# Evaluating The Role Of HoloGRAFT One Assay to Assess Changes in Dd-cfDNA Levels In Kidney Transplant Recipients and Predicting Delayed Graft Function

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## Background

We aim to assess the efficacy of HoloGRAFT One assay for monitoring donor derived cell-free DNA (dd-cfDNA) in blood from kidney transplant recipients. Initial studies show good correlation between dd-cfDNA and organ dysfunction, but the trajectory of these changes during initial months post-transplant is unclear. Moreover, high testing costs have limited the use of dd-cfDNA testing as a diagnostic assay. Therefore, dd-cfDNA assessment during this period will help establish better baselines and might help reduce testing costs.

Our lab is validating HoloGRAFT One assay for its ease of use, cost and accuracy in monitoring dd-cfDNA in kidney patients, post-transplant. The study titled *Sequential Measurement of Donor Derived cfDNA (dd-cfDNA) (Holograft) and Correlation with Patient Outcomes Post Kidney Transplant,* aims to evaluate changes in dd-cfDNA for 60 patients. Streck Cell-Free DNA BCT® tubes are used for patient blood collection with samples collected at 6 timepoints including: day 2; 1-, 2- and 6- weeks; 4- and 6- months post-transplant. cfDNA is extracted from plasma using QIAamp MinElute ccfDNA Midi Kit. dd-cfDNA concentrations are measured in patient plasma using Omixon HoloGRAFT ONE kit and Qiagen QIAcuity One dPCR (digital Polymerase Chain Reaction).In HoloGRAFT assay's dPCR, the patient cfDNA is divided into thousands of partitions and Polymerase Chain Reaction (PCR) amplification occurs in each partition. Quantification of target DNA molecules in each partition makes it highly efficient in detecting lower concentrations of dd-cfDNA in patient blood. The assay provides absolute quantification of dd-cfDNA using 48 multiplexed copy number variant (CNV) markers (long stretches of DNA inherited in zero, one or two-copy forms).

Donor and patient pairs are first screened for the presence of unique CNVs in their genomic DNA (gDNA). Patient's plasma samples are then screened for the presence and concentration of these unique donor specific markers. The results from each patient's dd-cfDNA% are compared to clinically acceptable markers of renal function including creatinine levels as well as tissue biopsies.

Changes in the levels of dd-cfDNA in patient blood during first 6 months post transplant will assess the efficacy of HoloGRAFT assay in predicting organ rejection, before chronic rejection is confirmed by biopsies, in kidney transplant patients.

## Methods

Consent 60 patients post kidney transplant

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Blood collection
(Day 2, weeks 1, 2, 6; 4 months and 6 months)

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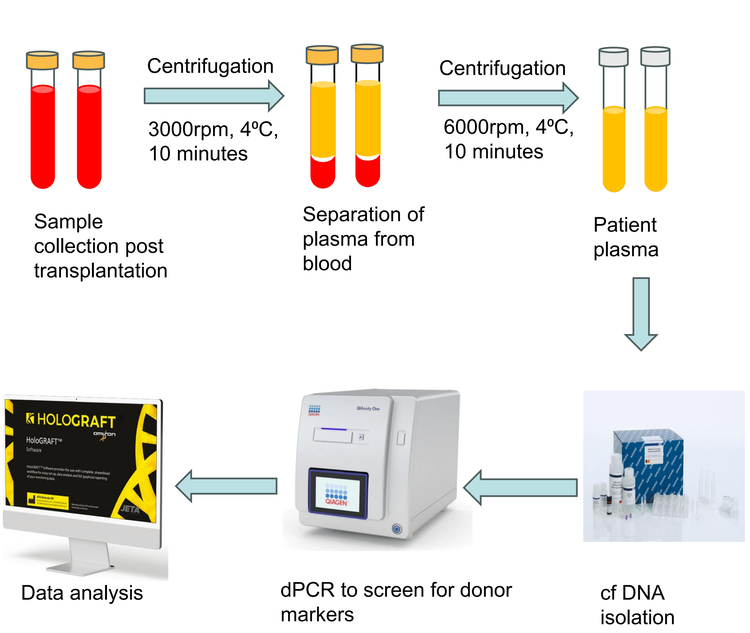
Plasma separation from blood

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Isolation and quantification of dd-cfDNA using Omixon HoloGRAFT ONE and Qiagen QIAcuity ONE dPCR

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Comparison of dd-cfDNA levels with clinically accepted markers

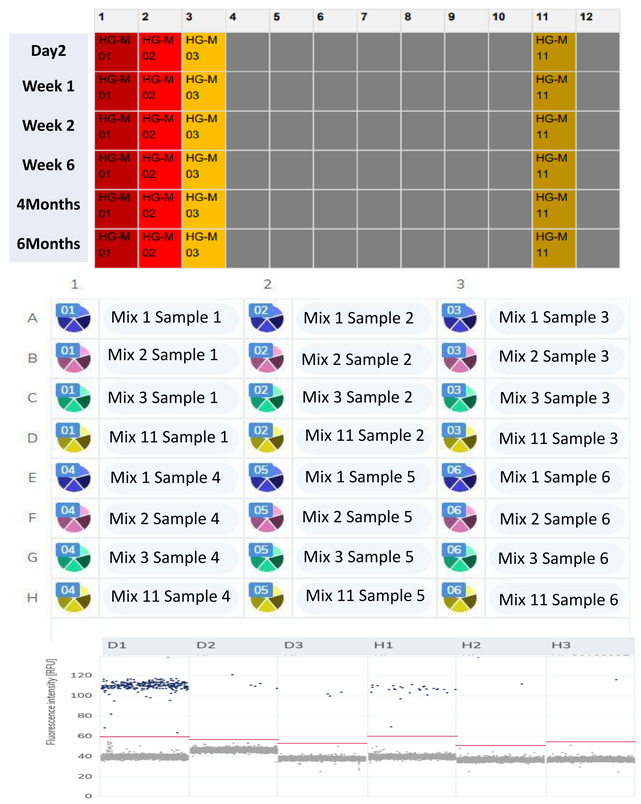



Patient blood is collected in cell preparation Streck tubes followed by separation of plasma through centrifugation. cfDNA in the plasma samples is isolated using QIAamp MinElute ccfDNA kit. dd-cfDNA% in patient blood is measured using Omixon HoloGRAFT ONE kit and Qiagen QIAcuity ONE dPCR (digital Polymerase Chain Reaction). HoloGRAFT ONE software analyses the preliminary run for donor and recipient specific informative markers in their genomic DNA (gDNA), following which the successive cfDNA samples are only tested for the concentration of identified informative markers.

## Results

During our preliminary findings, dd-cfDNA levels in 20 transplant patients were measured using HoloGRAFT One assay and compared to the results from our center's send-out test or biopsy findings. For patients monitored using send out test/ biopsies, our results aligned with lower levels of dd-cfDNA% in send-out tests or biopsies negative for rejection, respectively.
To evaluate if higher dd-cfDNA levels measured immediately post-transplant were indicative of graft function, patients' dd-cfDNA% levels were compared to their graft function at day2 post-transplant. 70% of the patients (12 out of 17) with delayed graft function (DGF) showed dd-cfDNA% >0.5% following a transplant, indicating a possible link between dd-cfDNA and DGF.

| | Assay Mix 1 | Assay Mix 2 | Assay Mix 3 | Assay Mix 4 | Assay Mix 5 | Assay Mix 6 | Assay Mix 7 | Assay Mix 8 | Assay Mix 9 | Assay Mix 10 | Assay Mix 11 | Assay Mix 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | - | - | - | + | + | - | + | - | + | + | - | + |
| Donor | + | + | + | + | - | - | - | - | + | - | + | - |




| | dd-cfDNA >0.5% | Delayed Graft Function |
|---|---|---|
| Pt1 | 1 | 0 |
| Pt2 | 1 | 1 |
| Pt3 | 0 | 0 |
| Pt4 | 1 | 1 |
| Pt5 | 1 | 1 |
| Pt6 | 1 | 0 |
| Pt7 | 1 | 0 |
| Pt8 | 1 | 1 |
| Pt9 | 1 | 1 |
| Pt10 | 0 | 1 |
| Pt12 | 1 | 0 |
| Pt11 | 1 | 0 |
| Pt13 | 1 | 1 |
| Pt14 | 1 | 1 |
| Pt15 | 1 | 1 |
| Pt16 | 1 | 1 |
| Pt17 | 1 | 1 |
| Pt18 | 0 | 0 |
| Pt19 | 1 | 0 |
| Pt20 | 1 | 1 |
| | n=17 | n=12 |

| | Deceased donor Txp (n=14) | Living Related Txp (n=3) | Living Unrelated Txp (n=3) |
|---|---|---|---|
| dd-cfdNA > 0.5% | 13 | 1 | 3 |
| DGF | 11 | 1 | 0 |

DGF: Delayed Graft Function

| | dd-cfDNA >0.5% | Delayed Graft Function | Elevated creatinine at day 2 |
|---|---|---|---|
| Number of Patients | 17 | 12 | 6 |

## Conclusion

With results comparable to send-out tests and biopsy findings, HoloGRAFT One assay seems to be a viable option for on-site testing of dd-cfDNA in clinical HLA laboratory. Our preliminary results also show that changes in dd-cfDNA levels during initial days post-transplant can be used as a good indicator of DGF. However, more testing is needed in this direction to better understand the correlation between dd-cfDNA and DGF.

**References:** www.omixon.com/products/holograftone; www.qiagen.com

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