Automation of a magnetic immuno-PCR on a centrifugal point-of-care analyzer

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ABSTRACT

For the first time we demonstrate automation of an Immuno-PCR (IPCR) assay on the centrifugal microfluidic platform. All necessary assay steps are integrated on a single test carrier ("LabDisk") that is processed on a centrifugal point-of-care analyzer: A one-step kinetics-based immunoreaction; two-fold washing; thermal elution of DNA-conjugated antibodies; and a realtime PCR. Processing solely requires loading of the sample and immuno-reagents. The entire process is automated from thereon using a programmed rotational protocol. As a model study we demonstrate quantification of human high-sensitivity C-reactive protein (CRP).

KEYWORDS: Immuno-PCR, centrifugal-microfluidics, C-reactive protein, point-of-care

INTRODUCTION

The IPCR format combines the high versatility of enzyme-linked immunosorbent assays (ELISA) with the high sensitivity of PCR amplification, implementing a powerful method for detecting low quantities of antigens [1]. IPCR is applied e.g. for detection of low concentrated tumor markers, viral proteins, pathogens or toxins [1]. IPCR excels in improved limit of detection, less required sample volume and compatibility with complex biological matrices. Despite these advantages, IPCR is limited by complicated multistep protocols and increased assay duration compared to ELISA. Automated workstations enable IPCR assays with reduced hands-on time, yet are expensive and cannot be applied in point-of-care settings. We introduce a novel IPCR technology that only requires a mobile centrifugal analyzer and a cost-efficient test carrier (LabDisk) for easy-to-use, automated analysis.

THEORY

Conventional ELISAs use detection antibodies that are conjugated to enzymes for conversion of substrate solutions thereby generating a detection signal. In contrast, IPCR uses specific detection antibodies that are conjugated to a double-stranded DNA label. Upon immuno-complex formation and extensive washing, the DNA label is used for signal generation by quantitative PCR [2]. The highly sensitive nature of PCR may lead to a 100- to 10.000-fold increase in sensitivity, compared to ELISA [3]. Furthermore the dynamic range of antigen quantification of up to six orders of magnitude can be realized by IPCR [3].

EXPERIMENTAL

All experiments were conducted using LabDisks of COP (Zeon Chemicals, USA), fabricated by micro-thermoforming [4]. CRP concentrations of 50 ng/mL, 10 ng/mL and 2 ng/mL (pathophysiological range of CRP: 3-80 ng/mL) in spiked human serum were used as sample. For analysis, the immuno-reagents and the sample were loaded at the LabDisk inlets (see Figure 1): Inlet-1: 30 µL Capture-antibody-conjugated magnetic beads (AB-Beads), 30 µL DNA-conjugated detection antibodies (DNA-AB) and 30 µL sample; Inlet-2 and 3: 100 µL washing buffer (PBS, 0.05% Tween 20); Inlet-4: 60 µL PCR buffer. Afterwards, the automated analysis was started on the centrifugal analyzer (LabDisk player) that conducts the centrifugation protocol, gas-transfer-magnetophoresis (GTM)-based actuation [5,6] of the AB-beads, thermal elution, PCR thermocycling and fluorescence signal readout. The temperature

control for DNA elution and PCR thermocycling is thereby realized by air heating and cooling within the processing chamber of the LabDisk player which holds the rotation axis and the LabDisk test carrier.

RESULTS AND DISCUSSION

The complete workflow, depicted in Figure 1, was integrated and automated on a single LabDisk test carrier. In short, upon centrifugation, all liquids are pumped into the reaction chambers. AB-Beads are incubated with sample and DNA-ABs to form a sandwich immunocomplex (immuno-reaction). Using the GTM principle [5,6], AB-Beads are transported through the washing buffers (where unbound molecules are removed) into the PCR buffer. b) AB-Beads and the PCR buffer are pumped into the thermal elution module by centrifugation at 30 Hz. Here, the PCR buffer is heated to 95°C for 2 min to thermally release the DNA-ABs into the liquid phase. c) Finally the PCR buffer (including the released DNA-AB) is pumped by pneumatic actuation to the aliquoting structure, where the solution is split into 5 sub-volumes of 10 µL each (Note: 5 reactions are conducted for higher reproducibility of real-time PCR data). Real-time PCR is then conducted and the signal is read out by the LabDisk player.

The fluorescence data for the spiked samples and a reference serum (background CRP < 10 ng/mL) are depicted in Figure 2. Table 1 shows the total number of cycles carried out (35 thermocycles) subtracted by the cycle-of-quantification (C_q), showing a clear correlation to the CRP concentrations in the samples. The analysis time was 2 hrs. (30 min. for immunoreaction, washing and thermal elution;1 hr 30 min for real-time PCR) with ~ 5 min hands-on time.

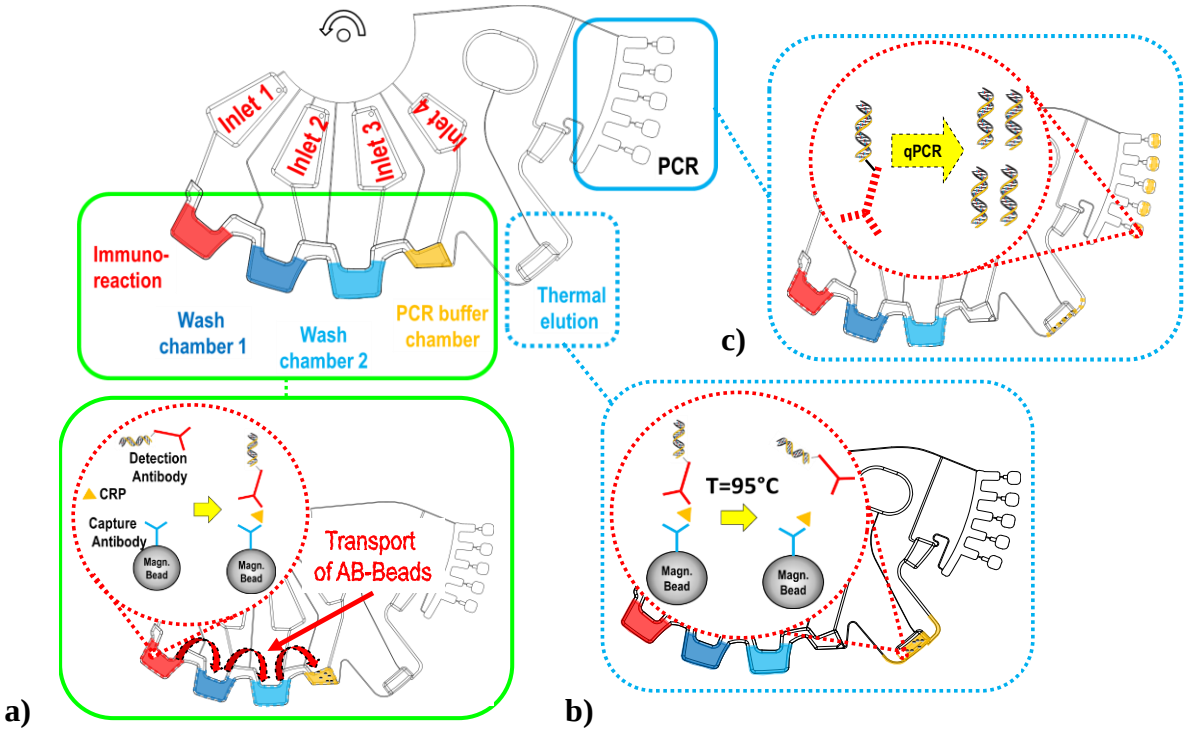

Figure 1: The IPCR workflow on the LabDisk.

CONCLUSION AND OUTLOOK

We demonstrate full automation and integration of IPCR on the LabDisk platform, enabling highly sensitive, automated quantification of biomarkers at the point-of-care. In the future, the system will be equipped with additional antigen specific antibody-conjugated magnetic beads and detection antibodies conjugated to different DNA labels for implementation of multiplex immuno-PCRs. Thereby different antigen-specific magneto-immuno-complexes are formed and its different DNA labels can be detected using different primers in the amplification chambers for multiplex detection. Furthermore, removal of all manual handling steps by pre-storage of all liquid buffers will further reduce manual handling to one single step - the loading of the sample.

Table 1. IPCR quantification data

Samples *	C_q	35 cycles - C_q
50 ng/mL CRP	17.1	**17.9**
10 ng/mL CRP	18.9	**16.1**
2 ng/mL CRP	21.7	**13.3**
(Normal serum)	22.9	**(12.1)**

** Human serum was spiked with CRP*

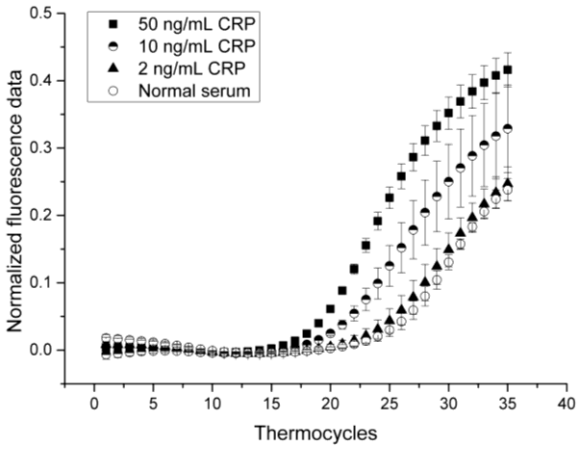

Figure 2 - Amplification curves *for the IPCR assay for 50 ng/mL, 10 ng/mL and 2 ng/mL of CRP spiked into human serum. The error bars represent the standard deviation of the fluorescence signal in five detection cavities.*

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