Automated detection of human C-reactive protein on centrifugal microfluidics-based LabDisk platform

G. Czilwik[1], V. Klein[1], O. Strohmeier[1], G. Roth[2 3], F. von Stetten[1 2],R. Zengerle[1 2 3], S. K. Vashist[1 2] and D. Mark[1]

[1] Institut für Mikro- und Informationstechnik (HSG-IMIT), 79110 Freiburg, Germany
[2] Laboratory for MEMS Applications, IMTEK - Department of Microsystems Engineering, University of Freiburg, 79110 Freiburg, Germany
[3] BIOSS – Centre for Biological Signalling Studies, University of Freiburg, 79110 Freiburg, Germany

Abstract

We have developed an **automated magnetic immunoassay** for human C-reactive protein (CRP) and integrated it into the **centrifugal microfluidics**-based `**LabDisk**` platform, enabling automated processing at the **point-of-care** (POC). It enables **rapid detection of human CRP** in human serum with an analysis time of just 25 min.

Motivation

Processing of conventional immunoassays on microtiter plates can take up several hours and includes multiple labor-intensive manual handling steps that require skilled personnel. Complex automated workstations are available, but their costs, complexity and bulkiness prevent their use in POC settings. Our LabDisk platform provides a rapid and cost-effective solution for automated POC detection. The automated magnetic immunoassay was demonstrated by the detection of CRP, an inflammatory biomarker with the highest reference for use in clinical practice as identified by the American Heart Association/Center for Disease Control [1].

Automated immunoassay processing

A magnetic immunoassay protocol is automated using a specific centrifugal processing protocol, where the magnetic beads acts as a mobile phase that are subsequently transported through liquid buffers by interplay of magnetic- and centrifugal forces. The immunoassay workflow is described in Figure 1. The only manual handling step is the loading of liquid immunoassay reagents before starting the centrifugation protocol on our portable `LabDisk Player`.

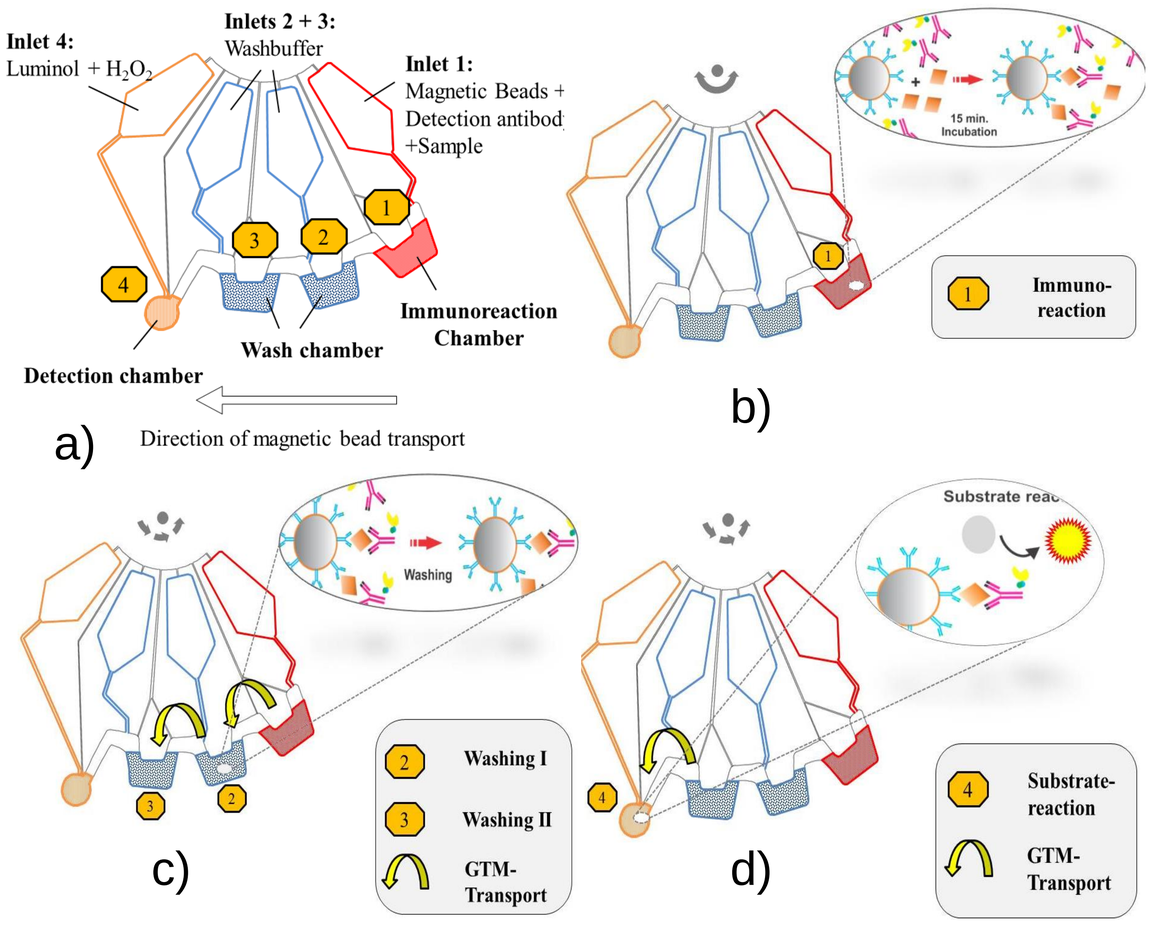

Fig. 1 – Immunoassay principle. Liquid reagents are loaded at the inlet ports of the Disk. a) A first centrifugation step drives the liquids into the reaction cavities. b) Capture-antibody-functionalized magnetic particles are incubated with the sample and an enzyme-conjugated detection antibody simultaneously for 15 minutes. Thereafter, an immune complex is formed on magnetic particles. c) This magneto-immune complex is washed twice in two subsequent washing chambers. d) Finally, an enzymatic reaction is conducted in a final detection chamber, where a luminescence signal is acquired using a detector, integrated in the processing device.

Magnetic bead transport mechanisms

The transport principle uses microfluidic chambers, which are isoradially arranged on the LabDisk. Adjacent chambers are interconnected by an air split (Figure 2). Transportation of magnetic beads from the liquid in a first chamber (i.e. immunoreaction chamber) through the air split into the liquid in a second chamber (i.e. washing chamber) is achieved by incremental rotation of the LabDisk with respect to a non-rotating permanent magnet, which is integrated in the processing device.

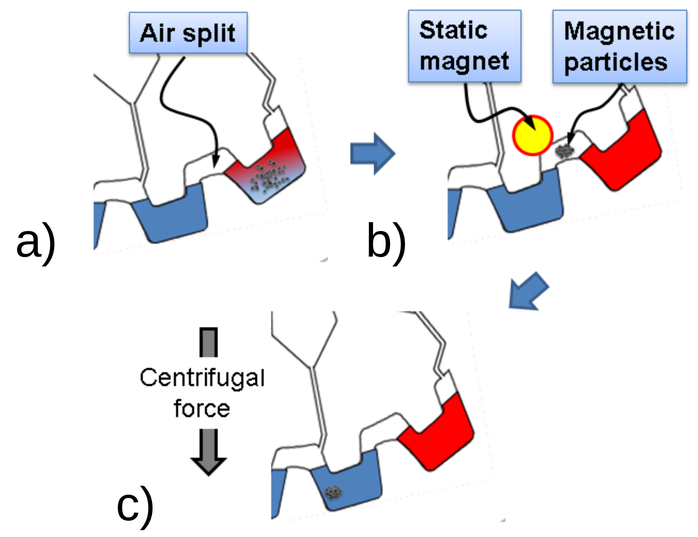

Fig. 2 – Automated transport principle of magnetic beads. a) Magnetic beads are incubated in the first chamber. b) The LabDisk is stopped with respect to a stationary magnet that pulls the beads into the air split. By incremental rotation, the beads move towards the next chamber. c) The LabDisk is rotated and the magnetic beads are transfered into the next chamber by centrifugal force.

Results

The test is fully automated after the initial loading of sample and immunoreagents at the inlet ports. It detects human CRP in the range of 0.3 - 81.0 ng/mL with a limit of detection of 0.8 ng/mL and an analytical sensitivity of 0.9 ng/mL, respectively.

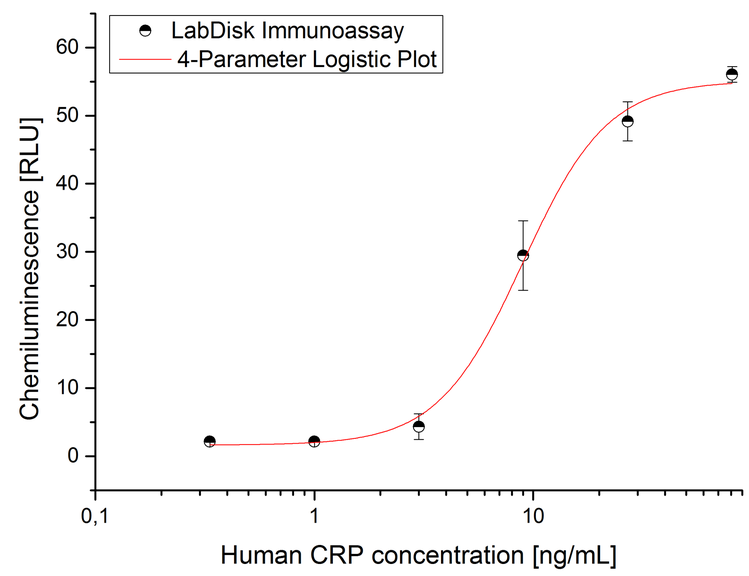

Fig. 3 - Assay curve of the developed CRP immunoassay

Conclusion and outlook

We used a modular principle for automated transport of magnetic particles to implement an immunoassay for the quantification of CRP within the physiological range. The integration of the module in combination with multiplex detection and prestoarage of reagents is currently under development.

References

[1] G. L. Myers *et al*, Circulation, 2004, 110, e545–e549.