Novel Procedure for Rapid Isolation of ccfDNA from Plasma or Serum

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Background

Circulating cell-free DNA (ccfDNA) fragments in blood have huge potential for the analysis of genetic markers without the inconvenience and risks of tissue biopsies or fine needle aspirates. For example, nucleic acids found in plasma and serum enable the specific detection of tumor types in patients, while cell-free fetal DNA circulating in maternal plasma gives valuable insights in genetic diagnostics. Due to the low concentrations of ccfDNA in plasma, large sample volumes (≥ 4ml) of plasma are often required to extract sufficient material for analysis, leading to slow and cumbersome extraction procedures.

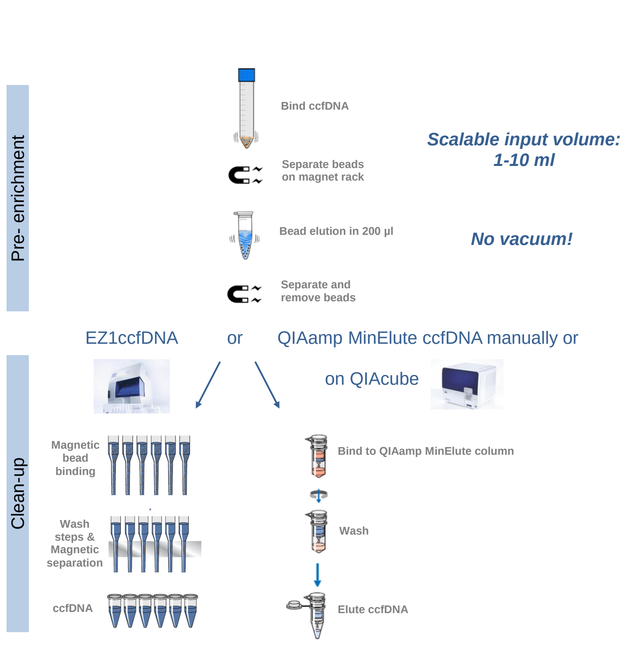
Materials & Methods

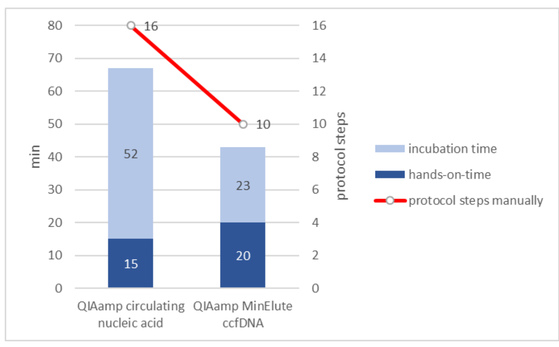
Volumes up to 10 ml of plasma were incubated with magnetic beads in order to bind free circulating DNA during end-over-end shaking. Bound nucleic acid was then eluted from the beads in a small volume, and processed by either a manual or automated clean-up procedure. Eluted DNA was evaluated for size distribution, yield, and usability in healthy donors and breast cancer patients.

Results

The new ccfDNA extraction method scaled linearly at all volumes of input plasma, with constant ng/ml plasma results. The entire process of extracting DNA from up to 10ml of plasma can be accomplished in under an hour. Total yields were equivalent to both gold standard and classical methods (QIAamp Circulating Nucleic Acid Kit and phenol-based extraction). This could also be shown with automated versions of the new method on the QIAcube or EZ1. In metastatic breast cancer patients, the amount of ccfDNA was shown to be elevated compared to healthy or non-metastatic donors.

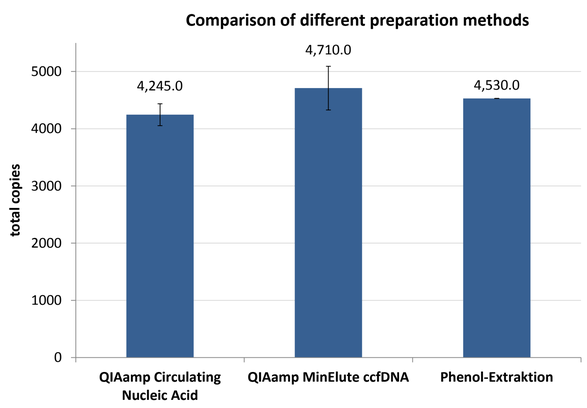
Pre-concentration procedure simplifies ccfDNA isolation

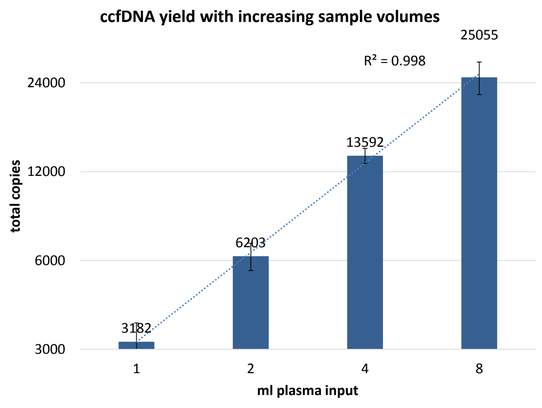




The new procedure to purify ccfDNA from blood uses magnetic beads to specifically bind low-abundant ccfDNA from flexible amounts of fresh, frozen or stabilized plasma (e.g. with the PAXgene Blood ccfDNA Tube®). After separation from the remaining sample, the pre-enriched DNA is eluted from the beads and cleaned up via different automation solutions or in a manual bind-wash-elute process.
This method saves about one third of the time and manual steps needed for usual isolation methods.

Scalable isolation of ccfDNA from plasma

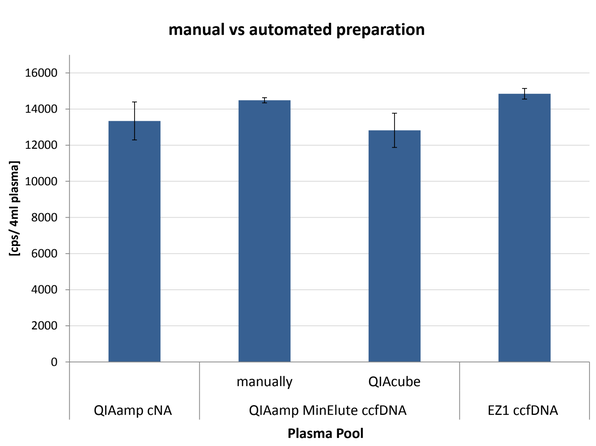


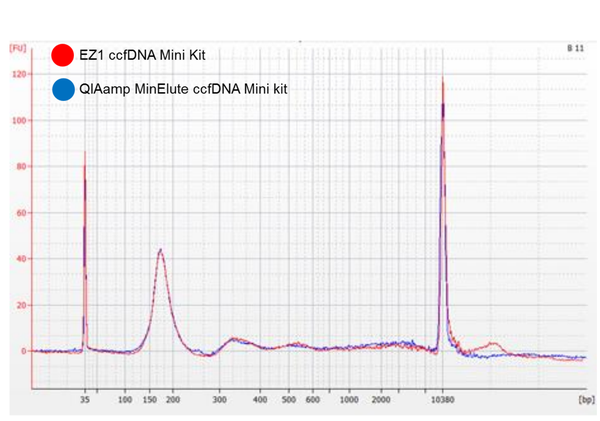


ccfDNA was isolated from human plasma using the new method. Although the isolation procedure was markedly faster and less tedious, the yield was comparable to the gold standard methods: QIAamp circulating nucleic acid kit and phenol-based extraction. Yield was determined using qPCR for a 66bp target from the 18S rRNA gene.

Due to the low concentrations of ccfDNA in plasma (1-100 ng/ml), it is often necessary to process large volumes of plasma in order to isolate sufficient material for analysis. To evaluate the scalability of the new method, 1ml, 2ml, 4ml, and 8ml of plasma was processed. The recovered yield of ccfDNA was directly proportional to the sample volume, proving reliable scalability.

Efficient recovery of ccfDNA from human plasma using manual or automated preparation

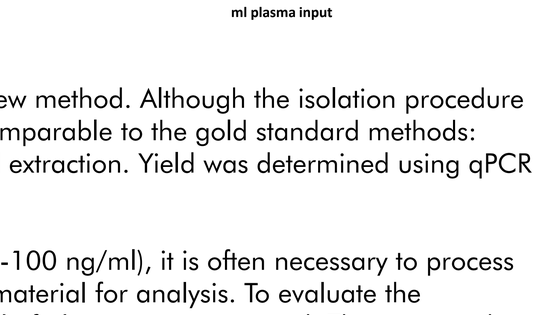




The new method gives three different cleanup possibilities – either manual, or automated on QIAcube or EZ1 Advanced XL. In preparations of 4ml of human plasma, all showed amounts of ccfDNA that were comparable to the standard QIAamp Circulating Nucleic Acid Kit.

In addition, size distribution of ccfDNA isolated using the new manual kit was analyzed in comparison to the automated version on the EZ1 Advanced XL. The same size distribution of DNA fragments was visualized on electropherograms, further proving equivalent ccfDNA recovery.

Increased amounts of ccfDNA can be an indicator for medical conditions



Plasma or serum from breast cancer patients carrying PI3K mutations either with primary tumors or with metastasis was compared to plasma from healthy donors using the new method. Yields were measured by 18S rRNA-PCR and Bioanalyzer peak analysis. ccfDNA amounts of patients suffering from metastasis were up to 50fold higher than ccfDNA amounts of healthy donors and non-metastatic patients with PI3K mutated primary tumors. This indicates potential correlations between amounts of ccfDNA and certain medical conditions.

Conclusion

Cell-free circulating DNAs usually are contained in varying, often low concentrations in plasma. Current isolation methods have fixed, predefined sample-volumes and include tedious vacuum-based, manual steps. In order to enable scalability of sample volume and yield, and to allow an easier isolation of the valuable targets, we have developed a bead and column based method with the following characteristics:

- Compatible with fresh, frozen, or stabilized plasma (e.g. in PAXgene Blood ccfDNA Tube®)
- Flexible plasma input volumes with constant extraction efficiency, allowing each user to select precisely the amount of plasma needed for their application
- Flexible and small (20 µl) elution volumes for maximum concentration of ccfDNA eluates

Sample to Insight