124

Blutspendedienst des Bayerischen Roten Kreuzes

Evaluation of a new PCR-based CMV assay for blood donor screening

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Introduction / Background

According to German Guidelines, the use of leukocyte-depleted blood components is an effective strategy for reducing the risk of transfusion-transmitted cytomegalovirus (CMV). However, there is a residual risk of transfusion-transmitted infections by non cell-associated CMV. We tested a new PCR-based CMV assay developed for high-throughput blood donor screening. The approval process for the PoET CMV assay by the notified body is still ongoing.

Methods

CMV detection by nucleic acid amplification testing (NAT) was performed in pools up to 48 samples of EDTA-plasma (0.1 ml per sample). Sample preparation was performed by *PoET Instrument* with the PoET CMV-Kit (GFE)[1)]. 1,300 µl plasma of each pool was processed using PoET reagents for extraction.
Analytical limit of detection (95 %) is specified with 12.5 IU/ml (confidence interval 9.3 – 19.0 IU/ml), while the limit of detection in pools with 48 samples is 600 IU/ml per tested sample. For evaluation of the assay, dilution series of CMV reference material (1st WHO IS for human CMV 09/162) were performed.

Results

Sensitivity

Sensitivity was tested by 3 concentrations (12.5, 25 and 50 IU/ml) of CMV reference material. Each dilution was tested in 48 replicates.

Table 1. Hit-rates of diluted samples

IU/ml CMV	number of tested samples	number of reactive samples	hit-rate
12.5	48	45	94%
25	48	48	100%
50	48	48	100%

94 % of tested samples with 12.5 IU/ml CMV IS were tested positive. Samples with 25 IU/ml and 50 IU/ml CMV IS were tested all positive.

Precision

Intra-assay variability was tested by extraction of 8 replicates of a low-positive sample with 18.75 IU/ml CMV IS (1.5-fold LoD). The average positive point (PP) is 33.5 ± 0.71 (day 1, Table 2).
Intra-laboratory variation was tested by extration of 8 replicates of a low-positive sample (18.75 IU/ml) on 3 days on 3 different testing platforms (*PoET Instrument*) carried out by 3 different staff members. The average PP of tested samples was 33.4 ± 0.12.

Table 2. Intra-assay variability and intra-laboratory variation

	day 1	day 2	day 3	intra-laboratory variation
average (PP)	33.5	33.3	33.5	33.4
standard deviation	0.71	1.56	0.71	0.12
coefficient of variation	0.02	0.05	0.02	0.004

Robustness

Robustness was tested by extraction of 48 positive samples with 25 IU/ml (2-fold LoD) and 50 IU/ml (4-fold LoD). All samples were tested reactive.

Table 3. Results of testing robustness

IU/ml CMV	tested samples	number of reactive samples	failure rate
25	48	48	0
50	48	48	0

Reproducibility (proficiency testing)

Plasma samples of proficiency testing (Instand e.V.) were tested as predetermined by the proficiency testing provider as single samples. Results were equivalent to the declaration of the provider (Table 4).

Table 4. Results of proficiency testing

CMV-DNA (qual.)	PP-value	result	evaluation
365-230314-01	27	reactive	correct
365-230314-02	23	reactive	correct
365-230314-03	neg	not reactive	correct
365-230314-04	25	reactive	correct

Screening of whole blood donations

84 pools representing 4,014 whole blood donations were tested for the presence of CMV-DNA in a routine-like setting. Out of them 3 pools were initially reactive (PP 34), but could not be confirmed.

Two pools were tested initially reactive (Figure 1). Resolution of the pools by testing rows and columns identified two reactive sample. The PCR result was confirmed by repeat testing of the single sample. In addition, the PCR-positive results were confirmed with an alternative PCR assay (cobas® CMV).

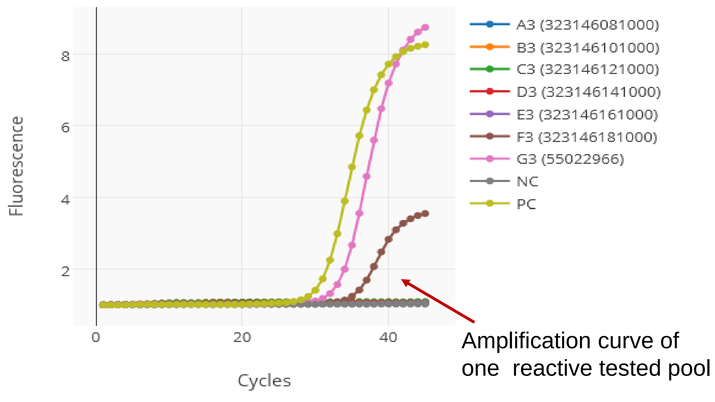

Figure 1. Amplification plot of CMV-PCR

Conclusions

PoET CMV on the *PoET Instrument* is a new and sensitive method for CMV infectivity screening of blood donors.

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References

1) *PoET CMV* Gebrauchsanweisung V0 (nur für Leistungsbewertungszwecke) GFE