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ASM Microbe 2019 Abstract Summary

Karius Microbial Cell-free DNA Sequencing for Quantitative Detection of CMV, EBV, and BKV

Poster Session CPHM-10—Diagnostic Virology
Sunday | June 23 | 11:00 AM–1:00 PM

Poster CPHM-921

Evaluation of Karius Plasma Next Generation Sequencing of Cell-free Pathogen DNA to Detect and Quantitate Cytomegalovirus, Epstein-Barr Virus, and BK Virus

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Study Design

Residual frozen plasma samples from clinical testing by PCR for CMV (n=124; Roche Cobas® 6800/8800), EBV (n=49) and BKV (n=23) (3M Focus/Diasorin Integrated Cycler) were tested by Karius microbial cfDNA sequencing. Comparison between individual viral PCR tests and Karius results are reported.

Results

High sensitivity and strong quantitative correlation was observed between Karius microbial cell-free DNA sequencing and single analyte PCR tests for CMV, EBV, and BKV.

1. **CMV**— Karius microbial cell-free DNA sequencing showed 100% agreement with the cobas CMV testing across 124 samples, including 25 CMV-negative samples and 99 CMV-positive samples. Quantitative correlation was strong (r=0.95) across more than 5 orders of magnitude.

2. **EBV**— Karius demonstrated 100% agreement with EBV-positive samples having viral titer >1500 IU/mL (n=42), and 80% agreement with EBV-negative samples (n=10), with strong quantitative correlation (r=0.89) across four orders of magnitude.

3. **BKV**— Karius had 100% agreement with BKV-positive samples (n=31) and 90% agreement with EBV-negative samples (n=10), with strong quantitative correlation (r=0.95) across three orders of magnitude.

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ABSTRACT

Introduction

Cytomegalovirus (CMV), Epstein-Barr virus (EBV), and BK polyomavirus (BKV) can cause serious complications in transplant recipients, including pneumonitis and colitis, post-transplant lymphoproliferative disorder, and polyomavirus-associated nephropathy in kidney transplant recipients. Viral monitoring is widely used to monitor disease caused by these viruses, but each virus is currently measured separately using a single-analyte PCR assay. Microbial cell-free DNA (mcfDNA) sequencing can quantify hundreds of viruses simultaneously direct from plasma. We compared quantitative microbial cell-free DNA sequencing to individual qPCR tests for CMV, EBV, and BKV viruses in transplant recipients.

Methods

Residual plasma samples from transplant recipients submitted for routine testing by qPCR for CMV (n=125; Roche cobas 6800/8800), EBV (n=76), and BKV (n=47) (DiaSorin Integrated Cycler) were sent frozen to Karius for mcfDNA sequencing. Samples ranged in viral load from undetectable to 5,000,000 IU/mL. Plasma samples from 507 healthy individuals were also analyzed. Microbial cfDNA was extracted from 250 uL of plasma and sequenced. Human reads were removed and remaining sequences were aligned to a curated microbial database. Results were reported as molecules of viral cfDNA per microliter of plasma (MPM). The Pearson correlation and linear regression analyses were performed on log10-transformed values from viral PCR and mcfDNA sequencing for all samples quantified by comparator tests.

Results

Viral cfDNA was detected in 100% (90/90), 90.5% (38/42), and 100% (31/31) of samples with quantifiable CMV, EBV, and BKV detection by PCR, respectively. All samples with CMV, EBV, or BKV viral titers above 1,500 IU/mL (90/90) were detected by mcfDNA sequencing. Pearson correlation coefficients of paired results from PCR and mcfDNA sequencing were 0.95, 0.89, and 0.95 for CMV, EBV, and BKV, respectively. The number of cfDNA fragments detected per international unit varied by virus, with approximately 1,267 CMV, 36 EBV, and 29 BKV cfDNA fragments detected per IU of virus based on linear regression. Microbial cfDNA sequencing of plasma from 507 healthy individuals showed undetectable CMV, EBV, and BKV cfDNA in 95.9%, 91.1%, and 100% of samples, respectively, with all viral cfDNA concentrations in the remaining samples correlating to viral titers <1 IU/mL.

Conclusions

Microbial cell-free DNA sequencing of plasma from transplant recipients enabled multiplexed quantification of CMV, EBV, and BK polyomavirus with sensitivity similar to single-analyte PCR, and strong quantitative correlation. These data show the potential utility of mcfDNA sequencing as a single test for quantitative monitoring of multiple viruses in transplant recipients.

Sample Processing and Workflow

RESULTS

Figure 1: Performance of Karius Cell-free DNA Sequencing Versus Single-Analyte qPCR Testing in Transplant Recipients

	Single-Analyte PCR > 1500 IU/mL virus	Single-Analyte PCR > titer minimum virus	Single-Analyte PCR < titer minimum virus
Karius CMV cfDNA	100% Sensitivity (49/49)	100% Sensitivity (90/90)	100% Sensitivity (9/9)
Karius EBV cfDNA	100% Sensitivity (18/18)	90.5% Sensitivity (38/42)	55% Sensitivity (11/20)
Karius BKV cfDNA	100% Sensitivity (23/23)	100% Sensitivity (31/31)	83.3% Sensitivity (5/6)

Agreement between Karius cell-free DNA detection and positive single-analyte qPCR tests. The titer minimum refers to the published limit of quantitation. The cobas CMV 6800/8800 titer minimum was 34.5 IU/mL. The DiaSorin Integrated Cycler EBV titer minimum was 500 IU/mL. The DiaSorin Integrated Cycler BKV titer minimum was 200 IU/mL.

Single-Analyte PCR Not Detected	Untested Healthy Plasma Donors
100% Specificity (25/25)	95.9% Negative (486/507)
80% Specificity (8/10)	91.1% Negative (462/507)
90% Specificity (9/10)	100% Negative (507/507)

Agreement between Karius cell-free DNA detection and negative single-analyte qPCR tests or untested healthy plasma donors. Any detection of cell-free DNA fragments mapping uniquely to the virus of interest were scored as positive by Karius cfDNA sequencing.

Figure 2: Correlation of Karius cfDNA Sequencing with Single-Analyte qPCR

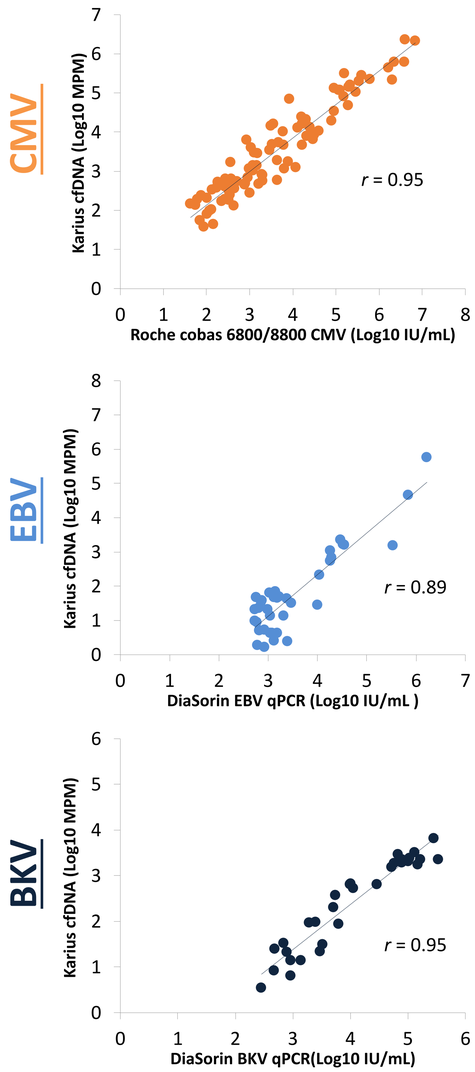

Figure 3: Distribution of cell-free DNA concentrations in various categories

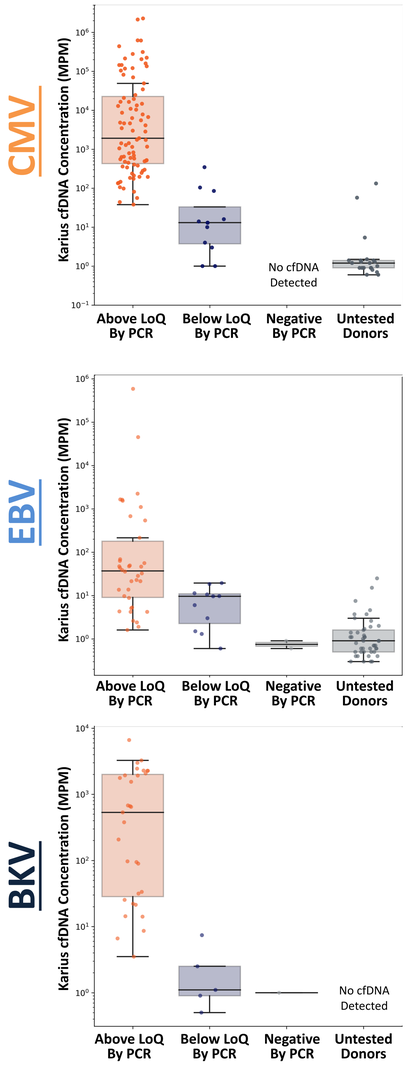

Figure 4: Summary Statistics for Quantitative Correlation

	CMV	EBV	BKC
Pearson Correlation (Karius MPM vs. qPCR)	0.95 (N=90)	0.89 (N=38)	0.95 (N=31)
cfDNA Fragments per International Unit	1267	36	29
Cell-free genomes per International Unit	~50%	~2%	~50%

CONCLUSIONS

1. Karius microbial cell-free DNA sequencing showed sensitivity similar to single-analyte PCR for detection of CMV, EBV, and BKV from plasma.
2. The concentration of viral cfDNA fragments measured by Karius was strongly correlated with single-analyte qPCR measurements of viral titer.
3. Tens to thousands of viral cfDNA fragments per international unit of virus are detected in plasma by microbial cfDNA sequencing.
4. The concentration of viral cfDNA in plasma was extremely low, when detected at all, in samples negative by PCR and in untested healthy plasma donors.
1. These data show the potential utility of mcfDNA sequencing as a single test for quantitative monitoring of multiple viruses in transplant recipients

Conflict of Interest Disclosures:

This study was funded by Karius, Inc. TB, CH, HS, DH, LB, and DKH are employees of Karius, Inc.