A novel extraction method for enrichment of circulating cell-free DNA

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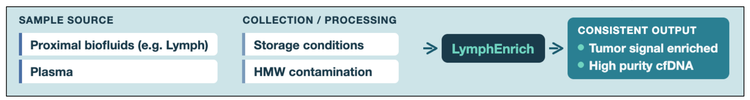
Introduction

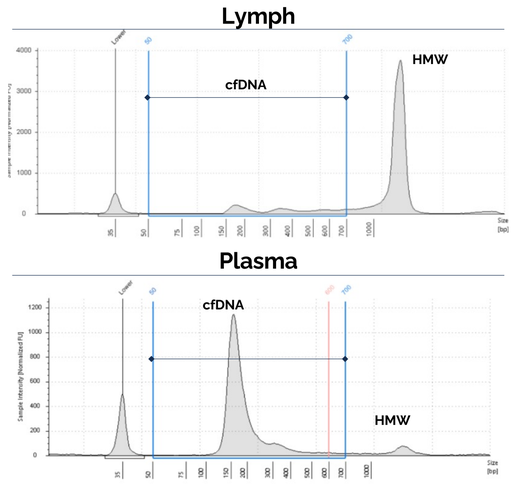
Proximal biofluids have shown enriched sensitivity for liquid biopsy but present unique preanalytical challenges. Even in plasma, variations in collection or processing can affect cell-free DNA (cfDNA) isolation and size profiles. These differences may affect downstream sensitivity.

Our lab has pioneered the use of lymphatic exudate ("lymph") from surgical drains as a novel proximal liquid biofluid for MRD detection. We have previously shown that lymph collected 24 hours after surgery identified molecular residual disease (MRD) in head and neck squamous cell carcinoma (HNSCC). This biofluid contains lymphatic fluid, blood, and interstitial fluid, and differs markedly from blood or plasma. cfDNA in lymph displays distinct nucleosomal distribution, including prominent mono- and dinucleosome peaks ("cfDNA fraction"), but most extracellular DNA is >700 bp ("HMW fraction"). Our analysis revealed that tumor-derived DNA is more abundant within the < 700 bp cfDNA fraction of post-surgical lymph.

To negate the signal-diluting effects of the HMW fraction, we developed a novel extraction method called LymphEnrich, that simultaneously maximizes cfDNA recovery and selectively depletes HMW DNA. The method utilizes Proteinase K treatment for protein removal, selective bead-based removal of the HMW fraction, and purification of the enriched cfDNA from the supernatant.



Lymph cfDNA distribution is distinct from plasma

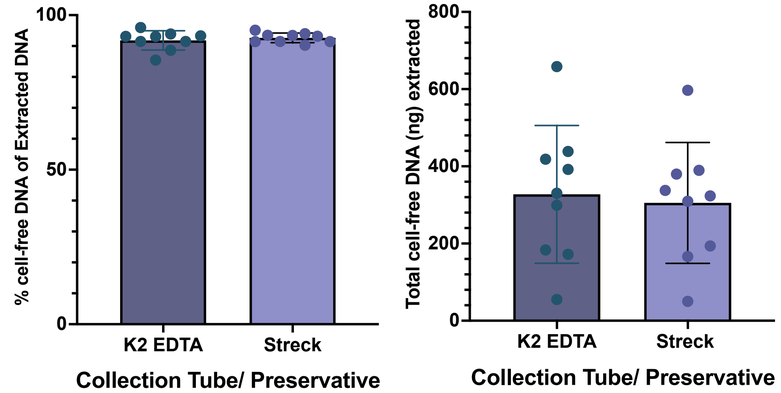


Raw lymph cfDNA extract was extracted using a modified version of Droplet's extraction method (lacking HMW depletion). Lymph cfDNA displays distinct nucleosomal distribution from plasma., with prominent mono- and di-nucleosome peaks and the dominant high-molecular weight (>700 bp) fraction across all surgical drain types.,

In contrast, plasma cfDNA (visualized after commercial kit extraction) is primarily mononucleosomal with minimal di-nucleosomal or HMW peaks.

Current commercial solutions do not enrich lymph cfDNA, which causes variation in downstream NGS applications due to low purity.

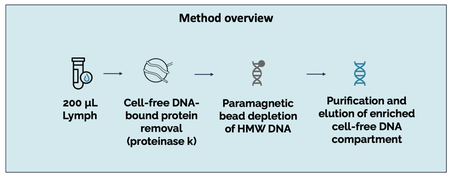
LymphEnrich is compatible across collection tubes



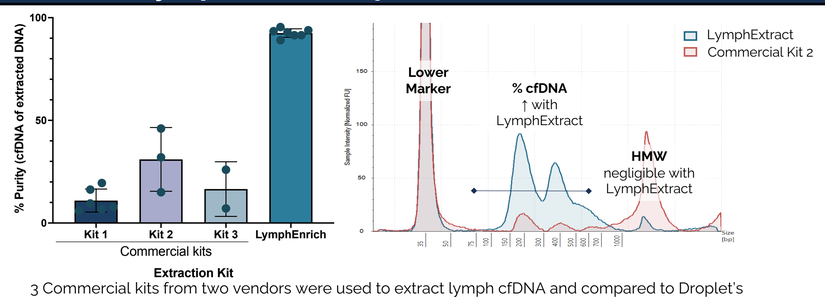
Lymph was collected 24 hours after surgery in both K_2EDTA and Streck tubes (n=9). LymphEnrich was applied to 200 µL of processed lymph and yielded comparable cfDNA purity and concentration across both tube types. Differences in yield were patient-dependent, not tube-dependent.

Methods and Materials

Lymph was collected 24-hours after surgery in K2EDTA/Streck blood collection tubes, centrifuged to remove cells and debris, and the supernatant stored at −80 ˚C (n=36). 36 patient samples were processed using our novel extraction method; matched subsets were used for commercial kit comparisons (3 commercial cfDNA extraction kits). cfDNA concentration and size distribution were analyzed using Qubit dsDNA and Tapestation cfDNA assays respectively, with cfDNA defined as fragments 50-700 bp.

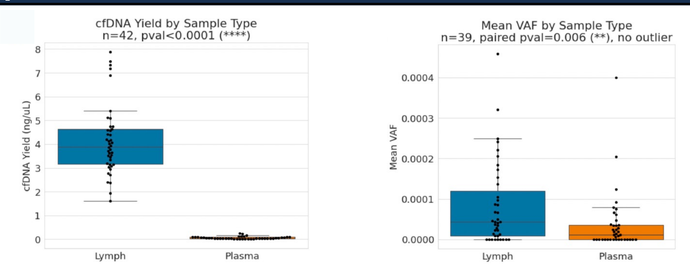


LymphEnrich improves cfDNA isolation



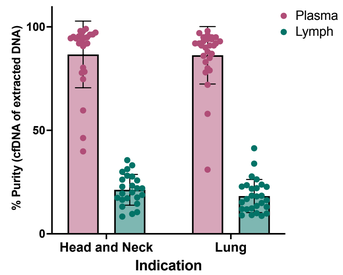
3 Commercial kits from two vendors were used to extract lymph cfDNA and compared to Droplet's extraction method, LymphEnrich. While total yield varied, LymphEnrich was the only extraction method to achieve both high purity and yield.

Droplet's extraction enables sensitive ctDNA detection



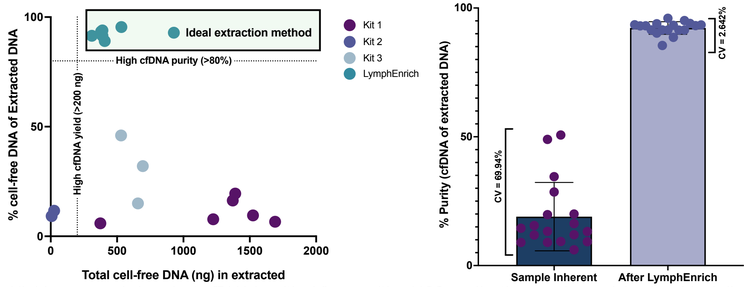
cfDNA in lymph extracted with LymphEnrich was 65 times more concentrated than plasma (mean 4.14 ng per µL of lymph and mean 0.06 ng per µL of plasma, P < 0.0001). Mean ctDNA allelic fraction as measured by NGS was two times higher in lymph than in plasma samples (lymph = 0.004 ± 0.036%; plasma = 0.002 ± 0.078%; P = 0.03, n = 42). The LymphEnrich method enabled sensitive detection of ctDNA in lymph.

Commercial kits yield low cfDNA purity for lymph



The same commercial extraction kit was used to extract matched plasma and lymph from HNSCC (n=25) and Lung cancer (LC) patients (n=28). Lymph cfDNA was underrepresented in the extracted cfDNA (mean cfDNA = 21% (HNSCC) and 18% (LC)) compared to plasma (mean %cfDNA = 86% in both HNSCC and LC).

LymphEnrich improves sample consistency



Highly pure and abundant cfDNA is critical for sensitive NGS applications such as minimal residual disease detection. Compared to commercial kits, LymphEnrich was the only one that yielded both high cfDNA amounts (>200 ng) and high purity (>85%). It also produced consistent purification across samples despite inherent differences in lymph cfDNA composition (n=18).

Conclusions and Future Work

We present LymphEnrich - an optimized cfDNA extraction method which consistently yields high purity, concentrated cfDNA from biofluids with substantial HMW fractions, dramatically increasing the effective ctDNA yield compared to standard commercial protocols. This technical advancement resolves a critical preanalytical challenge for assays using lymphatic exudate, enabling sensitive clinical monitoring with this readily available proximal biofluid.

More information

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Please visit posters: **#5155** Accelerating minimal residual disease (MRD) detection through GPU-accelerated genomic analysis using NVIDIA Parabricks, **#6137** Multi-omics in post-operative lymphatic exudate in HPV-negative head and neck cancer, **#7382** Lymphatic exudate is a novel source of tumor-associated immune cells and **#7833** A novel extraction method for enrichment of circulating cell-free DNA