Evaluation of cfDNA Extraction Methods for One Lambda™ Devyser Accept cfDNA Assays

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Abstract

Purpose: Monitoring the health of an organ following transplantation is crucial to the selection of timely and optimal treatment strategies. The One Lambda™ Devyser Accept cfDNA kit enables detection of cell-free DNA (cfDNA) from up to 3 genotypes in circulation following kidney transplantation. To accurately measure the genotype-of-origin proportions of circulating cfDNA, cfDNA isolated from plasma must be of sufficient purity and concentration. The objective of this study was to assess the compatibility of different cfDNA extraction methods with the One Lambda™ Devyser Accept cfDNA kit.

Methods: Three cfDNA extraction kits- EZ1&2 ccfDNA Kit (Qiagen), MagMAX Cell-Free DNA Kit (ThermoFisher), and Maxwell RSC ccfDNA LV Plasma Kit (Promega), automated on corresponding instruments – were utilized. Each extraction platform was evaluated using three plasma samples from 10 donors, and artificial plasma (Biochemazone) spiked with sheared genomic DNA and interfering substances. Quality and yield of cfDNA were assessed, and libraries were generated for downstream sequencing. Variant allele frequency (VAF) compliance and substance interference were analyzed.

Results: cfDNA samples obtained with the Qiagen EZ1 kit passed extraction and sequencing quality control, with occasional bead carry-over requiring removal. cfDNA purified with Thermo Fisher MagMAX / KingFisher Flex passed criteria but required elution in maximum volume for consistent top quality library generation. cfDNA obtained with Promega Maxwell passed criteria with proteinase K pre-treatment implemented, and a 1:1dilution with elution buffer improved PCR amplification. All kits produced cfDNA suitable for downstream NGS library generation and accurate measurement of cfDNA proportions.

Conclusion: The study demonstrates that the Qiagen EZ1 kit requires no modifications, while slight adjustments are needed for MagMAX and Maxwell kits to produce consistent high-quality cfDNA for downstream NGS analysis. These findings support the use of different extraction methods with the One Lambda Devyser Accept cfDNA assay.

Introduction

Cell-free DNA (cfDNA) is a form of degraded genomic DNA released into circulation following necrosis or apoptosis. Many studies have demonstrated that donor-derived cell free DNA (dd-cfDNA) released from donated organs is a marker of graft allograft injury[1,2]. The One Lambda™ Devyser Accept cfDNA kit accurately detects dd-cfDNA fractions with high sensitivity. Here, we demonstrated that the One Lambda™ Devyser Accept cfDNA kit is compatible with popular cfDNA isolation protocols with and without the presence of common interfering substances.

Materials

Sample Preparation

Blood samples for this study were taken from healthy donors and collected in Streck Cell-Free DNA Blood Collection tubes by Discovery Life Sciences (Huntsville, AL, USA). Each blood collection tube contained 8 mL of blood, which yielded 4 mL of plasma following double centrifugation. 4 mL plasma samples were then subjected to cfDNA purification using the Qiagen EZ1&2 ccfDNA kit , ThermoFisher MagMAX kit on a 24-well format KingFisher Flex instrument, or Promega Maxwell RSC ccfDNA LV Plasma Kit.

Interfering substance testing was performed by dissolving interfering substances to final concentrations (Table 1) in artificial plasma (Biochemazone; Leduc, Alberta, CAN). Samples were spiked with 5% donor /95% recipient sheared genomic DNA (~166 bp) at a final concentration of 0.01875 ng/µL.

Methods

Test Methods

Testing was divided into two phases: 1) test whether each cfDNA extraction method was compatible with the One Lambda™ Devyser Accept cfDNA kit using real blood samples and 2) test whether interfering substances spiked into each sample interfered with the One Lambda™ Devyser Accept cfDNA assay when extracted using each tested cfDNA extraction method.

For real blood testing, 3 tubes from 10 donors were used as input. To pass cfDNA extraction testing, at least 90% of samples had to passed acceptance criteria which was defined as: =>80% cfDNA purity measured by an Agilent Cell-free DNA ScreenTape Analysis and => 0.1 ng/µL yield measured on a ThermoFisher Qubit Fluorometer. Upon passing cfDNA extraction testing, 5 samples from different donors were used to generate sequencing libraries and sequenced as “Screening” samples on Illumina sequencers.

For interfering substances testing, 9 interfering substances were testing across all extraction methods (Table 1). Each interfering substance was tested in triplicate with 4 mL used as input to each cfDNA extraction method. cfDNA extraction testing was assessed for quality using the same metrics as the real blood testing. However, all samples were made into sequencing libraries and sequenced as “Monitoring” samples by Illumina sequencing.

Table 1. Interfering Substance Dilutions

Interfering Substance	Final Substance Concentration (mg/mL)
Albumin	60
Hemoglobin	10
Bilirubin, conjugated	0.4
Bilirubin, unconjugated	0.4
Intralipid-20%	15
EDTA	0.00339
Cyclosporine	0.0018
Mycophenolic acid	0.042
PBS	NA (20% by volume)

Data Analysis

Both real blood and interfering substance samples were analyzed using Advyser Solid organs software.

For real blood samples, the variant allele fraction (VAF) was analyzed to ensure that each sample had allele ratios within the normal range. A passing VAF range was defined as >99% or <1% allele fraction for a homozygous locus and 40%-60% allele fraction for a heterozygous locus.

For interfering substance testing, substances were assessed by analyzing the coefficient of variation (CV) of percentage donor results relative to PBS spike-in control samples. An interfering substance that resulted in a < 5% CV relative to PBS-spiked controls was considered acceptable.

Results

cfDNA extraction kits provide high quality cfDNA

Each cfDNA extraction platform that was tested was able to reliably extract high quality cfDNA from human plasma samples. All kits provided adequate cfDNA yield (Figure 1) and quality (Figure 2) as defined in Test Methods.

The Qiagen EZ1&2 ccfDNA Kit required no modifications from the standard protocol. The ThermoFisher MagMAX Cell-Free DNA Kit run on the ThermoFisher KingFisher Flex (24 well format) also required no modifications to the standard protocol but performed most reliably when samples were eluted in 100 uL. The Promega Maxwell RSC ccfDNA LV Plasma Kit required additions to the standard protocol to ensure reliable cfDNA extraction. First, 4 mL plasma samples were incubated at 60C for 20 minutes with 60 µL Proteinase K (20 mg/mL) and 200 µL 20% SDS solution. Second, cfDNA extraction samples were diluted 2X in elution buffer following the final elution step. As cfDNA yields were consistently high, dilution ensured that no carry-over substances would interfere with the Devyser Accept cfDNA assay while yields of cfDNA remained greater than 0.1 ng/µL.

All samples tested had normal VAF balances at Deviser least 94% of alleles.

Figure 1. cfDNA extraction yield. 3 technical replicates from 10 distinct blood donors were used to extract cfDNA using the Qiagen EZ1 (blue), ThermoFisher MagMAX (red), and Promega Maxwell (yellow) cfDNA extraction kits. The dashed line shows the minimum cfDNA yield of 0.1 ng/µL required for input into the One Lambda™ Devyser Accept cfDNA kit. At least 90% of samples (9/10) pass cfDNA yield standards across all kits.

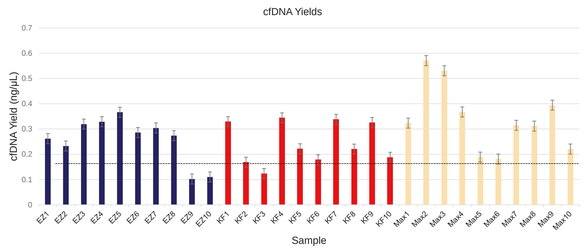
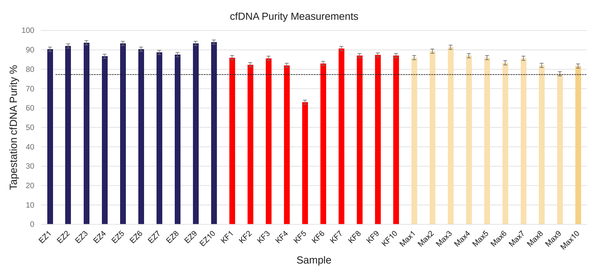


Figure 2. cfDNA extraction purity assessed by Agilent Tapestation. 3 technical replicates from 10 distinct blood donors were used to extract cfDNA using the Qiagen EZ1 (blue), ThermoFisher MagMAX (red), and Promega Maxwell (yellow) cfDNA extraction kits. The dashed line shows the minimum cfDNA quality threshold of 80% required for accurate read out from the One Lambda™ Devyser Accept cfDNA kit. At least 90% of samples (9/10) pass cfDNA purity standards.



Results

cfDNA extraction and One Lambda™ Devyser Accept cfDNA kits are robust to interfering substances

Table 2. CV of interfering substance samples relative to PBS controls.

Interfering Substance	EZ1	MagMAX/Kingfisher	Maxwell
Albumin	1.70	1.09	3.83
Hemoglobin	0.23	0.96	1.37
Bilirubin, conjugated	0.97	1.83	4.44
Bilirubin, unconjugated	0.74	3.14	3.32
Intralipid-20%	2.10	2.97	0.22
EDTA	0.65	1.05	2.18
Cyclosporine	3.13	0.05	2.68
Mycophenolic acid	1.55	1.91	3.51

Conclusions

- All cfDNA extraction methods tested produce cfDNA of sufficient quality and yield for the One Lambda™ Devyser Accept cfDNA kit.
- The One Lambda™ Devyser Accept cfDNA kit is robust to the presence of the tested interfering substances in plasma samples when cfDNA is extracted with the tested extraction kits.

References

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