



EliGene® HCV LC

REF 90059-LC (for 50 samples)

Kit components:

5 x 200 µl HCV LC Mix
1 x 80 µl Enzyme Mix
5 x 100 µl IC RNA 01
1 x 100 µl HCV QS1 (1 x 10¹ IU/µl)
1 x 100 µl HCV QS2 (1 x 10² IU/µl)
1 x 100 µl HCV QS3 (1 x 10³ IU/µl)
1 x 100 µl HCV QS4 (1 x 10⁴ IU/µl)
1 x 100 µl HCV QS5 (1 x 10⁵ IU/µl)
Instruction for Use

Storage and shelf life after first opening:

All components of the kit must be transported and stored at -20 °C.
Kit must be stored at -20 °C in a dark.

Intended use

The EliGene® HCV LC kit is intended for detection and quantification of hepatitis C virus RNA.

Principle of the method

This kit is based on RealTime PCR method. In this kit primers and labeled probes (FAM and HEX) for the detection of HCV RNA and for the detection of internal control are used.

Introduction

Hepatitis C virus is enveloped RNA virus classified to *Flaviviridae* family. Virus genome consists of 9379 nucleotides. HCV represents heterogeneous group of viruses divided into 6 genotypes (groups) and more than 40 sub-types. EliGene® HCV LC Kit gives the positive results with genotypes 1a, 1b, 2a, 2b, 3a, 4a, 6 and 6b.

Today, the world prevalence about 175 millions persons infected by hepatitis C virus is assessed. Virus is disseminating mainly by parenteral way, the sexual transfer is observed rare. In our conditions, significantly risk group is represented by the injection drug consumers.

Primary sample collection, handling and storage

Serum, plasma

Manual: Chemagic Viral DNA/RNA Kit (Chemagen-PerkinElmer)

QIAamp DSP Virus Kit (Qiagen)

Automatic: MAGNETO BodyFluid DNA/RNA isolation kit (Elisabeth Pharmacon)

WARNING: To keep the sensitivity of the test we recommend follow strictly the pre-analytical procedures mentioned in this instruction. Specially do not change the recommended procedures of isolation as for stated amount of specimen. For RNA isolation other RNA isolation kits can be used but pre-analytical procedures for storage and transport of samples must be followed. During isolation from smaller quantity of sample it is necessary to make elution into adequate volume of elution buffer for keeping of method sensitivity. E.g. in case of isolation from 200 microliters of sample to 50 microliters of elution buffer, the sensitivity of the method would be 5 times lower.



Serum, plasma:

According to standard protocol take the sample of the serum or plasma into sterile tubes. Samples must be stored and transported at 4 °C. It is recommended to isolate RNA from the sample during the day of taking sample or store at -20°C maximally for one month. For RNA isolation at least 200 µl of sample must be used.

Manual isolation:

Add 20 µl of Proteinase K (Chemagic Viral DNA/RNA Kit) and 10 µl of Internal Control RNA 01 (IC RNA 01) to 200 µl of the specimen and then continue according to the standard protocol of Chemagic Viral DNA/RNA Kit or QIAamp DSP Virus Kit with final elution step to 25 µl of molecular grade water or elution buffer. Isolated RNA use immediately for the detection or store it at -70 °C for longer period.

Automatic isolation:

Add 10 µl of Internal Control RNA 01 (IC RNA 01) to 1.0 ml of the specimen and isolate RNA from the sample by using Prepito NA Body Fluid Kit according to protocol for plasma samples with elution to 50 µl of molecular grade water. Isolated RNA use immediately for the detection or store it at -70 °C for longer period. In the case of smaller quantity of the serum specimen fill up specimen to 1.0 ml by molecular grade water.

Additional required equipment

- Automatic pipette 5–20 µl and sterile tips with filter DNA-, RNA- free, DNase-, RNase- free (we recommended plastic with CE certificate for diagnostic purposes).
- Sterile stand DNA-, RNA- free, DNase-, RNase- free.
- Equipment for RealTime PCR – the kit is designed for RealTime Systems ABI7500FAST (Applied Biosystems), LightCycler 2.0, LightCycler 480 and LightCycler Nano (Roche), RotorGene 6000 or RotorGene Q (Qiagen), CFX96 Touch Real-Time PCR Detection System (Bio-Rad), MIC qPCR Cycler (Bio Molecular Systems).
- Sterile plastic (strips, plates, tubes) DNase-, RNase- free compatible with given RealTime PCR system.
- Lab safety gloves.

Configuration of Real Time instrument

- For HCV detection the probe labeled with FAM is used (exc. 494 nm – em. 518 nm).
- For Internal Control the probe labeled with HEX is used (similar to VIC – exc. 525 nm – em. 548 nm, similar to JOE exc. 520 nm – em. 548 nm).
- Reaction Mix includes passive reference control dye ROX for signal normalization.

LightCycler® 2.0 (Roche):

For reaction use glass capillaries only.

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

| | | | |
|------|--------|--------------------|-------------------------|
| 55°C | 15 min | Ramp rate (20°C/s) | Acquisition mode "None" |
|------|--------|--------------------|-------------------------|

Step 2 - Analysis mode "None", 1 Cycle

| | | | |
|------|-------|--------------------|-------------------------|
| 95°C | 5 min | Ramp rate (20°C/s) | Acquisition mode "None" |
|------|-------|--------------------|-------------------------|

Step 3 - Analysis mode "Quantification", 50 Cycles

| | | | |
|------|-----|--------------------|-------------------------|
| 95°C | 5 s | Ramp rate (20°C/s) | Acquisition mode "None" |
|------|-----|--------------------|-------------------------|



| | | | |
|------|------|--------------------|---------------------------|
| 60°C | 20 s | Ramp rate (20°C/s) | Acquisition mode "Single" |
| 72°C | 10 s | Ramp rate (20°C/s) | Acquisition mode "None" |

The complete temperature profile can be up-loaded from Run Template "HCV_LC_LC2.0_v01.ix0". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

LightCycler® 480 (Roche):

For reaction use white plates only. The usage of natural plates can lead to decreased sensitivity of the kit. Do not reuse plates; the contamination of your laboratory could occur during the manipulation with plates.

In option Detection format choose "Dual Color Hydrolysis probe".

Set up the following temperature profile:

Step 1 - Analysis mode "None", 1 Cycle

| | | | |
|------|--------|---------------------|-------------------------|
| 55°C | 15 min | Ramp rate (4.4°C/s) | Acquisition mode "None" |
|------|--------|---------------------|-------------------------|

Step 2 - Analysis mode "None", 1 Cycle

| | | | |
|------|-------|---------------------|-------------------------|
| 95°C | 5 min | Ramp rate (4.4°C/s) | Acquisition mode "None" |
|------|-------|---------------------|-------------------------|

Step 3 - Analysis mode "Quantification", 50 Cycles

| | | | |
|------|------|---------------------|---------------------------|
| 95°C | 5 s | Ramp rate (4.4°C/s) | Acquisition mode "None" |
| 60°C | 20 s | Ramp rate (2.2°C/s) | Acquisition mode "Single" |
| 72°C | 10 s | Ramp rate (4.4°C/s) | Acquisition mode "None" |

Step 4 - Analysis mode "None", 1 Cycle

| | | | |
|------|-------|---------------------|-------------------------|
| 40°C | 1 min | Ramp rate (2.2°C/s) | Acquisition mode "None" |
|------|-------|---------------------|-------------------------|

The complete temperature profile can be up-loaded from Run Template "HCV_LC_LC480_v01.ix0". The Run Template can be imported to the software in menu "Navigator" by clicking to icon "Import" from the CD included in the kit.

LightCycler® Nano (Roche):

For reaction use clear strips only.

In "Run Settings" menu choose "Hydrolysis Probes" option and "Normal Quality" option.

In "Profile menu" set up the following temperature profile:

Step 1 - Hold

| | | |
|------|--------|-------------------|
| 55°C | 15 min | Ramp rate (5°C/s) |
|------|--------|-------------------|

Step 1 - Hold

| | | |
|------|-------|-------------------|
| 95°C | 5 min | Ramp rate (5°C/s) |
|------|-------|-------------------|

Step 2 – 3-Step Amplification, 50 cycles

| | | |
|------|------|------------------------------------|
| 95°C | 5 s | Ramp rate (5°C/s) |
| 60°C | 20 s | Ramp rate (4°C/s) "Acquire" signal |
| 72°C | 10 s | Ramp rate (5°C/s) |

Step 3 - Hold

| | | |
|------|-------|-------------------|
| 40°C | 1 min | Ramp rate (4°C/s) |
|------|-------|-------------------|

In "Samples" menu click in window "Targets" (upper right window) on icon "+" and choose FAM dye as "Target"



1". Then click once again on icon "+" and choose HEX dye as "Target 2". In window "Samples" (upper left window) click on icon "+" and add your samples. Then assign the samples with positions and Targets FAM and HEX as an Unknown sample (Samples) or Standard.

The complete temperature profile can be up-loaded from Run Template "HCV_LC_LCNANO_v01.ppf". The Run Template can be copied from the CD included in the kit.

RealTime System ABI7500FAST (Applied Biosystems):

Use the program module for absolute quantification (Plate Type "Quantitation-Standard Curve" experiment for ABI 7500FAST). In case of ABI7500FAST use "7500 (96wels)" instrument type.

Set up the following temperature profile:

Holding stage

55°C 15 min

Holding stage

95°C 5 min

Cycling stage – 50 cycles

95°C 5 s

60°C 20 s Data collection ON

72°C 10 s

Collect emission signal at the second step – 60°C.

The complete temperature profile can be up-loaded from Run Template "HCV_LC_ABI7500_v01.edt". The Run Template can be copied from the CD included in the kit.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Startup Wizard Create a new Experiment for CFX96 instrument and Create New Protocol.

Set up the following temperature profile:

Step 1 55°C 15 min

Step 2 95°C 5 min

Step 3 95°C 5 s

Step 4 60°C 20 s + Plate Read

Step 5 72°C 10 s

Step 6 GOTO Step 3 50x

Step 7 40°C 60 s

Enter the Sample Volume 30 µl

Collect emission signal at the Step 3 at 60° C.

For filter settings use the "Scan Mode" All Channels but in Plate Manager select for the samples only fluorophores FAM and HEX. Then assign the samples with positions and Targets FAM and HEX as an "Unknown" sample or "Standard".

MIC qPCR (Bio Molecular Systems Pty Ltd):



In Run Setup Create a new Run Profile for the MIC instrument. Enter the Sample Volume of 30 µl and Temperature Control "Fast TAQ (v3)".

Set up the following temperature profile:

Hold

95°C 15 min

95°C 5 min

Cycling – 50 cycles

95°C 5 s

60°C 20 s Acquire on "Green" and "Yellow"

72°C 10 s

40°C 60 s

RotorGene 6000 or Q (Qiagen):

In the "New Run" window choose "Three Step" run

Choose the appropriate "Rotor Type" and click "Next".

Set up the following temperature profile:

Holding stage

55°C 15 min

Holding stage

95°C 5 min

Cycling stage – 50 cycles

95°C 5 s

60°C 20 s Acquiring in channels "Green" and "Yellow"

72°C 10 s

For the Gain optimization in both channels select option an "automatic gain optimization before first acquisition". The complete temperature profile can be up-loaded from Run Template "HCV_LC_RG6000_v01.ret". The Run Template can be copied from the CD included in the kit.

Reagent preparation

- To avoid the contamination keep all tubes closed and follow the instructions.
- Before the usage, all reagents must be completely thawed, briefly mix on vortex and shortly spin.
- Isolate RNA according to standard protocol.

Preparation of Reaction Mix

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

1. Preparation of MasterMix: Take one microtube with HCV LC Mix and let it thaw. Consequently, take off from freezer microtube with Enzyme Mix, shortly spin and pipette 15 µl of Enzyme Mix into the microtube HCV LC Mix. Immediately after pipetting put the tube with Enzyme Mix back to the freezer otherwise it might be lowered the sensitivity of subsequent analyses.



2. Detection: Take one microtube with MasterMix and pipette 20 µl of MasterMix to amplification microtubes, plate or glass capillaries and add 10 µl of isolated RNA. During the pipetting of samples be careful to avoid cross-contamination of samples. Prepared MasterMix should be used in 30 minutes and cannot be used repeatedly.
3. Quantification Standards: Take one microtube with MasterMix and pipette 20 µl of MasterMix to microtubes, plate or glass capillaries and add 10 µl of the quantification standard of given concentration. Thaw the standard perfectly and warm up to room temperature (warm it in your hand), after thawing, vortex the standard properly and spin on centrifuge shortly. Repeat this procedure with all other standards of different concentrations. During the pipetting of standards be careful to avoid contamination of other samples. Use separate pipette for standards, the pipette for positive control can be used. Prepared MasterMix should be used in 30 minutes and cannot be used repeatedly.

Insert the microtubes, plate or glass capillaries with samples to the RealTime PCR instrument and run the program according to chapter "Configuration of Real Time instrument" above.

Result viewing

LightCycler® 2.0 (Roche):

Analyze real-time PCR results in channels 530 and 560 (LightCycler® Software 4.X) respectively, using the Absolute quantification module of the LightCycler® Analysis Software. Enter the concentrations of HCV Standards in FAM channel that are mentioned on single microtubes.

Positive result for HCV: The positive result is characterized by amplification and growth of signal in FAM channel (530). In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (560). Cp value corresponds to the quantity of positive result. The value of "Score" must be higher than 4.5.

LightCycler® 480 (Roche):

In "Sample Editor" menu choose "Abs Quant" workflow. Enter the concentrations of HCV Standards in FAM channel that are mentioned on single microtubes.

In menu "Analysis" choose "Abs Quant/2nd Derivative Max" option.

In Analysis window click to "Color Comp" icon and choose Universal CC FAM (510)-VIC (580) calibration. Analyze results by clicking to icon "Calculate".

Positive result for HCV: The positive result is characterized by amplification and growth of signal in FAM channel (465-510). In a case of negative results the amplification will not occur. The values of Concentration correspond to the quantity of positive result; "Negative" means negative result.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (533-580).

LightCycler® Nano (Roche):

In "Samples" enter the concentrations of HCV Standards in FAM channel that are mentioned on single microtubes.

In "Analysis" menu click in window "Select Analysis" on icon "+" and choose "Automatic Quantification".

HCV analysis

For HCV analysis enter in "Settings" for FAM channel following values:

Excluded early cycles: 3; Min. Relative Amp: 0.1; Min. Amp. Quality: 5

Positive result for HCV: The positive results are characterized by amplification and growth of signal in FAM



channel (510-528). In a case of negative results the amplification will not occur.

Internal control Analysis

For Internal Control analysis enter in “Settings” for HEX channel following values:

Excluded early cycles: 3; Min. Relative Amp: 0.01; Min. Amp. Quality: 1

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (530-548).

The values of Concentration correspond to the quantity of positive result; “Negative” means negative result. Positive result is characterized by increasing of fluorescence signal in selected channel.

RealTime System 7500FAST (Applied Biosystems):

In menu “Setup” enter the concentrations of HCV Standards in FAM channel that are mentioned on single microtubes in the software of RealTime instrument.

In “Analysis Settings” choose “Automatic Treshold” and “Automatic Baseline” option and analyze results.

Positive result for HCV: The positive results are characterized by amplification and growth of signal in FAM channel (em. 518 nm). In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (em. 548 nm).

The values of Qty correspond to the quantity of positive result, “result undet.” means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

CFX96 Touch Real-Time PCR Detection System (Bio-Rad):

In Data Analysis window choose “Quantification”. In “Settings” menu choose option “Baseline Threshold” and select “Baseline Cycles” option as “Auto Calculated” and Single “Threshold” option as “Auto Calculated”.

In Data Analysis window select a single fluorophore (FAM or HEX) by the clicking the box next to the fluorophore name located under the amplification chart and read the results for individual samples.

Positive result: The positive result is characterized by the growth of fluorescence signal in FAM channel (em. 518 nm). In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in HEX channel (em. 548 nm).

MIC qPCR (Bio Molecular Systems):

To view and analyze acquired data, click the small cross next to Cycling under Analysis module and then select the target – Non-Assay Green (FAM) and Non-Assay Yellow (HEX). In the down right corner **increase “Fluorescence Cut-off Level” to 20 %**. A quantification cycle (Cq) value is displayed for each positive well. This is the cycle number at which the sample fluorescence rises above the threshold (the red horizontal line near the bottom of the amplification curve chart). In the Samples panel on the right, click sample names to hide or display the associated amplification curves and result data.

Positive result: The positive result is characterized by the growth of fluorescence signal in Green channel. In a case of negative results, the amplification will not occur.

The Internal Control must be amplified in each sample. The Internal Control amplification is characterized by the growth of fluorescence signal in Yellow channel.



RotorGene 6000 or Q (Qiagen)- version 1.7 and higher:

Click to “Edit Samples” icon in the menu and choose for Quantitation Standards Type “Standard” and for samples Type “Unknown”. Enter the concentrations of HCV Standards that are mentioned on single microtubes.

Click to “Analysis” icon in the menu and choose Analysis option “Quantitation”. In “Quantitation Analysis” window choose “Dynamic Tube” and “Slope Correct” option.

For HCV analysis (Green channel) use Threshold value of 0.1 and for internal control analysis (Yellow channel) use Threshold value of 0.05.

Positive result for HCV: The positive result is characterized by amplification and growth of signal in FAM channel (Green). In a case of negative results the amplification will not occur.

The Internal Control is amplified every time. The Internal Control amplification is characterized by amplification and growth of signal in HEX channel (Yellow).

The values of “Calc. conc.” correspond to the quantity of positive result; “Negative” means negative result. Positive result is characterized by increasing of fluorescence signal in given channel.

Interpretation of results

Negative result: If the increasing of amplification signal in FAM channel does not appear before the cycle number 45, the result of test should be interpreted as probably HCV negative. The signal for internal control must be positive. This result does not exclude the occurrence of HCV infection because results of this test are dependent on proper sample collection and elaboration. Results are also dependent on enough quantity of analyzed RNA.

Positive result: Amplification signal in FAM channel appears before cycle number 45. HCV RNA was detected in the sample. The sample is HCV RNA positive.

WARNING: The contamination in laboratory space is also possible. Use separate pipette for MasterMixes, separate pipette for positive controls and separate pipette for samples. Follow all recommendations for laboratories of DNA analyses.

Inhibited sample:

In the case that increasing of amplification signal in FAM channel that is specific for HCV and also increasing of amplification signal in HEX channel (specific for internal control) is not observed, it is necessary to repeat the analysis. The best would be to use new RNA prepared by new extraction process.



Control procedure

EliGene® HCV LC Kit involves Internal Control RNA 01 (IC RNA 01) and HCV quantification standards. Internal Control follows the quality of RNA isolation and detects impurities in the isolation process. It detects the occurrence of an inhibition of reverse transcription and amplification process. In the case that the sample is HCV RNA negative, the Cp of internal control must be Cp < 40. In the case of strongly positive samples usually the internal control amplification is not detected.

HCV quantification standards follow the proper function of MasterMix. Minimal Cp of the lowest HCV quantification standard (HCV QS1) must be 38 or less. The Cp higher than 38 can't be accepted and analysis must be repeated. In the case of repeatedly higher Cp contact manufacturer ELISABETH PHARMACON.

Use negative control for each run. As negative control use 10 times diluted IC RNA 01 in the water for molecular biology used in your laboratory (1 µl IC RNA 01 + 9 µl water). For negative control use the pipette for DNA samples.

Usage the HCV quantification standards:

To generate a standard curve on Instruments, all 5 quantitation standards should be used and defined in the "Edit Samples" dialog box as standards in FAM channel with the specified concentrations (see the instrument user manual).

Applied Biosystems RealTime System 7500 and RotorGene 6000 or RotorGene Q (Qiagen) instruments will perform the correlation coefficient calculation of standard curve labeled r by entering the appropriate values of calibrators. The value r of the correlation coefficient of standard curve must be higher than 0.9.

LightCycler® 480 and 2.0 instruments will perform the standard error calculation of standard curve labeled "Error" by entering the appropriate values of calibrators. The value "Error" of standard curve must be lower than 0.1.

In this case, the device managed on the basis of the measured results of calibrators build usable calibration line, whereby precisely subtract the results of other analyzed samples. Otherwise, it is necessary to repeat the analysis. Insufficient value of correlation coefficient or error can be caused by bad pipetting, insufficient vortexing of thawed calibrators or inappropriate storage of calibrators.

The quantitation standards are defined as IU/µl. The following equation has to be applied to convert the values determined using the standard curve into IU/ml of sample material:

$$\text{Result (IU/ml)} = \frac{\text{Result (IU/}\mu\text{l)} \times \text{Elution Volume (}\mu\text{l)}}{\text{Sample Volume (ml)}}$$

Use negative control for each run. As negative control use 10 times diluted IC RNA 01 in the water for molecular biology used in your laboratory (1 µl IC RNA 01 + 9 µl water). For negative control use the pipette for DNA samples.

Reference material:

To monitor the all examination process covering RNA isolation and RealTime PCR detection is possible to use reference viral material. The positive material is possible to order from the Acrometrix company (Life Technologies).

Troubleshooting:

1. If there is no amplification of Internal Control RNA, there is some problem in the isolation of RNA or the kit is after the expiration date or there is RealTime instrument breakdown.



- If there is no amplification of Standards, the kit is after the expiration date or there is RealTime instrument breakdown.

Performance characteristics

Analytical performance characteristics:

The Limit of detection (LOD) in consideration of the purification (Prepito Viral DNA/RNA1k Kit) of the EliGene[®] HCV LC Kit was determined using a dilution series of the HCV Specimen with different genotypes No. 68 (1a), 173 (3a) and 194 (1b) from 50 000 to nominal 5 IU/ml HCV spiked in HCV negative clinical plasma specimen. These were subjected to RNA extraction using the Prepito Viral DNA/RNA1k Kit (extraction volume: 1.0 ml, elution volume: 50 µl). Each of the five dilutions was analysed by the LightCycler 480 instrument (Roche, DE) with the EliGene[®] HCV LC Kit on three replicates. The analytical detection limit in consideration of the purification of the EliGene[®] HCV LC Kit is 50 IU/ml for Prepito Viral DNA/RNA1k Kit. This means that 100% sensitivity can be declared for EliGene[®] HCV LC Kit for the concentration of 50 IU/ml of serum.

Analytical sensitivity of the method is 50 IU/ml of serum.

Analytical specificity of the method was analyzed by comparison of primers and probes with all known DNA sequences in GenBank database. Analytical specificity was also analyzed by the addition of DNA from EBV, HSV1, HSV2, VZV, MTB, *Borrelia sp.*, *C. trachomatis*, *E. coli*, *A. niger*, *C. albicans* to the reaction mix. These DNA did not give false positive result for HCV (see table below).

| Control group | HCV (FAM) | IC DNA 01 (HEX) |
|--|-----------|-----------------|
| Human herpesvirus 1 (Herpes simplex virus 1) | - | + |
| Human herpesvirus 2 (Herpes simplex virus 2) | - | + |
| Human herpesvirus 3 (Varicella-zoster virus) | - | + |
| Human herpesvirus 4 (Epstein-Barr virus) | - | + |
| Human herpesvirus 5 (Cytomegalovirus) | - | + |
| <i>Mycobacterium tuberculosis</i> | - | + |
| <i>Borrelia sp.</i> | - | + |
| <i>Chlamydia trachomatis</i> | - | + |
| <i>Escherichia coli</i> | - | + |
| <i>Aspergillus niger</i> | - | + |
| <i>Candida albicans</i> | - | + |

Clinical specificity was tested on human serum samples. 200 samples of different human DNA isolated from the serum did not give false positive result.

Performance characteristics:

Within the frame of testing the functional characteristics of EliGene[®] HCV LC Kit overall 500 clinical specimens were analyzed. From these specimens, 300 HCV positive specimens and 200 HCV negative specimens were verified by COBAS AMPLICOR HCV MONITOR Test, version 2.0 (v2.0). The EliGene[®] HCV LC Kit diagnosed as HCV positive 299 specimens. One specimen was determined by the EliGene[®] HCV LC Kit as negative.

The calculation of the sensitivity and the specificity of the EliGene[®] HCV LC Kit are shown in table below. The sensitivity and specificity of EliGene[®] HCV LC Kit is 99,7 % and 100%, respectively.

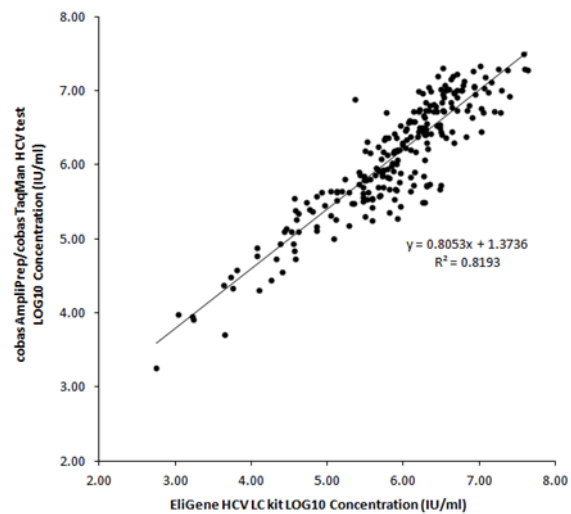


| | |
|----------------------------|----------------------------|
| A = 300 Really positive | B = 0 False positive |
| C = 1 False negative | D = 200 Really negative |

Sensitivity = $A/(A+C) = 300/(300+1) = 99,7\%$

Specificity = $D/(D+B) = 200/(200+0) = 100\%$

The correlation of the quantitative results of EliGene[®] HCV LC Kit and COBAS AMPLICOR HCV MONITOR Test, version 2.0 (v2.0) was analysed by linear regression, when the logarithmised results of both kits were plotted as scatterplots against each other (Figure 1). Correlation coefficient was calculated to $r=0.81$.



Comparison of the COBAS AMPLICOR HCV MONITOR Test (v.2.0) against the EliGene[®] HCV LC Kit

Measuring interval

The linear range (analytical measurement) of the EliGene[®] HCV LT Kit was determined by analysing a dilution series of a HCV quantitation standard ranging from 1×10^5 IU/ml to 50 IU/ml. Each dilution has been tested in three replicates using the EliGene[®] HCV LC Kit on the LightCycler[®] 480 instrument (Roche, DE). The linear range of the EliGene[®] HCV LC Kit has been determined to cover concentrations from 10 IU/ml to at least 1×10^5 IU/ml (Figure 1).

Internal control of quality

As an internal control of quality the Internal Control RNA for checking the process of RNA isolation together with HCV quantification standards for functional control of MasterMix and as a reference sample is used.

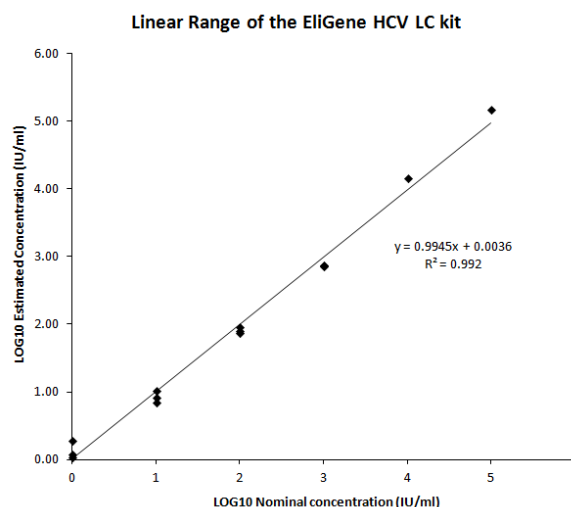




Figure 1. Calculation of the linear range. The straight was determined by linear regression of the log₁₀ calculated concentrations with the log₁₀ nominal concentrations. The equation of the regression line is included in the figure.

Limitation of the examination procedure

The sensitivity of kit depends on handling with specimen (isolation of RNA). It is strictly recommended to use isolation kits and procedures mentioned above.

Biological reference intervals

Not applicable information for this kit.

Warning

After mixing, MasterMix is stable for 30 minutes. Discard remaining MasterMixes – do not freeze again! Do not mix components of the kits of different lots.

Warnings and general precautions

- Handle and dispose of all biological samples as if they were capable of transmitting infective agents. Avoid direct contact with the biological samples. Avoid splashing or spraying. The materials that come into contact with biological samples must be treated with 3% sodium hypochlorite for at least 30 minutes or autoclaved at 121 °C for one hour before disposal.
- Handle and dispose of all reagents and all assay materials as if they were capable of transmitting infective agents. Avoid direct contact with the reagents. Avoid splashing or spraying. Waste must be treated and disposed of in compliance with the appropriate safety standards. Disposable combustible materials must be incinerated. Liquid waste containing acids or bases must be neutralized before disposal.
- Wear suitable protective clothing and gloves and protect eyes/face.
- Never pipette solutions by mouth.
- Do not eat, drink, smoke or apply cosmetic products in the work areas.

- Wash hands carefully after handling samples and reagents.
- Dispose of leftover reagents and waste in compliance with regulations in force.
- Read all the instructions provided with the kit before running the assay.
- Follow the instructions provided with the kit while running the assay.
- Do not use the kit after the expiry date.
- Only use the reagents provided in the kit and those recommended by the manufacturer.
- Do not mix reagents from different batches.
- Do not use reagents from other manufacturer's kit.



Warnings and precautions for molecular biology

- Molecular biology procedures, such as extraction, reverse transcription, amplification and detection of nucleic acids, require qualified staff to prevent the risk of erroneous results, especially due to degradation of the nucleic acids contained in the samples or due to sample contamination by amplification products.
- It is necessary to have 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 designed for extraction/preparation of amplification reactions.
- It is necessary to have lab coats, gloves and tools which are exclusively employed in the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never transfer lab coats, gloves or tools from the area designed for the amplification/detection of amplification products to the area designed for the extraction/preparation of the amplification reactions.
- The samples must be exclusively employed for this type of analysis. Samples must be handled under a laminar safety box. Tubes containing different samples must be never opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Reagents must be handled under PCR box or laminar flow box. The reagents required for amplification must be prepared in such a way that they can be used in a single session. The pipettes employed to handle the reagents must be used exclusively for this purpose. The pipettes must be of the positive displacement type or be used with aerosol filter tips. The tips employed must be sterile, free from DNases and RNases, free from DNA and RNA.
- Amplification products must be handled in such way as to reduce dispersion into the environment as much as possible, in order to avoid the possibility of contamination. Pipettes used to handle amplification products must be employed exclusively for this specific purpose.

Warnings and precautions specific to components of the kit

- The tubes containing mixes (HCV LC Mix) are disposable and therefore must be used once only in the preparation of the reaction mixture.
- The tubes containing IC RNA 01 are disposable and therefore must be used once only in the preparation of the reaction mixture.
- These mixes carry the following safety warnings (P):

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P281 Use personal protective equipment as required.

Literature

Barbara A. Bannister, Norman T. Begg and Stephen H. Gillespie: Infectious Disease. Blackwell Science, 2th Ed., 2000

Symbols



Catalog number



Upper limit of temperature



Batch code



Use by (last day of month)



in vitro diagnostic medical device



Fulfilling the requirements of European Directive 98\79\EC for *in vitro* diagnostic medical device.



Contains sufficient for "N" tests



Attention, consult instructions for use



Manufacturer

Manufacturer

ELISABETH PHARMACON Ltd.

Rokycanova 4437/5, 615 00 Brno, Czech Republic Tel.: +420 542 213 851, +420 542 213 827

E-mail: info@elisabeth.cz