strains and investigate laboratori performance**. Journal of Clinical microbiology, Apr. 2011, p1234-1239

Baylis SA, Terao E, Hanschrmann KM, Collaborative Sudy to establish the 1st **WHO International Reference Panel for Hepatitis E Virus RNA genotypes for Nucleic Acid Amplification technology (NAT) based assays.** WHO Report 2015, WHO/BS/2015.2264

TECHNICAL ASSISTANCE

For any question and support please contact our Technical support:

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phone: +39 02 56814413

IVD	*In vitro* diagnostic device
	Read the instruction's manual
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
LOT	Lot (xxxx)
REF	Code
	Manufacturer
Σ	Contains sufficient for <n> tests

EDMA code: 15020540
CND: W0105020507

The ***quanty HEV*** kit is CE marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/CE.

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CE IVD
for *in vitro* diagnostic use

ST. RT41-ENG.8
Revision 15th May 2018