

# A semi-automated procedure for monitoring of cytomegalovirus

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## Background

Monitoring of cytomegalovirus (CMV) in transplanted and other immunocompromised patients can efficiently be performed using molecular diagnostics based on real-time PCR, such as the CE-labelled affigene<sup>®</sup> CMV trender assay (Cepheid AB, Bromma, Sweden).

In high throughput laboratories it is not always possible or convenient to use manual sample preparation systems for the analysis of large amount of samples. Today, there are a number of automated sample preparation systems on the market. An interesting procedure for automated sample preparation is the Nuclisens<sup>®</sup> easyMAG<sup>™</sup> from Biomérieux (Lyon, France) which is CE-labelled for in-vitro diagnostic use.

## Objectives

The aim of this study is to evaluate the easyMAG<sup>™</sup> instrument in combination with affigene<sup>®</sup> CMV trender.

## Results

### LOD

The LOD of the semi-automated system was 57 c/mL (95% confidence interval 36-134 c/mL), see table 1.

### Quantitative range and imprecision

The quantitative range of the system is from at least 100 c/mL to  $2 \times 10^7$  c/ml with a total imprecision of less than 0.2 log standard deviation, see table 2 and Fig 1.

### Clinical samples

The clinical samples were quantified with excellent correlation between the CE-labelled process including the affigene<sup>®</sup> DNA extraction and the easyMAG<sup>™</sup> procedure, see Fig 2.

## Conclusions

Automation of sample preparation, using Nuclisens<sup>®</sup> easyMAG<sup>™</sup>, in combination with the standardized affigene<sup>®</sup> CMV trender PCR assay is an attractive option for quick CMV monitoring in laboratories requiring high throughput. The procedure is now CE-labelled.

Sample	Expected c/mL	Positivity rate
LOD1	100	24/24 (100%)
LOD2	50	22/24 (92%)
LOD3	25	19/24 (79%)
LOD4	10	14/24 (58%)
LOD5	5	7/24 (29%)

Table 1. Limit of detection study. Five different CMV levels. Each level prepared and amplified in 24 replicates.

Sample	Expected	Mean observed (log(c/mL))	Within-assay precision (log)	Total imprecision (log)
High	7.3	7.41	0.05	0.07
Low	2.0	2.01	0.14	0.17

Table 2. Imprecision study. Two different CMV levels were prepared and amplified at three different occasions. At each occasion 4 replicates were analysed.

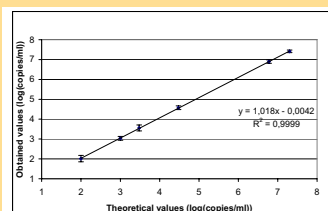


Fig 1. Quantitative range. Six different levels of CMV were each prepared in twelve replicates and subsequently amplified. The standard deviation for each concentration is shown using bars

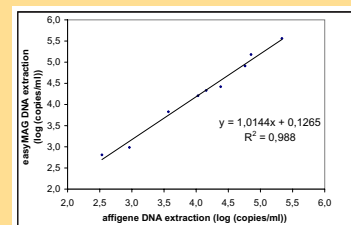


Fig 2. Comparison of clinical samples prepared using easyMAG<sup>™</sup> and affigene<sup>®</sup> DNA extraction. The prepared samples were amplified using affigene CMV trender.

## Materials and Methods

### Material

For performance of the complete procedure different concentration levels were prepared in CMV negative human plasma using the CMV strain AD169 from VQC Acrometrix (The Netherlands) and ATCC (USA).

To verify the procedure 20 clinical plasma samples from a number of patients were included in the analysis.

### Limit of Detection (LOD)

Five levels of CMV in the range from 5 c/ml to 100 c/mL were each prepared in 24 replicates and subsequently amplified. The probit statistical analysis was used for determination of LOD.

### Quantitative range

Six different levels of CMV, in the range from 100 c/mL to  $2 \times 10^7$  c/mL were prepared in twelve replicates each and subsequently amplified.

### Imprecision

The imprecision was determined by analysing four replicates at two different CMV levels in three consecutive runs.

### Clinical samples

The clinical samples were prepared in parallel using the easyMAG<sup>™</sup> and the affigene<sup>®</sup> DNA extraction procedures. Each preparation was subsequently amplified using affigene<sup>®</sup> CMV trender and the results were compared.

### Sample preparation

DNA was prepared from 500  $\mu$ L plasma on the easyMAG<sup>™</sup> and from 200  $\mu$ L plasma using affigene<sup>®</sup> DNA extraction kit according to manufacturers recommendations. The samples were eluted into 60  $\mu$ L eluent.

### Amplification

Amplification using affigene<sup>®</sup> CMV trender was performed in 50  $\mu$ L reactions (25  $\mu$ L mastermix and 25  $\mu$ L prepared sample) on the Mx3000P<sup>®</sup> instrument (Stratagene, La Jolla, USA).