MOVING THE SOLID PHASE: A STATIONARY MICROFLUIDICS PLATFORM TECHNOLOGY FOR CARTRIDGE BASED SANDWICH IMMUNOASSAYS

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ABSTRACT

We report on a stationary liquid phase microfluidic based platform which is applicable to various immunoassay formats. It employs magnetic beads which constitute both the solid phase for immobilizing capture molecules and in addition are used for magnetic field activated assay processing steps. Externally applied magnetic fields enable a comprehensive set of assay procedures such as active incubation, accumulation, dispersion, and transport between different assay solutions. This thus, allows for flexible adaptation of individual assay steps. The target molecules are detected through a fluorescence sandwich immunoassay.

Assays for a protein biomarker (interleukin 8) and a viral pathogen (Hepatitis A Virus) were established and yielded a limit of detection of 1.49 pg/mL (0,186pM) and 10^4 virus particles/ml, respectively.

KEYWORDS: microfluidic cartridge, immunoassay, magnetic particle, Point-of-Care Testing (PoCT)

INTRODUCTION

Microfluidic cartridge based assays hold great promise for application in Point-of-Care Testing (POCT) as they enable complex multi-step biochemical reactions with minimal user interference. Therefore, they may also be employed by untrained users. Ideally, assay components, sample and waste are contained in the cartridge and disposed of together with the cartridge once the assay is completed thus avoiding any risk of contamination. On the other hand such a scheme requires a maximum of automation to be integrated in the system operating the cartridge based assay. Exchange of fluids between system and cartridge would require pumps, valves, and reservoirs to be included in the peripheral system, thus increasing the risk of malfunction and also involve more complex regulatory requirements to be met. In contrast, moving the solid phase by using magnetic beads and applying suitable magnetic fields enables performance of complex assays yet completely avoids fluid handling between system and cartridge. In magnetic actuation schemes previously investigated by other research groups, magnetic beads were used for separation [1], [2] or mixing [3] but were not exploited for the integration of a complete assay procedure which is at the core of the system discussed in this report.

EXPERIMENTAL

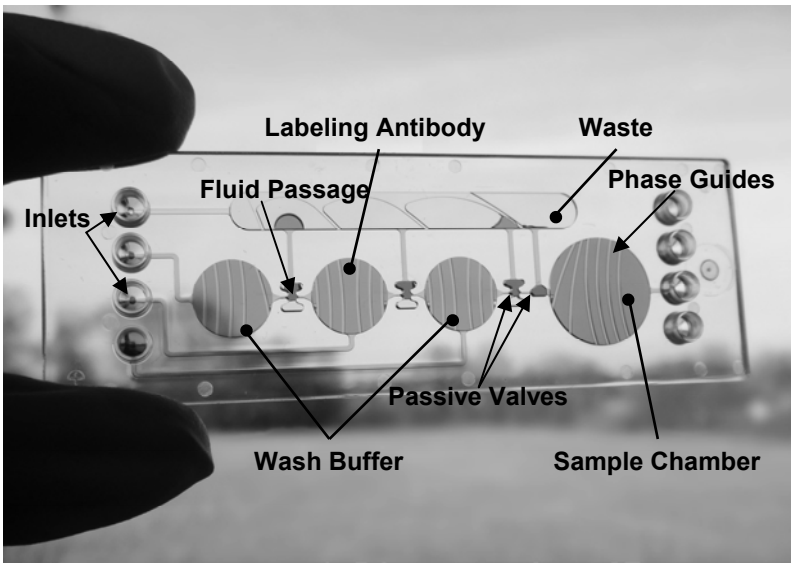

Figure 1: Cartridge comprising reservoirs for sample, wash buffers, detection antibody, and waste. Phase guides provide for robust and reproducible filling. Capillary stop valves help metering of fluid and enable transfer of particle aggregates by external magnetic actuation.

The cartridge was injection molded from cyclic olefin polymer (COP) and comprises several chambers separated by capillary valves (Figure 1). Chambers contain the assay reagents, through which the beads are manipulated via externally applied magnetic fields. Active incubation is made possible by magnetic field induced self-assembly of the beads into microstirrers [4] and systematically scanning them through a chamber (Fig.2a). The beads are transported by focusing them to form an aggregate which subsequently is dragged through the valves (Figure 2b). Notably, these valves avoid the requirement of an oil phase to separate chambers and prevent intermixing of fluids, in contrast to previous reports by other groups [1], [5]. Once the aggregate enters a chamber, it is re-dispersed and magnetic actuation is used to re-assemble the beads into microstirrers. The assay protocol involves an incubation of sample with antibody-coated magnetic beads, followed by steps for washing or separation, labeling with fluorescent detection antibody and finally fluorescence detection.

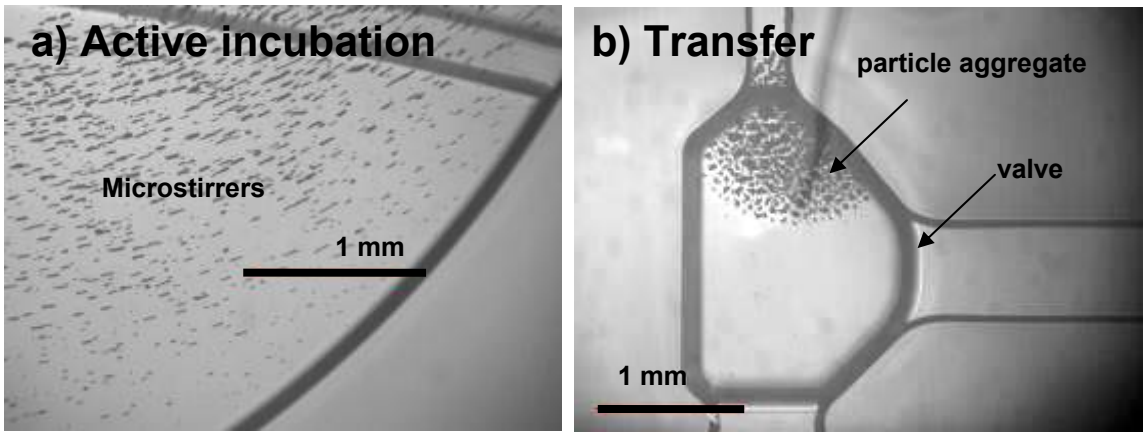

Figure 2: Manipulation of magnetic beads by externally applied magnetic fields: a) fields oriented parallel to the plane of chip induce formation of "pearl chain" arrangements of beads used as microstirrers during active incubation with sample. b) a focused magnetic field causes beads to form an aggregate which is transferred through a capillary valve to the subsequent chamber.

RESULTS AND DISCUSSION

An interleukin-8 assay served as a model for evaluating the system and a concentration as low as 1.49 pg/ml (0.186 pM) was successfully detected (Figure 3a). Furthermore, hepatitis A virus as a model pathogen was detected with an LOD of 2×10^4 virus particles / ml (Figure 3b).

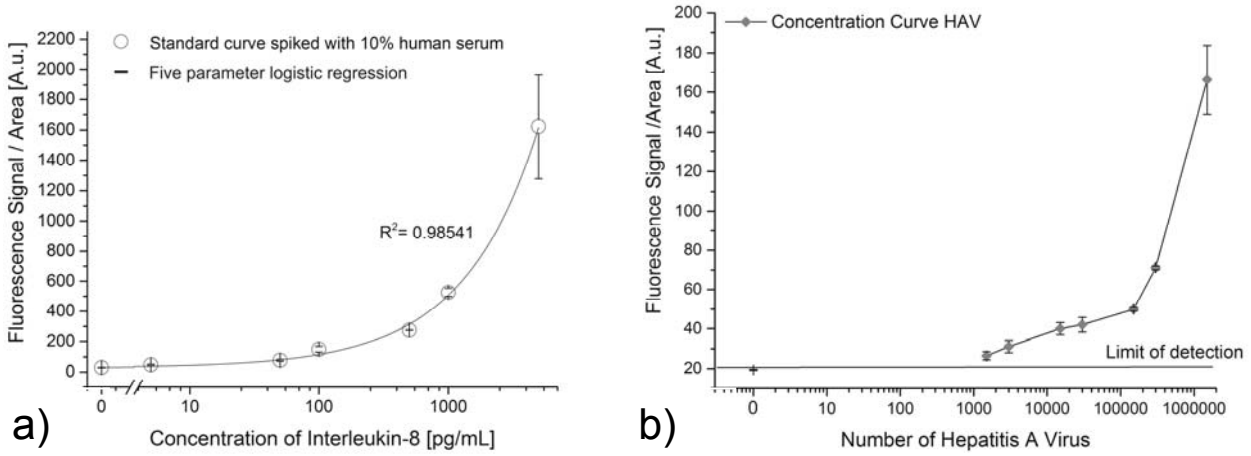

*Figure 3: a) IL-8 assay calibration curve fitted using a five parameter logistic model, showing a limit of detection (LOD) of 1.49pg/ml. b) Detection of hepatitis A virus, LOD =2*10^4 HAV /ml*

CONCLUSION

The platform presented shows potential to be developed into a diagnostic tool to be used in a PoCT setting. "Moving the solid phase" is a paradigm that enables comprehensive and robust assay performance in a cartridge, without resorting to external peripheral devices for fluidic control to run the assay. This substantially simplifies the assay procedure and enhances its robustness.

The cartridge was used as fabricated without any further preparation and achieved sensitive detection, as shown by the results obtained. Apparently, blocking or pretreatment steps of the cartridge are not necessary for the concentration ranges tested. An important feature of this platform is its compatibility with commercially available buffers, even including those containing detergents. The actuation parameters can also be adapted to a given assay by means of facile changes to a software protocol. These features can be exploited to further simplify the transfer of additional assay parameters to this system.

ACKNOWLEDGEMENTS

Funding for this research was obtained with the Spitzencluster microtec Südwest from the Federal Ministry for Education and Research of Germany through grant no. 16SV5111, project management was by VDI/VDE-IT, Berlin.

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