Gesellschaft zur Forschung, Entwicklung
und Distribution von Diagnostika
im Blutspendewesen mbH

Instructions for use

PoET HCV

For use with PoET Instrument

In vitro diagnostic medical device

REF **P2A-28-30**

IVD CE 0483

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1. Intended use

1.1. Abstract

The PCR kit *PoET HCV* by the *Gesellschaft zur Forschung, Entwicklung und Distribution von Diagnostika im Blutspendewesen mbH* (hereinafter referred to as GFE) is a real-time PCR kit for the qualitative detection of hepatitis C virus RNA (HCV RNA).

1.2. Intended use

The PCR kit *PoET HCV* is an *in vitro* diagnostic test kit for the qualitative detection of hepatitis C virus RNA (HCV RNA) in human plasma samples taken in the context of blood donations. *PoET HCV* is CE-marked according to IVD Directive 98/79/EC.

The PCR kit *PoET HCV* is intended for the screening of individual samples and sample pools comprised of aliquots of individual samples.

In addition, the PCR kit *PoET HCV* is suitable for the qualitative detection of HCV in individual human plasma samples.

The processing of the PCR kit *PoET HCV* is carried out with *PoET Instrument* from GFE.

2. Test principle

The safety of blood and blood products requires the determination of donor suitability and the testing of donations in order to minimize the risk of a potential transmission of viral pathogens during the transfusion of blood and blood components. But, even serological screening cannot eliminate the risk of transmission of viral infections by transfusion. A residual transmission risk exists from blood donations drawn during the seroconversion window period [1]. Testing for viral nucleic acid using NAT (nucleic acid amplification technology), shortens the diagnostic window of fresh infections and substantially reduces the risk of transmission. [1]

The detection of HCV-specific RNA in human blood with the ready-to-use PCR kit *PoET HCV* is carried out by a *real-time polymerase chain reaction* (real-time PCR) with *PoET Instrument.*

During PCR, one target sequence of HCV is amplified with the PCR kit *PoET HCV.* The sequence area is located in a conserved region of the HCV genome.

Samples are processed on *PoET Instrument* using the PCR kit *PoET HCV* together with *PoET Internal Control,* which monitors the entire process from sample preparation to result evaluation. The *Internal Control* (IC) is available as a separate accessory kit.

The evaluation of the data collected during PCR is performed fully automated on *PoET Instrument* by the integrated software *Calliope*.

3. Information on the pathogen HCV

Hepatitis C virus (HCV) is an enveloped, ss(+)RNA virus with a genome of about 9.6 kb and the causative pathogen of hepatitis C. HCV is taxonomically correctly referred to as *Hepacivirus C* and is currently the only human pathogenic representative of the genus *Hepacivirus* in the *Flaviviridae* family [2][3].

Currently, 8 genotypes with various sub genotypes and recombinants of HCV are known [2][4][5]. The diversity of the individual genotypes to each other is over 30 %. As an RNA virus, the genome of HCV is very variable. The virus is to be considered as a quasispecies. This variability is one of the main reasons why no vaccine against HCV has been developed so far.

HCV is predominantly transmitted parenterally by blood-infected needles, e.g. during drug use and tattooing, more rarely also by sexual transmission pathways [3]. Due to the introduction of HCV blood donation testing in Germany, the previously frequent transmission of HCV through blood transfusions and blood products has become very unlikely. The diagnostic window, which is unusually wide for HCV with an average of 60 days, was significantly shortened by the introduction of HCV NAT testing [6].

Acute hepatitis, which can usually develop 6 to 8 weeks after infection with HCV, usually shows only a mild course of the disease. About 75 % of acutely HCV-infected persons develop a chronic HCV infection when left untreated. This chronic infection can often develop into liver cirrhosis and subsequently into primary liver cell carcinoma in the further course of the disease. Currently, there is no vaccination against HCV available. However, an effective antiviral therapy leads to a cure in most cases [3].

4. Test procedure

The PCR kit *PoET HCV* is used after sample preparation with the fully automated *PoET Instrument* during the subsequent PCR amplification and detection. The detection of viral nucleic acids with *PoET HCV* is based on real-time reverse transcription (RT)-PCR technology. The data and result management are conducted via the software *Calliope*.

The process on *PoET Instrument* is grouped into the following steps:

- Sample preparation
- PCR setup
- Amplification and detection
- Evaluation and report

Sample preparation

The sample material used is human EDTA plasma. At the beginning of the process, *PoET Internal Control* (available separately) is added to the sample material as a process control for extraction and PCR amplification.

Virus particles and nucleic acids are released by lysis and the nucleic acids are absorbed to magnetic particles. Unbound molecules such as proteins and other impurities are removed with subsequent washing steps. The nucleic acids are then eluted from the magnetic particles with elution buffer. The elution buffer contains the RNA of the IC and possibly existing viral nucleic acids to be detected.

PCR setup:

The PCR master mix set up by *PoET Instrument* consists of a universal *enzyme mix* and a specific *oligo mix.* The *oligo mix* contains virus-specific oligonucleotides (primers and probes) that bind to highly conserved regions of the viral nucleic acids if HCV is present in the sample. *PoET HCV* is supplemented with a second heterologous non-competitive amplification system. In addition to the virus-specific oligonucleotides, the *oligo mix* contains primers and probes for amplifying the Internal Control (IC) sequence.

To avoid contamination with amplicons of previous HCV PCR reactions, the *enzyme mix* contains a heat-labile uracil DNA glycosylase (UNG) and dUTP in the mixture of dNTPs. Any contaminating amplicons from previous PCR reactions are destroyed by the UNG at room temperature before the start of the RT-PCR. During the reverse transcription step, the UNG is inactivated by the increased reaction temperature of 55 °C. Newly generated amplicons are not destroyed.

Reverse transcription:

The RNA molecules of HCV and Internal Control (inactivated recombinant Sendai virus) are subject to reverse transcription by a recombinant variant of the enzyme M-MLV reverse transcriptase. During reverse transcription, a sequence-specific cDNA copy of HCV RNA and Internal Control is produced.

Amplification:

Amplification is carried out on the basis of the cDNA produced by reverse transcription. The reaction mixture is heated to separate the double-stranded DNA into single-stranded DNA templates ("denaturation"). When cooling the mixture, probes and primers attach themselves to the DNA individual strands ("annealing"). In the presence of Mg^{2+} ions and excess deoxy nucleoside triphosphates (dNTPs) the primers are extended along the target templates

("elongation") by the enzyme "*Thermus aquaticus* (Taq) DNA polymerase". In each cycle, new double-stranded DNA molecules are generated.

This process is repeated until 45 cycles are reached, where each cycle increases the amount of target DNA.

Detection:

The detection is carried out via oligonucleotide probes, which are coupled with a fluorescent dye (“reporter”) at the 5’-end and with a quencher at the 3’-end. As long as the probe is intact when excited by an external light source, the fluorescence signal of the reporter dye bound to the probe is suppressed by fluorescence resonance energy transfer (FRET) due to the spatial proximity to the quencher. During amplification, the sequence-specific probe hybridizes to the template DNA strain in the sequence region between the forward and reverse primer binding sites. During the elongation of the forward primer, the hybridized probe is cleaved by the 5’-3’-exonuclease function of the Taq DNA polymerase, whereby the reporter dye is released and thus the fluorescence signal is emitted. The fluorescence signal increases in relation to the number of amplicons produced.

The resulting signals are sequence-specific, since probe molecules can only hybridize to complementary DNA-strands of the target region and are cleaved by the Taq DNA polymerase.

The reporter dye used for HCV differs from the reporter dye of the IC and thus also the respective fluorescence emission spectrum. A successful amplification of HCV and the IC can therefore be detected by the signal increase in two different fluorescence channels.

Evaluation and report:

After the PCR run on *PoET Instrument,* the evaluation is carried out fully automated by *Calliope*. Further details on the evaluation are described in the operator’s manual of *PoET Instrument*.

5. Reagents and materials

The content of one PCR kit *PoET HCV* includes 30 reagent tubes of each, *enzyme mix* and *oligo mix.*

PoET HCV			
GFE Catalogue number	P2A-28-30		
Number of reactions per test (rxn)	28		
Number of tests per kit	30		
Total number of reactions	840		
Kit component:	**Volume [µL]**	**Identifier**	**Cap color**
enzyme mix	1130	EM v1	white
oligo mix HCV	148	O_C v2	blue

5.1. Storage and handling of reagents

The PCR kit is shipped on dry ice. The product should be checked after receipt (i.e. frozen state of reagents, integrity of packaging, completeness).

The PCR kit *PoET HCV* is stored at ≤ 18 °C and can be used until the date indicated on the label. After expiry of the declared shelf life, the reagents may no longer be used.

	Expired reagents are recognized and excluded by *PoET Instrument* using the reagent barcodes.
	The reagents are intended for single use and not for repeated freezing and thawing. Any remaining reagents must be discarded after application.
	The *oligo mix* is sensitive to light and should be stored protected from light during test preparation.
	Within 5 hours after removal of the reagents from the freezer the analysis has to be started on *PoET Instrument*. If the tubes were stored without cap for several hours, the functionality is no longer guaranteed depending on the duration and degree of evaporation.

5.2. Disposal

- The components *enzyme mix* and *oligo mix* of the PCR kit *PoET HCV* do not contain any hazardous substances or biohazardous substances. The safety data sheets are available on request from GFE Customer service.
- Used disposables and PCR reagent residues can be disposed of into standard commercial waste.
- For the disposal of the nucleic acid extraction reagents and their residues the instructions for use of the extraction kits *PoET Extraction* and *PoET Prep Reagent* have to be followed.

6. Required equipment

6.1. Devices and software

Fully automated *PoET Instrument* including software *Calliope* and operator's manual.

6.2. Required consumables for *PoET HCV* on *PoET Instrument*

These consumables for the PCR kit *PoET HCV* on *PoET Instrument* are available separately from GFE:

Name	Description	Catalogue number
PCR Plates Frame Star® 96 (cut corner A12)	4titude from Brooks Life Sciences FrameStar® 96 (cut corner A12): 96-well semi-skirted PCR plate, black wells, clear frame, bar-coded	SP-0362
Film roll	4titude from Brooks Life Sciences Heat Sealing film roll: "Clear Weld Heat Seal Mark 2"	❶

❶: The *Film roll* is changed as part of *PoET Instrument* maintenance by GFE Customer service.

The material required for the use of the accessory and control kits (Chapter 6.3) can be found in the associated instructions for use and in the operator's manual of *PoET Instrument*.

The use of other than the specified consumables on *PoET Instrument* is not permitted.

6.3. Accessory and control kits for use with *PoET instrument*

- *PoET Extraction* [Catalogue number P1A-24-04]
- *PoET Prep Reagent* [Catalogue number P1B-24-20]
- *PoET Internal Control* [Catalogue number P1C-1440-60]
- *PoET Negative Control* [Catalogue number P3A-500-30]
- *PoET Master Positive Control* [Catalogue number P3B-360-30]

6.4. Additional equipment required

- Centrifuge for the extraction of plasma from primary tubes (EDTA-K2 blood collection systems with gel barrier) meeting the specifications of the tube manufacturer. See also operator's manual of *PoET Instrument.*

7. Warnings and precautions

Good laboratory practice

- Wear personal protective equipment (laboratory coat, safety glasses, laboratory gloves).
- Do not eat, drink or smoke in the laboratory.
- Treat the samples as potentially infectious as described in "*Biosafety in Microbiological and Biomedical Laboratories*" [7] and CLSI document M29A4 [8].
- If sample material is spilled, immediately disinfect with a suitable agent. Treat contaminated materials as biologically hazardous.
- Disinfect and wash your hands thoroughly after handling the samples and reagents.
- Clean and disinfect all work surfaces with suitable disinfectants, e.g. listed by German Robert Koch Institute (RKI)[1]
- Eliminate potential nucleic acid contamination with DNA-ExitusPlus™ (AppliChem GmbH) or a comparably effective agent according to the manufacturer.

General information on use

- Use the PCR kit *PoET HCV* only in combination with *PoET Instrument* and the described accessory and control kits as well as consumables.
- Use all reagents for *in vitro* diagnostics only
- *PoET Instrument* shall only be operated by qualified personnel trained by GFE.
- In order to prevent cross-contamination of samples or controls, all material containing samples or controls must be handled in the laboratory in accordance with the regulations for safe work.
- Store samples, controls and PCR kits separately.
- For the safe handling of the used and sealed *Extraction Plates* and *PCR Plates,* follow the instructions in the operator's manual of *PoET Instrument.*
- Dispose all materials that have come into contact with potentially infectious samples, according to the relevant regional and national regulations (see in particular also instructions for use of the PoET accessory kits).
- Use the PCR kit *PoET HCV* in the range of +15 °C to +30 °C.

Reagent handling

- Remove the caps of the reagents before positioning on the carrier of the *PoET Instrument. PoET Instrument* does not have a device for the automated removal of caps ("Decapper").
- Carry out the loading and unloading of the *PoET Instrument* reagent carriers with PCR reagents according to the specifications in the operator's manual of *PoET Instrument*. This also applies to the correct preparation of samples and controls. Any deviation from the specified procedures may affect the test performance.

[1] or other suitable guidelines, e.g. William A. Rutala, Ph.D., M.P.H., David J. Weber, M.D., M.P.H., and the Healthcare Infection Control Practices Advisory Committee (HICPAC): Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008; Update: May 2019

- Avoid mixing up tube caps, as this can lead to contamination.
- The PCR kit *PoET HCV* is designed for single use. Do not reuse reagent residues.
- Do not exchange or combine reagents of different batch numbers of the PCR kit *PoET HCV.*
- Do not use reagents after their shelf life has been expired.

8. Collection, handling and storage of plasma samples

8.1. Sample material

- In the validation studies of the PCR kit *PoET HCV,* human EDTA plasma was used as sample material. All performance-related information is based on this material, which is therefore recommended for use with *PoET Instrument*.
- Citrated plasma is not validated for use with the PCR kit *PoET HCV*.
- Blood samples taken from heparin blood collection tubes, as well as samples from heparinized persons, may not be used, as heparin can impair the PCR analysis [9].

Treat all samples as potentially infectious.

8.2. Sample drawing & pretreatment

- The venipuncture is to be carried out with commercially available EDTA-K2 blood collection systems with gel barrier (e.g. Sarstedt or Becton Dickinson) according to the manufacturer's specifications.
- The EDTA blood tubes (primary blood tubes) have to be mixed immediately by inverting five to eight times according to the manufacturer's specifications
- The whole blood samples in the EDTA-K2 gel barrier blood collection tubes must be separated by centrifugation into the cellular and plasma components within 48 hours according to the manufacturer's specifications.
- *PoET Instrument* requires a volume of up to 1.5 ml plasma for processing. Depending on the test method, significantly lower volumes can be used. Further information can be found in the operator's manual of *PoET Instrument*.

The primary tubes must be filled sufficiently. Take care to ensure that no gel components or blood cells contaminate the plasma. This can lead to an impairment of the performance of the test procedure.

8.3. Sample transport

Sample material has to be shipped exclusively in shatterproof transport containers in order to reduce the risk of leakage of sample material and, as a result, the risk of infection. Sample material must be packed and shipped in compliance with applicable national or international regulations covering the transport of medical samples.

The permissible time and temperature of the transport for the samples have to comply with the storage conditions (see Chapter 8.4).

8.4. Sample storage

The samples can be transported and stored at a temperature of 0 °C to +35 °C until separation.

The EDTA plasma can be kept at +2 °C to +8 °C for up to 7 days without measurably changing the HCV viral load.

The test performance may be affected by freezing and thawing or prolonged storage of the samples.

8.5. Provision of samples for *PoET Instrument*

Sample material stored in the refrigerator can be used and analyzed directly. The handling of frozen and thawed sample material has not been validated. Therefore, no information is available for frozen and thawed sample material. If frozen plasma is to be used, it is recommended to thaw the plasma at +37 °C in a water bath to prevent the formation of precipitates that could affect the test performance.

9. Processing of samples on *PoET Instrument*

General information for working with *PoET Instrument*:

The handling of *PoET Instrument* is described in detail in the operator's manual of *PoET Instrument.* The following is the summarized test procedure for *PoET HCV* with *PoET Instrument:*

- Before starting the run: turn on the device and PC and carry out maintenance program according to the instructions on the screen
- Running the test for *PoET HCV*:
  - Select processing mode
  - Load samples
  - Assign testing orders (test type and test parameters)
  - Load *PoET Instrument* with reagents and consumables
  - Start run
  - Check results
  - Unload consumables and disposal of waste

Depending on the test plan of a run on *PoET Instrument,* the PCR results are available about 3.5 hours after the start of the run.

10. Control procedures

10.1. Quality control measures

The automated overall process consisting of sample preparation and PCR analysis is monitored by several controls:

Control type	Product	Function
Internal Control (IC)	*PoET Internal Control*	The IC monitors the processing from extraction to the result. For each HCV non-reactive sample, the IC indicates whether the result is valid.
PCR Positive Control (PC)	*PoET Master Positive Control*	The PCR Positive Control contains viral nucleic acids of HCV, HBV, HIV, HAV and B19V. It indicates that the process on *PoET Instrument* from the setup of the PCR reaction, through the sealing of the *PCR Plates* to the execution of the PCR has been executed correctly.
PCR Negative Control (NC)	*PoET Negative Control*	*PoET Negative Control* indicates that the PCR reagents have been set up without contamination. The NC corresponds to a "*No Template Control*" (NTC).

11. Evaluation and validity of the results

The evaluation is performed automatically by the software *Calliope*. The software analyzes the fluorescence signals of all PCR reactions, including the controls, and evaluates whether the overall result is valid for the parameter HCV and for each individual sample.

If one of the criteria of the validity check for the PCR controls is not met, the PoET run will be assessed as invalid for HCV.

If the run is evaluated as valid based on the results of the PCR controls, the individual sample results are evaluated according to the following scheme:

Case	HCV channel	IC channel	Assessment	On the report
1	not reactive	invalid*	Result is invalid	invalid
2	not reactive	valid**	Result is valid and not reactive for HCV	not reactive
3	reactive	valid**	Result is valid and reactive for HCV	reactive
4	reactive	invalid*	Result is valid and reactive for HCV	reactive

*) not reactive or values outside the IC limits

**) reactive and values within the IC limits

12. Procedural limitations

- The PCR kit *PoET HCV* has been validated exclusively for use with the reagents *PoET Extraction, PoET Prep Reagent, PoET Internal Control, PoET Negative Control* and *PoET Master Positive Control* with *PoET Instrument*.
- The detection of HCV RNA depends on the amount of virus-specific nucleic acids contained in the sample. In the case of a very low viral load (below the detection limit of the assay), this cannot be reliably detected by the PCR kit *PoET HCV*.

- Incorrect sample collection, untested interference substances and improper sample storage and preparation can negatively affect the stability of the virus and nucleic acids and impair the result of PCR. In addition, the plasma may contain inhibiting agents that interfere with extraction or PCR.
- Blood samples taken from heparin blood collection tubes, as well as samples from heparinized individuals, shall not be used because heparin can impair PCR.
- For samples with a very high albumin content (> 100 g/L), a reliable test result is not ensured.
- Mutations within the highly conserved regions of the viral genome may affect oligonucleotide binding resulting in failure to detect the presence of virus.
- Despite sequence matching and verification of the primers for detecting the genotypes of HCV, a newly discovered genotype may not be detected with the PCR kit *PoET HCV.*
- Cross contamination during sample handling and processing cannot be excluded for samples with very high viral load. When detecting a PCR result with an early amplification signal, further samples in the same run can thus show weakly reactive results.

13. Performance characteristics

The performance characteristics were determined using the "5th WHO International Standard for HCV NAT" (NIBSC Code 14/150) or internal reference material quantified based on the WHO Standard.

13.1. Analytical performance characteristics

Limit of detection HCV

The determination of the 95 % limit of detection (95 % LOD) for HCV with the PCR kit *PoET HCV* was carried out with a sample volume of 1.3 mL based on the extraction and detection of diluted virus standards in plasma. The sensitivity was determined by performing a PROBIT analysis (log10) with the software *IBM SPSS Statistics* on the basis of the hit rates of the serial dilutions of the virus standards.

Standard	**HCV WHO 14/150**
95 % LOD	9.1 IU/mL
Confidence interval	6.6 - 15 IU/mL

Limit of detection for smaller sample volumes

If samples with a plasma volume in the range of ≥ 40.5 µL and < 1300 µL are used in the test (e.g. in the case of pool aliquots or individual samples with a lower starting volume), *PoET Instrument* automatically replenishes the sample volume to 1.3 mL total volume with *sample diluent* (SD), a component of the kit *PoET Extraction.* As part of the validation of the PCR kit *PoET HCV*, it was confirmed that replenishing with SD has no effect on the limit of detection of *PoET HCV.*

A correct configuration of the required sample formats in *Calliope* must be ensured. Further information can be found in the operator's manual of *PoET Instrument*. If other settings are desired, contact GFE Customer service.

13.2. Diagnostic specificity

For the purposes of determination of the diagnostic specificity of the PCR kit *PoET HCV,* 512 HCV-negative samples were examined using single plasma donations from gel barrier blood collection tubes.

Tested sample number	Inhibited samples	Valid non-reactive samples	False reactive samples	Specificity
512	0	512	0	100 %

In the 512 samples tested, no false-reactive sample could be observed. Thus, for the PCR kit *PoET HCV*, a specificity of 100 % can be assumed.

13.3. Whole system failure rate

The determination of the system failure rate leading to false negative results (in percent non-reactive samples) of the overall system (short "failure rate") of the PCR kit *PoET HCV* was carried out for 276 samples. For this test negative human plasma was spiked with HCV in the concentration of less than threefold 95 % LOD.

No failure was observed in the 276 analyses. This results in a failure rate of 0 %.

13.4. Genotype verification

During PCR development, the amplification of diverse genotype sequences was tested by synthetic nucleic acids fragments.

In addition, the analytical detection of relevant genotypes was tested using samples of known genotypes for HCV, if available. These samples represent a large part of the previously known geno- and subtypes.

The genotype samples were analyzed using approximately the fivefold 95 % LOD (if sample concentration was specified). The following table summarizes the results:

HCV genotype	Number of samples	Hit rate [reactive / total]
1	5	5 / 5
1a	6	6 / 6
1b	11	11 / 11
Total GT 1	**22**	**22 / 22**
2	6	6 / 6
2a	2	2 / 2
2a/c	3	3 / 3
2b	11	11 / 11
2c	2	2 / 2
2i	2	2 / 2
Total GT 2	**26**	**26 / 26**

HCV genotype	Number of samples	Hit rate [reactive / total]
3	5	5 / 5
3a	13	13 / 13
3b	1	1 / 1
Total GT 3	**19**	**19 / 19**
4	9	9 / 9
4a	2	2 / 2
4a/4c/4d	1	1 / 1
4c/4d	7	7 / 7
Total GT 4	**19**	**19 / 19**
5	12	12 / 12
5a	5	5 / 5
Total GT 5	**17**	**17 / 17**
6	11	11 / 11
6a	1	1 / 1
Total GT 6	**12**	**12 / 12**

Genotypes 1 to 7 can be detected with the PCR kit *PoET HCV.* The detection is ensured by bioinformatic sequence comparisons. It has been shown that the sequences of genotypes 1 to 7 do not differ in the amplicon region of HCV PCR. The successful analytical detectability of genotypes 1 to 6 for which sample material was available is shown in the table above. For genotypes 7 to 8, no sample material was available at the point of testing. For genotype 8 [5] there is currently no sequence data available for the target sequence of HCV PCR.

13.5. Seroconversion panels

Ten commercially available seroconversion panels for HCV were tested.

Each panel member was tested in up to three different sample types: a) 1:6 diluted with plasma, b) 1:96 diluted with plasma and c) undiluted (if necessary). Following the analysis with the PCR kit *PoET HCV,* a comparison of the results with the information provided in the accompanying documents of the panels was carried out. As an expected result, with the PCR kit *PoET HCV* the samples that have been determined as reactive by the NAT test procedure (CE-IVD-marked reference NAT for HCV) named in the respective documents are determined as reactive as well.

In those cases where the 1:6 diluted samples were reactive on day 0 of a panel, the testing of undiluted samples was omitted, as no change in the overall result for this panel was expected. Otherwise, these panel members were tested without prior dilution using 1.3 mL of sample material.

For HCV, all samples from the preseroconversion phase that were above the 95 % detection limit of the PCR kit could be clearly detected.

On average, the PCR kit PoET HCV provides reactive results for HCV 26 days earlier than the respective anti-HCV reference test (reduction of the diagnostic window).

When testing the panels with samples diluted in a ratio of 1:6, there was no effect on the diagnostic window. When testing samples diluted in a ratio 1:96, an extension of the diagnostic window by about half one day was observed.

The testing of the seroconversion panels thus points out the higher sensitivity of NAT techniques to the serological test methods. Compared to the reference NAT tests, the PCR kit *PoET HCV* detects the RNA of HCV comparably well.

13.6. Investigations on limitations of the detection method

13.6.1. Analytical specificity - interfering substances

The influence of interfering substances on the PCR kit *PoET HCV* was investigated by means of the extraction of different samples and detection of HCV. In one section of the samples, HCV-negative plasma was only spiked with the respective substance. Another section of the samples was additionally spiked with virus standard at about fivefold 95 % LOD. Endogenous and exogenous interfering substances were tested.

Endogenous interfering substances

To assess the influence of hemolysis and increased bilirubin, albumin and triglyceride content on HCV-PCR, plasma samples were spiked with the respective endogenous substance in several concentrations up to abnormal high levels.

Results of the testing of endogenous substances:

Endogenous substance	Concentration	Observation
Bilirubin	20 – 50 mg/L	No influence
Hemoglobin	250 – 2000 mg/L	No influence
Triglycerides	2.5 – 40 g/L	No influence
Albumin	60 – 100 g/L	No influence
	> 100 g/L	Reliable test result not ensured

The analyzed endogenous substances (albumin, bilirubin, hemoglobin, triglycerides) showed no false-non-reactive or false-reactive results for the concentrations tested. For albumin

concentrations up to 100 g/L, no influence on the test results could be observed. For concentrations above 100 g/L, a reliable test result cannot be ensured.

Exogenous interfering substances

The tests for assessing the influence of exogenous substances (drugs taken before blood donation) were carried out on the basis of the information in the directive "EP7A2 Interference Testing in Clinical Chemistry". The selection of the medicaments and their concentration are derived from this guideline.[10]

Results of testing exogenous substances:

Exogenous substance	Effect	Concentration	Observation
Ascorbic acid	Antioxidant	60 µg/mL	No influence
Acetaminophen / Paracetamol	Painkiller	200 µg/mL	No influence
Aspirin	Painkiller	652 µg/mL	No influence
Ibuprofen	Painkiller	500 µg/mL	No influence
Naproxen	Painkiller	500 µg/mL	No influence
Phenylephrine HCl	Decongestant	82 µg/mL	No influence
Atrovastatin	Statin	335 µg/mL	No influence
Loratadine	Antihistamine	0.3 µg/mL	No influence
Fluoxetine	Antidepressant	3.5 µg/mL	No influence
Paroxetine	Antidepressant	1.0 µg/mL	No influence
Sertraline	Antidepressant	0.6 µg/mL	No influence

The tested exogenous substances did not show any false-non-reactive or false-reactive results in the respective concentration.

13.6.2. Analytical specificity – other viruses

Sequence comparisons of the primers and probes with potentially cross-reactive human pathogenic virus sequences and an optimized PCR design minimize the risk of unwanted PCR derived by-products.

As part of the validation, the influence of genomic nucleic acids from selected viruses on the PCR kit *PoET HCV* was investigated. For this purpose, negative human plasma (NHP) was spiked with standards for the viruses to be tested, extracted and amplified. In addition, HCV-positive plasma was spiked with standards for the viruses to be tested and analyzed. The HCV-positive samples were spiked with an HCV load of about fivefold 95 % LOD.

Results of the test of cross-reactivity:

Virus	Nucleic acid	Observation
Adenovirus type I	DNA	No influence
BK virus (Human Polyomavirus 1)	DNA	No influence
Cytomegalovirus	DNA	No influence
Hepatitis-A virus	RNA	No influence
Hepatitis-B virus	DNA	No influence
Hepatitis-D virus	RNA	No influence
Hepatitis-E virus	RNA	No influence
Herpes simplex virus type-1	DNA	No influence
Human immunodeficiency virus-1	RNA	No influence
Human immunodeficiency virus-2	RNA	No influence
Parvovirus B19	DNA	No influence
Varicella Zoster virus	DNA	No influence
West Nile virus	RNA	No influence

For the viruses tested, no influence on the PCR kit *PoET HCV* was observed. All reaction batches showed reactive results for the IC and no false-reactive or false-non-reactive results for HCV.

14. Changes in analytical procedure and performance

In the case of significant changes in the analytical procedure and / or in the analytical performance of the reagents, corresponding information will be passed on by the manufacturer to the users immediately. This also applies to the measures resulting from these changes. If necessary, this may include the recall of the *in vitro* diagnostic medical devices.

15. Explanation of the symbols

LOT	Symbol for "Batch code"
REF	Symbol for “Catalogue number”
YYYY-MM	Symbol for "Use by date" (year-month)
Σ 840	Symbol for "Sufficient for <n>tests" (n = total number of IVD tests)
-18°C	Symbol for "Upper limit of temperature"
	Symbol for "Consult instructions for use"
	Symbol for "Caution" Indication of safety-related information such as warning or precaution
	Symbol for “Do not re-use”
	Symbol for "Keep away from sunlight"
IVD	Symbol for "*In vitro* diagnostic *medical device*"
CE 0483	Symbol for conformity to the European Directive 98/79/EC (IVDD) on *in vitro* diagnostic medical devices and identification number of the notified body
	Symbol for “Manufacturer”
	GFE manufacturer logo

16. List of abbreviations

95 % LOD	Detection limit at 95 % probability
cDNA	Complementary or "Copy" DNA
DNA	Deoxyribonucleic acid (DNA, deoxyribonucleic acid)
dNTP	Deoxyribonucleoside triphosphates
dUTP	Deoxy uridine triphosphate
EDTA	Ethylenediaminetetraacetic acid
EM	enzyme mix
HCV	Hepatitis C virus
IC	Internal Control
IU	International units
M-MLV	Moloney Murine Leukemia Virus
NAT	Nucleic acid amplification technology
NC	*PoET Negative Control*
NTC	No Template Control
OM	oligo mix
PC	PCR Positive Control; *PoET Master Positive Control*
PCR	Polymerase Chain Reaction
RKI	Robert Koch Institute
RNA	Ribonucleic acid (NS, ribonucleic acid)
RT	Reverse transcription
rxn	Reactions
SD	Sample Diluent (filling medium for samples)
UNG	Uracil DNA glycosylase
WHO	World Health Organization

17. Technical Service

Questions regarding the PCR kit *PoET HCV* can be directed to GFE Customer service:

Email: service@gfeblut.de

Web: https://www.gfeblut.de/contact-us/

18. References

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19. Exclusion of liability and trademark protection

- The SuperScript® III reverse transcriptase included in the PCR kit *PoET HCV* is a product manufactured and licensed by Life Technologies Corporation.
- During the application of the PCR kit *PoET HCV,* the *PCR Plates* "FrameStar® 96 (cut corner A12)" with barcode [article number SP0362] are used. These are subject to the following license limitation: "FrameStar® is covered by one or more of the following US patents or their foreign counterparts, owned by Eppendorf AG: US Patent Nos. 7,347,977 and 6,340,589. FrameStar® is a registered trademark owned by 4titude® Ltd".
- Other registered names, trademarks, etc. used in this document are not to be considered legally unprotected, even if they are not specifically marked.

20. Change history

Version	Date [YYYY-MM-DD]	Remarks
Version 1	2020-09-14	Initial release

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