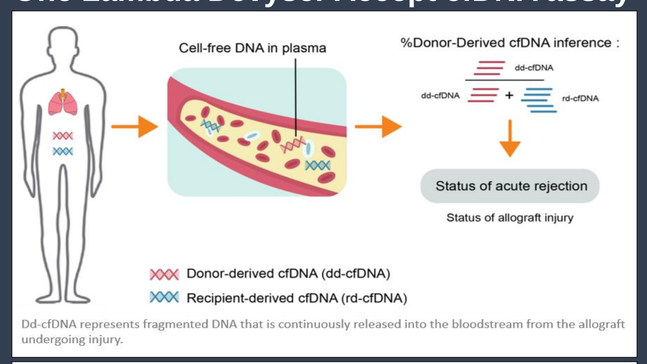
Performance assessment of the One Lambda Devyser Accept cfDNA assay in lung transplantation

- The repeatability, reproducibility, linearity, LoB, LoD, and overall performance of the One Lambda Devyser Accept cfDNA assay found to be within acceptable ranges.
- Monitoring of increasing amounts of donor derived cell-free DNA (dd-cfDNA) in plasma may serve as an important biomarker for graft integrity follow-up.
- Fluctuations in recipient cfDNA (e.g., due to excessive inflammatory responses) can affect the results of dd-cfDNA fractional determination. This can be addressed using absolute dd-cfDNA quantification.

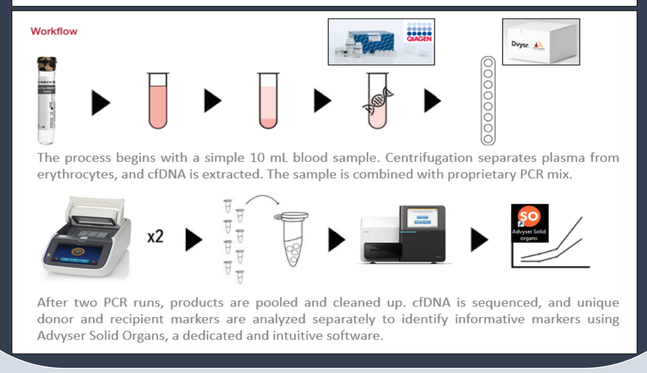
INTRODUCTION

Lung transplantation (LT) is a life-saving procedure which is nonetheless associated with limited long-term survival, partly due to rejection. This diagnosis is often delayed, hence, there is a major need for improved diagnostics. Dd-cfDNA in plasma is a promising non-invasive biomarker that may serve as an early indicator of graft injury following LT, potentially enhancing the diagnostic accuracy of transbronchial biopsy, the current standard of care. Our study examines the performance of the One Lambda Devyser Accept cfDNA assay in the context of LT.

One Lambda Devyser Accept cfDNA assay



Dd-cfDNA represents fragmented DNA that is continuously released into the bloodstream from the allograft undergoing injury.



The process begins with a simple 10 mL blood sample. Centrifugation separates plasma from erythrocytes, and cfDNA is extracted. The sample is combined with proprietary PCR mix.

After two PCR runs, products are pooled and cleaned up. cfDNA is sequenced, and unique donor and recipient markers are analyzed separately to identify informative markers using Advyser Solid Organs, a dedicated and intuitive software.

METHODS

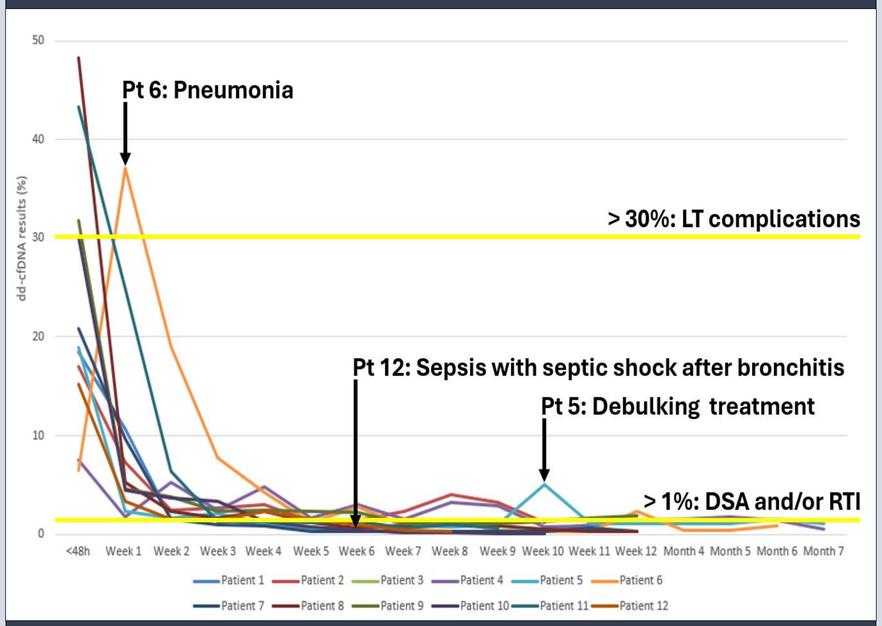
We investigated the performance in artificially generated samples (3 dilution series ranging from 30% to 0.1% dd-cfDNA, DNA size 166bp, concentration 0.6 ng/µL) and clinical samples from LT patients (pts) with plasma collection at different time points (24-48h, weekly for 3 months, monthly for up to 1 year).

RESULTS

Analytical performance of the One Lambda Devyser Accept cfDNA assay

Repeatability CV% (expected %dd-cfDNA)	• 4.6 (0.1) • 14.3 (0.5) • 2.3 (20)
Reproducibility CV% (expected %dd-cfDNA)	• 7.7 (0.1) • 22.2 (14.1 CV% without outlier) (0.2) • 4.9 (0.3) • 8.4 (0.4) • 15.8 (0.5) • 9.0 (1) • 1.4 (10) • 6.1 (20) • 5.4 (30)
Linear regression	• R^2 = 0.997 • Intercept = 0.06 • Quadratic term in a polynomial regression not significant (P=0.27)
Limit of Blank (LoB)	0.06% dd-cfDNA
Limit of Detection (LoD)	0.1% dd-cfDNA
Limit of Quantitation (LoQ)	0.2% dd-cfDNA
Marker reliability	Heterozygous markers (N=420) (screening samples): median absolute deviation of 3.4% from the expected 50% VAF (range of absolute deviations, 0% to 14.8% ; of which 4 outside One Lambda boundaries)
	Homozygous markers (N=480) (screening samples): median absolute deviation of 0% from the expected 100% VAF (range of absolute deviations, 0% to 1.49%; of which 2 outside One Lambda boundaries)

%dd-cfDNA results of patients at different time points after LT



- >30% LT complications: high % dd-cfDNA within 24-48 hours after LT with the highest values associated with LT complications,
- Rapid decrease after 1-2 weeks except LT complications (up to 5 weeks),
- >1% DSA and/or respiratory tract infection (RTI): Subsequently a mean value of 0.6% for pts without complications and a mean value of 1.9% for pts with DSA and/or RTI,
- Pt 12: Sepsis after bronchitis, died of septic shock. The %dd-cfDNA values were low which may indicate that relative quantification may be affected by an excessive inflammatory response,
- In single LT, values were doubled.

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