Performance of an NGS-based metagenomics assay to simultaneously detect and quantify multiple DNA viruses

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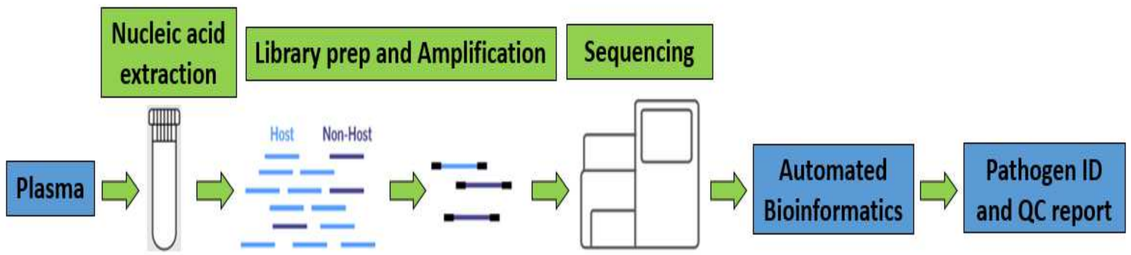
Introduction

Viral infections in solid organ and bone marrow transplant recipients can cause outcomes ranging from severe illness to transplant failure and even death (1). Current diagnostic assays use PCR to measure qualitative and quantitative presence of a target virus enabling clinical assessment of progression and the efficacy of treatment (2), however these require multiple reactions and fail to detect non-targeted co-infections, which are common in immunocompromised patients. Metagenomic next-generation sequencing (mNGS) has the potential to determine the presence and abundance of transplant-related viruses, as well as identify co-infections, in an unbiased manner.

Galileo™ Pathogen Solution is a sample to report mNGS pipeline for comprehensive pathogen detection that eliminates the need to query specific pathogens independently. In this RUO beta study, we demonstrate the use of an mNGS pipeline using Arc Bio Galileo™ Pathogen Solution (GPS) for identification of DNA viruses in residual EDTA plasma specimens by comparing with standard of care qPCR assays (Figure 1).

Figure 1. Overview of the Galileo Pathogen Solution Next Generation Sequencing pipeline



Materials and Methods

Patients, samples and qPCR

Thirty residual EDTA whole blood samples were selected from SOT/HCST patients admitted at Great Ormond Street Hospital for Children, London that were positive by routine PCR testing for one or more of the target viruses (listed in Table 1). Plasma was separated and screened by real-time PCR to confirm the presence and quantity of target viruses. Twenty three plasma samples were positive for the virus of interest and taken forward for sequencing.

Extraction, library prep and sequencing

Total nucleic acid was extracted from 400 µl plasma using the EZ1 Virus Mini Kit 2.0 (QIAGEN Ltd), followed by DNA library preparation with pathogen enrichment and human background depletion using the GPS kit (Arc Bio, LLC, Cambridge, MA). Sequencing was performed on the Illumina® NextSeq 500 with a high output kit. Data were analyzed using the Galileo™ Analytics web platform. In brief, sequencing reads were filtered based on sequence quality and queried against a curated database of over 350 reference sequences from the viruses in Table 1.

Table 1. Viruses included in sequence analysis database

Transplant related viruses			
Cytomegalovirus (CMV)	Herpes Simplex Virus 1 (HSV1)	Varicella Zoster Virus (VZV)	Parvovirus B19 (B19)
Epstein-Barr Virus (EBV)	Herpes Simplex Virus 2 (HSV2)	BK Virus (BKV)	Torque Teno Virus (TTV)
Human Adenovirus (ADV)	Human Herpesvirus 6 (HHV)	JC Virus (JCV)	

Table 2. qPCR vs. Galileo™ Pathogen Solution. Additional viruses detected by sequencing are underlined. Viruses missed by sequencing are shown in **bold**

Virus detected by targeted qPCR	qPCR Ct value (viral load where available)	Virus(es) detected by Galileo™ Pathogen Solution
EBV HHV6	36 (11,803 cp/ml) 32	**EBV not detected** HHV6
BKV	25	BKV JCV, CMV, TTV
VZV	25	VZV, TTV
EBV	36 (12,545 cp/ml)	**EBV not detected**
HAV CMV BKV	32 (377,851 cp/ml) 33 (45,196 cp/ml) 32	HAV CMV BKV JCV, TTV
HAV	25 (86,374,800 cp/ml)	HAV TTV
EBV	26 (12,376,100 cp/ml)	EBV TTV
HAV CMV BKV	30 (1,430,440 cp/ml) 34 (22,234 cp/ml) 29	HAV CMV BKV JCV, TTV
HAV	32 (387,283 cp/ml)	HAV
EBV HAV	27 (7,286,590 cp/ml) 37 (5,036 cp/ml)	EBV HAV TTV
HAV	19 (4,841,490,000 cp/ml)	HAV
EBV HAV BKV	39 (1,689 cp/ml) 35 (37,542 cp/ml) 22	**EBV not detected** HAV BKV JCV, TTV
HHV6	26	HHV6
HHV6	27	HHV6
EBV HAV	27 (5,522,430 cp/ml) 37 (5,969 cp/ml)	EBV HAV TTV
HHV6	38	**HHV6 not detected**
EBV HHV6	36 (4,224 cp/ml) 37	**EBV not detected** HHV6 B TTV
EBV HAV	32 (172,058 cp/ml) 36 (19,190 cp/ml)	EBV HAV TTV
EBV HAV	32 (194,654 cp/ml) 28 (4,997,800 cp/ml)	EBV HAV TTV
BKV JCV	31 37	BKV **JCV not detected** TTV
CMV	32 (79,838 cp/ml)	CMV
EBV BKV	34 (17,554 cp/ml) 31	EBV BKV JCV, TTV
EBV CMV	37 (3,281 cp/ml) 32 (34,071 cp/ml)	**EBV not detected** CMV

Results

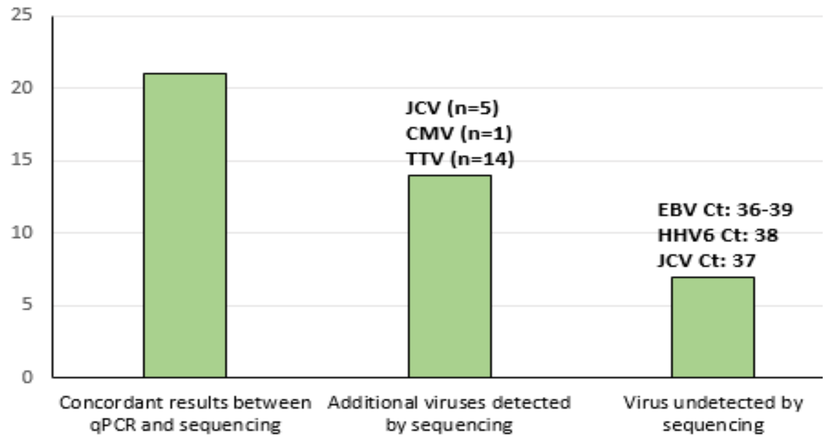
Sequencing outcomes

22/23 libraries processed had total >30M reads per sample (average number of reads generated 54M per sample). The Galileo™ Analytics web platform provided a Pathogen Detection report and a Quality Control report for all libraries processed. All samples (23/23) had detectable internal control and normalization controls.

Sequencing vs qPCR results

21/23 samples had concordant results between PCR and sequencing (Table 2). For 14/23 samples additional viruses were detected by sequencing that had not been identified by PCR. For 7/23 samples a virus was detected at low level in plasma by PCR but was not detectable by sequencing (Figure 2).

Figure 2. Sequencing vs qPCR results



Conclusions

This proof of concept dataset suggests that routine application of mNGS can enable the detection of multiple viral targets in peripheral blood. The provision of all reagents and analysis tools in the GPS kit facilitates mNGS in non-expert laboratories. In the majority of samples GPS identified the same or more viruses that were detected using qPCR, demonstrating the un-biased nature of metagenomics. Confirmatory testing is ongoing for samples with low viral load that were not detected by GPS and for JC virus that was not detected by PCR.

The inclusion of normalisation controls in each sample gives the potential to determine the viral load from the sequencing data; further work is ongoing to investigate the utility of GPS for viral load monitoring.

References
1. *J. A. Fisherman. Am J Transplant 17, 856-879 (2017).*
2. *M. M. Abecassis et al. Transplantation 63, 275-279 (1997).*