PoET CMV

Qualitative nucleic acid test
for use with *PoET Instrument*

For *in vitro* diagnostic use

REF **P2G-28-30**

IVD CE 0123

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1 Intended use

1.1 Intended purpose

PoET CMV is a PCR kit for professional use for automated *in vitro* testing of human plasma specimens from blood donors. It is used for the qualitative detection of human cytomegalovirus (CMV) DNA by real-time PCR in screening of individual specimens or pools of aliquots of individual specimens.
In addition, it is intended to confirm the results of samples tested in screening.
PoET CMV is processed on *PoET Instrument*.

1.2 Intended users

The application has to be carried out by qualified laboratory personnel who have been instructed and trained in *in vitro* diagnostic procedures and have successfully completed the operator's training on *PoET Instrument*.

2 Background

2.1 Pathogen information

The human cytomegalovirus (HCMV, CMV or HHV-5) belongs to the family of herpesviridae and to the subfamily beta herpesviridae. Another seven known viruses like *e.g.* herpes simplex belong to the same subfamily (1).
The virus's name derives from its ability to enlarge infected cells (cytomegaly). Its genome is embedded in a capsid. In addition, there is a protein layer called Tegument between the capsid and the viral envelope (2). The genome consists of a linear double-stranded DNA (236 kbp) and only one serotype or genotype is known for CMV, whose genome differs in various virus isolates. Eight viral glycoproteins are used to distinguish between the isolates (3) which phylogenetically do not represent genotypes.
CMV is transmitted via body fluids (*e.g.* saliva, urine, and genital secretions). Transmission trough blood, transplants and breast milk is of particular relevance. Breast milk plays an essential role as a reservoir of the viruses and is a basis of the natural epidemiology of CMV (4). CMV is spread worldwide and its endemic dissemination varies from 50 % to almost 100 %.
The incubation period of the virus is about four to six weeks. Symptoms rarely occur in immunocompetent individuals and manifest predominantly in non-specific symptoms such as common cold and cough. More severe courses are observed for immuno-incompetent or immunosuppressed individuals, including in particular transplant recipients, newborns or those infected with HIV. While growth and neural disorders occur in newborns, the infection often manifests itself as fever, colitis, retinitis or fatal pneumonia in AIDS patients. The virus is considered to be placenta-permeable and poses a risk to the unborn child if the mother is infected. It is the most common viral pathogen for embryo- and fetopathies (5). There is currently no vaccination available against CMV, but it is possible to administer virostatic drugs in severe cases. Like all herpes viruses, CMV enters a latent phase (6).

2.2 Rationale for testing

CMV can be transmitted via transfusion of blood and blood products. These transfusion transmitted CMV (TT-CMV) infections are rare but may cause complications, if the recipients are immunosuppressed or immuno-incompetent. In order to minimize the risk of TT-CMV for this group of patients it is often recommended by federal institutes for Health Care, to use only CMV-tested blood products for transfusions.

The global seroprevalence of CMV in blood donors is high (83.16 % CMV IgG) (7). In a seroprevalence study conducted with German blood donors, antibodies were detected in about 30 % of young adults and in 70 % of people over fifty years of age (8).

To protect high-risk recipients against TT-CMV, it is reasonable to test the blood reserves by nucleic acid amplification technology (NAT) procedures in order to prevent severe courses (2) (5).

2.3 Assay principle

The PCR kit *PoET CMV* is comprised of a real-time PCR (polymerase chain reaction) to detect CMV-specific DNA in human blood plasma. *PoET CMV* amplifies three distinct target sequences of the CMV genome. The target sequences are located in the well conserved regions of the UL83, UL123 and US9 genes.

All PoET PCR kits contain, in addition to the virus-specific oligonucleotides, a second heterologous non-competitive amplification system for amplifying the internal control sequence (*PoET Internal Control*, 'IC', available separately). The IC is added to each sample at the beginning of sample preparation.

3 PoET system overview

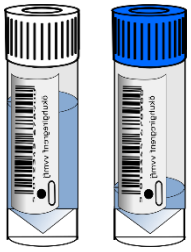
The PoET system is a fully automated solution for the extraction, amplification and detection of nucleic acids of pathogens in human body fluids in IVD high throughput screening or in individual samples. It consists of several different products which are available separately.

PoET system		
PoET Instrument	**PoET reagents**	**disposables**
	• PoET PCR kits • PoET controls • PoET extraction reagents	• PCR plates • extraction plates • sample tubes • pipette tips

4 Reagents

The PCR kit *PoET CMV* consists of two components, one tube containing *enzyme mix* (EM) and one tube containing *oligo mix CMV* (OM). You need both tubes to perform CMV testing.

PoET CMV	
Reference number	P2G-28-30
Basic UDI-DI	42623533720LZ
Total number of reactions	840



Kit component	Identifier	Primary packaging	Reagent ingredients	Quantity per kit
enzyme mix	EM v1	tube with screw cap (white)	H_2O, < 1 % master mix containing dUTP, dATP, dGTP, dCTP, dTTP and Taq polymerase, < 0.001 % uracil-N-glycosylase, < 0.002 % MMLV reverse transcriptase, < 0.01 % RNase inhibitor	30 x 1130 µL
oligo mix CMV	O_CM v1	tube with screw cap (blue)	Tris buffer, < 0.3 % forward and reverse CMV and IC primers, < 0.1 % fluorescent-labeled CMV probes, < 0.05 % fluorescent-labeled IC probes	30 x 148 µL

4.1 Reagent handling conditions

Material	Storage	Transport	Use
PoET CMV	≤ -18 °C	≤ -18 °C	+15 °C to +30 °C

The reagents are intended for single use. Any reagents remaining after application must be discarded.

The *oligo mix* is sensitive to light and should be stored protected from light during test preparation.

The analysis on *PoET Instrument* has to be started at the latest 5 hours after taking the reagents out of storage. Functionality of the reagents cannot be guaranteed, if the reagents have been stored open for several hours.

Do not use expired reagents. *PoET Instrument* monitors reagent barcodes and will not allow to start a run with expired reagents.

4.2 Additional materials required

These reagents and disposables for use on *PoET Instrument* are available separately from GFE:

Material	Reference number
PoET Extraction	P1A-24-04
PoET Prep Reagent	P1B-24-20
PoET Internal Control	P1C-1440-60
PoET CMV Positive Control	P3G-180-30
PoET Negative Control	P3A-500-30
1000µL-CO-RE II Tips	235905
300µL-CO-RE II Tips	235903
Extraction Plate Set	43001-0703
PCR Plate	SP-0362
*13 mL Tube & Cap**	60.541.004 & 65.714

*Optional. Please refer to the operator's manual of *PoET Instrument* for additional information about primary and secondary tubes.

The use of other disposables on *PoET Instrument* is not permitted.

4.3 Instrumentation and software required

Material	Reference number
PoET Instrument incl. software *Calliope*	P9A

5 Warnings and precautions

5.1 General precautions

- Use for *in vitro* diagnostics only.
- Use only in combination with *PoET Instrument* and the associated reagent kits and disposables.
- Clean and disinfect all work surfaces according to the 'Guideline for Disinfection and Sterilization in Healthcare Facilities' (9) or comparable methods.
- Eliminate potential nucleic acid contaminations with DNA-ExitusPlus™ (AppliChem GmbH) or a comparably effective agent according to the manufacturer.
- Treat the specimens as potentially infectious as described in 'Biosafety in Microbiological and Biomedical Laboratories' (10) and CLSI document M29A4 (11). If specimen material is spilled, immediately disinfect with an appropriate agent. Treat contaminated materials as biologically hazardous.
- If spillages of samples or reagents occur on *PoET Instrument*, follow the instructions in the operator's manual of *PoET Instrument* to clean and decontaminate its surface.
- Dispose of all materials that have come into contact with potentially infectious specimens and/or reagents, according to the relevant regional and national regulations.
- Material safety data sheets (MSDS) are provided by GFE.
- Wear personal protective equipment (laboratory coat, eye protection, laboratory gloves). Do not eat, drink or smoke in designated work areas.
- Disinfect and wash your hands thoroughly after handling the specimens and reagents, and after removing the gloves. Gloves must be changed between handling of specimens, controls and reagents. Avoid contaminating gloves when handling specimens and controls.

5.2 Reagent handling

- Handle all reagents, controls, and specimens according to good laboratory practice in order to prevent carryover of specimens or reagents.
- Store specimens, controls and PCR kits separately.
- Store all reagents, controls and specimens upright and at specified temperatures.
- PoET PCR kits and controls are shipped on dry ice. For safe handling and disposal follow the local instructions and guidelines.
- Check the product(s) after receipt (i.e. frozen state of PCR kits and controls, integrity of packaging, completeness). If there is any evidence of thawed reagents or damage, do not use these products for testing.
- PCR reagents are photosensitive. Take care to store and handle them protected from light sources.
- Avoid interchanging tube caps to prevent cross-contamination.
- The reagents are designed for single use. Do not reuse reagent residues.
- Do not combine different batches of the same reagents.
- Do not use reagents after their shelf life has been expired.
- Extraction reagents contain hazardous substances. Store, handle and dispose of them safely, according to the MSDS and chapter 7.13 of this IFU.

6 Process description

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

1. Sample preparation
2. PCR setup
3. Amplification and detection
4. Evaluation and report

6.1 Sample preparation

The specimen material used is human plasma (EDTA or CPD).
Samples can be provided as individual donor specimens or as pooled specimens consisting of aliquots of individual donations.
PoET Instrument offers the possibility to generate pools of 6 individual donations. For more information on available sample formats and pooling options please refer to the operator's manual of *PoET Instrument* (chapter 6).

Step	Description
Addition of IC	At the beginning of the process, *PoET Internal Control* (IC, available separately) is added to the samples as a process control for extraction and PCR amplification of each individual sample.
Lysis and binding	Virus particles are lysed to release the nucleic acids. In a second step the nucleic acids are adsorbed on magnetic particles.
Washing	Proteins and other impurities are removed by two washing steps.
Elution	The nucleic acids are then eluted from the magnetic particles with elution buffer. The recovered elution buffer contains the RNA of the IC and the viral nucleic acids to be detected.

6.2 PCR setup

During PCR setup, the PCR master mix, the eluates and the PCR controls (positive and negative control) are pipetted into the PCR plate(s).
The PCR master mix consists of a universal *enzyme mix* (EM) and a specific *oligo mix* (OM). The OM contains virus-specific oligonucleotides (primers and probes) that bind to highly conserved regions of the viral nucleic acids. In addition, the OM contains a second heterologous non-competitive amplification system with primers and probes for amplifying the internal control (IC) sequence. In the absence of the target virus a successful IC PCR reaction indicates correct PCR conditions and validates non-reactive target virus results.
To avoid contamination with PCR products of previous reactions, the EM contains a heat-labile *uracil DNA glycosylase* (UNG) and *deoxyuridine triphosphate* (dUTP) in the mixture of *deoxynucleotide triphosphates* (dNTPs). Any contaminating PCR products from previous reactions are eliminated by the UNG at room temperature before the start of the current RT-PCR. Afterwards the UNG is inactivated by the elevated temperature during reverse transcription.

6.3 Amplification and detection

Before PCR amplification starts, the RNA of the IC undergoes reverse transcription to produce cDNA copies of the RNA templates. Reverse transcription is carried out by a recombinant variant of the enzyme *Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase*, which is contained in the *enzyme mix* (EM). The PCR reaction then amplifies the cDNA copies of the IC and the DNA of the target virus in parallel.

The reaction mixture is heated to separate the double-stranded DNA into single-stranded DNA ('denaturation'). When cooling the mixture, probes and primers hybridize to the complementary DNA strands ('annealing'). In the presence of Mg^{2+} ions and excess dNTPs the primers are extended along the target sequences ('elongation') by the enzyme *Thermus aquaticus* (Taq) DNA polymerase. The hybridized probes are cleaved by the 5'-3'-exonuclease activity of the Taq DNA polymerase during elongation.

In each cycle, new double-stranded DNA molecules are generated ('amplicons'). The PCR reaction is carried out for 50 cycles. Starting with the sixth PCR cycle, a fluorescence light measurement is taken for each cycle.

Probes add an additional layer of specificity, since probe molecules can only hybridize to complementary DNA strands of the target region and probes are only cleaved by the Taq DNA polymerase, when hybridized to the complementary DNA strand.

To allow detection of the number of amplicons produced, the probes are coupled with a fluorescent dye ('reporter') at the 5'-end and with a quencher at the 3'-end. Due to the spatial proximity of the reporter to the quencher, the fluorescence emission of the reporter is suppressed by *fluorescence resonance energy transfer* (FRET). As long as the probe is intact, no fluorescence is emitted when excited by an external light source.

During elongation, the reporter dye is separated from the quencher and thus the fluorescence signal is emitted. The fluorescence signal increases in relation to the number of amplicons produced.

Different reporter dies with specific fluorescence spectra are used for target viruses and the IC. Successful amplification of target viruses and the IC can therefore be detected by the signal increase in two different fluorescence channels.

6.4 Evaluation and report

After the PCR run on *PoET Instrument*, the analysis and evaluation is carried out automatically by the software *Calliope*. Further details on the evaluation are described in chapter 7.9.

7 Performing the test

7.1 Requirements for performing the test

- Only personnel trained and qualified as proficient in the use of PoET products and in handling of infectious materials should perform this procedure.
- Closely follow the procedures and guidelines provided to ensure that the test is performed correctly. Any deviation from the procedures and guidelines may affect test performance.
- Use this product only for its intended purpose.
- Use only the specified reagents and disposables.
- Use in the temperature range of +15 °C to +30 °C.

7.2 Specimen material

- In the validation studies of the PoET product line, human EDTA and CPD plasma from living donors was used as specimen type. All performance-related information is based on those materials, which is therefore recommended for use with *PoET Instrument*.
- Blood specimens taken from heparin blood collection tubes, as well as specimens from heparinized persons, may not be used, as heparin can impair PCR (12).

Treat all specimens as potentially infectious.

7.3 Specimen drawing & pre-treatment

7.3.1 EDTA plasma

- 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 whole blood specimens in the EDTA-K2 gel barrier blood collection tubes must be separated into the cellular and plasma components within 48 hours according to the manufacturer's specifications.
- Depending on the test method, *PoET Instrument* requires a volume of up to 1.5 mL per specimen. Further information can be found in the operator's manual of *PoET Instrument* (chapter 6).

The primary tubes must be filled sufficiently. Take care to avoid gel components or blood cells contaminating the plasma. Otherwise, this may lead to an impairment of the performance of the test procedure.

7.3.2 CPD plasma

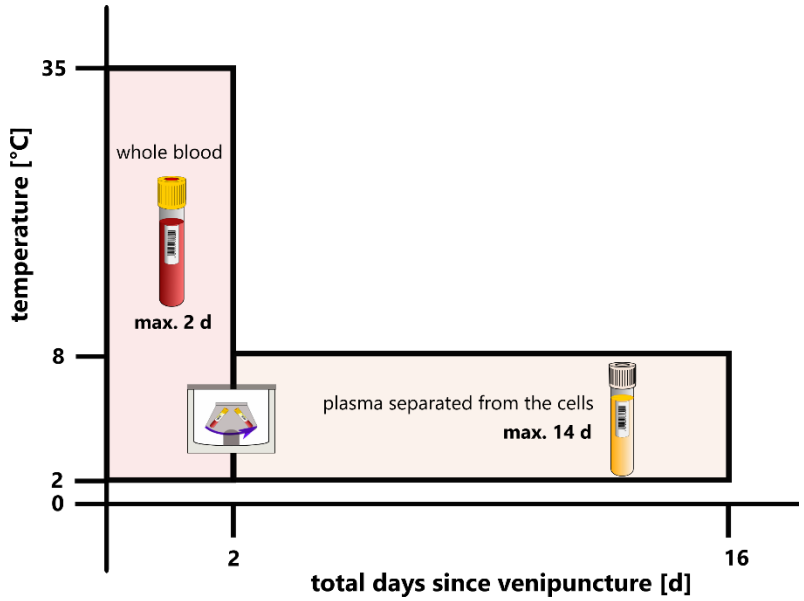
- Specimen drawing and CPD plasma production is to be carried out using blood bag collection systems (e.g. Fresenius-Kabi or Maco Pharma) following the sample collection bag manufacturer's instructions.
- Depending on the test method, *PoET Instrument* requires a volume of up to 1.5 mL per specimen. Further information can be found in the operator's manual of *PoET Instrument* (chapter 6).

7.4 Specimen transport

Transport durations and conditions have to comply with the storage conditions (see next chapter).

7.5 Specimen storage

7.5.1 EDTA plasma



- The specimens can be transported and stored for 48 hours at +2 °C to +35 °C until separation.
- After separation from the cells, EDTA plasma can be kept in primary or secondary tubes at +2 °C to +8 °C for up to 14 days without measurably changing the CMV viral load.
- Plasma specimens can be stored frozen for 60 days at ≤ -18 °C
- Frozen plasma specimens must be thawed in a water bath at 37 °C to avoid precipitates.
- Thawed plasma specimens must be refrozen immediately after taking an aliquot for testing.
- Do not apply more than three freeze / thaw cycles

The test performance may be affected by exceeded storage of the EDTA plasma.

Do not freeze whole blood specimens.

7.5.2 CPD plasma

- The frozen CPD plasma bags can be stored at ≤ -30 °C until the declared shelf-life of the individual bag is reached.
- Frozen plasma specimens must be thawed in a water bath at 37 °C to avoid precipitates.
- Thawed plasma specimens cannot be stored and must be aliquoted and refrozen immediately after taking an aliquot for testing.

The test performance may be affected by exceeded storage of the CPD plasma.

7.6 Processing of samples on *PoET Instrument*

The handling of *PoET Instrument* is described in detail in the operator's manual of *PoET Instrument* (chapters 7 and 8).
The following is a summary of the test procedure:

Step	Action(s)
1	Turn on *PoET Instrument* and PC
2	Carry out the maintenance program according to the instructions on the screen
3	Prepare the run: • Select the processing mode • Load samples onto the instrument • Assign testing orders (test types and test parameters) Load reagents and consumables
4	Start run
5	Check results
6	Remove sample tubes. If needed, cap the tubes for future use. Unload disposables, reagent tubes and reagent troughs and dispose of the waste.

Depending on the chosen test parameters and number of samples, the PCR results of a sample batch are available about 3.5 hours after the start of the run.

7.7 Preparing samples for a *PoET Instrument* run

For information about primary and secondary sample tubes accepted on the instrument please refer to chapter 4.2 'Additional materials required' and the operator's manual of *PoET Instrument*.
EDTA specimens stored in the refrigerator can be used and analyzed directly. Frozen EDTA and CPD specimens have to be thawed in a water bath at +37 °C.

7.8 Preparations before use

- Completely thaw the required number of *PoET Internal Control* at +15 °C to +30 °C before use.
- PCR kits and PCR controls can be loaded frozen on *PoET Instrument*.
- *PoET Extraction* and *PoET Prep Reagent* can be used directly.
- Before use, visually inspect each reagent tube to ensure that there are no signs of leakage. If there is any evidence of leakage, do not use that tube for testing.
- Remove the caps of the reagent tubes and the peel-seal films of the extraction reagent troughs before positioning them on the carriers of *PoET Instrument*. *PoET Instrument* does not have a device for the automated removal of caps ('Decapper') or the piercing of films.
- In order to avoid evaporation of reagents, remove tube caps and peel-seal films only shortly before use. Remove the peel-seal films of the reagent troughs carefully to avoid spilling of reagents.
- Take care that no liquid residues remain in the tube caps or on the tube walls.

- During positioning of the sample and reagent tubes on the carriers, make sure that the barcode labels are visible through the openings on the side of the carriers. Refer to the operator's manual of *PoET Instrument* for barcode specifications.
- Carry out the loading and unloading of the *PoET Instrument* reagent carriers according to the specifications in the operator's manual of *PoET Instrument* (chapter 7).
- Disposables are for one time use only. Do not reuse.
- Please refer to the operator's manual of *PoET Instrument* for proper instrument maintenance.

7.9 Calculation of results

The evaluation of the PCR raw data is performed using the *Calliope* software. Each individual amplification curve is analyzed (evaluated) using GFE's proprietary algorithm, and reactive curves are assigned 'positive points' (PP). As a second value for result evaluation, the algorithm calculates the 'quotient value' (Q) for each individual curve. This value results from the highest fluorescence value of the last cycles divided by the lowest fluorescence value of the first cycles. It represents the signal strength of the individual curve.

7.10 Quality control measures and validity of results

The entire process from sample preparation to PCR analysis is monitored by several controls:

Control type	**Product**	**Function**
Internal control (IC)	*PoET Internal Control*	IC is added to each sample at the beginning of the process. For each non-reactive sample, IC indicates whether the processing from extraction to the result is valid.
PCR positive control (PC)	*PoET CMV Positive Control*	PCs are set up as individual reactions. The PCR positive control contains synthetic nucleic acids of CMV. The PC proves that the reagents involved in the amplification of CMV are functional.
PCR negative control (NC)	*PoET Negative Control*	NCs are set up as individual reactions. The PCR negative control indicates that the PCR reagents are free of contaminations, which might act as templates for the respective target virus amplification.

Based on the PP and Q values of the controls *Calliope* evaluates, whether the overall result is valid for the sample batch and for each individual sample.

7.10.1 Validation of PCR negative controls (NCs)

PoET Negative Control consists of an aqueous buffer solution and is set up as a separate reaction. It is used as a PCR negative control to demonstrate that the reagents involved in the amplification reaction are not contaminated with the nucleic acids to be detected.
NC results have to be 'not reactive' for the target virus and IC.
If more than one NC reaction is set up on one PCR plate, only one NC is allowed to be reactive for either the virus parameter or IC.

Case	NC result for target virus and IC	Assessment
1	All NC on the same PCR plate are not reactive.	Overall NC result is valid
2	Only in case of > one NC per target virus on the same PCR plate: One NC is reactive.	Overall NC result is valid
3	≥ Two or all NC on the same PCR plate are reactive.	Overall NC result is invalid

In case of an overall invalid result of NC, the PoET run will automatically be assessed as invalid for the corresponding sample batch.

7.10.2 Validation of PCR positive controls (PCs)

PCR positive controls are set up as separate reactions. They serve to demonstrate that the reagents involved in the amplification reaction of the nucleic acids of the respective target virus are functional.
PC results have to be 'reactive' for the target virus and must meet the predefined limits for PP and Q values. The limit values are stored in the software *Calliope*.
If more than one PC reaction is set up for the same target virus on one PCR plate, only one PC is allowed to be 'not reactive' for the target virus or to exceed the PP value limit or to stay below the Q value limit.

Case	PC result for target virus	Assessment
1	All PC on the same PCR plate are reactive and meet PP and Q limit values.	Overall PC result is valid
2	Only in case of > one PC per target virus on the same PCR plate: One PC is not reactive or does not meet PP and/or Q limit values.	Overall PC result is valid
3	≥ Two or all PC on the same PCR plate are not reactive or do not meet PP and/or Q limit values.	Overall PC result is invalid

In case of an overall invalid PC result, the PoET run will automatically be assessed as invalid for the corresponding sample batch.

7.10.3 Validation of internal control (IC) and result assessment

PoET Internal Control is added to each sample before starting the processing. The samples thus contain an additional analyte which undergoes the entire sample processing. IC is used to evaluate the validity of the results of the samples tested. As IC runs through the entire process, it serves to functionally monitor the reagents used and the *PoET Instrument* employed.

The analysis of IC validity is only performed if the overall results of the PCR controls (NC, PC) are valid for the corresponding sample batch.

If samples are 'not reactive' for the target virus, IC results have to be reactive and meet the predefined limit values for PP and Q. The limit values are stored in the software *Calliope*.

If the IC is 'not reactive', exceeds the PP value limit or stays below the Q value limit, the respective sample position on the PCR plate will be assessed as invalid.

In case of a 'reactive' result for the target virus, IC performance is not relevant and may be or may not be reactive.

Case	IC	Assessment
1	not reactive or does not meet PP and/ or Q limit values	IC result is invalid
2	reactive and meets the limit values for PP and Q	IC result is valid

7.11 Interpretation of results

A valid sample batch in an individual *PoET Instrument* run may include both valid and invalid sample results.

Sample results are only valid if the respective PCR controls (PC, NC) of the corresponding sample batch are valid and no processing errors occurred. Invalid samples require repeat testing.

Valid sample results can be either 'reactive' or 'not reactive':

Sample result	Interpretation
Reactive	Target virus is reactive and IC may or may be not reactive/ meet the PP and Q limit values
Not reactive	Target virus is not reactive and IC meets the PP and Q limit values
Invalid	Target virus is not reactive and IC is not reactive or does not meet the PP and Q limit values or Result is invalidated due to processing errors, *e.g.* pipetting errors or invalid PCR controls

Depending on the configuration, *Calliope* either provides the results automatically to a laboratory information management system (LIS, LIMS) or the results have to be reviewed and transmitted manually to a LIS. Please refer to the operator's manual of *PoET Instrument* (chapters 4 and 8) for more details.

7.12 Procedural limitations

- The detection of viral nucleic acids is concentration dependent. Viral nucleic acids concentrations below the detection limit of the assay, cannot be reliably detected by the PCR kit.
- Incorrect specimen collection, untested interference substances and improper specimen storage and preparation can negatively affect the stability of the virus and nucleic acids and impair the PCR results.
- Blood specimens taken from heparin blood collection tubes, as well as specimens from heparinized individuals, shall not be used because heparin can impair the PCR.
- For CPD specimens with albumin contents > 60 g/L, reliable test results are not ensured.
- Pre-exposure prophylaxis preparations or other virostatic medication taken by blood donors can lead to low concentrations of viral nucleic acids that accordingly cannot be reliably detected (see above).
- Despite sequence matching and verification of the primers for detecting the variants relevant for blood donation, mutations within the highly conserved regions of the viral genome may affect oligonucleotide binding and thus virus detection.
- Carryover during sample handling and processing cannot be excluded for specimens with very high viral loads. When detecting a PCR result with a very low PP value, samples in the same run can thus show weakly reactive results.

7.13 Disposal

- *PoET Extraction lysis buffer* contains guanidine thiocyanate. Avoid contact of the reagent with the skin, eyes or mucous membranes. If contact does occur, immediately wash with generous amounts of water. Do not allow *lysis buffer* to get in contact with sodium hypochlorite solution (bleach). This mixture can produce a highly toxic gas.
- Used extraction and PCR plates are sealed at the end of the process. Dispose of all materials that have come into contact with reagents, according to the relevant regional and national regulations.
- Dispose of reagent residues according to the relevant regional and national regulations.
- Dispose of all materials that have come into contact with potentially infectious specimens according to the relevant regional and national regulations.

8 Performance characteristics

The performance characteristics of *PoET CMV* were determined using the '1st WHO International Standard for Human Cytomegalovirus for Nucleic Acid Amplification Techniques; NIBSC code: 09/162'.

8.1 Key test features

Specimen type	EDTA or CPD plasma
Required specimen volume range	200 – 1500 µL*
Processed sample volume range	40.5 – 1300 µL*
Limit of detection	12.5 IU/mL
Specificity	100 %
Test duration	Depending on the test plan of the PoET run, results are available approximately 3.5 h after loading the samples on the instrument.

* Depending on the required sensitivity for testing. Please refer to the operator's manual of *PoET Instrument* or contact your GFE representative for more information.

8.2 Analytical sensitivity

8.2.1 Limit of detection (LoD)

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

Standard	CMV NIBSC code: 09/162
95 % LoD	12.5 IU/mL
Confidence interval	9.3-19.0 IU/mL

For CPD plasma, the success rate for virus concentration was determined based on the 95 % LoD of EDTA plasma. CPD plasma has no negative influence on the sensitivity of *PoET CMV*.

8.2.2 LoD for smaller sample volumes

If samples with a plasma volume < 1.3 mL are used in the test (*e.g.* in the case of sample pool aliquots or individual donations with a lower starting volume), *PoET Instrument* automatically replenishes the volume to 1.3 mL total volume with *sample diluent* (SD), a component of *PoET Extraction*. The LoD of *PoET CMV* is reduced according to the dilution factor.

As part of the validation of the PCR kit *PoET CMV*, it was confirmed that replenishing with SD instead of plasma has no effect on the LoD of *PoET CMV*. Further information about sample input volumes can be found in the operator's manual of *PoET Instrument* (chapter 6).

8.2.3 Seroconversion panels

Three commercially available seroconversion panels for CMV were matching the requirements of the Common Specifications (CS). Each panel member was tested in two different sample types: a) 1:6 diluted with EDTA plasma and b) 1:96 diluted with EDTA plasma. Following the analysis with the PCR kit *PoET CMV*, a comparison of the results with the information provided in the accompanying documents of the panel was carried out. As expected, the PCR kit *PoET CMV* also determines samples as reactive that were determined as reactive with the NAT test method (CE-IVD-labelled reference NAT for CMV) mentioned in the respective documents.

For only 1 out the 3 CMV seroconversion panels NAT reference data was available. One sample within the progression of the infection was clearly detected by *PoET CMV* in a 1:6 und 1:96 dilution whereas the reference NAT showed a non-reactive result.

For all other seroconversion panels, only serological results were listed in the panel description. However, with *PoET CMV* some samples that were still serologically assessed as CMV-negative could already be detected as CMV-reactive in each panel.

Conversely, it can be observed that as soon as the serological results are positive and therefore a decrease in the viral load can be expected, the samples can no longer be detected with *PoET CMV*.

Based on limited availability of seroconversion panels, on average the PCR kit *PoET CMV* provides reactive results approximately 5 days earlier than the respective serological CMV reference test, even in sample pools of ≥ 6.

The testing of the seroconversion panels thus points out the higher sensitivity of NAT techniques compared to the serological test methods (see table).

Panel	**Abbott Architect**	**Abbott Architect**	**Roche cobas® TaqMan**	***PoET CMV***	***PoET CMV***
	CMV IgM Test first positive day#	**CMV IgG Test first positive day#**	**Reference-NAT first positive day#**	**Sample pool [1] (n=6) first positive day#**	**Sample pool [1] (n=96) first positive day#**
0615-0039 (SeraCare)	15	na	15	7	15
SCP-CMV-002 (Biomex)	33	29	na	26	26
SCP-CMV-005 (Biomex)	6	6	na	0	0

na: not available

Day 0 corresponds to the time of collection of the first blood sample

[1] The test used sample dilutions corresponding to a 6 or 96 sample pool

8.2.4 Genotype verification

Testing of PCR inclusivity of different genotypes is not applicable, because CMV does not have phylogenetically different genotypes.

8.3 Analytical specificity

8.3.1 Experimental design

The influence of interfering substances on the PCR kit *PoET CMV* was investigated by means of the extraction of different samples and detection of CMV. In one part of the samples, CMV-negative plasma was only spiked with the respective substance. Another part of the samples was additionally spiked with a CMV virus standard at 2.5-fold 95 % LoD. Endogenous and exogenous interfering substances were tested.

8.3.2 Cross-reactivity and clinical conditions

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 by-products.
As part of the validation, the influence of genomic nucleic acids from selected viruses on the PCR kit *PoET CMV* was investigated. For this purpose, negative human EDTA plasma (NHP) was spiked with standards for the viruses/bacteria to be tested, extracted and amplified. In addition, CMV-positive plasma was spiked with standards for the viruses/bacteria to be tested and analyzed. The CMV-positive samples, were spiked with CMV at 5-fold 95 % LoD.

Test results for cross-reactivity:

Species	**Domain**	**Nucleic acid**	**Observation**
Hepatitis B Virus (HBV)	Virus	DNA	No Interference
Hepatitis C Virus (HCV)	Virus	RNA	No Interference
Hepatitis E Virus (HEV)	Virus	RNA	No Interference
Human Immunodeficiency Virus 1 (HIV-1)	Virus	RNA	No Interference
Human Immunodeficiency Virus 2 (HIV-2)	Virus	RNA	No Interference
Parvovirus B19 (B19V)	Virus	DNA	No Interference
Hepatitis A Virus (HAV)	Virus	RNA	No Interference
West Nile Virus, lineage 2 (WNV lineage 2)	Virus	RNA	No Interference
Hepatitis D Virus (HDV)	Virus	RNA	No Interference
Herpes Simplex Virus type 1 (HSV1)*	Virus	DNA	No Interference
Herpes Simplex Virus type 2 (HSV2)*	Virus	DNA	No Interference
Human Herpesvirus 6B (HHV 6b)*	Virus	DNA	No Interference
Human Herpesvirus 7 (HHV 7)*	Virus	DNA	No Interference
Varizella Zoster Virus (VZV)*	Virus	DNA	No Interference
Epstein-Barr Virus (EBV)*	Virus	DNA	No Interference
BK Virus (BKV)	Virus	DNA	No Interference
Adenovirus	Virus	DNA	No Interference
Serratia marcescens	Bacterium	DNA	No Interference
Klebsiella pneumoniae	Bacterium	DNA	No Interference
Streptococcus pyogenes	Bacterium	DNA	No Interference

*) Herpesviridae

For the viruses and bacteria tested, no influence on the PCR performance of *PoET CMV* was observed. All PCR reactions showed reactive results for the IC and no false-reactive or false-non-reactive results for CMV. Especially no cross-reactivity to other viruses of the Herpesviridae family was found.

8.3.3 Endogenous interfering substances

To assess the influence of hemolysis and increased bilirubin, albumin and triglyceride content on *PoET CMV*, plasma samples were spiked with the respective endogenous substance in several concentrations up to abnormally high levels. This validation included EDTA and CPD plasma specimens.

Test results for endogenous substances using EDTA plasma:

Endogenous substance	Concentration	Observation
Bilirubin	≤ 50 mg/L	No influence
Hemoglobin	≤ 2000 mg/L	No influence
Triglycerides	≤ 40 g/L	No influence
Albumin	≤ 120 g/L	No influence

The various concentrations of the spiked interfering substances did not affect the detection of positive or negative EDTA samples with *PoET CMV*.

Test results for endogenous substances using CPD plasma:

Endogenous substance	Concentration	Observation
Bilirubin	≤ 50 mg/L	No influence
Hemoglobin	≤ 2000 mg/L	No influence
Triglycerides	≤ 40 g/L	No influence
Albumin	≤ 60 g/L	No influence
	≥ 80 g/L	Interference was observed

The various concentrations of Bilirubin, Hemoglobin, and Triglycerides did not affect the detection of positive or negative CPD plasma specimens with *PoET CMV*.

For CPD plasma specimens with albumin concentrations up to 60 g/L, no influence on the test results occurred. For CPD plasma specimens with albumin concentrations > 60 g/L, reliable test results are not ensured.

8.3.4 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 provided in the directive 'EP7A2 Interference Testing in Clinical Chemistry' (13). The selection of drugs and their concentrations are derived from this guideline. These validation tests were done with EDTA plasma only.

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 at the respective concentrations.

8.4 Whole system failure rate

The determination of the system failure rate leading to false non-reactive results (in percent non-reactive samples) of the overall system (short 'failure rate') of the PCR kit *PoET CMV* was carried out by testing 288 samples for CMV. For this test, negative human plasma was spiked with CMV in threefold 95 % LoD.

No failure was observed in the 288 analyses. This results in a failure rate of 0 %.

8.5 Clinical performance

8.5.1 Diagnostic sensitivity

Diagnostic sensitivity was determined using available panels of donor samples (sample positive on reference NAT test) and initially negative individual donations spiked with virus to simulate positive individual donations.

Samples of the seroconversion panels were also considered as positive donor samples and are included (1 panel = 1 donor) in the calculation of the total number of samples.

Number of tested samples	Number of reactive samples	Diagnostic sensitivity
128	128	100 %

With a total of 128 samples used for testing, a diagnostic sensitivity of 100 % was achieved. All samples were reliably detected with *PoET CMV*.

8.5.2 Diagnostic specificity

To determine the diagnostic specificity of the PCR kit *PoET CMV*, CMV-negative samples were examined using individual EDTA plasma donations from blood collection tubes with gel barriers.

Number of valid non-reactive samples	Number of false reactive samples	Specificity
938	0	100%

Among the 938 valid samples, no false-reactive sample could be observed. Thus, for the PCR kit *PoET CMV*, a specificity of 100 % when using EDTA plasma can be assumed.

The specificity of *PoET CMV* was also tested using CPD plasma from 47 independent donors.

Number of valid non-reactive samples	Number of false reactive samples	Specificity
47	0	100%

No false reactive sample could be detected among the 47 samples. Thus, for the PCR kit *PoET CMV*, a specificity of 100 % when using CDP plasma can be assumed.

9 Overview of reagents and materials

Material	Manufacturer	Reference number	Storage conditions
PoET CMV	GFE	P2G-28-30	≤ -18°C
PoET CMV Positive Control	GFE	P3G-180-30	≤ -18°C
PoET Extraction	GFE	P1A-24-04	+2°C to +8°C, upright
PoET Prep Reagent	GFE	P1B-24-20	+2°C to +8°C, upright
PoET Internal Control	GFE	P1C-1440-60	≤ -18°C
PoET Negative Control	GFE	P3A-500-30	≤ -18°C

Material	Manufacturer	Reference number
PoET Instrument incl. software *Calliope*	GFE	P9A
1000 µL CO-RE II Tips	Hamilton Bonaduz AG	235905
300 µL CO-RE II Tips	Hamilton Bonaduz AG	235903
Extraction Plate Set	GFE	43001-0703
PCR Plate	Azenta Life Sciences	SP-0362
13 mL Tube & Cap	Sarstedt AG & Co.	60.541.004 & 65.714

Please refer to the operator's manual of *PoET Instrument* for additional information. All items are supplied by GFE.

10 Manufacturer and service contacts

Gesellschaft zur Forschung, Entwicklung und Distribution von Diagnostika im Blutspendewesen mbH
Altenhöferallee 3, 60438 Frankfurt/Main, Germany
Phone: +49 (0) 69 / 400 5513 0
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Questions concerning PoET products and training courses can be addressed to your local GFE representative:

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

10.1 Reporting

Inform your local competent authority and GFE if any serious incidents occur when using this product. The summary of the safety and performance report can be found using the following link: https://ec.europa.eu/tools/eudamed. Until the EUDAMED database is fully functional, please contact your local GFE representative.

11 Trademarks and patents

- *PoET* and *Calliope* are registered names owned by GFE.
- The *SuperScript® III reverse transcriptase* included in the PCR kits is a product manufactured and licensed by Life Technologies by Thermo Fisher Scientific.
- During the application of the PCR kits, the PCR plates (*PCR Plates*) '*FrameStar® 96 (cut corner A12)' with barcode* [Reference 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 Azenta Life Sciences*'.
- Other registered names, trademarks etc. used in this document are not to be considered legally unprotected, even if they are not specifically marked.

12 References

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13 Symbols

The following symbols are used in labeling of GFE products:

LOT	Batch code	SN	Serial number
REF	Reference number		Date of manufacture
YYYY-MM	Use by date (year-month)	UDI	Unique device identifier
Σ 840	Contains sufficient for <n> tests (n = total number of IVD tests)		GFE manufacturer logo
-18°C	Upper temperature limit value		Manufacturer
+ 2°C + 8°C	Temperature limits		Protect from sunlight
	Consult instructions for use	IVD	*In vitro* diagnostic medical device
	Attention Indication of safety-related information such as warning or precaution	CE	Conformity to the European Requirements on *in vitro* diagnostic medical devices
	Do not re-use	CE 0123	Conformity to the European Requirements on *in vitro* diagnostic medical devices and identification number of the Notified Body (0123)

14 Revision history

Version	Document ID	Date [YYYY-MM-DD]	Remarks
1	001170	2023-03-09	initial release