27th ECCMID Vienna, Austria 22 – 25 April 2017

The congress of ESCMID

Session: P042 Evaluation of novel tests in clinical virology

Category: 1g. Diagnostic virology (other than hepatitis & HIV)

23 April 2017, 13:30 - 14:30
P0928

Standardization of four types of human adenovirus in the absence of higher order standards

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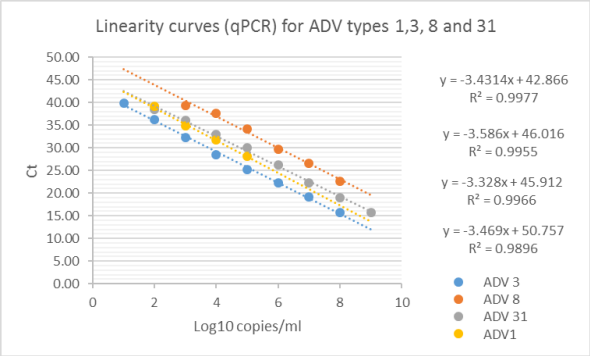
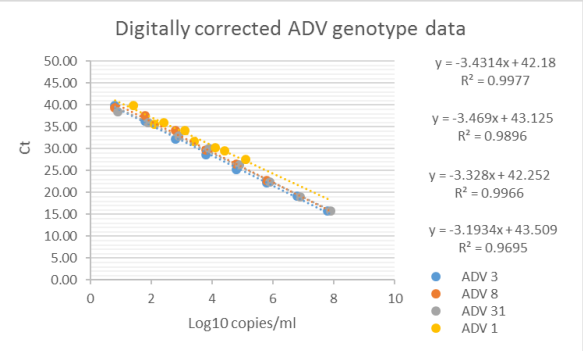
Background: Accurate viral load determination is critical in clinical diagnostics when monitoring patients' response to treatment and disease progression. Adenoviruses (ADV) comprise 70 types and are responsible for a variety of infectious diseases, particularly in the immunocompromised where infections 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. In the absence of an ADV International Standard true transferability of results is limited due to the lack of inter-laboratory standardisation. Assay variation obscures meaningful comparison of results. The use of materials which have been well characterised would allow for objective comparisons between laboratories and assays used. Objective; This study evaluated the use of digital PCR (dPCR) to standardise ADV quantitation PCR (qPCR) by comparing four ADV types: 1, 3, 8 and 31 before and after standardisation.

Material/methods: The four stocks materials were prepared as dilution series based on the values provided in the certificate of analysis. The dilution series were initially assessed by qPCR to establish performance and linearity. Subsequently droplet digital PCR, (BioRad QX200) was used to establish calibrator independent quantitation for the four materials. The digitally assigned values were reapplied to the stock materials and the data replotted.

Results: The initial qPCR data shows viral load determination may vary depending on the ADV type or calibrator used for standardisation by qPCR. Characterisation of the dilution series by digital PCR and the subsequent use of this data to normalise the qPCR values results in harmonisation of the standard curves and equivalent quantitation independent of the type used (See Figure 1).

Figure 1 shows the standard curves for ADV types 1, 3, 8 and 31 from qPCR (left) and the harmonised standard curve when the data has been calibrated to the values determined by dPCR (right).





The results indicate that digital PCR can be used to harmonise viral load quantitation for other ADV types and provide a viable method by which to standardise PCR assays for ADV load determination.

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. Digital PCR allows calibration of materials so the data is harmonised allowing inter-laboratory comparison.