Laboratory validation of CE-marked AlloSeq cfDNA to measure donor-derived cell-free DNA in transplant patients.

Thierry Viard 1, Zuleika Calderin-Sollet 2, Simon Mathers 3, Georgia Christopoulou 4, Stavroula Samara 5, Alkaterini Oikonomaki 6, Deanna Lee 1, Amanda Tilgren 1, Natali Gulbahce 1, Timothy O’Connor 3, Marica Grskovic 4, Pantelis Constantoulakis 4, Thanasis Athanasopoulos 4, Natalia Diaz Burlinson 1, Jean Villard 2
1CareDx, Brisbane, USA, 2Geneva University Hospital, Geneva, Switzerland, 3Manchester Royal Infirmary, Manchester, United Kingdom, 4SafeBlood BioAnalytica, Athens, Greece, 5Genotypos Science Labs MSA, Athens, Greece

Introduction
In solid organ transplant recipients, circulating donor-derived cell-free DNA (dd-cfDNA) is a noninvasive biomarker of transplant allograft injury2,3 that may enable more frequent, quantitative, and safer assessment of allograft rejection and injury status. We have developed a targeted next-generation sequencing (NGS) assay that utilizes 202 single-nucleotide polymorphisms located throughout the genome to measure the dd-cfDNA in plasma of transplant recipients. The assay combines a clinical laboratory compatible protocol with streamlined workflow and a fully automated analysis software to accurately quantify dd-cfDNA without separate genotyping of either donor or recipient. Performance validation was conducted with more than 40 samples across a total of 30 runs and established a Limit of Detection (LoD) of 0.23%. Limit of Blank (LoB) and limit of Quantification (LoQ) were determined at 0.18% and 0.23%, respectively. NCV for within run and between run of < 15% when tested with 1% dd-cfDNA. In tests conducted in parallel across three independent laboratories, AlloSeq cfDNA accurately and reproducibly quantified the fraction of dd-cfDNA in 12 samples of various types, including raw plasma samples that were extracted on site.

Methods and Materials
dd-cfDNA Quantification Assay
The concept of the CareDx AlloSeq cfDNA assay is centered around a single multiplex PCR followed by sequencing to determine the fraction of donor-specific nucleotides at 202 selected SNP loci, allowing the quantification of dd-cfDNA with or without prior genotyping of the donor or recipient (figure 1).

The kit includes a locus-specific oligonucleotide primer pool to amplify the targeted regions of interest. In combination with index adapters, a unique cycling protocol amplifies the locus-specific regions and indexes the libraries concurrently. Indexed samples are subsequently pooled and purified together to prepare for sequencing.

After sequencing is complete, an analysis report provides precise percentage of dd-cfDNA present in each sample, in less than 24 hours after sample receipt and cfDNA extraction. Library preparation is completed within four hours of which only one hour is hands on (figure 2).

Next, two plasma samples (tubes A & B) were obtained from each of six kidney transplant patients and were processed for cfDNA extraction at four different laboratories (figure 6b). Again, no significant differences were observed between tubes A (purified and tested at CareDx) and tubes B (purified and tested at the external laboratories), demonstrating the high reproducibility of the results generated by the AlloSeq cfDNA solution.

Results
During this study across three independent laboratories, a total of 238 libraries were generated using a DNA input of 10ng with a first pass rate of 96.6% (222/238). Analytical performance was confirmed and measurement reproducibility between sites was evaluated across the entire workflow, from blood same to dd-cfDNA results, in two experiments. In the first experiment, two independent cfDNA extractions (Extraction A and Extraction B) were carried out on a same plasma sample from each of four kidney transplant patients at CareDx. cfDNA samples from extraction A were tested at CareDx and cfDNA samples from extraction B were sent to three different laboratories for AlloSeq cfDNA testing (figure 6a). No significant difference in dd-cfDNA % were observed between the two independent extractions.

Conclusions
This noninvasive streamlined sequencing assay requires minimal hands-on time and can be completed within 24 hours post obtaining recipients cfDNA, on site, providing a critical practical turnaround time preferred in post transplantation surveillance (figure 7). AlloSeq cfDNA is a CE mark approved solution and is available to transplant patients and clinicians around the world.

References