ON-CHIP PROCESSING AND DNA EXTRACTION FROM LARGE VOLUME URINE SAMPLES FOR THE DETECTION OF HERPES SIMPLEX VIRUS TYPE 2

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ABSTRACT

We present a sample introduction interface that allows direct on-chip processing of crude, large volume urine samples for the detection of Herpes Simplex Virus 2 (HSV-2). Sample introduction, cell lysis, DNA purification and sample volume reduction from a 600 µL urine sample down to a 10 µL elution volume were achieved in 7 min, offering great potential for use in point-of-care diagnostic devices.

KEYWORDS

DNA extraction, urine analysis, herpes simplex virus

INTRODUCTION

Herpes simplex virus type 2 (HSV-2) is the major cause of Genital Herpes and one of the most common sexually transmitted infection worldwide [1]. HSV-2 infection often remains undiagnosed as it is often asymptomatic. Transmission can therefore unknowingly occur to sexual partners or during labour to neonates, for whom it carries a significant mortality risk [2]. Furthermore, genital herpes has been associated with an increased risk of HIV acquisition.

Fast and efficient diagnosis therefore plays a major role in treating HSV-2 infection and minimising its spread. Microfluidic devices offer great advantages for Point-of-care (POC) diagnostics due to increased analysis speed and sensitivity, reduced reagent use and full automation. Despite their great potential, the development of a realistic real-world sample introduction interface remains a challenge to be overcome. Current published integrated devices use simulated samples or require excessive off- or on-chip sample pre-treatment to achieve desired specimen volume reduction and target concentration. Simulated samples comprise of a few µL of a highly concentrated bacterial cell culture [3] which very rarely represents target concentrations and purities found in clinical samples. Zhang *et al.* [4] processed 5 µL of whole blood on a microfluidic device, but amplified a genomic gene whose abundance is higher than that of infection titres. Samples with lower target concentrations therefore require the use of larger sample volumes to assure sensitivity. This results in the need for excessive off-chip sample pre-treatment, such as centrifugation steps [5], or complicated on-chip sample preparation and concentration modules.

Chen *et al.* [6] presented an integrated microfluidic cassette for nucleic acid detection of pathogens from saliva. 100 µL of saliva specimen could be processed on-chip but required an on-chip vibrating disc powered mixing chamber to achieve effective mixing of the sample with the chaotrope. Baier *et al.* [7] processed 3 mL cervix sample on a microfluidic chip but needed to include an on-chip filtration step for concentration. Although effective, such measures add to the overall complexity of the chip, which should be as simple as possible for use in a POC setting.

Immiscible filtration assisted by surface tension (IFAST) is a method that relies on immiscible phase filtration for DNA purification [8]. Here we exploit and modify the IFAST principle to develop a sample introduction interface that allows the direct processing of large volume urine samples and includes all the steps from sample addition, lysis, purification, concentration and elution.

EXPERIMENTS

Chip fabrication: Polydimethylsiloxane (PDMS, Dow Corning® Sylgard®) was mixed in a 10 : 1 ratio with silicone elastomer (Dow Corning® Sylgard®) and de-gassed for 1 h. The resulting mixture was then poured over an aluminium mould and cured at 80 ˚C for 30 min.

Extraction procedure: Extractions were performed from 400 µL real or artificial urine samples [9] containing 5 M GuHCl (Promega), 1 µg carrier RNA (Invitrogen) and, if not otherwise stated, 0.5 ng of HSV-2 plasmid (Randox). Dynabeads MyONE™ Silane paramagnetic particles (PMPs) (Invitrogen) and MagneSil® PMPs (Promega) were used as the solid phase. DNA binding was achieved in 5 min under constant mixing of the sample and the PMPs with a handheld NdFeB magnet. The PMPs were then locally concentrated and pulled through the immiscible barrier, followed by a 2 min elution step in the PCR chamber under constant stirring.

DNA analysis: The extracted DNA was amplified using the GoTaq® Hot Start Polymerase assay (Promega) according to the manufacturer's instruction, with the addition of 0.2 µg μL^{-1} BSA, 0.01% (w/v) PVP and 0.1% (v/v) Tween20. HSV-2 primer sequences were provided by Randox and the forward primer labelled with ROX at the 5' end. The resulting amplicon was analysed using the ABI3500 capillary electrophoresis system (Applied Biosystems).

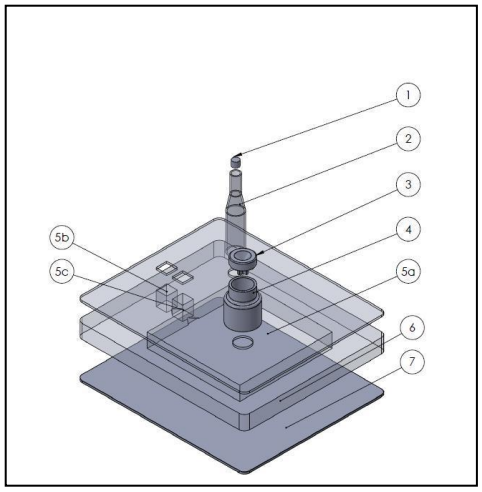

Figure 1: Real-world interface design consisting of filter (1), vent (2), septum (3), septum seat (4), all attached to an optical adhesive cover layer. Sample introduction and binding chamber (5a), wash chamber (5c) and elution chamber (5b).

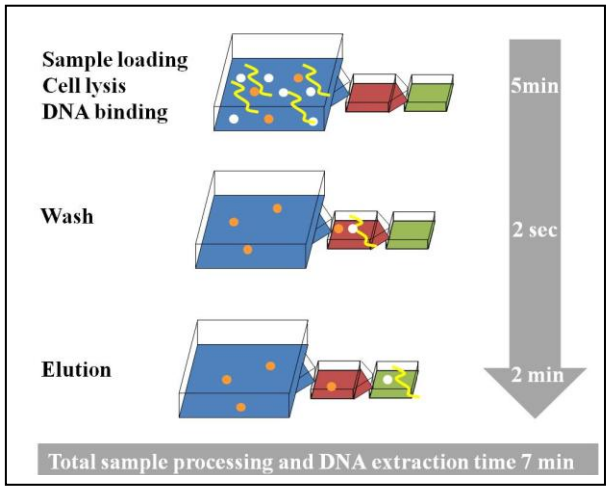

Figure 2: Schematic of the process. Upon sample loading, cell lysis and DNA (yellow) binding to PMPs (white) was achieved on-chip. The PMPs were then transferred through the immiscible barrier, leaving contaminants (orange) behind.

RESULTS

Interface design: The integrated device consisted of three chambers that were arranged in a linear configuration (Fig. 1). The device was made of PDMS and sealed with a double and single layer of optical adhesive on the bottom and top, respectively. The sample chamber was connected to a septum and vent to facilitate sample loading. It could accommodate a sample volume of up to 400 µL urine, whereas the wash and PCR chamber held a volume of 10 µL. The three chambers were interconnected by two trapezoidal microfluidic conduits that allowed the formation of stable aqueous/organic liquid interfaces [8]. The sample chamber was filled with the urine sample, GuHCl as the lysing agent and silica coated PMPs, whereas the elution chamber was filled with milliQ water. Mineral oil was loaded into the wash chamber as the immiscible barrier. A handheld permanent magnet was used to mix the magnetic particles with the sample and to draw the magnetic particles through the immiscible barrier. A schematic of the whole process is shown in Fig. 2.

Nucleic acid extraction performance: The extraction procedure was assessed by monitoring the amplification efficiency of downstream PCR reactions of HSV-2 plasmid DNA purified from artificial and real urine samples. Extraction experiments were performed using PMPs from two manufacturers, Dynabeads MyONE™ Silane PMPs (Invitrogen) and MagneSil® PMPs (Promega). The amount of particles used in the device was restricted by the geometry of the microfluidic conducts. A maximum of 0.32 mg of Dynabeads MyONE™ Silane PMPs and 0.24 mg of MagneSil® PMPs could be transported across the immiscible phase without particle loss or blocking the device. Extraction experiments using artificial urine as a sample matrix were therefore performed using 0.04 mg, 0.16 mg and 0.32 mg of Dynabeads MyONE™ Silane PMPs and 0.12 mg and 0.24 mg of MagneSil® PMPs (Fig. 3). The HSV-2 target was successfully amplified using both types of PMPs. However, MagneSil® PMP extracted DNA showed qualitatively superior amplification results, with higher overall PCR yields and reliable consistency. Similar results were obtained when extractions were performed from real urine samples using either 0.32 mg of Dynabeads MyONE™ Silane PMPs or 0.24 mg of MagneSil® PMPs (Fig. 4). The latter was therefore chosen for further experiments.

Extraction limit: The extraction limit of the system was defined as the plasmid concentration processed, which reproducibly amplified in downstream PCR analysis. Target concentrations ranging from 3.6×10^8 down to 36 plasmid copies mL^{-1} were tested. DNA extracted with the system produced reproducible results down to 3.6×10^4 copies mL^{-1}. Lower target concentrations resulted in inconsistent amplification results and were considered as below extraction limit.

DISCUSSION

LOC devices are of great potential for use in POC settings as they can be fully automated, allowing for minimum user intervention and reagent use as well as reduced analysis times. Despite recent advances in microfluidic-based integrated diagnostic systems, the sample introduction interface, especially in regards to large volume samples, has been neglected.

The presented device exploits IFAST to realise a real-world interface that allows direct processing of crude large volume urine samples in 7 min. IFAST, as a concept, has been described for DNA and cell purification purposes. We significantly improved the method by Berry *et al.* to adapt it to the requirements of the real-world interface. Firstly, our design includes a large sample reservoir that accommodates 600 µL of total sample volume. Sample loading was facilitated by an incorporated septum and vent connected to the sample loading chamber (Fig 1). Secondly, cell lysis and DNA binding to the solid phase was achieved on-chip by preloading the chamber with the chaotrope and the

PMPs; steps that were previously performed off-chip. Thirdly, instead of bonding the PDMS layer containing the microfluidic restrictions to a glass substrate, we opted for an optical adhesive, conventionally used in real-time PCR applications, double layer as the bottom substrate of the chip. This very simple and fast bonding approach had the added benefit that the hydrophobic surface properties of the optical adhesive substrate allowed the transfer of the magnetic particles through the immiscible barrier without the addition of detergents to lower the interfacial energy. The resulting device enabled the direct processing of large crude urine samples, which allowed DNA purification and volume reduction within 7 min without the need for pre-concentrating or filtering the specimen. Furthermore, effective mixing of the sample and through chaotrope mixture was achieved through PMPs movement, avoiding the need for an additional mixing system.

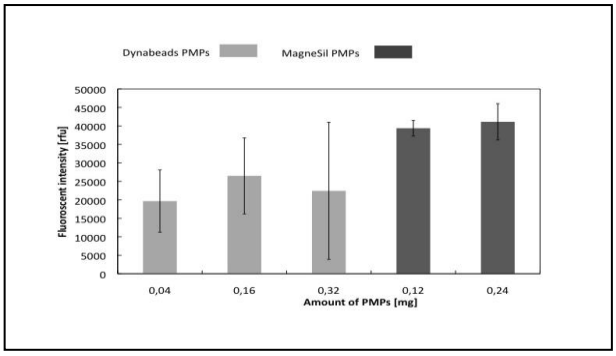

Figure 3: Amplification of the target isolated from artificial urine samples spiked with 3.6 x 10^8 mL^{-1} HSV-2 target sequence copies.

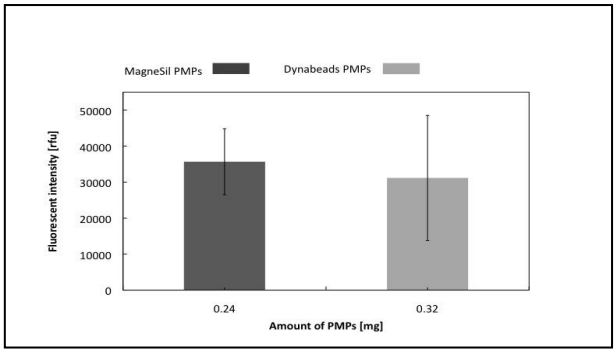

Figure 4: Amplification of the target isolated from real urine spiked with 3.6×10^8 mL^{-1} HSV-2 target sequence copies.

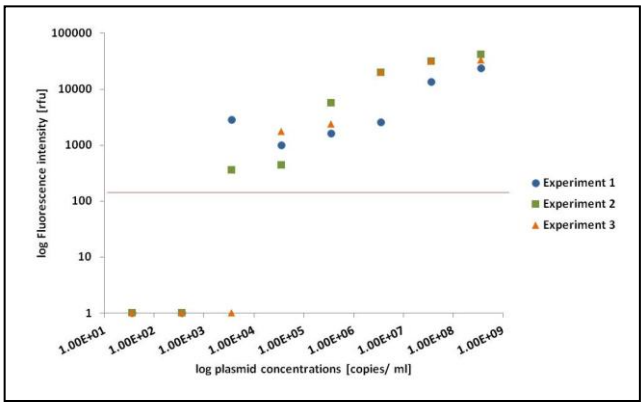

Figure 5: Detection limit of the demonstrated sample introduction interface. Horizontal red line represents background noise level of the capillary electrophoresis system.

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