

# Standardisation of quantitative real-time PCR for CMV and EBV

Z. Rayment, K. Hannon, R.H. Cave, M.A. Diggle, Z. Aiken, K. Levi

Clinical Microbiology, Nottingham University Hospitals NHS Trust, Queen's Medical Centre, Nottingham

## Introduction

The availability of World Health Organisation (WHO) international standards for CMV and EBV will allow accurate inter-laboratory comparison of quantitative real-time PCR results and enable consensus therapeutic guidelines to be defined. In order to transfer to quantitation in international units (IU)/mL from quantitation in copies/mL a conversion factor is required which will be dependent on sample matrix and extraction platform.

## Methods

The WHO CMV international standard was extracted on the easyMAG (bioMerieux) and serially diluted. Extracts were tested in triplicate on ABI 7500 platform with two commercially available kits: Alert Q-PCR (results in copies/mL) (ELITech Molecular Diagnostics) and RealStar (results in IU/mL) (Altona Diagnostics) kits (Fig.1)

The same process was followed using the WHO EBV international standard.

Whole blood samples (n=204) for CMV viral load testing were also extracted on the easyMAG using the on-board Specific B protocol and tested with both real-time PCR kits. An initial sample set (n=17) of EBV whole blood specimens were selected for testing based on the copies/mL result.

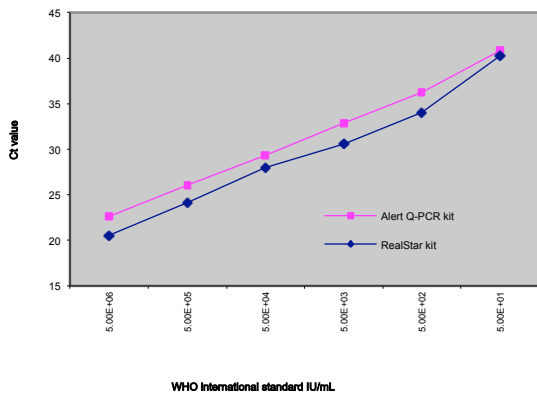


Figure 1. Serial dilution of extracted WHO CMV international Standard with Alert Q-PCR and RealStar PCR kits

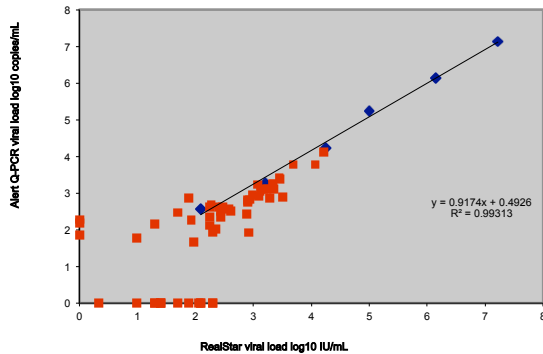


Figure 2. Quantification of WHO CMV international standard and clinical samples using the Alert Q-PCR and RealStar PCR kits

## Results

Results from both kits were used to calculate a regression equation from the  $\log_{10}$  data (demonstrated for CMV in Fig1). Improved sensitivity was demonstrated for CMV and EBV detection with the RealStar kits compared to the Alert Q-PCR kits.

### CMV:

Whole blood samples (n=204) for CMV viral load were tested in parallel with both kits. Of these, 162 were undetected by the Nanogen kit, but 14 were given a quantitative result by the RealStar kit ( $C_t$  values 36.6 – 43.4). Three samples had quantitative results with the Nanogen kit ( $C_t$  values 38.2, 38.9, 39.1) but were undetected by the RealStar kit.

Where results were available from both kits (n=39) (Fig. 2), the regression equation was used to calculate the predicted result in copies/mL from the measured result in IU/mL and was compared with the measured result in copies/mL. In total, 29 predicted results were  $<0.5 \log_{10}$  of the measured result (Table 1). Of the ten samples with predicted results  $>0.5 \log_{10}$  of the measured result, six of these had results  $<500$  copies/mL (i.e. below the quantifiable limit of the Alert Q-PCR kit), three had results  $<1000$  copies/mL and one result was 1305 copies/mL.

### EBV:

Fifteen samples had a measurable viral load with both kits (Fig.3) The regression equation was used to calculate the predicted result in copies/mL from the measured result in IU/mL and was compared with the measured result in copies/mL. Six samples had predicted results  $<0.5 \log_{10}$  of the measured result, nine samples had predicted results  $>0.5 \log_{10}$  of the measured result (Table1). Four of these nine samples had results  $<500$  copies/mL (i.e. below the quantifiable limit of the Alert Q-PCR kit),

Table 1. Difference between the predicted and measured viral loads for CMV and EBV in copies/mL

	$<0.5 \log_{10}$	$0.5-1.0 \log_{10}$	$>1.0 \log_{10}$
CMV (n=39)	29 (74.4%)	9 (23.1%)	1 (2.6%)
EBV (n=15)	6 (40%)	8 (53.3%)	1 (6.7%)

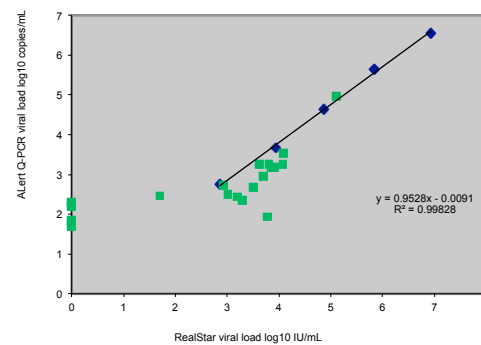


Figure 3. Quantification of WHO EBV international standard and clinical samples using the Alert Q-PCR and RealStar PCR kits

## Conclusions

- RealStar CMV and EBV real-time PCR kits were shown to be more sensitive using the WHO international standard
- A linear regression equation can be used to predict results in copies/mL to aid the transition process for service users, within the quantifiable limits of the assays

Thanks to Alere for supplying the RealStar kits for this evaluation