Standardisation of quantitative real-time PCR for CMV and EBV

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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 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, values 36.6 – 43.4). Three samples had quantitative results with the Nanogen kit (C, 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 (Table 1). 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

<table>
<thead>
<tr>
<th></th>
<th>&lt;0.5 Log_{10}</th>
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<tr>
<td>CMV (n=39)</td>
<td>29 (74.4%)</td>
<td>9 (23.1%)</td>
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<td>EBV (n=15)</td>
<td>6 (40%)</td>
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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:
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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