Investigation into the application of Droplet Digital PCR to cross-validate and standardise four human Adenovirus types in the absence of higher order standards

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Background

Adenoviruses (ADV) comprise 70 types and are responsible for a variety of infectious diseases, particularly in the immunocompromised where infections can develop and progress quickly, resulting in high mortality. Monitoring of ADV infections is critical to initiating antiviral treatment promptly in order to improve outcome. Accurate viral load determination is critical in clinical diagnostics when monitoring patients' response to treatment and disease progression. Where available, International Standards have helped to facilitate data comparison but where there is no standard or Certified Reference Material available assay variation obscures meaningful comparison of results at the technology and laboratory level. In the absence of an ADV International Standard true transferability of results is limited due to the lack of inter-laboratory standardisation.

Droplet digital PCR (ddPCR) permits the characterisation of control materials without the requirement of a standard or certified reference material thereby allowing direct comparison of assays and results between laboratories. **Objectives** This study evaluated the use of ddPCR to standardise ADV quantitation PCR (qPCR) by comparing four ADV types: 1, 3, 8 and 31 before and after standardisation.

Methods

4 stocks materials were prepared as 10-point dilution series based on the values provided in the certificate of analysis. The dilution series were initially assessed by in-house qPCR to establish performance and linearity. Droplet digital PCR (ddPCR), (BioRad QX200) was used to perform a series of experiments to establish calibration independent quantitation for the four materials (Figure 1). The digitally calibrated values (digital copies/ml) were reapplied to the stock materials and the data replotted.

Range Finder	Duplicate	Triplicate
• Dilution series tested • Working range identified on digital	• Minimum of 3 points within working range tested in duplicate • Reproducibility of digital results determined	• Minimum 3 points selected from working range • Each point extracted once and tested in triplicate • Assess impact of extraction on dPCR

Figure 1 : Process flow diagram detailing series of experiments performed by digital PCR

Results

The 4 stocks showed similar performance with the assay but variation in viral load determination was observed, dependent upon the ADV type used for calibration. See Figure 2.

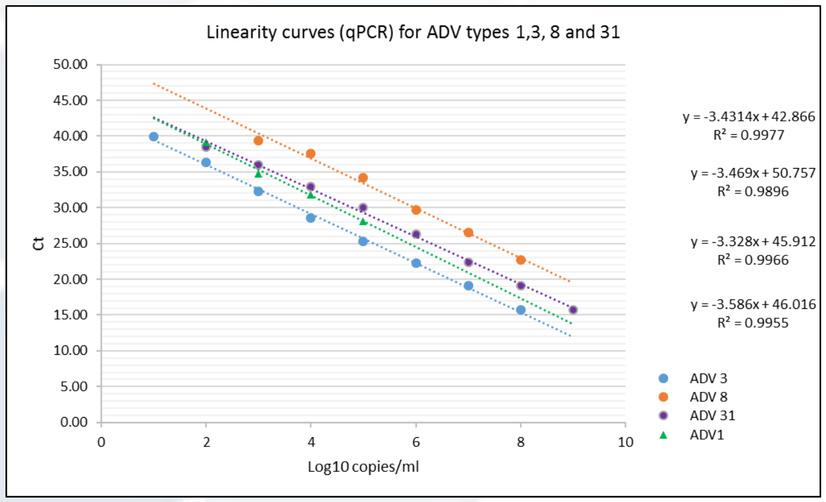

Figure 2 : Standard curves for ADV types 1, 3, 8 and 31 from qPCR

Results continued

Digital PCR was used to requalify the titre of the 4 dilution series to determine the digital copies/ml (dc/ml) value for the different ADV types. The data was replotted (Figure 3). Digital PCR was shown to unify viral load determination across the 4 types.

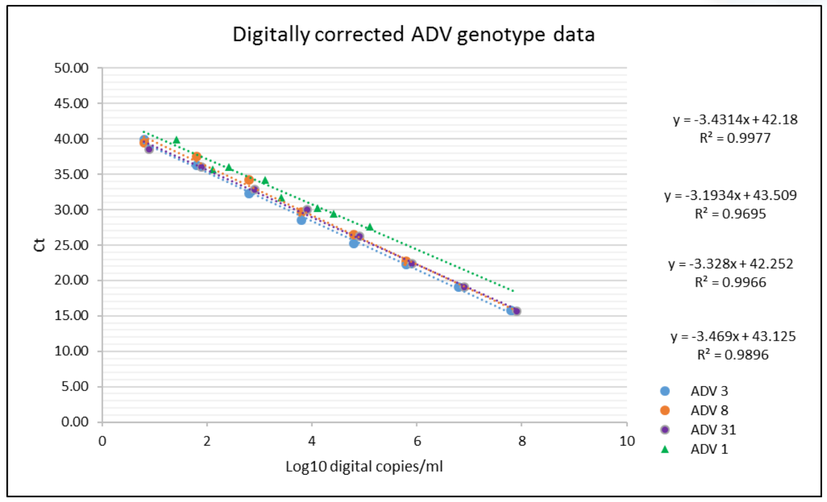

Figure 3: Standard curves for ADV types 1, 3, 8 and 31 following requalification by ddPCR

Conclusions

The comparison of inter-laboratory data for ADV load determination is limited in the absence of international standards or certified control materials due to assay variation. The results presented show type dependent variation in determining the viral load of ADV material when tested by qPCR. The results clearly show that ddPCR can be used to harmonise viral load quantitation for different ADV types and provides a viable method by which to standardise PCR assays for ADV load determination. Digital PCR allows standardisation of ADV materials and provides a method by which to quantify materials without a calibrated standard curve.

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