Small Samples, High Stakes: Addressing the Technical Challenges for Measuring Donor-Derived Cell-Free DNA for Pediatric Solid Organ Transplant Recipients

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BACKGROUND

- Laboratory tests for measuring donor-derived cell-free DNA (dd-cfDNA) are being used to detect graft injury [1,2].
- Total cell-free DNA concentrations are often <10 ng/mL plasma [3] and clinically relevant dd-cfDNA levels can be 0.2- 1% dd-cfDNA.
- For pediatric patients it is important to be able to test small blood volumes which can be safely collected.
- To determine feasibility of using the **One Lambda™ Devyser Accept cfDNA** [4] for pediatric patients, performance characteristics for were determined the using as little as 1 ml plasma.

METHODS

- Blood was collected from healthy controls and transplant recipients into Cell-Free DNA BCT (STRECK).
- Plasma was isolated by a two-step centrifugation protocol.
- cfDNA extraction was performed using the QIAamp MinElute ccfDNA Mini Kit.
- Total cfDNA levels were quantified by fluorometry (Qubit High sensitivity assay).
- **One Lambda™ Devyser Accept cfDNA assays** were performed following vendor's recommendations.

RESULTS

1 ml plasma provides >4ng cfDNA

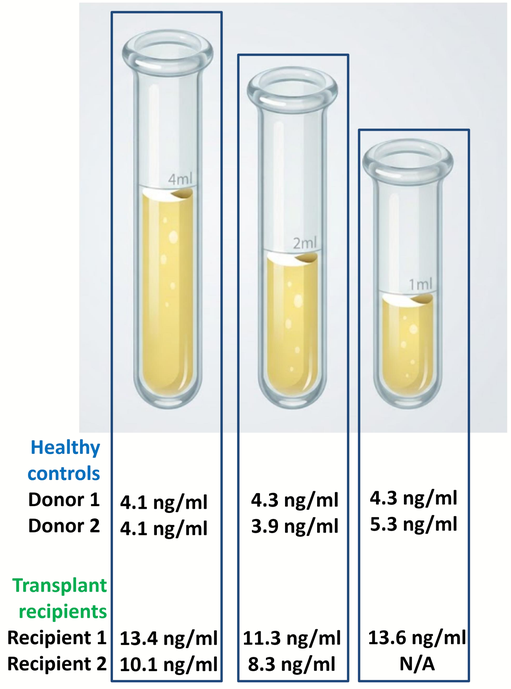


Figure 1. Total cfDNA levels (ng/ml of plasma) measured in healthy controls and pediatric transplant recipients.

Accuracy for input of 4 ng DNA

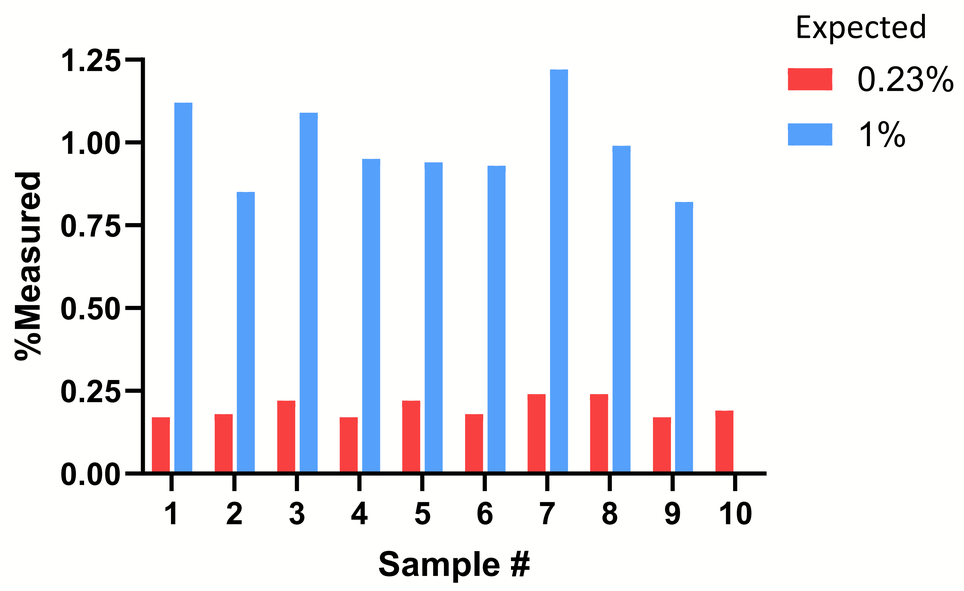


Figure 2. Assay accuracy with 0.23% and 1% dd-cfDNA (simulated) for a total DNA input of 4 ng.

CONCLUSIONS

- The **One Lambda™ Devyser Accept cfDNA assay** accurately measured %DD-cfDNA at thresholds associated with graft injury, with DNA input levels expected from small samples.
- Assay performance improved with increased DNA input and %dd-cfDNA.
- This assay shows promise for testing small samples that can be safely obtained from pediatric patients.

REFERENCES & ACKNOWLEDGMENTS

1. Edwards RL, Menteer J, Lestz RM, Baxter-Lowe L. *Cell-free DNA as a solid-organ transplant biomarker: technologies and approaches*. Biomark Med.2022;16(5):401-15.
2. Edwards RL, Menteer J, Lestz RM, Baxter-Lowe, L. *Overview of Laboratory Techniques For Measuring Donor-Derived Cell-Free DNA in Solid Organ Transplantation*. ASHI Quarterly Q1. 2026; 22–27.
3. North PE, Ziegler E, Mahnke DK, Stamm KD, Thomm A, Daft P, Goetsch M, Liang HL, Baker MA, Vepraskas A, Rosenau C, Dasgupta M, Simpson P, Mitchell ME, Tomita-Mitchell A. Cell-free DNA donor fraction analysis in pediatric and adult heart transplant patients by multiplexed allele-specific quantitative PCR: Validation of a rapid and highly sensitive clinical test for stratification of rejection probability. PLoS One. 2020 Jan 13;15(1):e0227385.
4. Pettersson L, Westerling S, Talla V, Sendel A, Wennberg L, Olsson R, Hedrum A, Hauzenberger D. *Development and performance of a next generation sequencing (NGS) assay for monitoring of dd-cfDNA post solid organ transplantation.* Clin Chim Acta. 2024. 552: 117647.

ACKNOWLEDGMENTS: THIS WORK WAS SUPPORTED BY THE GOFARR FUND. REAGENTS SUPPLIED BY ONE LAMBDA, A THERMO FISHER BRAND. IRB APPROVED STUDY.