Assessing Different DNA Extraction Methods for Cell-Free DNA Testing

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Aim

Donor-derived cell-free DNA (dd-cfDNA) detection is rapidly becoming a standard in post-transplant monitoring of allograft rejection. While there are an increasing number of dd-cfDNA assays commercially available for in-lab testing, clinical validation of a dd-cfDNA test remains complex and expensive. Choosing a cell-free DNA (cfDNA) extraction method is the first step into establishing a dd-cfDNA test. Based on our expected test volume and current laboratory setup, we evaluated three cfDNA extraction kits for yield, purity, and inter-tech reproducibility to choose the optimal extraction method.

Methods

Five panel samples were extracted for each comparison performed. Sufficient plasma volume was collected in one draw for two technologists to perform one extraction using each of the cfDNA extraction kits. Each extraction used 4 mL of plasma. Samples were extracted using three different kits: two manual extraction kits, the QIAamp Circulating Nucleic Acid (NA) Kit (vacuum/column-based) and QIAamp MinElute ccfDNA Kit (bead/column-based) with both eluting in 40 µL, and an automated EZ1&2 ccfDNA Kit (bead-based) eluting in 45 or 75 µL. Yield and purity were assessed on the day of extraction using Qubit and TapeStation.

Table 1. Overview of Three QIAGEN cfDNA Extraction Kits.

	Circulating Nucleic Acid	MinElute	EZ1&2
Process	Manual	Manual	Automated
Type	Vacuum/Column	Bead/Column	Bead
Plasma volume	1 – 5 mL	1 – 10 mL	1 – 10 mL
Plasma volume used for study	4 mL	4 mL	4 mL
Elution volume	20 - 150 µL	20 - 80 µL	45 µL/75 µL (but ranges from 35–55 µL/60–80 µL)
Elution volume used for study	40 µL	40 µL	45 or 75 µL
Uses carrier RNA?	Yes	No	No

Figure 1. Methods Used by QIAGEN cfDNA Extraction Kits. Left image: Vacuum and columns used for the Circulating NA kit. Middle image: Magnet and spin column used for the MinElute kit. Right image: EZ2 instrument used for the automated EZ1&2 kit.

Results

Qubit measurements showed that the Circulating NA kit yielded 20% more DNA for all samples than the MinElute kit, which yielded slightly more DNA than the EZ1&2 kit (Figure 2). However, the purity for the Circulating NA kit, as measured by percent cfDNA, was consistently lower than the other kits (Figure 4). Although automated, the EZ1&2 kit lacked consistency in elution volumes, ranging from 31 to 54 µL (Figure 5). Inter-tech results were consistent for all metrics.

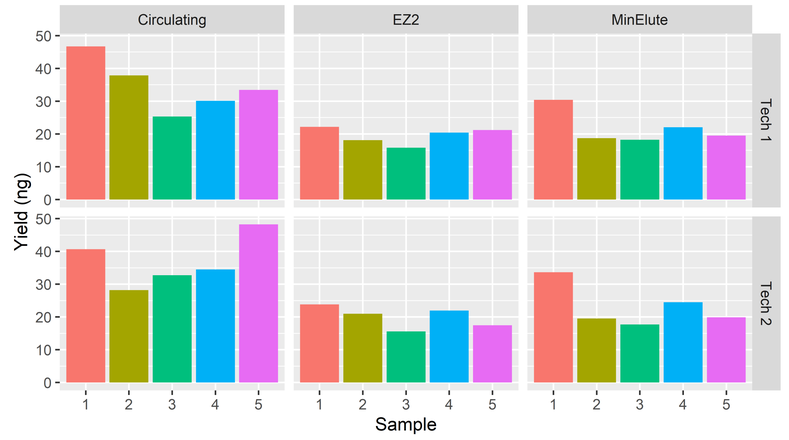

Figure 2. Total DNA Yield (ng) Obtained from Kit Extractions. The Circulating NA kit obtained the highest amount of DNA for all samples for both technologists. The MinElute and EZ1&2 kits were similar except for Sample 1, where the MinElute produced higher yields for both technologists.

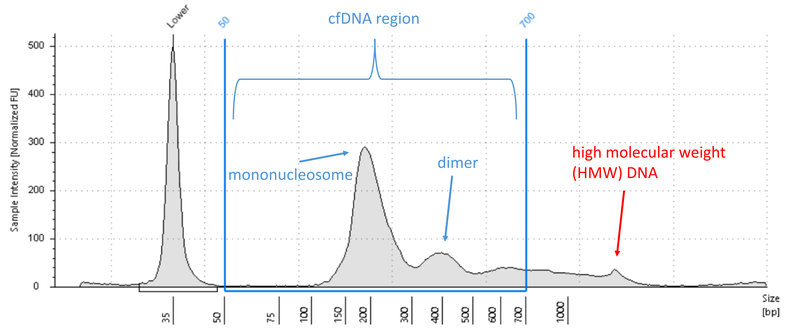

Figure 3. Electopherogram of a Typical cfDNA Extraction Using the Agilent cfDNA ScreenTape Assay. Percent cfDNA is calculated by assigning the 50-700 bp region as cfDNA compared to the total region, which includes HMW DNA.

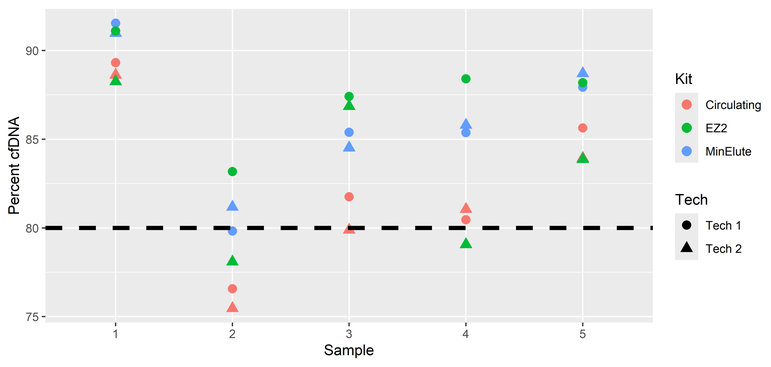

Figure 4. Samples Extracted Using the Circulating NA Kit Exhibit Lower cfDNA Purities. Accurate calculation of dd-cfDNA requires that the input material be free of HMW DNA, as its presence will lead to underestimation of the donor-derived fraction. Therefore, valid calculations assume a minimum cfDNA purity threshold of 80% (indicated by the dashed line). The MinElute kit produces the most consistent purity results.

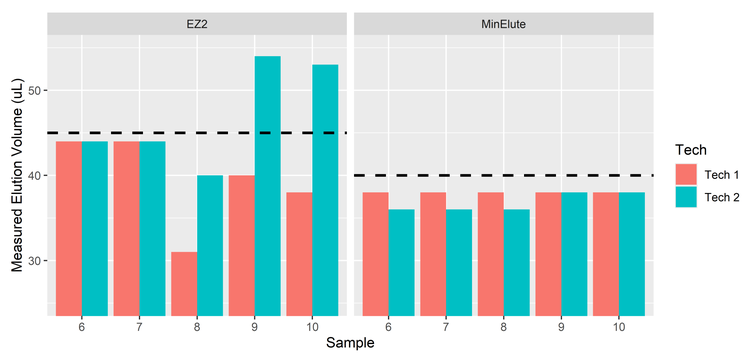

Figure 5. Increased Variability in Elution Volumes was Observed with the EZ1/2 Kit as Compared to the MinElute Kit. Comparison testing used an expected 45 µL elution volume for the EZ1&2 kit and a 40 µL elution volume for the MinElute kit (dashed lines). Elution volumes were manually measured to determine the exact amount obtained.

Conclusions

The MinElute kit showed the most consistent results for yield, cfDNA quality, and reproducibility. Based on this strong overall performance, our lab selected this kit for cfDNA extraction.