Evaluation of the Galileo™ Pathogen Solution Next-Generation Sequencing Pipeline for the Identification and Quantification of DNA Viruses in Transplant Patients

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Introduction

Solid organ and hematopoietic stem cell transplant recipients are uniquely susceptible to infections, often with increased severity, due to a number of common and opportunistic viruses. Specifically, viral infections including cytomegalovirus (CMV), Epstein-Barr virus (EBV), BK virus (BKV), human herpesvirus-6 (HHV6) and human Adenovirus (ADV) can cause outcomes ranging from severe illness to transplant failure and even death (1).

There are only handful of commercial assays available for detecting/ quantifying CMV, with the remainder of assays available as either ASRs or LDTs. These tests measure the presence of and/or viral load of the target virus, enabling clinical assessment of progression and the efficacy of treatment (2). However, all of these tests are run individually, and they fail to detect non-targeted co-infections, which are common in immunocompromised patients.

Next-generation sequencing (NGS) has the potential to enable clinicians to determine the presence and abundance of transplant-related viruses with unprecedented precision, as well as identify co-infections in an unbiased manner. Sequencing of clinical samples can reveal pathogens that targeted investigations miss, including unculturable or closely related species, all in a single test.

Arc Bio is developing the Galileo™ Pathogen Solution (Galileo™), a research use only sample-to-report next-generation sequencing pipeline to enable comprehensive testing and eliminate the need to query pathogens independently (Figure 1). In this proof of principle study, we demonstrate the first use of this pipeline for the identification and quantification of DNA viruses in residual EDTA plasma specimens.

Figure 1: Overview of the Galileo™ Pathogen Solution pipeline

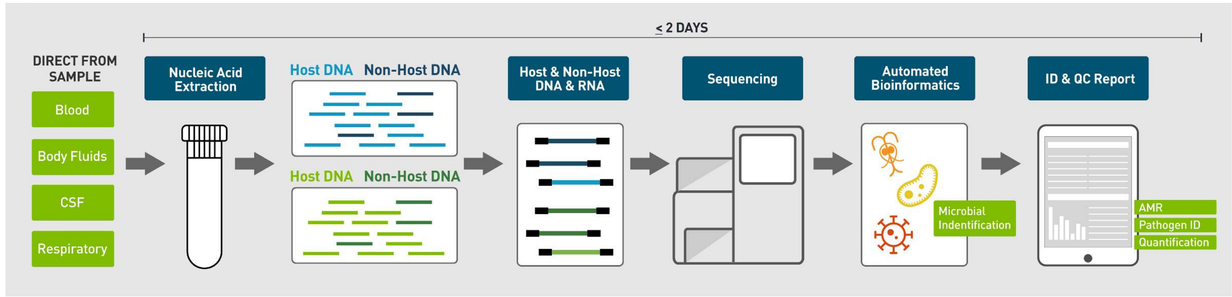


Figure 2: Transplant-related viruses detected

Transplant-related viruses detected by the Galileo™ Pathogen Solution pipeline
Cytomegalovirus (CMV)
Epstein-Barr Virus (EBV)
Human Adenovirus (ADV)
BK Virus (BKV)
Human Herpesvirus 6 (HHV6 A & B)
Herpes Simplex Virus 1 (HSV1)
Herpes Simplex Virus 2 (HSV2)
JC Virus (JCV)
Varicella Zoster Virus (VZV)
Parvovirus B19 (B19)
Torque Teno Virus (TTV)

Materials and Methods

Single- and multi-transplant virus linearity panels (9 individual viruses and 9 viruses combined; excluding HHV-6B, HSV2, and TTV) were prepared to determine linear range using donor EDTA plasma spiked with 10-fold dilutions of standard material from 10^2 to 10^5 cp or IU/mL (Exact Diagnostics). In brief, total nucleic acid was extracted from 0.4 mL of plasma using the EZ1 Virus Mini Kit 2.0 (QIAGEN), followed by DNA library preparation with pathogen enrichment/human background depletion, sequencing (NextSeq® 500, Illumina®), and automated data analysis. Sequencing reads were filtered based on sequence quality and queried against a curated selection of references. In addition, 24 residual EDTA plasma samples previously tested by qPCR (CMV=4, EBV=4, ADV=4, BKV=4, HHV6=4, negative=4) were sequenced.

Results

All panels were linear from 10^2 to 10^5 cp or IU/mL (Figure 3). At an average sequencing depth of 25 million paired-end (PE) reads, sensitivity for ADV, BKV, CMV, EBV, and HHV6 at 10^2 cp or IU/ml ranged from 57% to 100% depending on the virus, and at 10^3 cp or IU/ml sensitivity was 100% for all five viruses (Figure 4). For the 24 residual clinical samples at an average of 15 million PE reads, we obtained a positive percent agreement of 100%, with viral loads ranging from <135 (CMV) to 112,813 IU/ml (BKV) (Figure 5). An additional 27 potential co-infections were detected by the Galileo pipeline, with 5 CMV co-infections confirmed by reviewing patient medical records. Insufficient volume was available for further discordant testing. Furthermore, the qPCR titers of the residual clinical samples correlated well with the standard curve generated from the multiplexed linearity panel (Figure 6).

Figure 3: Linearity of mixed and single-analyte viral panels

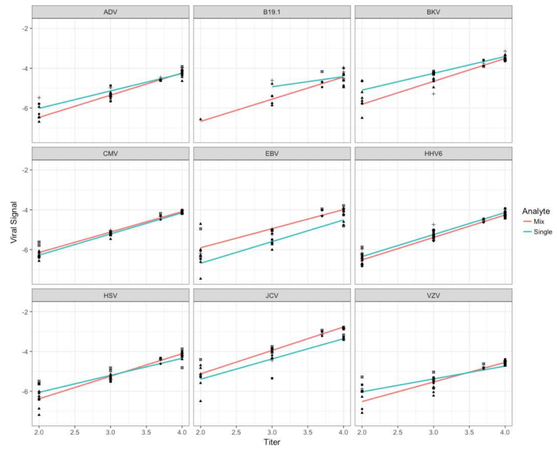


Figure 4: Sensitivity at 100 and 1,000 cp/mL or IU/mL

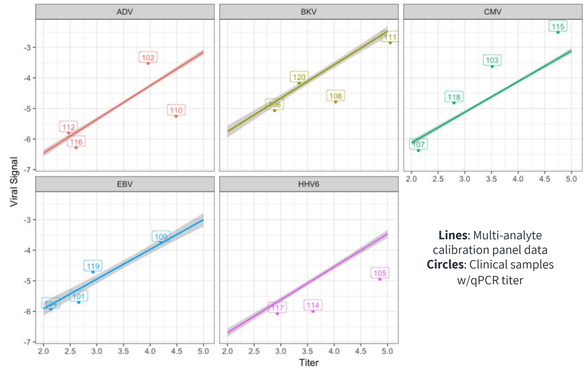
Spiked-in Virus	Titer (IU/mL or cp/mL)	Percent positive
ADV	100	4/7 (57.1%)
ADV	1,000	7/7 (100%)
BKV	100	4/7 (57.1%)
BKV	1,000	7/7 (100%)
CMV	100	6/7 (85.7%)
CMV	1,000	7/7 (100%)
EBV	100	6/7 (85.7%)
EBV	1,000	7/7 (100%)
HHV6	100	7/7 (100%)
HHV6	1,000	7/7 (100%)

Figure 5: Galileo™ results vs. qPCR

Targeted virus detected by qPCR	qPCR titer (IU/mL or cp/mL)	Virus(es) detected by Galileo™
ADV	295	ADVC, CMV*, TTV
ADV	409	ADVC, CMV*, TTV
ADV	9,148	ADVC, HHV6B
ADV	30,686	ADVF, CMV, TTV
BKV	765	BKV, CMV, TTV
BKV	2,209	BKV, TTV
BKV	10,645	BKV, CMV, TTV
BKV	112,813	BKV
CMV	<135	CMV, HHV6B, TTV
CMV	627	CMV
CMV	3,257	CMV, EBV, TTV
CMV	55,898	CMV, HHV6B, TTV
EBV	137	EBV, TTV
EBV	457	EBV
EBV	847	EBV
EBV	15,922	EBV
HHV6	249	HHV6B
HHV6	860	HHV6B
HHV6	4,000	HHV6B, EBV, TTV
HHV6	71,941	HHV6A, CMV*, TTV
Negative (BKV)	N/A	CMV*
Negative (BKV)	N/A	NONE
Negative (CMV)	N/A	TTV
Negative (CMV)	N/A	TTV

*Sample previously tested positive for CMV by qPCR

Figure 6: Quantification correlates with qPCR



Lines: Multi-analyte calibration panel data
Circles: Clinical samples w/qPCR titer

Conclusions

This proof of concept dataset suggests the Galileo™ Pathogen Solution assay may offer a promising solution for the comprehensive detection and quantification of common transplant-related DNA viruses at similar levels of sensitivity to standard-of-care qPCR methods, but from a single blood draw. Further work includes testing additional residual plasma samples at or around the qPCR lower limit of quantification, performing additional LOD and linear range experiments including HSV2, HHV6B, and TTV, and testing the performance of the assay with 50 additional plasma samples with sufficient volume to confirm any identified co-infections using orthogonal methods.

References and Acknowledgements

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

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