LABDISK INTEGRATED DNA EXTRACTION FROM WHOLE BLOOD USING MAGNETIC PARTICLES

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ABSTRACT

Integration of sample preparation is a key issue in the development of point of care diagnostic devices. We address the integration of a magnetic-particle based DNA extraction from 200 µl of whole blood into a centrifugal-microfluidic LabDisk. Quality of LabDisk extracted DNA was compared to DNA extracted by commercial spin columns as golden standard with four independent extractions from one blood sample. Experiments reveal comparable recovery of human growth factor gene determined by qPCR ($c_{1:10\ LabDisk}$ = 4.6 +/- 0.7 ng/µl vs. $c_{1:10\ spin\text{-}column}$ = 4.1 +/- 0.4 ng/µl), comparable purity (A260/A280 ~ 1,8 +/- 0.1), and slightly increased ethanol concentrations in LabDisk extracted DNA 5.9 +/- 2.4 % compared to 3.3 +/- 0.2 % in case of spin-columns.

KEYWORDS: Centrifugal microfluidics, DNA extraction, blood, diagnostics.

INTRODUCTION

Blood is a major sample matrix in genotyping applications. External-pump driven microfluidic chips for such DNA-purifications usually transfer the sample volumes to the microscale, thus loosing sensitivity of the assay [1,2]. DNA-purification from whole blood spiked with pathogens using a centrifugal microfluidic disk has been demonstrated [3], however, the technical requirements included laser valves and moveable magnets. An easier LabDisk based approach with static magnets and without need of laser valves was demonstrated in [4] by DNA-purification from an *E. coli* lysate. Here we demonstrate applicability of this approach to the extraction of DNA from whole blood samples, especially focusing on sample volumes of clinical relevance (200 µl).

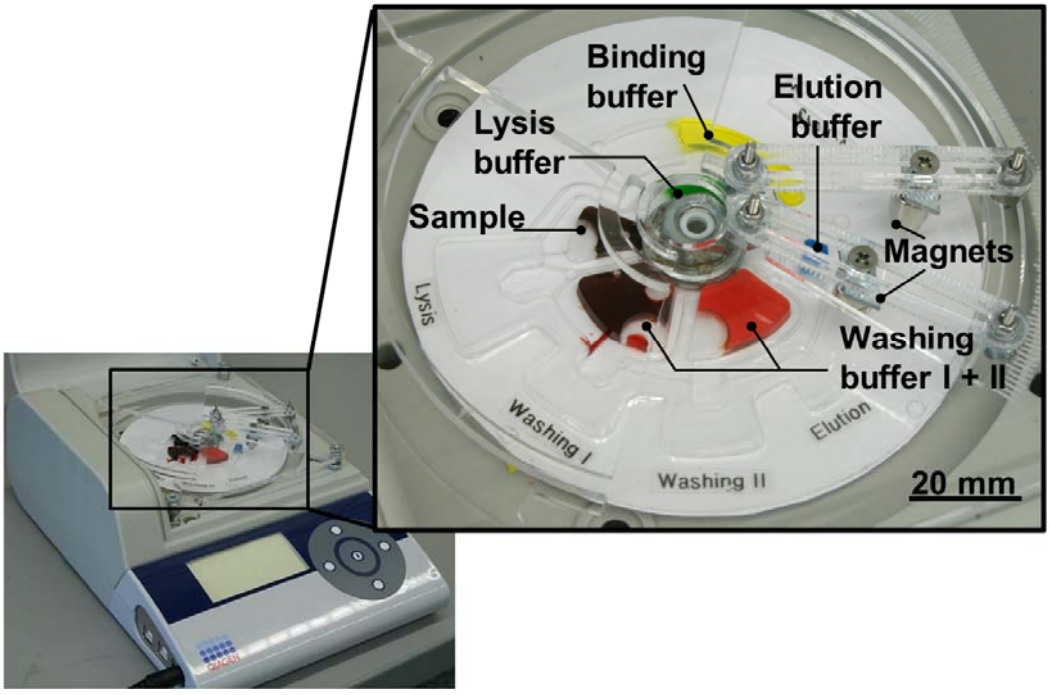

Figure 1: Prototype LabDisk processing device (Qiagen Lake Constance GmbH) with microfluidic LabDisk for DNA extraction. The disk is fixed at the motor of the player comprising a holder with two magnets for bead transfer. All reagents are loaded by pipetting before the automated protocol starts. Lysis, bead binding, washing and elution are automatically performed by rotation at different frequencies and halting.

PROCESSING DEVICE; LABDISK LAYOUT AND FUNCTION, AND EXPERIMENTAL SETUP

COP-foil based LabDisks are fabricated by blow thermoforming [5] and processed by a prototype LabDisk Player (Qiagen Lake Constance GmbH) (fig. 1). A LabDisk comprises chambers for reagent loading, blood cell lysis and DNA-to-bead binding, two washing steps, and elution. Bead-transfer between chambers is accomplished by two static external magnets and slow movement of the disk along the magnets (fig. 2). Reagents are pre-loaded prior to extraction and consist of buffers provided with the Qiagen DNA Blood Mini Kit (lysis-, binding-, washing- and elution buffers). The blood sample is mixed with magnetic beads and Proteinase K and loaded onto the disk. Then the automated extraction process is started. Afterwards, the eluted DNA is pipetted off the LabDisk. The eluate is analyzed for purity by absorbance measurement, recovery by real-time PCR and ethanol concentration by mass spectrometry. Results are compared to spin-column based reference extractions (Qiagen DNA Blood Mini Kit). Data were obtained from four independent extractions with each method and based on one human blood sample.

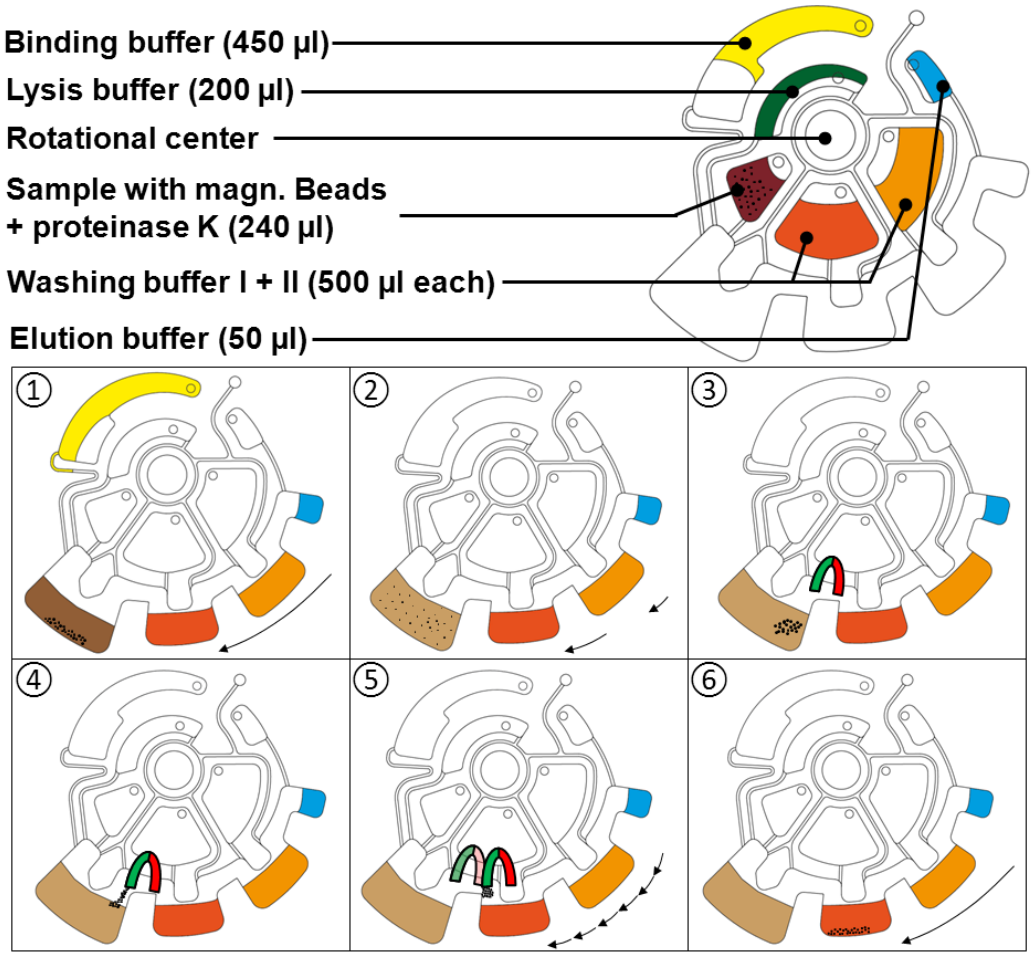

Figure 2: Microfluidic structure and protocol for DNA isolation. The LabDisk is loaded with sample and all reagents. Then the automated frequency protocol starts: ① Reagents and sample are transferred into corresponding reaction chambers and chemical lysis begins. ② Successive stopping and turning the disk enables binding buffer valving into the lysis chamber to bind the released DNA to silica magnetic beads. Following steps are repeated for two washings and the elution: ③ & ④ The disk stops to collect beads by external magnets. ⑤ Slow stepwise turning leads to bead transfer towards the next reaction chamber. ⑥ Final disk rotation releases the beads into the next chamber.

EXPERIMENTAL RESULTS

Purity of the eluates from the two different DNA extraction setups (A260/A280) was within the optimal range (1.8-2.0): 1.79 +/- 0.10 (LabDisk) and 1.81 +/- 0.11 (spin column). Remaining ethanol (measured by mass spectrometry) was slightly increased for the LabDisk: 5.9 +/- 2.4 % (LabDisk) vs. 3.3 +/- 0.2 % (spin column). Recovery of human growth factor gene (triplicate qPCR of 1:10-dilutions of the DNA eluates, using 1 µl of DNA per reaction) was determined by quantitative real-

time PCR in comparison to concentration standards from human genomic DNA. The quantities of DNA in the two independent extractions were comparable: 4.6 +/- 0.7 ng/µl (LabDisk) and 4.1 +/- 0.4 ng/µl (spin column) (fig. 3).

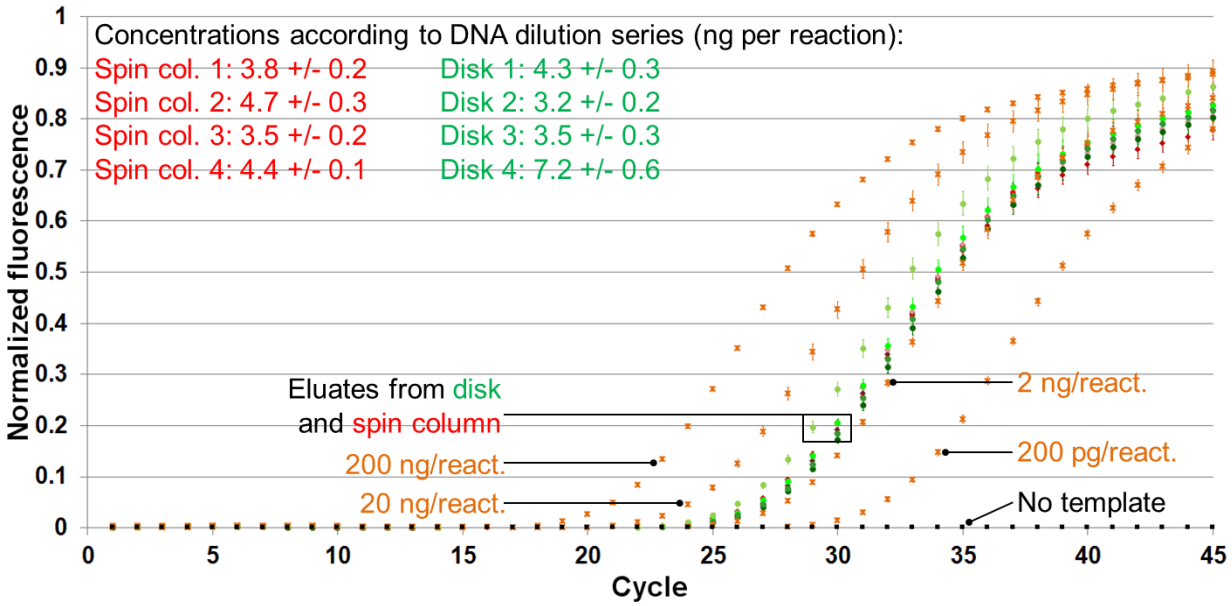

Figure 3: Quantitative PCR results. Amplification was performed in triplicates. Concentrations of human DNA in disk and spin column eluates (1:10 dilutions) were backcalculated according to cycle of quantification (C_q) values of known concentrations of human DNA (200 ng, 20 ng, 2 ng, 200 pg per reaction).

CONCLUSION AND OUTLOOK

DNA-extraction from 200 µL of whole blood on a LabDisk was compared to the golden standard of spin-column based extraction (Qiagen DNA blood mini kit). Good agreement was found in terms of purity and recovery. Residual ethanol was increased by (79 +/- 32) % in the LabDisk-based extractions but acceptable. The suggested approach for LabDisk integrated DNA-extraction from whole blood can be recommended for integration of sample-in-answer-out systems, including downstream DNA-amplification protocols such as diagnostic PCR, RPA or LAMP assays.

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REFERENCES

[1] Duarte GR, Price CW, Littlewood JL, Haverstick DM, Ferrance JP, Carrilho E, Landers JP, *Characterization of dynamic solid phase DNA extraction from blood with magnetically controlled silica beads.* Analyst, 135: pp. 531-537, (2010).

[2] Azimi S, Nixon G, Ahern J, Balachandran W, *A magnetic bead-based DNA extraction and purification microfluidic device.* Microfluid Nanofluid, 11: pp. 157-65, (2011).

[3] Cho YK, Lee JG, Park JM, Lee BS, Lee Y, Ko C, *One-step pathogen specific DNA extraction from whole blood on a centrifugal microfluidic device.* Lab Chip, 7: pp. 565-573, (2007).

[4] Strohmeier O, Emperle A, Focke M, Roth G, Mark D, Zengerle R, von Stetten F, *Magnetic bead based DNA purification on a disposable centrifugal microfluidic foil cartridge.* Proc.of the 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences (MicroTAS), Groningen, The Netherlands, pp. 402-404, (2010).

[5] Focke M, Stumpf F, Faltin B, Reith P, Bamarni D, Wadle S, Mueller C, Reinecke H, Schrenzel J, Francois P, Mark D, Roth G, Zengerle R, von Stetten F, *Microstructuring of polymer films for sensitive genotyping by real-time PCR on a centrifugal microfluidic platform.* Lab Chip, 10: pp. 2519-2526, (2010).

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