Performance of a novel NGS assay suitable for monitoring dd-cfDNA following

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BACKGROUND

One severe complication following solid organ transplantation is antibody- and/or cell mediated rejection of the graft. Early detection of rejection is therefore of paramount importance to improve outcome of solid organ transplantation. The present study was conducted to determine the analytical performance of the Devyser kit designed for sensitive detection of dd-cfDNA (donor-derived cell free DNA) following kidney transplantation.

MATERIAL AND METHODS

Three artificial dilution series (Series 7, Series 8 and Series 9) mimicking recipient and donor were manufactured to determine the analytical performance of the assay. The DNA samples were sheared either by sonication or enzymatic fragmentation (NEBNext dsDNA Fragmentase, New England Biolabs) to mimic cfDNA (~166 bp). The integrity of cfDNA was evaluated using Agilent TapeStation Cell-free DNA ScreenTape Analysis (figure 1).

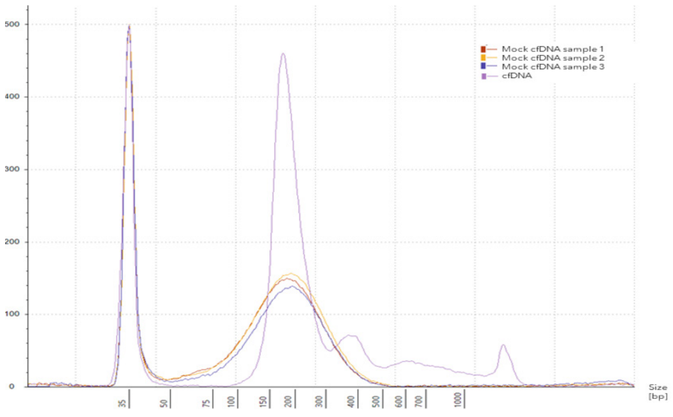
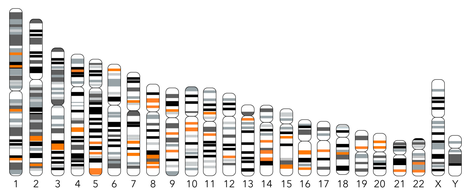


Figure 1. Chromatograms of three mock cfDNA which had been enzymatically sheared (red, yellow and dark purple) and one sample of extracted cfDNA (light purple). The main peaks of the generated mock-cfDNA were in line with the cfDNA sample (~166-180bp)

The dilution points for each dilution series were the following; 0.05%, 0.1%, 0.2%, 0.3% 0.4%, 0.5%, 1%, 10%, 20% and 30% dd-cfDNA.

Detection of dd-cfDNA were performed by sequencing using the Devyser kit. The kit is designed to include 50 population-independent indels (Figure 2), spread out over all the autosomal chromosomes, which are used to distinguish between donor and recipient and determination of % dd-cfDNA.

All samples were tested and sequenced on three different Illumina MiSeq instruments and the fastq files were analyzed using the ADVYSER software.



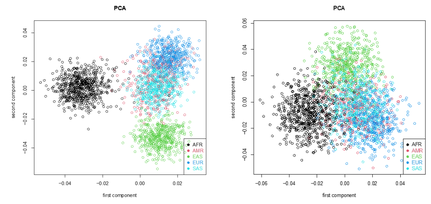


Figure 2. Markers spread out through all the 22 autosomal chromosomes; orange band indicate positioning of marker. PCA plots visualizing the first selection of markers and the sorted population-independent selection.

RESULTS

Regression analysis of the three artificial dilution series, Series 7, Series 8 and Series 9 all displayed excellent R2 . All series displayed linearity R2 >0.99 (Table 1 and figure 3). Run-to-run variation were performed by sequencing 10 replicates of dilution point 1 % dd-cfDNA from Series 7 (Figure 4 and table 2). The average % dd-cfDNA were 1.08%, with a standard deviation of 0.05 and a CV of 4.4%.

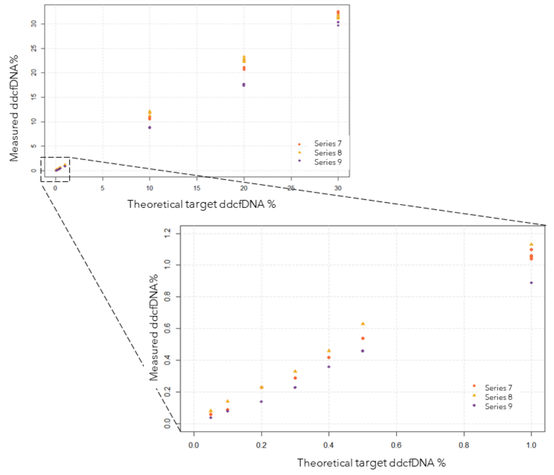


Figure 3. Established linearity of three artificial dd-cfDNA dilution series, Series 7, Series 8 and Series 9. Each series displayed excellent linearity in the range of 0.05- 30% dd-cfDNA, $R^2 = 0.99$. As well in the range of 0.05 – 1% dd-cfDNA, $R^2 = 0.99$.

	Series 7	Series 8	Series 9
0.05 - 30 % dd-cfDNA	0.999	0.996	0.991
0.05 - 1 % dd-cfDNA	0.998	0.995	0.996

Table 1. Regression analysis (R^2) on the three mock dd-cfDNA dilution series, Series 7, Series 8 and Series 9. Each series displayed excellent linearity in the range of 0.05 - 30% dd-cfDNA, $R^2 = 0.99$. As well in the range of 0.05 - 1% dd-cfDNA, $R^2 = 0.99$

Average	1.08
Standard deviation	0.05
CV %	4.4

Table 2. Ten (10) replicates of dilution Series 7, theoretical target dd-cfDNA at 1%.

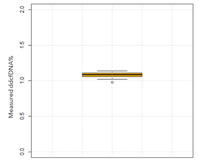


Figure 4. Ten (10) sequencing runs on three different MiSeq instruments of Series 7 dilution point 1 % dd-cfDNA displayed on average 1,08 % dd-cfDNA (table 2.)

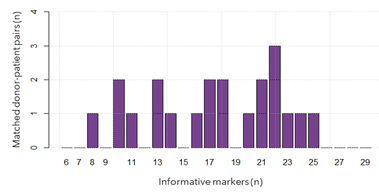


Figure 5. Number of informative markers in 21 donor-patient pairs. Screening was performed using 50 markers, on average 17.4 markers were assigned informative between donor and recipient of which 12.1 are heterozygous and 5.3 are homozygous.

21 patient-donor pairs were sequenced to evaluate marker performance with respect to generating informative markers, i.e. markers where the donor and recipient have different genotypes. On average 17.4 informative markers were generated of which 12.1 were heterozygous and 5.3 homozygous. (Figure 5)

The preliminary established Limit of Blank (LOB) is 0.047% when using both homozygous and heterozygous markers. LOB with the use of only homozygous markers is 0.034%. The current of Limit of Detection (LOD) is 0.072%. The results presented indicates a low LOQ.

CONCLUSION

Results of this study show that the new NGS-based assay developed for solid organ transplantation, displays excellent sensitivity, accuracy and precision suitable for monitoring dd-cfDNA in clinical samples from patients undergoing kidney transplantation.