Early-access testing of SOtrack: HLA-informed donor-derived cell-free DNA monitoring

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Introduction

- Monitoring donor-derived cell free DNA (dd-cfDNA) levels in whole blood after solid organ transplantation can enable early detection and treatment of graft rejection, which is pivotal to prevent graft loss.
- To quantify dd-cfDNA without the need for genotyping, a digital PCR (dPCR) prototype SOtrack assay was developed based on available donor information, including HLA and sex markers (Table 1).
- Additionally, the assay includes an internal reference assay and quality control (QC) for genomic DNA (gDNA) contamination, which facilitates reliable dd-cfDNA monitoring even in gDNA-contaminated samples (Figure 1).

Methods

- Three external sites isolated cfDNA from plasma of organ transplant recipients:
  1. Erasmus Medical Centre (Rotterdam, The Netherlands), using QIAamp Circulating Nucleic Acid Kit (QIAGEN).
  2. University Medical Centre Utrecht (Utrecht, The Netherlands), using EZ1&2 ccfDNA Kit for Automated cfDNA Extraction (QIAGEN).
  3. Department of Medical Sciences, University of Torino (Turin, Italy), using EZ1&2 ccfDNA Kit for Automated cfDNA Extraction (QIAGEN).
- All SOtrack measurements were performed at external sites according to the workflow (Figure 2):
  1. A donor-specific marker was selected, i.e. a mismatched HLA or Chromosome Y.
  2. DNA was diluted to 0.1 ng/µl, and 1 ng of DNA was amplified using the appropriate multiplex pre-amplification assay.
  3. The resulting amplicon was used for a triplex dPCR amplification (QIAcuity One 5plex). The selected donor-specific marker, total DNA based on reference gene, and gDNA are detected by FAM, HEX, and CY5 dyes, respectively.
  4. The donor-derived (dd) fraction in the sample and gDNA% are calculated, based on which the gDNA-corrected dd-cfDNA% is determined.
- The Cell-free DNA ScreenTape assay was performed in house using a TapeStation system (Agilent Technologies).

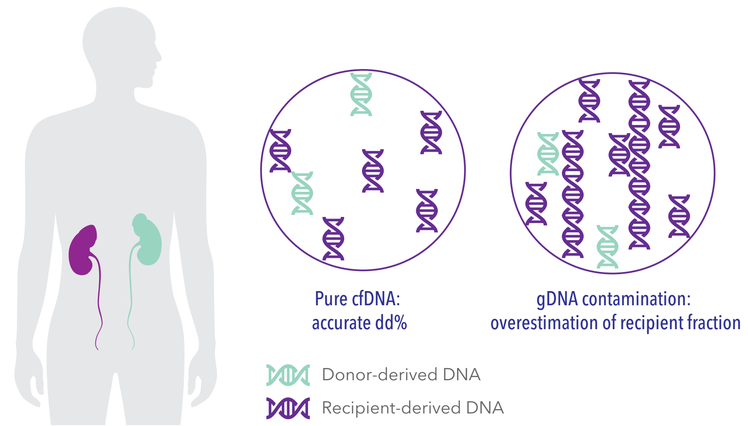


Figure 1. SOtrack QC assay quantifies gDNA contamination in cfDNA samples, which enables correcting for overestimation of the recipient fraction and therefore improves dd-cfDNA% accuracy.



Figure 2. SOtrack workflow comprises four steps with a turn-around time of <3.5 hours.

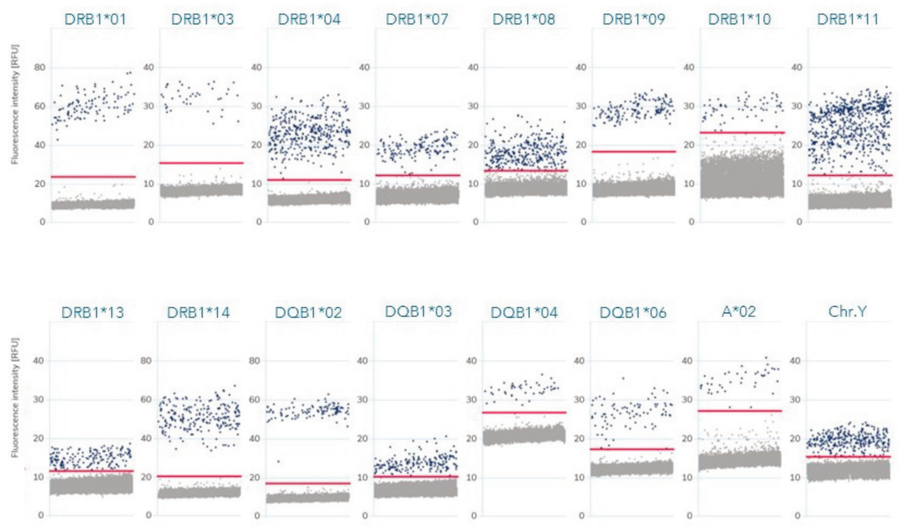


Figure 3. Donor-unique assays were compatible with cfDNA samples from organ recipients tested at external sites, with a clear separation between positive and negative partitions for each target. Representative dPCR plot for each donor-unique target tested on cfDNA at external site. Fluorescence intensity is shown in relative fluorescence units (RFU) on the y-axis. Threshold is shown as a red line.

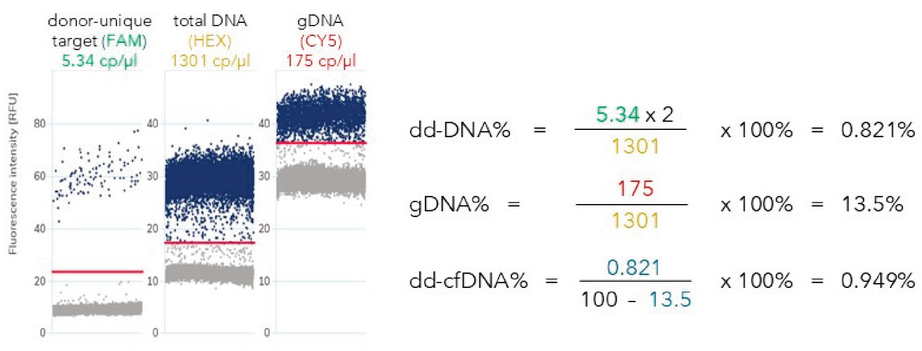


Figure 4. dd-cfDNA can be determined by triplex dPCR results using FAM, HEX and CY5 channels as illustrated for a heterozygous DRB1*01 target.

Table 1. Current maker panel of SOtrack prototype.

HLA-DRB1 markers	HLA-DQB1 markers
DRB1*01	DQB1*02
DRB1*03	DQB1*03
DRB1*04	DQB1*04
DRB1*07	DQB1*05
DRB1*08	DQB1*06
DRB1*09	
DRB1*10	
DRB1*11	
DRB1*12	**Additional markers**
DRB1*13	HLA-A*02
DRB1*14	HLA-C*07:01
DRB1*15/16	Chromosome Y

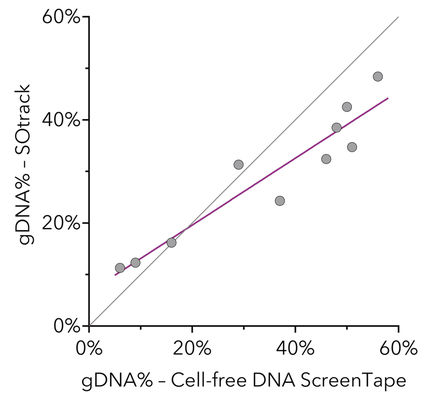


Figure 5. Strong correlation in gDNA between SOtrack QC assay and Cell-free DNA ScreenTape assay on cfDNA samples from organ recipients. Diagonal (y=x) is depicted in gray; linear fit with $R^2 = 0.893$ is depicted in purple.

Conclusion

- External testing confirmed that donor-specific makers of SOtrack prototype assay can be used with isolated cfDNA samples from organ recipients and dd-cfDNA % can be calculated corrected for gDNA% contamination.
- In summary, early-access testing of the SOtrack prototype assay at three independent sites strongly supports the suitability of the assay for reliable dd-cfDNA monitoring using HLA-based donor-specific markers.

Results

- SOtrack prototype was assessed at external sites using cfDNA samples isolated from plasma of organ transplant recipients.
  - These tests showed compatibility of donor-specific markers with cfDNA recipient samples (Figure 3).
  - From cfDNA samples, the dd-cfDNA % could be calculated (Figure 4).
  - SOtrack prototype assay can reliably correct for recipient-derived contamination of gDNA, as demonstrated on cfDNA samples isolated at one of the sites (Figure 5).

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