

PRE-STORAGE AND RELEASE OF PURIFICATION REAGENTS FOR FULL “HANDS-OFF” INTEGRATION OF DNA/RNA ASSAYS ON THE LABDISK PLATFORM

Markus Rombach^{1*}, S. Hin², O. Strohmeier¹, F. von Stetten¹, R. Zengerle^{1,2} and D. Mark¹

¹HSG-IMIT – Institut fuer Mikro- und Informationstechnik, Georges-Koehler-Allee 103, 79110 Freiburg, GERMANY

²Laboratory for MEMS Applications, Department of Microsystems Engineering - IMTEK, University of Freiburg, Georges-Koehler-Allee 103, 79110 Freiburg, GERMANY

ABSTRACT

For the first time we demonstrate **complete integration and pre-storage of all required components** for **nucleic acid extraction and purification** on the centrifugal microfluidic LabDisk platform. On the one hand pre-storage of large volume liquid reagents from 200 – 550 μL is realized in **stick-packs, that are stacked to decrease the footprint consumption** on the LabDisk and are **released automatically during the run** using centrifugal pressure ($p_{cent} = 1 \text{ bar}$). On the other hand magnetic beads are mixed with PEG8000 (ratio 2:1) and air dried into the final cavity over 12 h. **PEG8000 allows stable pre-storage and does not influence the extraction yield.**

KEYWORDS: LabDisk, reagent pre-storage, stickpack, magnetic beads, nucleic acid purification

INTRODUCTION

Nucleic acid extraction and purification is an essential sample preparation step for the quickly growing market of PCR-based diagnostics. The centrifugal microfluidic LabDisk is one promising platform for full automation of nucleic acid testing [NAT] [1,2]. However, they require manual pre-loading of all reagents, greatly increasing the manual workload and trained operators. Therefore the pre-storage of reagents is a crucial factor to reach the goal of a completely automated system with as little manual interaction as possible [3]. For the large field of NAT, this work solves the pressing need for totally integrated test carriers that are easily operable, can be stored long-term and eliminate the risk of handling errors.

PRE-STORAGE AND RELEASE OF MAGNETIC BEADS

Magnetic beads are pre-stored in an air-dried fashion using PEG8000 as a stabilizing and resuspension supporting agent. 20 μL of the magnetic bead solution (MagaZorb reagent, Promega, USA) is mixed with 10 μL PEG8000 solution (50 % in H₂O, Sigma-Aldrich, Germany) and air-dried for 12 h until a stable pellet is formed (figure 1). Resuspension is demonstrated according to the protocol in table 1 using 450 μL of binding buffer (MagaZorb DNA Mini Prep Kit, Promega, USA) (figure 2). A complete resuspension of the beads was observed in 3 out of 3 cases. The yield and PCR performance are not statistically significantly affected by this pre-storage method, the mean value is even slightly higher for air-dried beads (figure 3).

Table 1. Frequency protocol for the bead resuspension and transfer into the next downstream chamber by the binding buffer.

Step	Description	Frequency [rpm]	Duration [s]	Temperature [°C]
A	Siphon priming	1200	5	23
B	Pellet wetting	300	15	23
C	Incubation	120	30	50
D	Emptying	1500	30	23

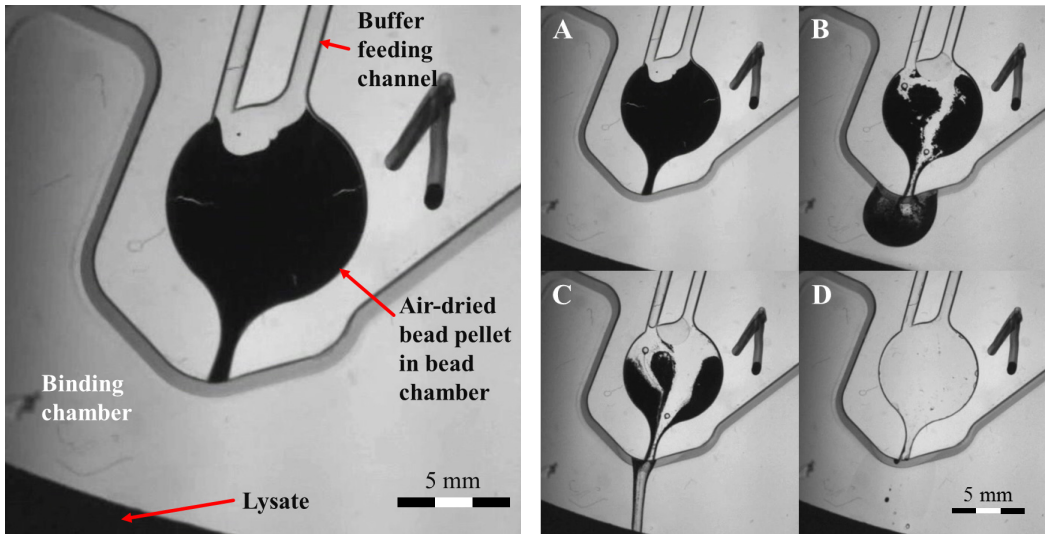


Figure 1 (left): Bead chamber with an air dried bead pellet right before resuspension by the binding buffer enabling the transfer of the beads into the binding chamber.

Figure 2 (right): Process of bead resuspension in 4 steps with the initial state (A), introduction of the binding buffer from radially inwards (B), incubation and PEG melting at 50 °C and 120 rpm (C) and complete emptying of the chamber at 1500 rpm (D).

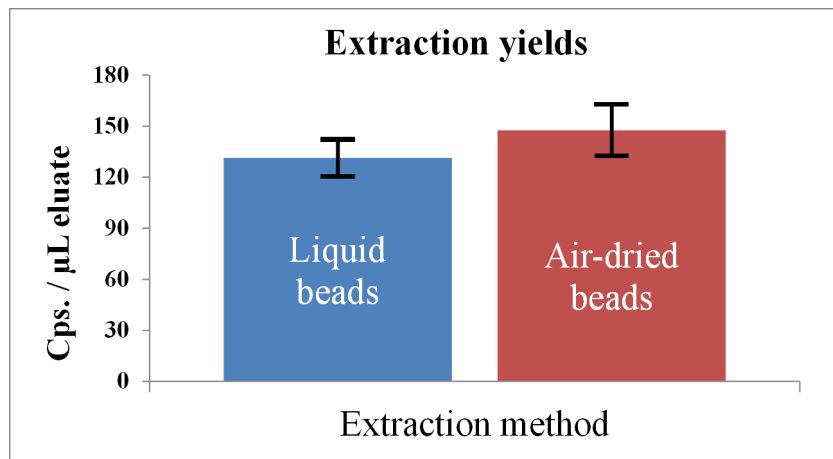


Figure 3: Comparison of the yields from purifying DNA out of a spiked sample with either liquid beads (blue bar) or air-dried beads (red bar) with PEG8000, whereas the bars represent 3 extractions each. The DNA was quantified in triplicates via real-time PCR reaction.

PRE-STORAGE AND RELEASE OF LIQUID REAGENTS

In 2013 our group published a paper on miniature stick-packaging of liquid reagents which can be opened using centrifugal force [4]. In order to save footprint (figure 4), we present the possibility of overlapping stickpacks. All stickpacks were produced on a stick-packaging machine (Type SBL-50, Merz-Verpackungsmaschinen GmbH, Germany) with a frangible seal that bursts at a centrifugal pressure of $p_{cent} = 1 \pm 0.1$ bar. For the test setup, a chamber comprising 3 stickpacks was designed, whereas the radially inward sides of the stickpacks overlap, displayed in figure 5. The frangible side of each stickpack is located radially outward and ends in a collection reservoir which can accommodate the complete volume of each. Liquids are released in parallel at 1200 rpm and collected in the collection reservoir, shown in figure 5. A total of 16 structures with 3 stickpacks each were tested successfully.

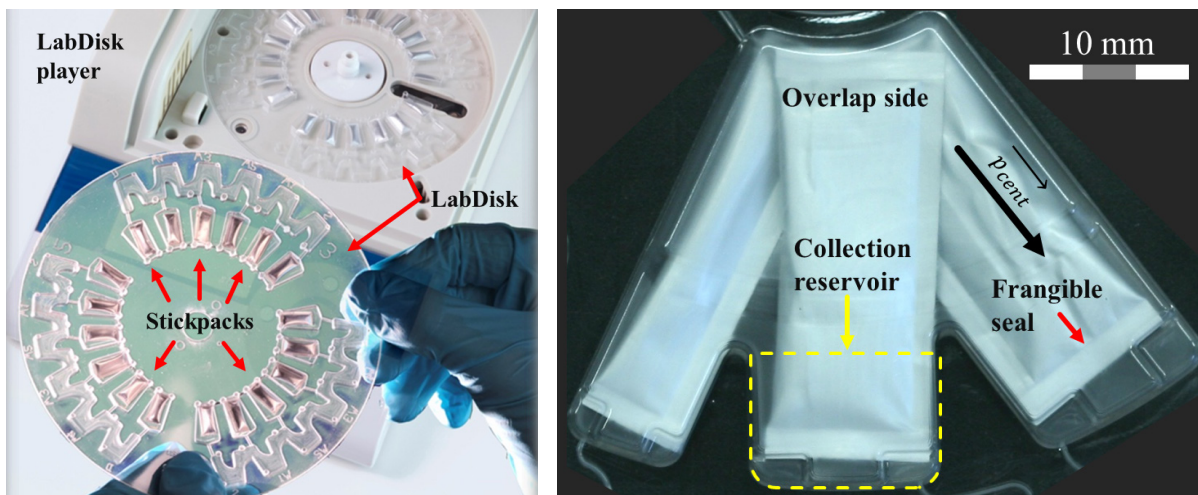


Figure 4 (left): The centrifugal microfluidic LabDisk platform: Disposable disk (in front) and LabDisk player (in the background). As shown, non-overlapping stickpacks require a large footprint (photomontage for illustration).

Figure 5 (right): Liquid pre-storage chamber with 3 overlapping stickpacks. The frangible seal is pointing radially outwards and is ending in separated collection reservoirs. Stickpacks are opened by using centrifugal pressure p_{cent} and collected in the reservoir before transferred to the destined chamber.

CONCLUSION AND OUTLOOK

For the first time pre-storage concepts that allow a complete integration of nucleic acid extraction reagents are presented. This constitutes a significant progress towards a fully automated analysis platform, enabling true hands-off sample-to-answer NAT as the next step.

ACKNOWLEDGEMENTS

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CONTACT

* M. Rombach; phone: +49-761-203-73222; Markus.Rombach@hsg-imit.de