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

Instructions for use

PoET Internal Control

For use with PoET Instrument

In vitro diagnostic medical device

REF P1C-1440-60

IVD CE

Table of contents

1. Intended use

1.1. Abstract

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

1.2. Intended use

Intended purpose

PoET Internal Control is an accessory for *in vitro* testing for nucleic acids of infectious agents with the corresponding PCR kits of the PoET product line.

PoET Internal Control is added to each sample before starting the processing. The samples thus contain an additional analyte which, together with the infectious agents, undergoes the entire sample processing. *PoET Internal Control* is used to evaluate the validity of the results of the samples tested.

As *PoET Internal Control* runs through the entire process, it serves to functionally monitoring the reagents used and the *PoET Instrument* employed.

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

PoET Internal Control is a CE marked class A *in vitro* diagnostic accessory for professional use in accordance with Regulation (EU) 2017/746.

Intended users

The application must be carried out by pre-trained and qualified laboratory personnel who have been instructed and trained in *in vitro* diagnostic procedures and have successfully completed GFE's training.

2. Test principle

PoET system

The kit *PoET Internal Control* is a stand-alone reagent product in the PoET system, consisting of the *PoET Instrument* and the PoET reagent kits for fully automated extraction, amplification and detection of nucleic acids (nucleic acid amplification technology, NAT) of infectious agents.

Principle of PoET Internal Control

PoET Internal Control fulfils the function of an internal control (IC).

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

PoET Internal Control has several control functions:

- Extraction control

  During sample processing on *PoET Instrument*, a defined amount of *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 a sufficiently high concentration. During result evaluation, the detection values of the IC are compared with defined limit values to evaluate the validity of the sample results.

In the 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. As a result, the IC cannot be used in the required concentration in the NAT test. In this case, the signals of the IC are outside the defined limit values. Results of 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 for the PCR reactions to be carried out.

  A successful amplification and detection of the IC indicates that all the necessary components for reverse transcription (RT) from RNA to DNA, the multiplication of DNA by PCR and the generation of suitable fluorescent reporter dyes were present in the reaction and fulfilled their functions. Since the oligonucleotides of the IC cannot be added to a reaction separated from the virus-specific oligonucleotides, the amplification and detection of the IC also shows that the virus-specific oligonucleotides were present in the reaction, even if no virus-specific signal was generated for a sample.

  In addition to the correct preparation of the PCR reagents, it is necessary that the nucleic acid extracts produced in the previous process phase have been added to the PCR reactions during PCR setup. If deviations or interferences lead to incomplete PCR setup, or if the reaction contains PCR inhibitors that could not be removed during nucleic acid extraction, the IC cannot be properly amplified and detected. In this case, the signals of the IC are outside the defined limit values. Results of samples that are not reactive for the tested virus parameter and whose IC signals are outside the limit values are declared invalid.

- Detection control

  In the PoET system, the PCR reactions are carried out with the help of the real-time PCR modules of *PoET Instrument*. The devices have 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 indicates that the real-time PCR modules are functional in terms of heating, cooling and fluorescence measurement.

  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 are outside the defined limit values. Results of 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 aforementioned extraction, amplification and detection control, the IC monitors the processing of the samples from sample preparation to the results. The analysis is carried out separately for each processed sample. If the virus-specific result of a sample is ‘non-reactive’, it is only to be regarded as valid if the result of the IC for the same sample meets the defined limit values. The IC thus displays the validity of ‘non-reactive’ results for the corresponding virus parameter.

Despite the extensive functions of *PoET Internal Control* and the associated significant reduced risk of false-negative results, these cannot be completely ruled out [1].

3. Reagents

One kit *PoET Internal Control* contains 60 tubes of *internal control* (IC).

Table 1: Labelling and content

PoET Internal Control			
GFE Reference number	P1C-1440-60		
Basic UDI-DI	42623533713M4		
UDI	(01)04262353370025(17)YYMMDDD(10)1CYYXX		
Usable volume per test (test unit)	1440 µL		
Number of tests per kit	60		
Total usable volume	86.4 mL		
Kit component	**Filling volume [µL]**	**Identifier**	**Primary packaging (closure type)**
internal control	2300	IC v1	Screw tube (white cap)

The UDI (Unique Device Identifier) consists of UDI-DI (Device Identifier) and UDI-PI (Production Identifier). It is composed as follows: (01) UDI-DI, (17) expiration date in YYMMDD format and (10) batch number in 1CYYXX format.

The symbols are explained in Chapter 12.

3.1. Transport and storage of reagents

The kit *PoET Internal Control* is shipped on dry ice. The product should be checked upon receipt (i.e. frozen state of reagents, integrity of packaging, completeness).

PoET Internal Control is stored at ≤ 18°C and is stable until the date stated on the label.

3.2. Handling of reagents

- Please check the filling of the tubes before use.
- Take care to ensure that no reagent drops have formed above the actual liquid level on the inner tube surface and/or caps of the tubes.
- The *internal control* (IC) must be completely thawed at room temperature (15-30°C) after removal from the freezer. Other methods of thawing are not recommended, as this can lead to precipitate formation. After thawing, remove the cap and place the screw tube on the appropriate position of the carrier system of the *PoET Instrument*.

	Allow the *internal control* (IC) to thaw completely before loading the *PoET Instrument*.
	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.

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.

3.3. Disposal of reagents

- The component *internal control* (IC) of the kit *PoET Internal Control* contains no hazardous substances or biohazard substances. The material safety data sheet is available on request from GFE Customer service.
- The contents and containers of the reagents as well as the *Extraction Plate Sets* that come into contact with the reagents during use must be disposed of in accordance with the relevant regional and national regulations. Further information on how to dispose of the *Extraction Plate Sets* can be found in the operator's manual of *PoET Instrument*.
- When using the kit *PoET Internal Control*, PCR plates and PCR reagent residues as well as consumables that have come in contact with them are produced. These must be disposed of in accordance with the relevant regional and national regulations. Further information 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 software *Calliope* and operator's manual.

4.2. Required consumables

The consumables for the application of *PoET Internal Control* on *PoET Instrument* are available separately from GFE.

The required consumables can be found in the corresponding instructions for use of the PoET extraction kits and PoET PCR kits as well as in the operator's manual of *PoET Instrument*.

The use of other than the consumables specified in the operator's manual of *PoET Instrument* is not allowed.

5. 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*' [2] and CLSI document M29A4 [3].
- 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 *PoET Internal Control* only with *PoET Instrument* and the associated reagent kits (PCR and accessory kits) and 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 materials 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 safe handling of the used and sealed *24well Extraction Plates* and *PCR Plates,* please follow the instructions in the operator's manual of *PoET Instrument*.
- Dispose of all materials that have come into contact with potentially infectious samples, according to the relevant regional and national regulations.
- Use *PoET Internal Control* in the temperature range from +15°C to +30°C.

Handling of reagents

- Place the completely thawed *internal control* (IC) on the appropriate position of the carrier of the *PoET Instrument*.
- 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 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.
- Avoid mixing up tube caps, as this can lead to contamination.
- *PoET Internal Control* is designed for single use. Do not reuse reagent residues.
- Do not use reagents after their shelf life has expired.

[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

6. Processing of samples on *PoET Instrument*

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

Depending on the test plan of a run on *PoET Instrument*, the PCR results are available about 3 hours after the start of the run.

7. Control procedures

The automated overall process consisting of sample preparation and PCR analysis is monitored by several controls. As an overall process control, IC represents a quality control of the analytical process.

Table 2: Control procedures

Control type	Product	Function
Internal control (IC)	*PoET Internal Control*	The IC indicates whether the processing from extraction to result was valid for each non-reactive[2] sample.
PCR positive control (PC)	Multi-parameter control kit (*PoET Master Positive Control*) or Single-parameter PCR positive control kits	The PC contains 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). It indicates whether 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 are free of contaminating nucleic acids. The NC corresponds to a 'no template control' (NTC).

8. Evaluation and validity of the results

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

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

If there is no detection value for internal control or a value outside the specified limits, the affected sample position on the PCR plate is evaluated as 'not valid' if, in addition, there is no reactive result for any of the viruses to be detected (target viruses) in this sample. If the sample

[2] Additional function for semi-quantification when using *PoET B19V*: reactive samples containing B19V concentrations above the cut-off value are evaluated with 'AboveCutOff', samples below the cut-off value with 'BelowCutOff'. Samples below the cut-off value are considered weakly reactive. For these samples, the validity assessment by the IC is also applied. Further explanations on semi-quantification can be found in the instructions for use for *PoET B19V*.

result is ‘BelowCutOff’[2] for the target virus, the sample result is ‘not valid’ as well. In those cases, the affected sample must be retested.

Because the amplification efficiency of internal control decreases in the presence of high concentrations of target virus, the signal for internal control is not evaluated if the affected sample has been evaluated as reactive or with ‘AboveCutOff’[2] for a target virus.

If one of the criteria of the validity check for the PCR controls (PCR positive control or PCR negative control) is not met, the PCR plate of the PoET run will be assessed as invalid for the affected test parameter.

For more information, refer to the instructions for use of the PoET PCR kits used with *PoET Internal Control*.

9. Procedural limitations

- *PoET Internal Control* is intended exclusively for use together with the PCR kits of the PoET product line (e.g. *PoET HCV*, *PoET HBV*, *PoET HIV*, *PoET HAV*, *PoET B19V*, *PoET HEV, PoET WNV*) and the accessories and consumables specified therein as well as the *PoET Instrument*.
- Mutations of the recombinant murine virus are not relevant. They would be detected in the context of product production through quality controls.
- Incorrect sampling, untested interfering substances and improper sample storage and preparation can negatively affect the stability of viruses and nucleic acids and impair the result of the PCR. In addition, the plasma may contain inhibiting substances that interfere with extraction or PCR reaction.

10. Performance characteristics

The performance characteristics of *PoET Internal Control* can only be determined together with the associated extraction kits *PoET Extraction* and *PoET Prep Reagent* and the GFE PCR kits used in combination. For this reason, the performance characteristics are described in the instructions for use of the respective PoET PCR kits.

11. Changes in analytical procedure and performance

In the event 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.

12. Explanation of symbols

LOT	Symbol for ‘Batch code’
REF	Symbol for ‘Reference number’
YYYY-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’
	Symbol for ‘Keep away from sunlight’
IVD	Symbol for ‘*In vitro* diagnostic medical device’
CE	Symbol of conformity with Regulation (EU) 2017/746 on *in vitro* diagnostic medical devices
UDI	Symbol for ‘Unique Device Identification’
	Symbol for ‘Manufacturer’
	GFE manufacturer logo

13. List of abbreviations

B19V	Parvovirus B19
DNA	Deoxyribonucleic acid
GFE	Gesellschaft zur Forschung, Entwicklung und Distribution von Diagnostika im Blutspendewesen mbH
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
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
RNA	Ribonucleic acid
RT	Reverse transcription
UDI	Unique Device Identifier
UDI-DI	UDI device identifier
UDI-PI	UDI production identifier
WNV	West Nile virus

14. Contact

14.1. Technical service

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

Email: service@gfeblut.de

Web: https://www.gfeblut.de

14.2. Reporting of serious incidents

Regulation (EU) 2017/746 requires all serious incidents involving the device to be reported to the manufacturer and the competent authority. Please send your notification in writing to us as manufacturer to the e-mail address given in Chapter 14.1.

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. Exclusion of liability and trademark protection

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

17. Version history

Version	Date [YYYY-MM-DD]	Remarks
1	2021-03-23	Initial release
2	2022-05-20	• Chapter 1 Intended use: Adaptation of intended use in accordance with Regulation (EU) 2017/746 • Chapter 2 Test principle: overview of PoET system inserted • Chapter 3 Reagents: UDI and reference to Chapter 12 (Explanation of symbols) inserted • Chapter 7 Control procedure: Insertion of a footnote explaining the assessment of the IC for the additional function ‘semi-quantification’ for B19V • Chapter 12 Explanation of symbols: reference to Regulation (EU) 2017/746 inserted at CE symbol; addition of UDI symbol • Chapter 14: Renamed into ‘Contact’, divided into Chapter 14.1: Technical service and newly added Chapter 14.2 Reporting of serious incidents

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