

# Automation of Silica Bead-based Nucleic Acid Extraction on a Centrifugal Lab-on-a-Disc Platform

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**Abstract.** We describe a centrifugal microfluidic ‘Lab-on-a-Disc’ (LoaD) technology for DNA purification towards eventual integration into a Sample-to-Answer platform for detection of the pathogen *Escherichia coli* O157:H7 from food samples. For this application, we use a novel microfluidic architecture which combines ‘event-triggered’ dissolvable film (DF) valves with a reaction chamber gated by a centrifugo-pneumatic siphon valve (CPSV). This architecture permits comprehensive flow control by simple changes in the speed of the platform innate spindle motor. Even before method optimisation, characterisation by DNA fluorescence reveals an extraction efficiency of 58%, which is close to commercial spin columns.

## 1. Introduction

The centrifugal microfluidic platform [1-7] has successfully demonstrated sample-to-answer automation of bioassays for a wide range of applications including biomedical point-of-care diagnostics [8], cell analysis [9, 10], environmental monitoring [11-13] and quality control of industrial processes such as the production of biologics [14]. The cartridge holding all liquids is typically of a similar geometry as optical data storage media (e.g. CD or DVD) and spun on a miniaturised system similar to a commercial Discman™.

The LoaD platform offers a number of benefits. The system-innate centrifugal force field can be harnessed for pumping, liquid handling operations such as metering, aliquoting and mixing, as well as for sample preparation such as high-purity plasma extraction from whole blood [15, 16]. The disc can also be loaded and operate while open to atmosphere; a simple pipette can be used to introduce sample as opposed to a cumbersome ‘world-to-chip’ interfacing based on liquid connectors. Similarly, low-cost spindle motors are rather ubiquitous and generally significantly cheaper than specialised pumps.

Furthermore, as it scales with the square of the spin rate, the centrifugal field can be easily varied over several orders of magnitude, thus providing a large range of forces on fluids and suspended particles. These features contribute to provide a compact, highly autonomous, rugged, portable, user friendly and affordable bioanalytical sample-to-answer system which is particularly suitable for decentralised point-of-use testing.

For nucleic acid purification and concentration according to the established Boom method [17], a sequence of sample, wash and elution buffers are run through a chip-integrated silica-based solid phase. Many recent advances using the LoaD for nucleic acid analysis incorporate sample purification, nucleic acid extraction and subsequent nucleic acid based amplification to identify specific markers



for pathogens such as *Salmonella enterica* [18], *Bacillus atrophaeus* [19], *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Vibrio parahaemolyticus* and *Listeria monocytogenes*[20]. However, due to the complexity of the laboratory unit operations (LUOs) [7] involved, typically Load systems use support instrumentation, such as external heating, to operate the valves required to automate these processes. These additional modules add to the complexity and cost of the systems, and thus tend to undermine the simple concept inherent to the Load platform.

In this work we combine our recently introduced event-triggered valves [21, 22], which are based upon dissolvable film (DF) technology [23], with a sample incubation chamber which is combined with a centrifugo-pneumatic siphon valve (CPSV) [16, 24-26]. The event-triggered valves, where liquid is released through one DF, called the 'Load Film' (LF) when a second DF located at a remote location, called the Control Film (CF), is wetted, offer performance akin to an electrical relay. As they are decoupled from external instrumentation and from the changes in spin rate, the number of serial LUOs which can be placed on the disc is practically only limited by the available disc real estate. Yet, a major drawback of the event-triggered approach is the dependence of valve actuation on the dissolution times of the DFs. Thus, the event-triggered valves are unsuitable to implement the long incubations required for many bio-analytical processes. Optimisation of process timing therefore entails the replacement of the formulation of the DFs to be integrated in the system. A possible solution is to place a CPSV on the outlet of the incubation chamber so the timing is controlled by the programmable rotation of the spindle motor rather than the material-specific dissolution time of the DF.

We demonstrate our technology by processing a dilution series of DNA standard through this disc. We incubate our DNA standard with silica beads, then wash this sample with a high concentration of ethanol (75% EtOH) to remove chaotropic salts, and then eluting the DNA from the silica beads using buffer adapted from a commercial kit.

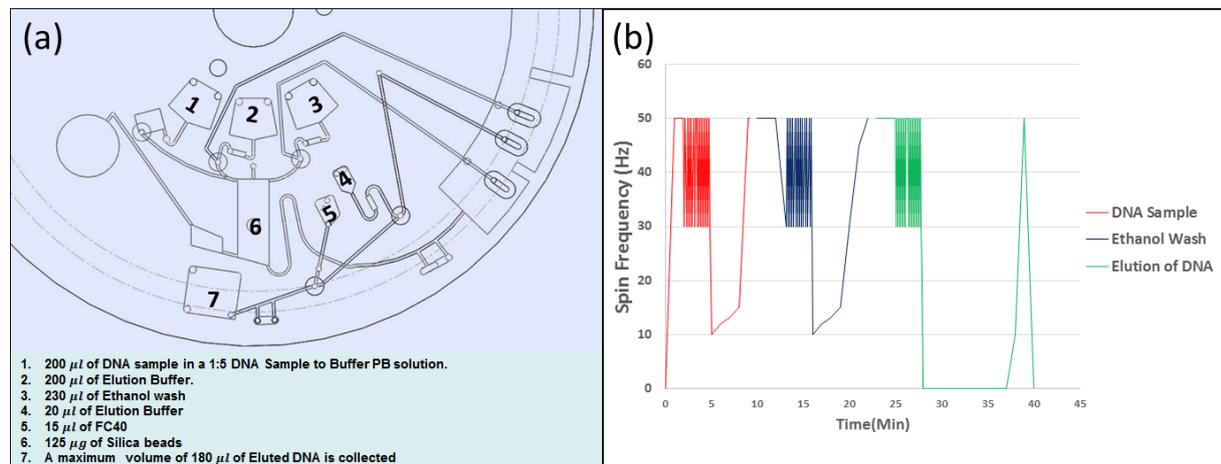
## 2. Material and Methods

### 2.1 Disc Manufacture and Assembly

The microfluidic cartridges (Fig. 1a) used in this study were assembled using multi-lamination methods from four layers of Poly(methyl methacrylate) (PMMA) and four layers of PSA (Pressure Sensitive Adhesive, Adhesives Research, Limerick, Ireland) [21]. Voids in the 86- $\mu\text{m}$  thick PSA layers [27] representing microchannels and other small features, were defined by a commercial knife-cutter (Graphtec, Yokohama, Japan). Larger features such as reservoirs for reagent storage and sample collection were created in 1.5-mm thick PMMA layers using a laser cutter (Epilog Zing, USA). The layers were aligned on a custom assembly jig. Between each alignment step, the attachment of layers was reinforced using a hot-roll laminator (Hot Roll Laminator, Chemsultant Int., US). Each DF was mounted on PSA tab using previously described methods [21] and then manually positioned within the disc during manufacture.

### 2.2 Experimental Test Stand

All discs in this study were imaged using an experimental test platform commonly referred to as a "spin stand" [21, 28]. Discs are mounted on a computer controlled motor (Faulhaber Minimotor SA, Switzerland) and spun according to programmed rates. An encoder embedded on the spindle provides two signals (1 pulse / rotation and 1000 pulses / rotation); custom hardware is used to define the angular position where a digital trigger activates a stroboscopic light source (Drelloscop 3244, Drello, Germany) and a sensitive, short-exposure time camera (Pixelfly, PCO, Germany). The discs are accelerated and slowed down at  $12.5 \text{ Hz s}^{-1}$ , except for the pneumatically driven disc which was decelerated at  $1 \text{ Hz s}^{-1}$  (Fig. 1b).



**Figure 1.** (a) Disc architecture. (b) Spin protocol for the disc-based nucleic acid extraction.

### 2.3 DNA Purification and Quantification

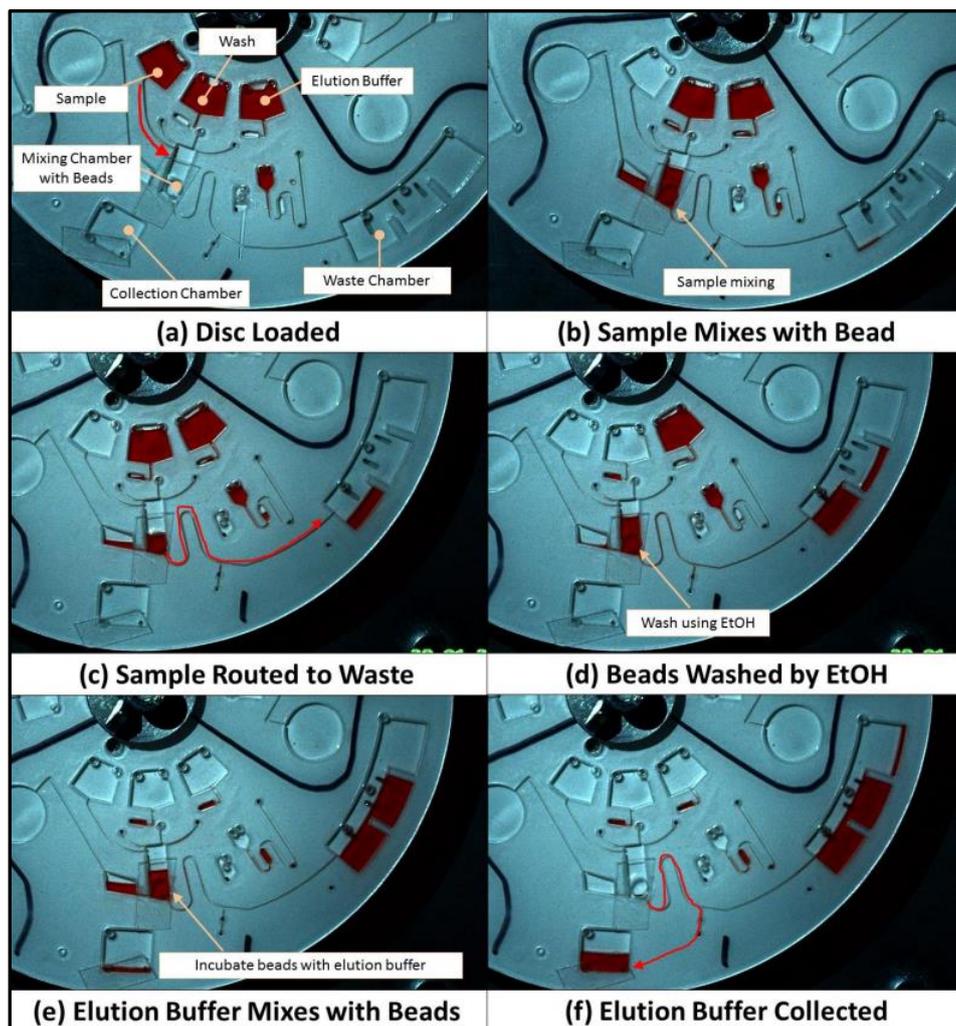
The DNA purification disc uses reagents adapted from QIAquick PCR Purification kit (Qiagen, Hilden, Germany); the extracted DNA in the eluate and was quantified using a Quant-iT™ PicoGreen® dsDNA (ThermoFisher). First, the Lambda DNA standard provided with the PicoGreen kit was diluted to a concentration of  $2 \mu\text{g mL}^{-1}$  using the method and buffer provided (TE Buffer). A working solution of Quant-iT™ PicoGreen® reagent is also created as per supplier's protocol.

For both, processing in the QIAquick spin column and on disc, the  $2 \mu\text{g mL}^{-1}$  is diluted 5:1 using buffer PB (provided in the QIAquick kit). A sample of  $60 \mu\text{l}$  is loaded. For the QIAquick columns, this sample is then washed using  $750 \mu\text{l}$  of PE Buffer and Ethanol (EtOH) based buffer provided in the QIAquick kit while on disc two  $100 \mu\text{l}$  washes are loaded into the chambers called EtOH #1 and EtOH #2. For both methods, the elution step is performed using  $60 \mu\text{l}$  of EB buffer (again provided in the QIAquick kit). For quantification,  $30 \mu\text{l}$  of the sample recovered from the QIAquick column, from the disc and from the reference DNA ( $2 \mu\text{g mL}^{-1}$ ) are each added to  $30 \mu\text{l}$  of the working solution of Quant-iT™ PicoGreen®. Fluorescence is measured using a commercial plate reader (Tecan) per the Quant-iT protocol and, for reporting, fluorescent signal is normalised relative to the reference ( $2 \mu\text{g mL}^{-1}$  DNA) fluorescent signal.

## 3. Disc Operation and Performance

### 3.1 Disc Architecture and Operation

A schematic of the disc architecture and the associate spin protocol are shown in Figure 1, a frame sequence of disc operation is portrayed in Figure 2. The disc is manufactured from eight layers of PMMA and PSA (as described previously) and the DFs are selected to be compatible with the reagents which are loaded in each chamber. The disc is loaded per the volumes described in Figure 1. Initially, the disc is accelerated to 50 Hz. At this point, the dense FC-40 liquid creates a plug in front of the route to the DNA collection chamber (Chamber 7) which ensures that the DNA sample and EtOH are both routed to the waste chamber. The sample flows into the incubation chamber, where it is transiently retained by a centrifugo-pneumatic siphon valve (CPSV). Due to the high spin-rate, centrifugal pressure forces some liquid into the dead end pneumatic chamber (which is a constituent part of the CPSV). By rapidly cycling the spin rate from 50 Hz to 30 Hz, part of this pneumatic chamber can be alternately emptied and refilled; this pneumatically enhanced 'shake mode' [29] mixing ensures that the DNA sample and the silica beads interact efficiently. After an arbitrary incubation time, defined by programming the spindle motor, the frequency of rotation is decreased to 10 Hz. This decrease in centrifugally induced pressure expels liquid from the dead end pneumatic chamber, increasing the liquid 'height' in the incubation chamber above the crest of the siphon and



**Figure 2.** Frame sequence of the disc operation. Dyed water is used for visualisation. (a)The disc is loaded, (b) the spin rate is increased to 50 Hz to deliver the sample into the incubation chamber where it mixes with acid-washed silica beads. The hydrodynamics in this chamber can be agitated by ‘shake mode’ mixing through rapidly alternating the rotational frequency between 30 Hz and 50 Hz. (c) The disc is decelerated to 10 Hz to prime the siphon. The disc is slowly accelerated to 15 Hz so the liquid empties to the waste (d). This protocol is repeated to wash the beads with Ethanol (75% EtOH). (e) The elution buffer is incubated with the beads. Simultaneously, the release of the FC-40 plug opens a route to the sample collection chamber. (f) Upon lowering the spin rate, the siphon is primed and now the elution buffer is routed to a collection chamber.

thus priming the siphon valve (CPSV). Next, the spin rate is increased so the content of the incubation chamber is emptied into the waste chamber. Here, the first CF is wetted, triggering the release of the 75% EtOH wash. In a manner similar to the DNA sample, the wash can again be incubated with the sample and be transferred to the waste chamber.

The 75% EtOH wash then wets and opens the second and third CFs located into the waste chamber. This releases the elution buffer, which again can be incubated with the bead substrate. Simultaneously, a liquid element is released which removes the FC-40 plug blocking the route to the sample collection chamber. Thus, upon deceleration of the disc, the elution buffer is preferentially routed away from the waste chamber to the sample collection chamber.

### 3.2 Disc Characterisation and Performance

The disc design underwent iterative optimisation to ensure proper function, manufacturability and correct process control. Changes were made to ensure correct volumes were used, proper siphon priming and efficient routing to the sample collection chamber. Once the reliable liquid handling was accomplished, the disc was tested using a DNA standard using the protocol described above. Normalised relative to the DNA standard ( $100\% \pm 7.2$ ,  $n = 3$ ), the commercial spin columns demonstrated an extraction efficiency of  $84.7 \pm 4.2\%$  ( $n = 3$ ) while we measured an extraction efficiency of the disc of  $58.4 \pm 7.5\%$  ( $n = 3$ ).

### 4. Conclusion

The disc architecture presented in this work presents several advantages compared to other methods: The combination of event-triggered valves and CPSVs permits good process control to be combined with a reliable and expandable reagent storage mechanism. The extraction efficiency is comparable to the state-of-the-art for LoAD. Performance might be improved, relative to the commercial platforms, by optimisation of reagents, bead concentration, surface treatments, and incubation times. Combined with appropriate nucleic acid amplification methods, such as LAMP, this platform will be used for multiplex serotyping of common *E. Coli* pathogens.

### Acknowledgements

This work was supported by the European Union under Grant number FP7-KBBE-2013-7-613908-DECATHLON and the Science Foundation Ireland under Grant No 10/CE/B1821.

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