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Multi-centre evaluation of the variability of adenovirus quantification by PCR

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INTRODUCTION

- Accurate nucleic acid amplification techniques (NAT) are critical for the diagnosis and monitoring of human adenovirus (HAdV) infections.
- The effective monitoring of HAdV by NAT depends upon the reliable quantification of all relevant HAdV types in different clinical specimens.
- This study aims to investigate the performance of different assays and the effectiveness of quantifying different HAdV types using a range of both patient and cultured virus samples.

MATERIALS AND METHODS

- HAdV DNA positive patient samples were sourced from clinical laboratories in
- Europe. Samples were typed by sequencing 600bp of the HVR-7 region of the hexon gene.
- HAdV cell culture types (from PHE Cultures Collection and Hannover Medical School) matching the patient samples types were propagated in Hep2C cells for 7 days.
- HAdV DNA was quantified using a LDT targeting the hexon gene and the Adenovirus R-gene® real-time PCR kit.
- Patient samples were diluted in HAdV-negative material. Cultured virus stocks were diluted in a universal buffer matrix and negative sample diluent and coded TCS 1-9. (Table 1)
- Four 10-fold dilutions of cell culture stocks were also prepared in HAdV-negative sample matrix corresponding to the patient samples (matrix TCS samples).
- 12 study participants received 2 vials each of TCS1-9, and 2 and 1 vials respectively of each patient sample and matrix TCS sample relevant to their HAdV NAT assay. Samples were tested using the participants routine quantitative HAdV assay.

Table 1. Study samples and codes (colour coded by HAdV type).

Tissue culture samples				Patients samples	
Study code	**HAdV type (species)**	**Matrix TCS sample diluent (-1 to -4)**		**Sample type**	**Sample diluent**
TCS1	41 (F)	Stool		Stool	Stool
TCS2	40 (F)	Stool		Stool	Stool
TCS3	31 (A)	Plasma		Plasma	Plasma
TCS4	14 (B)	Whole blood		Whole Blood	Whole blood
TCS5	7 (B)	Urine		Urine	Urine
TCS5	7 (B)	Nasal lavage		Nasal lavage	Normal saline
TCS6	5 (C)	Stool		Stool	Stool
TCS7	4 (E)	Eye swab		Eye swab	UTM
TCS7	4 (E)	-		Eye swab	UTM
TCS7	4 (E)	Sputum		Sputum	PBS
TCS8	2 (C)	Plasma		Plasma	Plasma
TCS8	2 (C)	Urine		Urine	Urine
TCS9	1 (C)	Plasma		Plasma	Plasma
TCS9	1 (C)	Serum		Serum	Serum

RESULTS: TISSUE CULTURE STOCKS

- Data were received from 17 quantitative real-time PCR assays (9 commercial and 8 LDT). The majority were 'pan-adenovirus' assays targeting the HAdV hexon gene.
- The SDs of $\log_{10}$ mean estimates for TCS1-9 for all assays ranged from 0.40-1.01 $\log_{10}$ copies/mL, with the highest variation reported for HAdV types 7, 31 and 41* (Table 2).
- The mean SDs (relative potencies) were reduced for the majority of types when expressed against other TCS samples (Table 2).
- The lowest relative potencies were reported for HAdV types 2 and 5 **. A low relative potency indicates a good material to act as a standard.

Study Code	HAdV type (species)	Mean	Range	SD (inter-laboratory)	Mean SD (relative potency)	SD (intra-laboratory)
TCS1	41 (F)	6.58	4.60-7.61	1.01*	0.88	0.22
TCS2	40 (F)	4.68	3.95-5.65	0.41	0.47	0.17
TCS3	31 (A)	8.79	7.48-9.70	0.58*	0.55	0.23
TCS4	14 (B)	7.70	7.18-8.82	0.40*	0.49	0.24
TCS5	7 (B)	7.35	5.71-8.20	0.72	0.57	0.27
TCS6	5 (C)	7.90	7.09-8.49	0.43	0.40**	0.21
TCS7	4 (E)	7.31	6.51-8.00	0.48	0.42	0.18
TCS8	2 (C)	7.55	6.77-8.15	0.47	0.40**	0.18
TCS9	1 (C)	7.68	6.91-8.37	0.51	0.41	0.21

Table 2. Overall laboratory mean estimates and range ($\log_{10}$ copies/mL) for cultured virus samples, SD of mean estimates (inter-laboratory variation) and SD of mean estimates of TCS1-9 relative to each TCS sample (relative potency). SD of mean estimates of duplicate samples within laboratories (intra-laboratory variation). Total of 17 datasets.

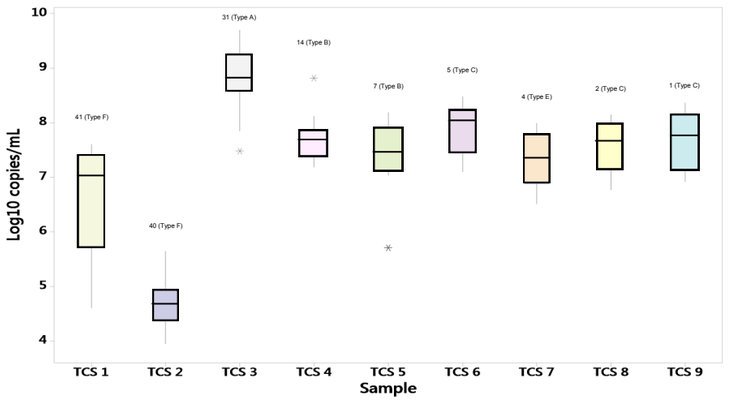

Figure 1. Shows the variability in the quantification of TCS 1-9 by the different assay methods. Some assays showed either under or over quantification compared to the overall mean estimate for each HAdV type (individual assay results not shown).

RESULTS: PATIENT SAMPLES

- The SDs of $\log_{10}$ mean estimates for patient samples for all assays ranged from 0.33-1.09, with the highest variation reported in urine samples
- For the majority of the patient samples the SD was reduced when the overall laboratory mean estimates are expressed relative to the matrix-TCS sample (SD relative potency compared to SD inter-laboratory) (Table 3).
- For all samples, the inter-laboratory variation was greater than the intra-laboratory variation ($p<0.01$) (Tables 2 and 3).

Table 3. Overall laboratory mean estimates and range ($\log_{10}$ copies/mL) for patient samples, SD of mean estimates (inter-laboratory variation) and SD of mean estimates relative to the respective matrix TCS -2 sample (relative potency).

Sample	HAdV type (species)	No. of data sets	Mean	Range	SD (inter-laboratory	SD (relative potency	SD (intra-laboratory)
Stool	41 (F)	7	8.45	7.93-8.85	0.33	0.46	0.06
Stool	40 (F)	6	7.67	6.51-8.38	0.27	0.43	0.06
Plasma	31 (A)	14	4.39	3.29-4.91	0.51	0.13	0.07
Whole blood	14 (B)	15	6.81	6.27-7.90	0.44	0.36	0.07
Urine	7 (B)	7	7.46	5.54-8.72	1.09	0.11	0.08
Nasal lavage	7 (B)	9	4.66	4.00-5.65	0.54	0.22	0.16
Stool	5 (C)	6	6.12	5.11-7.18	0.72	0.28	0.12
Eye swab	4 (E)	7	6.01	5.38-6.65	0.39	0.16	0.05
Eye swab	4 (E)	7	6.26	5.62-7.21	0.50	0.24	0.11
Sputum	4 (E)	7	8.07	7.60-8.51	0.38	0.41	0.16
Plasma	2 (C)	14	3.89	3.20-4.33	0.39	0.20	0.12
Urine	2 (C)	7	3.53	1.74-4.74	1.04	0.23	0.13
Plasma	1 (C)	14	7.04	6.39-7.55	0.38	0.31	0.11
Serum	1 (C)	6	9.41	8.98-9.99	0.41	0.17	0.13

CONCLUSIONS

- There is variability in HAdV quantification of different cultured HAdV types between laboratory assays.
- Inter-laboratory variability was significantly higher than the intra-laboratory variability, highlighting a need for standardisation HAdV of NAT assays.
- For the majority of cultured HAdV-types assay harmonisation was observed when viral loads were expressed relative to other HAdV types. The lowest relative potency was seen for types 2 and 5 (species C).
- For the majority of the patient samples assay harmonisation was observed when viral loads were expressed relative to the corresponding TCS sample in the same diluent.
- A proposal to develop the 1st WHO International Standard based on cultured virus for HAdV for the standardization of NAT assays has been endorsed by the WHO

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