Performance Characteristics of an Automated, Random-access Human Adenovirus (HAdV) qPCR Assay for Monitoring HAdV DNA in Plasma

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Background

HAdV is a common virus from the *Adenoviridae* family and is known for causing self-limiting infections in the conjunctiva, respiratory and gastrointestinal tracts. HAdV can remain latent and in immunocompromised individuals may reactivate and spread to multiple organs via the bloodstream. The importance of appropriate diagnostic HAdV monitoring in blood is underlined by the fact that the morbidity and mortality in immunocompromised patients with invasive infection can be very high, both in the pediatric and adult settings. Quantitative DNA measurements can contribute to the diagnosis of disseminated infection and act as surrogates that correlate with clinical response to therapy. qPCR may also be an effective screening modality to identify asymptomatic patients at risk for progressive adenovirus-associated disease.

Material and methods

Performance of the NeuMoDx® HAdV Quant Assay was characterized in plasma, at a specimen input volumes of 550 µl. Performance was demonstrated across key analytical metrics including analytical sensitivity (LoD), linearity, precision and specificity. Analytical sensitivity was characterized by testing a dilution series: 42 replicates at each dilution level were processed (positive samples) and 8 replicates for negative samples, per day. To determine LLoQ and ULoQ, a TAE ≤1.0 criterion was used for each of the HAdV target levels >95% detection. Analytical specificity was demonstrated by screening 23 organisms commonly found in plasma/serum, as well as species phylogenetically similar to HAdV for cross-reactivity. Precision was determined by testing two replicates of a 5-member panel of HAdV specimens twice daily, using one NeuMoDx 96 System for 20 days.

Analytical sensitivity

LoD

Analytical sensitivity of the NeuMoDx HAdV Quant Assay was characterized by testing a dilution series of the EDX AdV Verification Panel (Exact Diagnostics) in HAdV-negative plasma/serum samples to determine LoD on NeuMoDx Systems. LoD was defined as the closest target level above the concentration determined by Probit-style analysis with 95% CI. The study was performed over 3 days with multiple lots of NeuMoDx reagents. 42 replicates at each dilution level were processed (positive samples) and 8 replicates for negative samples, per day.

Positive detection rates for LoD determination of the NeuMoDx HAdV Quant Assay

Target conc. (Copies/ml)	Target conc. (Log_{10} copies/ml)	Plasma/serum (550 µl workflow)		
		No. valid tests	No. positives	Detection rate
200	2.30	42	42	100%
100	2.00	42	41	97.62%
70	1.85	42	39	92.86%
50	1.48	42	20	47.62%
NEG	0.00	24	0	0%

LoD of the NeuMoDx HAdV Quant Assay in plasma/serum (550 µl workflow) was 100 copies/ml (2 Log_{10} copies/ml) with 95% CI: 82.85 copies/ml.

LLoQ and ULoQ

Lower limit of quantitation (LLoQ) and upper limit of quantitation (ULoQ) are defined as the lowest and upper target levels at which >95% etection is achieved AND the total analytical error* (TAE) ≤1.0. To determine LLoQ and ULoQ, TAE was calculated for each of the AdV target levels >95% detection.

*TAE: Bias + 2*SD (Westgard Statistic)

NeuMoDx HAdV Quant Assay ULoQ and LLoQ, with bias and TAE

Target conc. (Copies/ml)	Target conc. (Log_{10} copies/ml)	Plasma/serum (550 µl workflow)				
		Average conc. (Log_{10} copies/ml)	Detection (%)	SD	Bias	TAE
3.23 x 10^8	8.5	9.11	100	0.16	0.61	0.93
200	2.30	2.46	100	0.15	0.16	0.46
100	2.00	2.23	97.62	0.26	0.23	0.75
70	1.85	2.13	92.86	0.31	0.28	0.91
30	1.48	2.08	47.62	0.22	0.61	1.04

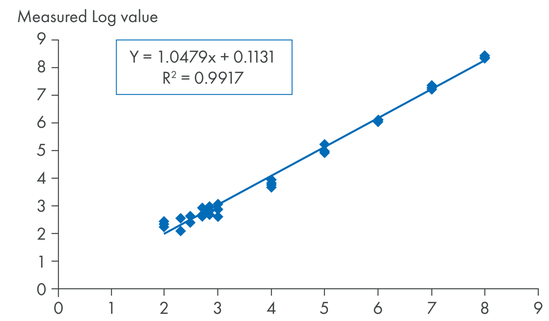
LoD and LLoQ of the NeuMoDx HAdV Quant Assay were both determined to be 100 copies/ml (2 Log_{10} copies/ml) for plasma/serum with the 550 µl workflow. ULoQ is 3.23 x 10^8 copies/ml.

Analytical sensitivity: linearity

Linearity was established in plasma/serum by preparing a dilution series using 11 serial dilutions of AdV Synthetic Plasmid prepared in HAdV-negative Base Matrix 53 spanning a concentration range of 8–2 Log_{10} copies/ml for 550 µl.

Linearity of the NeuMoDx HAdV Quant Assay across genotypes

Genotype	Linearity equation y = NeuMoDx HAdV Assay C_t x = dilution series	R^2
Reference sequence	y = –3.529x -0.7881	0.99
HAdV A	y = –3.626x + 1.348	0.99
HAdV B1	y = –3.449x + 1.1285	0.97
HAdV B2	y = –3.911x – 2.079	0.99
HAdV D	y = –3.384x + 3.9873	0.99
HAdV E	y = –3.687x – 1.2335	0.99
HAdV F	y= –3.036x + 5.28965	0.98



Linearity of the NeuMoDx HAdV Quant Assay for plasma/serum (550 µl workflow) specimens.

Analytical specificity: cross-reactivity and interfering substances

Cross-reactivity: Analytical specificity was demonstrated by screening 23 organisms commonly found in plasma/serum, as well as species phylogenetically similar to AdV for cross-reactivity. Organisms were prepared in pools of between 5/6 organisms and tested at a high concentration. Two organisms (*E. coli* and HCV) were analyzed in silico. No cross-reactivity was observed with any of the organisms tested, confirming 100% analytical specificity of the NeuMoDx HAdV Quant Assay.

Interference testing – exogenous agents (drug classifications)

Pool	Drug name	Classification
Pool 1	Valganciclovir	Antiviral
	Prednisone	Immunosuppressive
	Cidofovir	Antiviral
	Cefotaxime	Antibiotic
	Mycophenolate mofetil	Immunosuppressive
Pool 2	Vancomycin	Antibiotic
	Tacrolimus	Immunosuppressive
	Famotidine	Histamine antagonist
	Valacyclovir	Antiviral
	Leflunomide	Immunosuppressive

Interference testing – exogenous and endogenous agents

	Average conc.	Bias (absolute)
Endogenous (plasma/serum)	**Log_{10} copies/ml**	**Log_{10} copies/ml**
Triglycerides 500 mg/dl	2.03	0.46
Conjugated bilirubin (0.25 g/l)	2.21	0.28
Unconjugated bilirubin (0.25 g/l)	2.71	0.22
Albumin (58.7 g/l)	2.74	0.25
Exogenous (drugs)		
Pool 1: Valganciclovir, prednisone, cidofovir, cefotaxime, mycophenolate mofetil	2.83	0.08
Pool 2: Vancomycin, tacrolimus, famotidine, valacyclovir, leflunomide	2.52	0.23

Interfering substances: The NeuMoDx HAdV Quant Assay was evaluated in the presence of typical exogenous and endogenous interfering substances encountered in HAdV clinical plasma/serum specimens. Each substance was added to screened HAdV-negative Base Matrix 53 spiked with 2.5 Log_{10} copies/ml HAdV and samples were analyzed for interference. None of the exogenous and endogenous substances affected the specificity of the NeuMoDx HAdV Quant Assay.

Precision and reproducibility

Within-lab precision

Precision was determined by testing two replicates of a 5-member panel of AdV specimens prepared with HAdV plasmid twice a day, using one NeuMoDx 96 System for 20 days. The within-run, between-run, within-day and between-day precisions were characterized, and the overall standard deviation determined to be ≤0.30 Log_{10} copies/ml. Excellent precision was demonstrated across days and runs.

Lot-to-lot reproducibility

Determined using a 5-member panel of HAdV prepared with HAdV plasmid was used to assess performance on one NeuMoDx 96 Molecular System across three separate runs. Maximum overall bias was 0.39 Log_{10} copies/ml. Equivalent performance was demonstrated across lots as quantitation of all panel members was within tolerance specification.

Instrument-to-instrument reproducibility

Determined using three different systems (two NeuMoDx 288 Molecular System and one NeuMoDx 96 Molecular System). A 5-member panel of HAdV prepared with HAdV plasmid was used to assess performance. Testing was performed in parallel on the systems for 5 days. The variation within-day and between systems was characterized, and the overall standard deviation was ≤0.30 Log_{10} copies/ml. Equivalent performance was demonstrated across systems as SD in quantitation of all panel members was within tolerance specification.

Within-lab precision – NeuMoDx HAdV Quant Assay on NeuMoDx Systems

Sample	Within-day SD (Log_{10} copies/ml)	Between-day SD (Log_{10} copies/ml)	Within-run SD (Log_{10} copies/ml)	Between-run SD (Log_{10} copies/ml)	Within-lab SD (Log_{10} copies/ml)
	Plasma/serum specimen (input 550 µl)				
5.51 Log_{10} copies/ml	0.15	0.13	0.15	0.01	0.19
4.51 Log_{10} copies/ml	0.17	0.10	0.17	0.05	0.20
3.51 Log_{10} copies/ml	0.18	0.00	0.12	0.14	0.19
2.51 Log_{10} copies/ml	0.16	0.07	0.15	0.03	0.17
0 Log_{10}copies/ml	0.00	0.00	0.00	0.00	0.00

Lot-to-lot reproducibility – NeuMoDx HAdV Quant Assay

Sample	Absolute bias between Lot 1 and Lot 2 (Log_{10} copies/ml)	Absolute bias between Lot 1 and Lot 3 (Log_{10} copies/ml)	Absolute bias between Lot 2 and Lot 3 (Log_{10} copies/ml)
	Plasma/serum specimen ((input 550 µl)		
5.51 Log_{10} copies/ml	0.26	0.28	0.02
4.51 Log_{10} copies/ml	0.00	0.17	0.17
3.51 Log_{10} copies/ml	0.27	0.17	0.10
2.51 Log_{10} copies/ml	0.39	0.08	0.31
0 Log_{10} copies/ml	0.00	0.00	0.00

Instrument-to-instrument reproducibility – NeuMoDx HAdV Quant Assay

Sample	Repeatability SD (Log_{10} copies/ml)	Between-day SD (Log_{10} copies/ml)	Within-instrument SD (Log_{10} copies/ml)	Between-instrument (Log_{10} copies/ml)	Reproducibility SD (Log_{10} copies/ml)
	Plasma/serum specimen (input 550 µl)				
5.51 Log_{10} copies/ml	0.13	0.04	0.14	0.05	0.14
4.51 Log_{10} copies/ml	0.12	0.00	0.14	0.04	0.15
3.51 Log_{10} copies/ml	0.14	0.00	0.14	0.10	0.17
2.51 Log_{10} copies/ml	0.18	0.00	0.18	0.08	0.19
0 Log_{10} copies/ml	0.00	0.00	0.00	0.00	0.00

Conclusion

The NeuMoDx HAdV Quant Assay provides a rapid, easy-to-use and automated method for detection and quantification of HAdV DNA in plasma specimens.

The NeuMoDx HAdV Quant Assay is for in vitro diagnostic use.

Sample to Insight