Sample-To-Answer Analysis Of Cell-Free Dna Using Centrifugal Microfluidics And Multiplex Mediator Probe Digital Pcr - A Concept Study

Abstract Submitter: Christoph Stärk, Germany*

Co-Authors: Truong-Tu Truong, Yumi Kaku, Judith Schlanderer, Franziska Schlenker, Eva Dazert, Nikolas von Bubnoff, Sabrina Kartmann, Nils Paust, Tobias Hutzenlaub, Peter Juelg

*Hahn-Schickard

Abstract

Background: Clinical utility of cell-free DNA (cfDNA) is currently under investigation for cancer and other diseases. However, complex laboratory protocols cause long turnaround times from blood sampling to obtaining test results. Reducing the sample-to-answer time and complexity can be advantageous in multiple domains: (1.) Patients could benefit from faster initiation of therapy, e.g., in highly aggressive malignancies such as pancreatic cancer. (2.) Healthcare systems could benefit from accelerated workflows, e.g., reducing hospital stays. (3.) Liquid biopsy analysis could become accessible for a larger group of healthcare providers.

Objective: We present a concept for rapid sample-to-answer cfDNA analysis in an integrated device. This includes design and experimental verification of three microfluidic cartridges, covering all aspects from blood-plasma-separation and cfDNA extraction to mutation analysis by digital PCR (dPCR). With this concept study, we want to evaluate and discuss the opportunities and technical challenges of an integrated sample-to-answer system for liquid biopsy applications.

Methods: We developed and characterized three centrifugal microfluidic modules for blood-plasma-separation (centrifugation and microfiltration), cfDNA extraction (magnetic nanoparticles), and KRAS mutation analysis (4-plex mediator probe dPCR). As proof-of-concepts, we analyzed circulating tumor DNA markers associated with colorectal cancer (CRC), including patient samples and healthy controls.

Results: In our proof-of-concept studies, we were able to demonstrate fast blood-plasma-separation (< 10 min, with comparable cell clearance to manual 2-step centrifugation), efficient cfDNA extraction from plasma (about 95% recovery) and 4-plex dPCR quantification of CRC-associated point mutations (KRAS G12D, G12V, G12A; linearity $R^2 > 0.98$). Finally, we present a design study for integrating these modules in a single microfluidic cartridge.

Conclusions: We demonstrate the feasibility of all individual steps required for sample-to-answer analysis of cfDNA in centrifugal microfluidic cartridges. Hence, we conclude that centrifugal microfluidics in combination with multiplex dPCR could enable rapid cfDNA testing in a decentralized and automated fashion. Ultimately, reaching this goal requires further efforts toward developing an integrated system, as presented in our design study. Such a system could positively impact both, patient’s outcome and the healthcare economy.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.