Evaluation of a Next-Generation Sequencing Metagenomics Assay to Detect and Quantify DNA Viruses in Plasma from Transplant Patients

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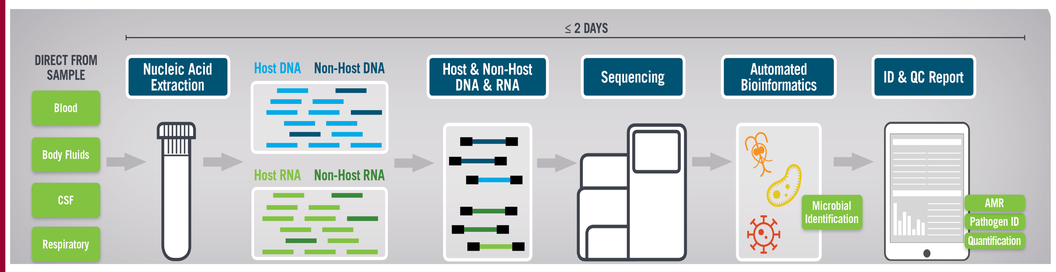
Background

- Viral infections are major causes of morbidity and mortality in solid organ and hematopoietic stem cell transplant patients
- Cytomegalovirus (CMV), Epstein–Barr virus (EBV) and BK virus (BKV) are among the most common infections after allogeneic transplantations and are individually tested using qPCR assays
- Metagenomics based on shotgun next generation sequencing allows an unbiased approach for the detection of nearly all potential pathogens in a single assay
- The Galileo Pathogen Solution (GPS, Arc Bio, LLC) RUO assay is a sample to result metagenomic pipeline designed to simultaneously detect and quantify 10 DNA viruses (CMV, EBV, BKV, HAdV, JCV, HSV-1, HSV-2, VZV, HHV-6 A and HHV-6 B) and to qualitatively detect B19 and TTV
- The objective of the study was to evaluate the performance characteristics of the GPS assay by comparing with standard of care qPCR assays using viremic plasma from transplant patients

Materials & Methods

- The performance of the GPS assay was evaluated for linear dynamic range using a calibration panel that consists of 11 viruses (CMV, EBV, BKV, HAdV, JCV, HSV-1, HSV-2, VZV, HHV6 A and B, B19; Arc Bio, LLC) at 100,000 IU or cp/mL, 10,000 IU or cp/mL, 5000 IU or cp/mL, 1000 IU or cp/mL and a negative plasma matrix tested in quadruplicate
- Retrospective and prospective residual viremic plasma samples, n=47 (CMV= 29 , BKV=17, HSV1-1) obtained for routine clinical testing were evaluated in the pilot study using beta reagents and software
- Total nucleic acid was extracted from 0.4 mL of plasma using the EZ1 DSP Virus kit (QIAGEN), followed by DNA library preparation with pathogen enrichment/human background depletion, sequencing (NextSeq® 500, Illumina®), and automated data analysis
- Four controls (positive external control, high control, low control and a negative matrix control) were included along with patient samples
- Sequencing reads were filtered based on sequence quality and queried against a curated selection of references
- Additional viruses were confirmed using qPCR assays including artus EBV and BKV (Qiagen) and RealStar® HHV-6 and JCV PCR Kits (Altona) on Rotor-Gene Q MDx platform (QIAGEN)

Fig 1: Workflow of Galileo Pathogen Solution (GPS) assay



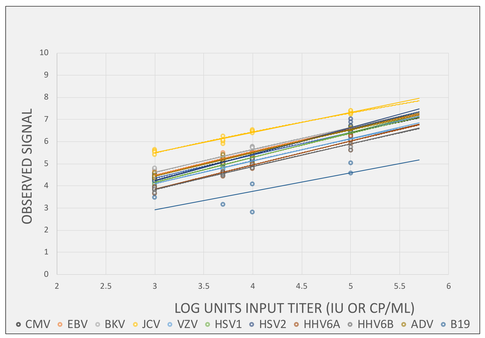
Results

- With an average depth of 34 million paired-end reads, the calibration panel was found to be linear with a 100% sensitivity with the exception of B19 which is only qualitatively detected by the assay
- The 47 residual clinical samples tested had an 100% agreement in detecting viruses and were accurately quantified using the standard curve generated from the calibration panel with viral loads ranging from 1.15-6.82 log10 IU/mL or cp/mL
- The mean difference between the GPS and CMV Qiagen Artus assay was 0.02 log_{10}IU/mL (SD±0.32 log_{10} IU/mL); mean difference between the GPS and CMV Abbott Real-time assay was 0.44 log_{10}IU/mL (SD±0.32 log_{10}IU/mL); mean difference between the GPS and BKV Qiagen Artus assay was -0.31 log10cp/mL (SD±0.36 log10cp/mL)
- The automated Galileo Analytics bioinformatics tool detected and quantified virus(es) with an average time of 30-45 minutes/sample

Table 1: Summary of the coefficient of correlation and slope values for each virus from linear range evaluation

Virus	Percent of replicates with recovered signal	R^2	Slope	Intercept
CMV	100.0	0.99	1.03	1.26
EBV	100.0	0.97	1.06	1.31
BKV	100.0	0.97	1.01	1.59
JCV	100.0	0.98	0.91	2.78
VZV	100.0	0.99	1.02	1.06
HSV1	100.0	0.95	1.13	0.76
HSV2	100.0	0.95	1.21	0.61
HHV6A	100.0	0.93	1.09	0.60
HHV6B	100.0	0.96	1.03	0.77
ADV	100.0	0.98	1.06	1.28
B19	37.5	0.57	0.83	0.42

Figure 2: Viral signal as a function of titer prediction for all viruses and replicates



Results

Table 2: Detection and quantification of CMV samples by GPS and Qiagen Artus/Abbott real-time assays

CMV Sample ID	GPS assay (Log10 IU/mL)	Real-time (Log10 IU/mL)	Log10 difference
ES1	4.61	4.40	0.21
ES2	4.53	4.54	0.01
ES3	6.82	6.52	0.3
ES4	3.62	3.34	0.28
ES5	5.24	4.94	0.3
ES6	4.10	3.85	0.25
ES7	3.90	3.85	0.05
ES8	3.91	3.66	0.25
ES9	3.80	3.62	0.18
ES10	3.64	3.45	0.19
SOS15	4.34	4.46	0.11
SOS16	5.08	5.29	0.21
SOS17	2.19	3.32	1.13
SOS18	3.86	4.00	0.14
SOS19	5.49	5.49	0
SOS20	5.42	5.36	0.06
SOS21	4.92	4.92	0
SOS22	4.12	4.16	0.04
SOS23	4.67	4.55	0.12
SOS24	4.50	4.67	0.17
SOS29	3.03	2.40	0.63
SOS30	2.27	2.42	0.15
SOS31	6.12	5.85	0.27
SOS33	4.16	3.48	0.68
SOS34	3.88	3.15	0.73
SOS35	3.35	2.80	0.55
SOS37	3.71	3.27	0.44
SOS38	3.64	2.86	0.78
SOS65	4.97	5.00	0.03

Table 3: Detection and quantification of BKV samples by GPS and Qiagen Artus assays

BKV Sample ID	GPS assay (Log10 cp/mL)	Real-time (Log10 cp/mL)	Log10 difference
SOS43	2.18	2.32	0.14
SOS44	3.13	2.35	0.78
SOS45	2.66	2.82	0.16
SOS46	3.12	2.98	0.14
SOS47	3.90	4.27	0.37
SOS48	4.53	4.81	0.28
SOS49	4.77	5.18	0.41
SOS50	5.26	5.86	0.60
SOS51	5.76	6.12	0.36
SOS52	5.80	6.27	0.47
SOS57	4.41	5.02	0.61
SOS58	4.31	4.64	0.33
SOS59	5.87	6.38	0.51
SOS60	3.62	4.22	0.60
SOS61	4.79	5.23	0.44
SOS63	2.12	2.16	0.04
SOS66	2.16	2.97	0.81

Figure 3: Comparison of GPS and Qiagen Artus assays by Bland-Altman analysis for the CMV samples

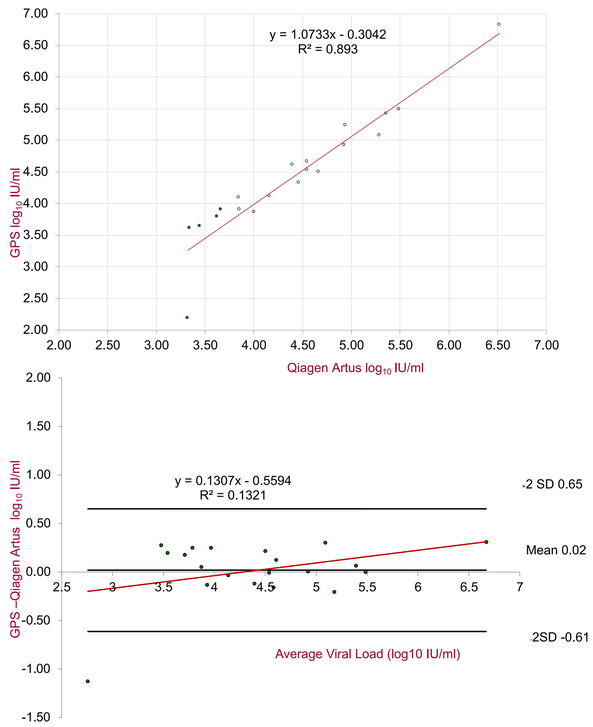


Figure 4: Comparison of GPS and Qiagen Artus assays by Bland-Altman analysis for the BKV samples

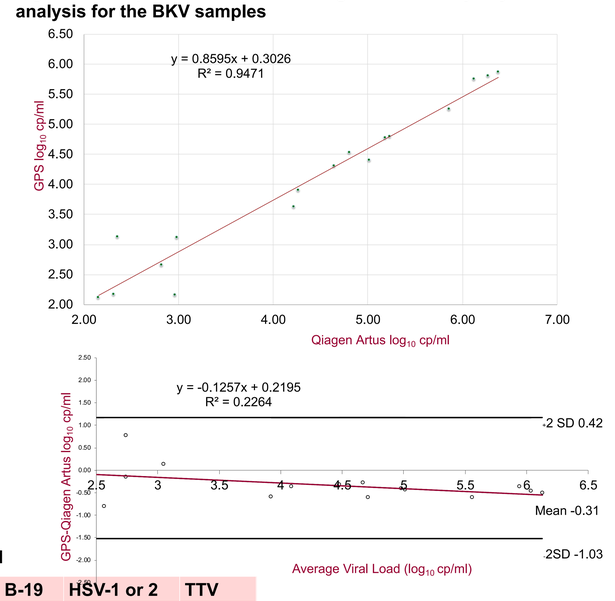


Table 4: Detection of additional viruses by GPS assay from the samples analyzed

Virus	EBV	BKV	CMV	VZV	HHV-6 A/B	JCV	HAdV	B-19	HSV-1 or 2	TTV
Number of samples	9	3	2	3	5	14	3	1	2	30

Conclusions

- The findings of this pilot study demonstrate that the GPS assay can simultaneously detect and quantify multiple viruses in transplant patients with results that are comparable to standard of care qPCR viral assays
- Further work will be performed to confirm the additional viruses detected (VZV, HAdV, B-19, HSV-1 and 2 and TTV)
- Future studies are required to evaluate the test on a larger number of clinical samples to better assess its use as a diagnostic tool for transplant recipients
- Potential advantages of metagenomics assays include, simultaneous detection and quantification from a single blood draw, ability to use the metagenomics data for antiviral resistance determination, and the presence of over 350 strains in the bioinformatics pipeline, which may circumvent challenges seen with qPCR and diverse viruses such as HAdV

Acknowledgements

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