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

Instructions for use

PoET Internal Control

For use on PoET Instrument

In vitro diagnostic medical device

REF P1C-1440-60

IVD CE

Content

1. Intended purpose

1.1. Short description

The control kit *PoET Internal Control (PoET IC*, IC) from *Gesellschaft zur Forschung, Entwicklung und Distribution von Diagnostika im Blutspendewesen mbH* (hereinafter referred to as GFE) serves as an internal control for quality monitoring of the analysis process.

1.2. Intended purpose

The control kit *PoET Internal Control* is CE marked according to IVD Directive 98/79/EC. It serves as an accessory for the *in vitro* diagnostic tests of the PoET product line for the detection of viral nucleic acids (DNA or RNA).

PoET Internal Control adds an additional analyte to the test procedure as an internal control (IC), which is extracted, amplified, and detected alongside the nucleic acid sequences to be detected. The IC is used to evaluate the validity of negatively tested samples.

The processing of the control kit *PoET Internal Control* is carried out with *PoET Instrument* from GFE.

2. Information and functionality of the test

The control kit *PoET Internal Control* contains an inactivated recombinant murine virus. Due to chemical virus inactivation, there is no risk of infection for the user.

The control kit *PoET Internal Control* fulfills several control functions within the PoET system, which can be categorized into three functional aspects:

- Extraction control

  During sample processing on the *PoET Instrument*, a defined amount of the *PoET Internal Control* is automatically added to each sample. In the subsequent nucleic acid extraction, the nucleic acids (RNA or DNA) of the viruses to be detected (if present) and the nucleic acids of the IC (RNA) are extracted and purified at the same time. All nucleic acids extracted from a sample are then amplified and detected in the respective NAT test – provided that the samples contain the corresponding nucleic acids in sufficiently high concentration. During result evaluation, the signals of the IC must fall within defined limit values for the results of the associated samples to be considered valid.

  In case of deviations or interferences in the process, the extraction does not proceed with the required efficiency and the yield of RNA of the IC is not high enough, so that the required concentration is not used for the NAT test, the IC signals will not fall within the defined limit values. Results from samples that are not reactive for the tested virus parameter and whose IC signals are outside the limit values are declared invalid.

- Amplification control

  All PoET PCR kits of the PoET system consist of an *oligo mix* and an *enzyme mix*. Each *oligo mix* contains all primers and probes required for the amplification and detection of the respective virus parameter and the IC. The *enzyme mix* contains all enzymes, cofactors, and salts required for the PCR reactions.

  Successful amplification and detection of the IC indicates that all necessary components for reverse transcription (RT) of RNA to DNA, amplification of DNA via PCR, and generation of suitable fluorescent reporter dyes were present in the reaction and functioned correctly. Since the oligonucleotides of the IC cannot be added separately from the virus-specific oligonucleotides to a reaction, the amplification and detection of the IC also demonstrate

that the virus-specific oligonucleotides were present in the reaction, even if no virus-specific signal was generated for a sample. To successfully amplify and detect the IC, it is necessary not only to set up the PCR reactions completely, but also to add the nucleic acid extracts generated in the previous process step to the PCR setups, as only these provide the required PCR templates.

If deviations or interferences in the process cause the PCR reaction to not be set up completely or if the setup contains PCR inhibitors that could not be removed during nucleic acid extraction, the IC cannot be properly amplified and detected. In this case, the IC signals will not fall within the defined limit values. Results from samples that are non-reactive for the tested virus parameter and whose IC signals are not within the limit values are declared invalid.

– Detection control

  In the PoET system, PCR reactions are performed using the real-time PCR modules of *PoET Instrument*. The devices are equipped with the necessary heating and cooling elements to adjust the respective reaction temperatures, as well as optical elements to detect fluorescence radiation.

  A successful amplification and detection of the IC indicate that the functions of the real-time PCR modules regarding heating, cooling, and fluorescence measurement are operational.

  If deviations or malfunctions in the process cause the real-time PCR modules not to function properly, the IC cannot be properly amplified and detected. In this case, the signals of the IC will not fall within the defined limit values. Results from samples that are not reactive for the tested virus parameter and whose IC signals are not within the limit values are declared invalid.

On the basis of the mentioned extraction, amplification, and detection controls, the IC monitors the processing of samples from preparation to results. The evaluation is performed separately for each processed sample. If the virus-specific result of a sample is "non-reactive," it is only to be regarded valid if the result of the IC for the same sample falls within the defined limits values. The IC thus indicates the validity of "non-reactive" results for the corresponding virus parameter.

Despite the extensive functions of the control kit *PoET Internal Control* and the associated significant reduction in the risk of false-negative results, these cannot be completely excluded [1].

3. Reagents and materials

The contents of the *PoET Internal Control* kit include 60 *internal control* tubes.

PoET Internal Control			
GFE reference number	P1C-1440-60		
Test unit	1440 µL		
Number of tests per kit	60	Total volume	86.4 mL
Kit component	**Volume [µL]**	**Identifier**	**Cap color**
internal control	2300	IC v1	White

3.1. Reagent storage

The control kit *PoET Internal Control* is shipped on dry ice. Upon receipt, the product should be checked for the following:

- Frozen state of the reagents
- Integrity of the outer packaging and individual reagent tubes
- Completeness regarding the number of reagent tubes

The control kit *PoET Internal Control* is stored at ≤ -18°C and is valid until the date indicated on the label.

3.2. Reagent handling

- Before use, check the reagent tubes for proper filling.
- Ensure that no drops of reagent are hanging above the actual liquid level on the walls and/or lid of the reagent tubes.
- The *intern control* (IC) must be completely thawed at room temperature (15-30°C) after removal from the freezer. Other thawing methods are not intended, as they may lead to precipitate formation. After thawing, remove the lid and place the IC tube in the appropriate position of the *PoET Instrument* carrier system.
- Reagents must not be used after the declared expiration date.

	Allow the *internal control* (IC) to thaw completely before loading on *PoET Instrument*.
	Expired reagents are recognized and excluded by *PoET Instrument* based on the reagent barcodes.
	The reagents are intended for single use only and not for repeated freezing and thawing. Any remaining reagents must be discarded after application.
	A maximum of 5 hours may elapse between removal from the freezer and the start of the analysis run on the *PoET Instrument*. If the tubes have been stored open for several hours, an adequate fill level may no longer be guaranteed depending on the duration and degree of evaporation.

3.3. Disposal

- The component *internal control* (IC) of the kit *PoET Internal Control* contains no hazardous substances or biohazardous substances. The material safety data sheet is available on request from GFE customer service.
- The contents and container of reagents must be disposed of in accordance with applicable regional and international regulations.
- The use of the control kit *PoET Internal Control* kit generates PCR plates and PCR reagent residues, as well as consumables that have come into contact with them. These must be disposed of in accordance with applicable regional and international regulations. Further instructions can be found in the instructions for use of the PoET PCR kits.

4. Required equipment

4.1. Devices and software

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

4.2. Required reagents and consumables for application

The consumables required for using *PoET Internal Control* on the *PoET Instrument* are available separately from GFE. Information about the required items can be found in the instructions for use of the PoET PCR kits, which are used in combination with the control kit *PoET IC*.

5. Warnings and precautions

Good laboratory praxis

- Ensure the use of personal protective equipment (lab coat, safety glasses, laboratory gloves).
- Do not eat, drink, or smoke in laboratory work areas.
- Treat samples as potentially infectious, as described in "*Biosafety in Microbiological and Biomedical Laboratories*"[2] and the CLSI document M29-A4 [3].
- If sample material is spilled, disinfect immediately with an appropriate agent. Treat contaminated materials as biological hazardous.
- After handling samples and reagents, disinfect and thoroughly wash hands.
- Clean and disinfect all work surfaces with disinfectants listed by the Robert-Koch-Institute (RKI).
- Eliminate potential nucleic acid contamination with DNA-ExitusPlus™ (AppliChem GmbH) or a similarly effective agent according to the manufacturer's specifications.

General usage notes

- Use the *PoET Internal Control* kit only in combination with the PoET PCR kits of the PoET product line.
- The *PoET Internal Control* kit is intended only for use with the *PoET Instrument* and the described accessory and other control kits, as well as consumables.
- Use all reagents for *in vitro* diagnostics only.
- The *PoET Instrument* shall only be operated by qualified personnel trained by GFE.
- In order to prevent cross-contamination of samples or controls, handle all materials containing samples or controls according to laboratory safety regulations.
- Store samples, controls, and PCR kits separately.
- For safe handling of used and sealed extraction plates and PCR plates, follow the instructions in the operator's manual of *PoET Instrument*.
- Dispose of all materials that have come into contact with potentially infectious samples in accordance with applicable regional and international regulations (see in particular the instructions for use of the sample preparation kits *PoET Extraction* and *PoET Prep Reagent*).
- Use the *PoET Internal Control* kit within a temperature range of +15°C to +30°C.

Handling of reagents

- Place the *internal control* of the *PoET Internal Control* on the appropriate position of the carrier system only when fully thawed.
- Remove the cap of the *internal control* before positioning it on the carrier systems of the *PoET Instrument*. The *PoET Instrument* does not have an automated cap removal device ("Decapper").
- Load and unload the *PoET Instrument* reagent carriers with PCR reagents according to the instructions in the operator's manual of *PoET Instrument*. This also applies to the correct preparation of samples and controls. Deviations from the specified procedures may impair test performance.
- Avoid mixing up tube caps, as this may lead to contamination.
- The IC of *PoET Internal Control* is designed for single use. Do not reuse reagent residues.
- Do not use reagents after their expiration date.

6. Processing of samples on the *PoET Instrument*

The procedure is described in detail in the operator's manual of *PoET Instrument*. The IC is positioned on the device along with the other reagents when loading the *PoET Instrument*. The use of the IC is automated by the *PoET Instrument*. For details on the procedure, please refer to the instructions for use of the respective PoET PCR kits and the operator's manual of *PoET Instrument*.

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

7. Control procedures

7.1. Quality control kits

The automated overall process, consisting of sample preparation and PCR analysis, is monitored by several controls. The *internal control* of the *PoET Internal Control* kit serves as one of the quality control measures for the overall process:

Control type	Product	Function
Internal control (IC)	*PoET Internal Control*	The IC indicates whether the processing from extraction to result was valid for each sample.
PCR positive control (PC)	Multiparameter control (*PoET Master Positive Control*) or single parameter PCR positive control	The PC contains viral nucleic acids of the parameters to be detected (e.g., multiparameter control *PoET Master Positive Control*: nucleic acids of HCV, HBV, HIV, HAV, and B19V) and indicates successful amplification, ensuring correct conditions for PCR from setup, sealing of PCR plates, to execution on the *PoET Instrument*.
PCR negative control (NC)	*PoET Negative Control*	The NC indicates that PCR reagents were set up contamination-free. The NC corresponds to a "No Template Control" (NTC).

8. Evaluation and validity of results

The evaluation is performed by the software *Calliope*. The software analyzes the fluorescence signals of all PCR reactions, including controls, and assesses whether the result is valid.

If the PCR controls (*PoET Master Positive Control* or alternatively corresponding single parameter PCR positive control and *PoET Negative Control*) do not meet the validity criteria, the PCR results of the samples for the affected test parameter on the PCR plate are rated as invalid.

When evaluating the results of the *internal control*, the fluorescence signals are used to calculate the parameters Positive Point (PP) and Quotient (Q). In order for IC results to be considered valid, they must not exceed the specified PP values and must not fall below the specified Q values. The IC limit values for each PCR parameter are stored in the *Calliope* software.

If there is no detection value for the internal control or if a value falls outside the specified limit values, the affected sample position on the PCR plate is evaluated as "invalid" if, in addition, no reactive result exists for any of the viruses (target viruses) to be detected in this sample. In this case, the affected sample must be retested.

Since the amplification efficiency of the *internal control* decreases in the presence of high concentrations of target virus, the signal for the *internal control* is not evaluated if the affected sample is rated as reactive for a target virus.

Further information can be found in the instructions for use of the PoET PCR kits used together with *PoET Internal Control*.

9. Procedure limitations

- The control kit *PoET Internal Control* is exclusively intended for use in combination with the GFE PCR kits of the PoET product line (e.g. *PoET HCV*, *PoET HBV*, *PoET HIV*, *PoET HAV*, *PoET B19V*, *PoET HEV*).
- Mutations of the recombinant murine virus are not relevant. They would be detected during product manufacturing through quality controls.
- Incorrect sampling, untested interfering substances, improper sample storage, and preparation can negatively affect the stability of viruses and nucleic acids and impair PCR results. In addition, plasma may contain inhibiting substances that can interfere with extraction or enter the PCR process.

10. Performance characteristics

The performance characteristics of *PoET Internal Control* can only be determined together with the associated PoET PCR kits. For detailed information, please refer to the instructions for use of the respective kits.

11. Changes in analytical procedure and performance

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

12. Explanation of symbols

LOT	Symbol for ‘Batch code’
REF	Symbol for ‘Reference number’
JJJJ-MM	Symbol for ‘Use by date’ (year-month)
-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’
IVD	Symbol for ‘*In vitro* diagnostic medical device’
CE	Symbol for conformity with European Directive 98/79/EG on *in vitro* diagnostic medical devices
	Symbol for ‘Manufacturer’
	GFE manufacturer logo

13. Abbreviations

B19V	Parvovirus B19
DNA	Deoxyribonucleic acid
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HEV	Hepatitis E virus
HIV	Human immunodeficiency virus
IC	Internal Control
IFU	Instructions for use
MPC	*PoET Master Positive Control* (Multiparameter PCR positive control)
NAT	Nucleic acid amplification technique
NC	*PoET Negative Control* (PCR negative control)
NTC	No Template Control
PC	PCR positive control
PCR	Polymerase Chain Reaction
PP	Positive point
Q	Quotient
RKI	Robert Koch Institute
RNA	Ribonucleic acid
RT	Reverse transcription

14. Technical Service

Questions regarding the product *PoET Internal Control* can be addressed to GFE customer service:

E-Mail: service@gfeblut.de

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

15. References

[1] Kleinman SH, Lelie N, Busch MP. Infectivity of human immunodeficiency virus-1, hepatitis C virus, and hepatitis B virus and risk of transmission by transfusion. Transfusion. 2009;49:2454-2489.

[2] Lewis & Wilson, Deborah. (2009). Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. HHS Publication No. (CDC) 21-1112 Revised December 2009

[3] Protection of Laboratory Workers From Occupationally Acquired Infections, 4th Edition; Clinical and Laboratory Standards Institute; May 2014; ISBN Number: 1-56238-962-9

16. Disclaimer and trademark protection

- All registered names, trademarks, etc., used in this document are not considered legally unprotected, even if they are not specifically marked.

17. Revision History

Version	Date [YYYY-MM-DD]	Remarks
Version 1	2021-03-23	First release

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