Microfluidic cartridges for DNA purification and genotyping processed in standard laboratory instruments

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ABSTRACT

Two microfluidic cartridges intended for upgrading standard laboratory instruments with automated liquid handling capability by use of centrifugal forces are presented. The first microfluidic cartridge enables purification of DNA from human whole blood and is operated in a standard laboratory centrifuge. The second microfluidic catridge enables genotyping of pathogens by geometrically multiplexed real-time PCR. It is operated in a slightly modified off-the-shelf thermal cycler. Both solutions aim at smart and cost-efficient ways to automate work flows in laboratories.

The DNA purification cartridge automates all liquid handling steps starting from a lysed blood sample to PCR ready DNA. The cartridge contains two manually crushable glass ampoules with liquid reagents. The DNA yield extracted from a 32 µl blood sample is 192 ± 30 ng which corresponds to 53 ± 8% of a reference extraction.

The genotyping cartridge is applied to analyse isolates of the multi-resistant *Staphyloccus aureus* (MRSA) by real-time PCR. The wells contain pre-stored dry reagents such as primers and probes. Evaluation of the system with 44 genotyping assays showed a 100% specificity and agreement with the reference assays in standard tubes. The lower limit of detection was well below 10 copies of DNA per reaction.

Keywords: lab-on-a-chip, lab-on-a-foil, centrifugal microfluidics, DNA purification, DNA genotyping

1. INTRODUCTION

So-called lab-on-a-chip systems enable miniaturisation, integration and automation of standard laboratory routines like production, purification or analysis of chemical or biochemical compounds. A recent approach to improve access to such beneficial systems is to develop specific microfluidic cartridges for common and well-established laboratory instruments. This approach makes development of own operating machines obsolete and makes work in laboratories more efficient since existing instruments can be "upgraded" by means of microfluidic integration. A further aspect is also to reduce barriers for a successful market entry of such systems.

Here, two independent lab-on-a-chip systems are presented that are implemented in existing laboratory instruments. Both disposable microfluidic cartridges automate laboratory protocols for processing of nucleic acids (DNA). Liquid handling is enabled by use of centrifugal forces. The first microfluidic cartridge enables purification of DNA from human whole blood and is operated in a standard laboratory centrifuge. Purification of nucleic acids is an important step in sample preparation prior to DNA analysis. DNA extraction is conventionally very time-consuming and requires several manual handling steps or large, expensive automated workstations.

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The second microfluidic cartridge enables genotyping of pathogens by geometrically multiplexed real-time polymerase chain reaction (PCR). It is operated in a slightly modified off-the-shelf thermal cycler. Genotyping is a common method to explore existance of a specific target gene in a given DNA sample. Multiple specfic reagents and numerous pipetting steps are required to set up a genotyping assay. Pipetting errors can easily lead to wrong results or even contamination of whole laboratories.

The presented systems allow to overcome problems associated with conventional laboratory protocols by integration of reagents and standardisation of liquid handling due to minimised requirement of manual pipetting.

2. DNA PURIFICATION IN A STANDARD LAB CENTRIFUGE

The disposable cartridge for DNA purification already contains essential liquid reagents and allows to automate all liquid handling steps starting from a lysed blood sample to PCR-ready DNA.

The following steps are integrated: Binding of DNA from a lysed blood sample to a silica membrane, two consecutive washing steps, and elution of the purified DNA from the silica membrane. Unbound sample residues and washing buffers are routed into a waste chamber. The purified eluate is directed into a separate elution chamber from which it can be collected and directly used in a DNA analysis.

The cartridge contains two glass capillaries with 100 µl of washing buffer 1 (AW1) and washing buffer 2 (AW2), respectively [1]. These aqueous buffer solutions are required for the process of DNA extraction. The glass containers are crushed manually to release the buffer solutions just before the DNA extraction starts. The eventual release of the liquids into the microfluidic network is then achieved by passive, time-controlled burst valves. These valves work by controlled delamination of the lid foil during fast rotation in the centrifuge. Local delamination of the lid foil opens a passage for the liquid into the adjacent microfluidic channel system [2].

The operator just adds elution buffer and a sample of lysed blood into the respective chambers. Subsequently, the DNA extraction procedure can be started. In contrast to a previously reported routing approach [3], a novel routing mechanism for separation of the eluate from waste liquids is applied in this approach: A liquid portion is directed to a venting channel to block an air vent. This prevents further filling of the waste chamber and forces the eluate to flow into the elution chamber for collection. The schematic microfluidic routing is displayed in figure 1.

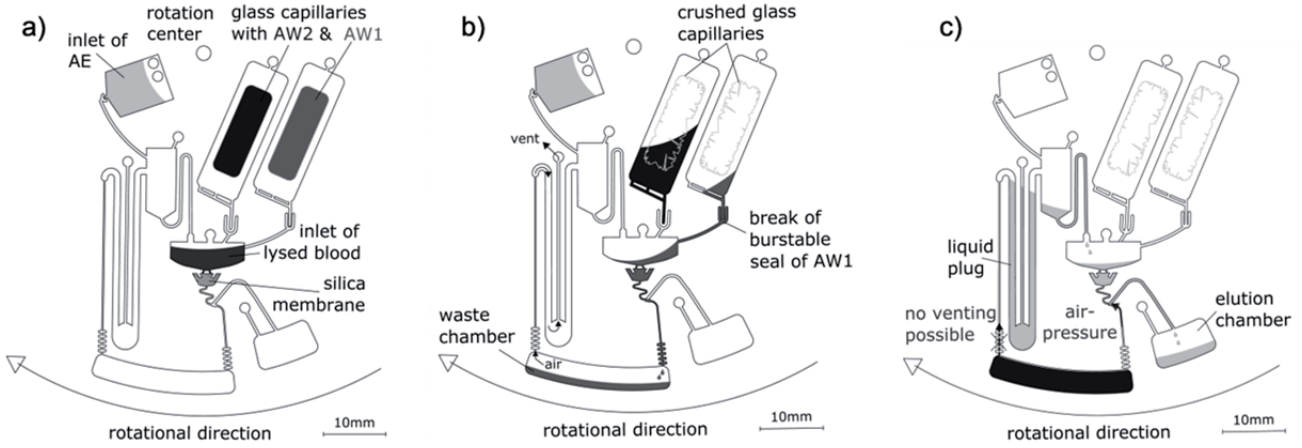

Figure 1. Microfluidic principle. a) Filling levels before rotation and uncrushed glass capillaries containing washing buffers AW1 and AW2; b) break of burstable seal of AW1 and centrifugation of AW1 into the waste chamber; subsequent automatic release of AW2; c) elution step after breakthrough of burstable seal of AE and blocking the switch channel by a liquid plug. Thereby the elution buffer AE is guided into the elution chamber, after collecting the purified DNA from the silica solid phase.

All experiments were implemented on a standard laboratory centrifuge (Figure 2) (Sigma 1-15, Sigma GmbH, Osterode am Harz, Germany) that was provided by the manufacturer with an automated control option via an RS232 interface. For fixation of the disk, a hematocrit rotor is used. DNA extraction reagents and the silica extraction matrix were taken from a QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany). Table 1 shows the protocol of rotational frequency in the lab centrifuge

Table 1. Frequency protocol of the automated DNA purification on disk after pipetting of blood and crushing of the glass capillaries containing both washing buffers

DNA extraction step	**Frequency /rpm**	**Time /min**
Centrifugation of lysed blood, AW 1 and AW 2 (100 µl each) into the waste chamber	6000	5.0
Breakthrough of burstable seal of AE (190 µl)	6000	57.0
Capillary filling of the siphon	0	1.0
Incubation step (optional for increased elution efficiency)	300 0	0.1 1.0
Elution step: centrifugation of the elution buffer in the elution chamber	900	3.0

An average of 192 ± 30 ng DNA has been extracted from 32 µl of blood in three independent experiments. This corresponds to a yield of 53 ± 8 % compared to a reference extraction with the conventional QIAamp Kit. Currently, the processing time for the complete extraction is about 66 minutes, since the opening time of the burst valves has not been optimized yet. Burst times of washing buffer AW1, AW2, and elution buffer are 50 ± 20 s, 105 ± 40 s, and 62 minutes(!) respectively, whereby the latter may be reduced to approximately 6 minutes with an adapted design of the time-controlled burst valves. In total, three out of four experiments with the completely integrated system were successful. Due to sealing failure in the fourth experiment the air vent of the waste chamber was not blocked so that the elution buffer flows directly in the waste chamber and the measurement of DNA-yield was not possible.

Figure 2. Microfluidic cartridge for DNA-Extraction operated in a standard laboratory centrifuge.

3. AUTOMATED GENOTYPING IN A MICROFLUIDIC CARTRIDGE

Genotyping of DNA samples plays an important role for decision making in the fields of food or infection control, in clinical settings as well as forensic scenarios. Here, the disposable genotyping cartridge is applied to analyse isolates of the multi-resistant Staphyloccus aureus (MRSA) by real-time PCR. The cartridge is made of a microstructured polymer foil ("Lab-on-a-Foil") [4,5]. This is for example advantageous as the thin sidewalls of this so-called blister cartridge support efficient thermocycling by fast heat transfer. Further, foil-based Lab-on-a-Chip systems suit perfectly as disposable consumables because they only require a minimum of material volume. In the future, such cartridges could be produced in fully integrated form-fill-seal production lines. Such production lines are long standard facilities for example in the field of packaging of pharmaceutical pills into blister packages.

Each foil cartridge contains four independent structures that allow to process up to four separate samples per cartridge (Fig. 3). The foil cartridge is designed to be processed in a slightly modified off-the-shelf thermal cycler (Rotor-Gene 2000, Corbett Research Ltd., Fig. 4). The modification of the instrument refers to an additional relay to increase the rotational speed discretely from standard 6.6 to 27.2 Hz. Further, the microtube holder is replaced by a custom-made, light-weight adaptor for fixation and alignment of microfluidic cartridges in the device.

The master mix (1x, RealMasterMix with ROX, 5Prime, Germany) containing the DNA sample is automatically aliquoted into individual reaction wells based on a centrifugo-pneumatic valving principle [6]. Reaction volumes of 9.8 µl are aliquoted with a precision of CV 3.4 % (32 wells).

The reaction wells contained pre-stored dry reagents such as primers and probes. This allowed to evaluate the system with 44 genotyping assays containing clinical isolates of 8 different genotypes of MRSA. The evaluation proved a 100% specificity in agreement with the reference assays in standard tubes in all 44 tests. The lower limit of detection was below 10 copies of DNA per reaction (N = 24 wells in 3 independent disks).

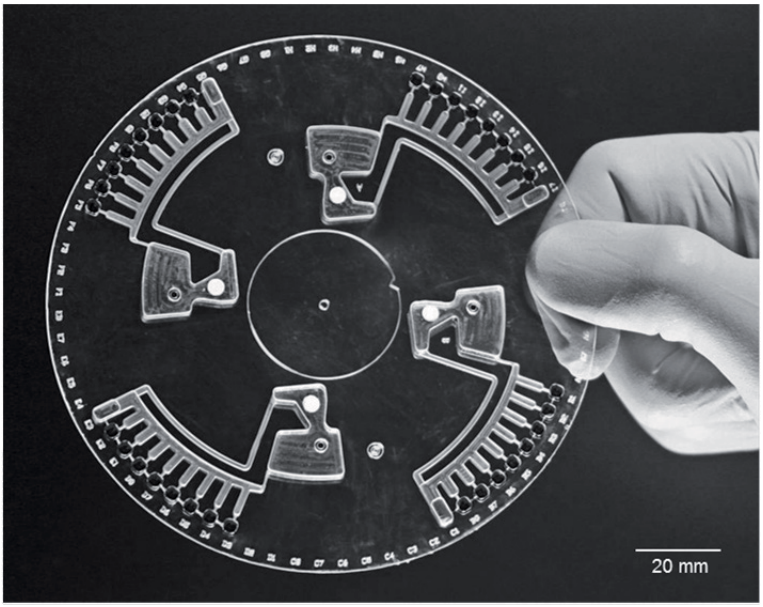

Figure 3. Microfluidic cartridge for genotyping of DNA. Up to four samples can be processed independently in each disk. On the outer diameter are four separate structures comprising eight reaction wells (highligthed with a dye for better visibility) and a reservoir for waste liquid.

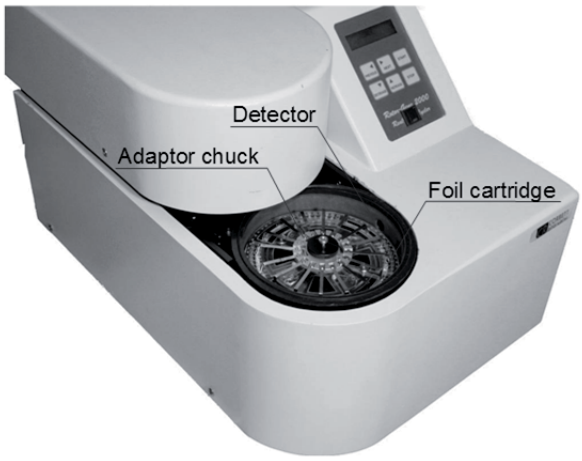

Figure 4. Microfluidic cartridge for genotyping processed in a slightly modified Rotor-Gene thermocycler

4. CONCLUSIONS

Two microfluidic cartridges for processing of DNA were demonstrated. The first cartridge was validated for miniaturised and automated DNA extraction. It has the potential of reducing DNA purification to a one-step automated protocol on an existing laboratory centrifuge. In order to complete the whole sample preparation process in the same cartridge, a microfluidic structure for cell lysis will be integrated. Further, the time-controlled burstable valves for controlled local delamination need redesign in order to reduce complete processing time down to approximately 8 minutes.

The second microfluidic cartridge proved suitable for automated aliquoting and performance of a genotyping assay by means of real-time PCR. The platform enabled handling of four independent samples and extremely sensitive detection of 8 different genes (current design). Numerous genotyping applications may be flexibly implemented by adopting the number of reaction wells in a design iteration and integrating multiplex assays in each reaction well.

In conclusion, it was demonstrated that microfluidic cartridges can be designed to be processed in standard laboratory instruments or instruments that only require minor modification. This creates added value for the supplier of standard lab equipment and reduces market entry barriers for organisations developing microfluidic solutions. Users of the microfluidic platforms only require minimal pipetting effort while profiting from pre-stored reagents and standardised assay performances.

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