Absolute quantification of dd-cfDNA using a novel NGS assay

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Introduction

The introduction of relative quantification of donor-derived cell-free DNA (dd-cfDNA) has provided the field of solid organ transplantation an important new biomarker for assessing graft injury. Interestingly, the total cfDNA level can vary among patients due to factors such as elapsed time from transplantation, their disease status or physical activity. These variations can influence the detected percentage of dd-cfDNA, highlighting the need for accurate quantification of dd-cfDNA copies. Additionally, the purity of the cfDNA sample can influence the relative quantification of dd-cfDNA. If the plasma sample contains genomic DNA (gDNA) from the patient, it may lead to a falsely negative %dd-cfDNA result. Therefore, there is a need for precise quantification of dd-cfDNA copies.

Aim

This study aims to assess performance of an innovative method for absolute quantification of cfDNA using Next-Generation Sequencing (NGS), complemented by the measurement of dd-cfDNA percentage as well as the assessment of the cfDNA purity.

Method

In four unique artificial sample sets (samples 1-4), an equal amount of donor plasma was mixed with different amounts of recipient plasma to simulate the variation in amount of cfDNA in the recipient. In addition, same amount of DNA fragments with pre-defined copy-numbers were added to all samples to enable absolute quantification, ensuring accurate measurement of dd-cfDNA copies. The samples were extracted using QIAGEN QIAamp MinElute ccfDNA and the cfDNA purity and concentration was evaluated using Agilent TapeStation and Qubit HS assay. The %dd-cfDNA was evaluated using the One Lambda Devyser Accept cfDNA NGS assay.

An addition to the One Lambda Devyser Accept cfDNA NGS assay design, a gDNA control design was included that encompasses several long amplicons (321 to 655 bp). If gDNA was present in the sample, the long amplicons would be formed and subsequently detected during sequencing.

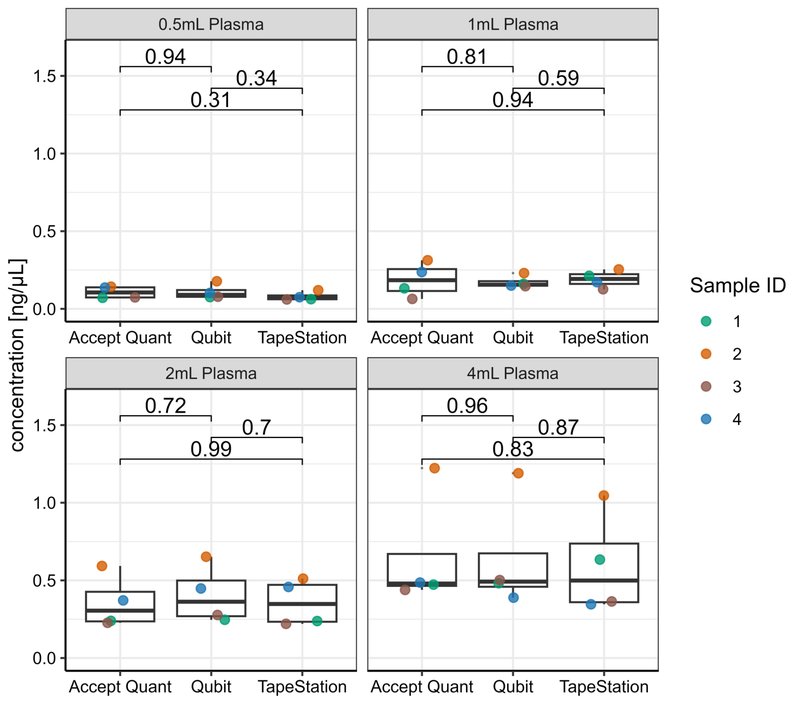

Figure 1. Quantification of total cfDNA extracted from different plasma volumes using Agilent TapeStation, Qubit HS and Accept Quant. No significant variance was observed between the three methods (numbers above box-plot show p=>0,05)

Results

We here show that quantification of total cfDNA determined by NGS (Accept Quant) correlates with measurements obtained by TapeStation as well as Qubit techniques (Figure 1). However, TapeStation and Qubit measurements tend to exhibit slightly lower DNA concentrations and total cfDNA copies, likely due to variances in extraction efficiency. Notably, the total count of cfDNA copies is dependent upon plasma volume, patient and the constant value of dd-cp/mL (donor-derived copies per milliliter of plasma) (Figure 2). In addition, the percentage of dd-cfDNA exhibits variations in relation to the total plasma volume used.

The cfDNA purity, assessed by the presence of gDNA using Accept Quant, shows ~~the~~ similar results as Agilent TapeStation (Figure 3).

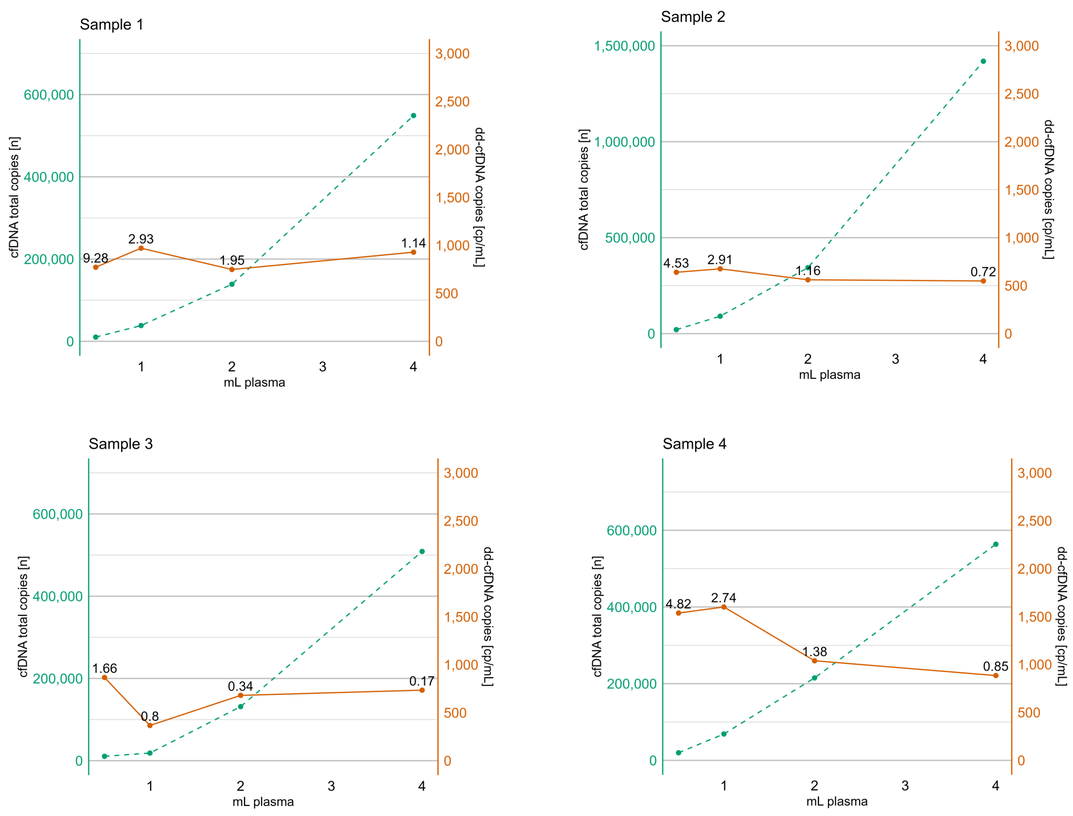

Figure 2. Total amount of cfDNA increases when plasma volume is increased (green, dotted), dd-copies/mL plasma is constant (orange) and the %dd-cfDNA (numbers attached to orange line) decreases when plasma volume is increased.

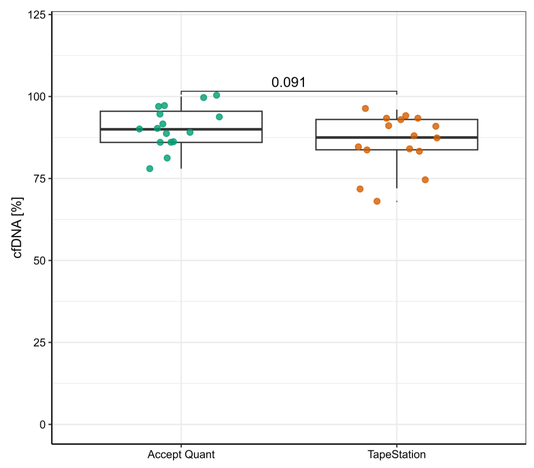

Figure 3. cfDNA purity in samples tested with TapeStation (green) and Accept Quant (orange). No significant difference was observed (p= >0,05)

Conclusion

In summary, our study demonstrates the effectiveness of NGS for precise quantification of cfDNA. This would especially to for measurement of dd-cfDNA in organ transplantation. Our findings show good correlation between NGS (Accept Quant), TapeStation and Qubit measurements. The dependence between cfDNA copy numbers and plasma volume, as well as the reproducibility in determining the number of dd-cp/mL is highlighted.

Variability in dd-cfDNA percentage underscores the need for new standardized protocols. Our study demonstrates a new approach for quantification of cfDNA using established NGS-methods, offering insights for improved organ transplant monitoring.

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