AUTOMATED ANALYSIS OF GENETIC MARKERS BY ISOTHERMAL RECOMBINASE POLYMERASE AMPLIFICATION IN A CENTRIFUGAL MICROFLUIDIC CARTRIDGE

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Summary

Recombinase polymerase amplification (RPA) is a **new isothermal DNA amplification method** that runs at **37 °C**, amplifies **single copies** in less than **15 minutes**, and allows **real-time fluorescence detection**. For the first time we **automated** this method by **microfluidic integration** into a **centrifugal lab-on-a-chip system**, comprising unit operations for reconstitution of reagents, mixing with the sample, and aliquoting to test cavities. As the cartridge contains all of the required **liquid and dry reagents** only the addition of sample DNA is required. The system was demonstrated by the qualitative **detection of < 10 copies** of the antibiotic resistance gene *mecA* in **less than 15 minutes**.

The presented disk-shaped Lab-on-a-Chip cartridge consists of a COP-foil structured by blow-molding [1]. The surface is coated with BSA for blocking and hydrophilization. Glass ampoules with liquid reagents and lyophilized dry reagents are inserted into reservoirs and the disk is sealed by an adhesive foil (fig 1A). When operated the sample is added to the cartridge, the liquid reagent ampoule is manually disrupted through the foil to release the reaction buffer, and the cartridge is placed into a centrifugal analyzer with integrated fluorescence detection (modified RotorGene 2000, Corbett Research, Australia). At spinning frequency of 27 Hz 50 µl of reaction buffer is transferred from the disrupted ampoule to the lyophilized RPA reagents. After dissolving the lyophilisate the solution is divided into 5 x 10 µL aliquots and transferred into the test cavities via a centrifugo-pneumatic valve (fig 1B). Each of the test cavities may contain a different primer and probe system. The RPA reaction is based on a special recombinase-primer-complex that allows strand displacement and amplification at 37 °C [2].

Experiments and Results

In first experiments the sensitivity of our approach was evaluated. Samples containing < 10, 20 and 200 copies of the *mecA* gene are directly pipetted into the reaction chambers and analyzed. The graph in fig. 2B shows the real-time amplification plot of the samples. The *ct*-values for > 10, 20, and 200 copies are already reached after 12, 10, or respectively 5 minutes. All curves show a "ditch" following the exponential rise of the signal intensity, a typical characteristics of the RPA reaction. In another experiment the reaction chambers are tested with respect to cross contamination. Therefore a sample containing 2000 copies is added in one reaction chamber while the adjacent reaction chambers contain no template controls (NTC). The graph in fig. 3 demonstrates that no DNA accesses the chambers with NTCs during the assay excluding cross-contamination.

A **fully integrated centrifugal Lab-on-a-Chip system** is presented for **isothermal amplification of DNA** using the **RPA reaction**. Our microfluidic cartridge features **prestorage of all reagents** required for the assay .The successful detection and amplification of **less than 10 copies** of the mecA gene out of a sample is shown **without cross-contamination** between adjacent reaction chambers.

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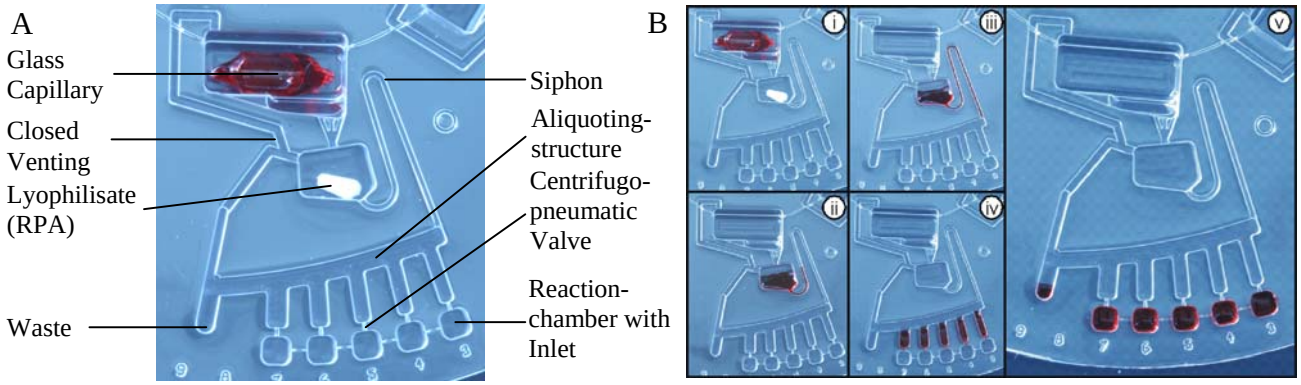

Fig. 1A) *The foil disk features a chamber with a glass capillary containing 50 µL of rehydration buffer and a chamber with a lyophilisate (RPA). A capillary siphon and a centrifugo-pneumatic valve are integrated for fluid control. An aliquoting structure splits the 50 µL buffer into 5 x 10 µL.* ***Fig. 1B)*** *i) The disk before operation. ii) The sample is added into the inlet. After the glass capillary is crushed the liquid is spun into the lyophilisate chamber. iii) A capillary siphon allows valving between lyophilisate chamber and aliquoting structure. iv) The 50 µL buffer volume is splitted into 5 x 10 µL aliquots. v) The fluid fills the reaction chambers via a centrifugo-pneumatic valve. For illustration the buffer is stained with red ink.*

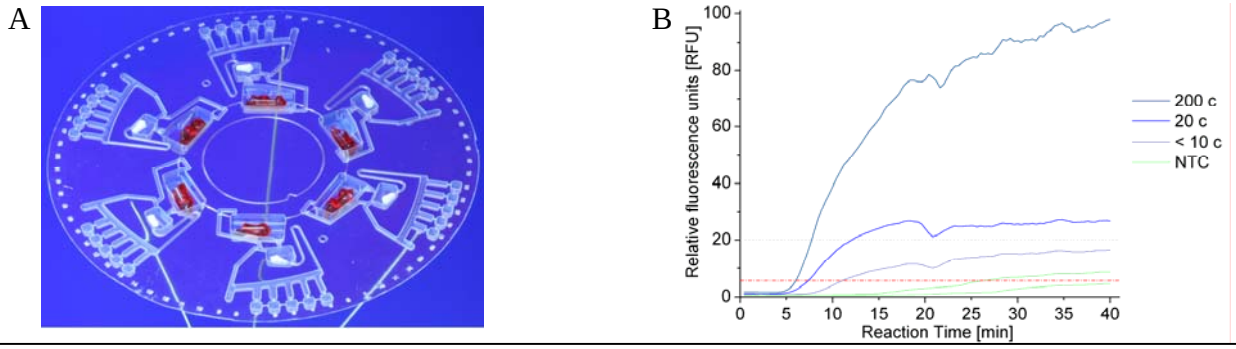

Fig. 2A) *Photograph of a foil disk assembled with all reagents featuring 6 fluidic structures each capable of processing 5 samples.* ***Fig. 2B)*** *Results of a sample dilution series processed in the RPA-disc. The amplification plot shows an exponential rise of the fluorescence within 5 to 10 minutes for all tested mecA gene concentrations and a RPA typical ditch following this exponential phase. The rise of the no-template control (NTC) is due to a detector drift.*

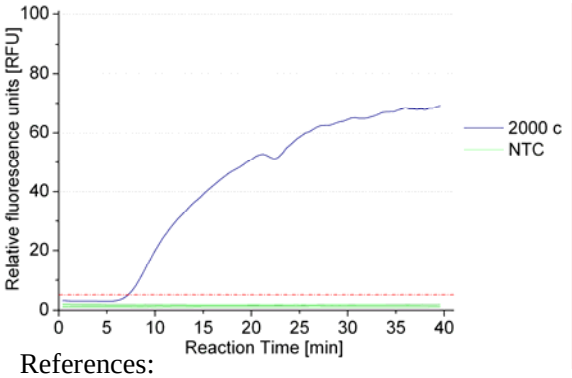

Fig. 3) *Results of a cross-contamination test of a foil disc. A sample containing 2000 copies of the mecA gene is transferred into the central reaction chamber while all surrounding chambers contain NTCs. No increase in the signal intensity can be seen for the NTCs while the 2000 copies show amplification proving that no cross-contamination between reaction chambers occurs.*

References:

1. “Blow moulding of polymer foils for rapid prototyping of microfluidic cartridges”, M. Focke et al., Proceeding of µTAS conference, San Diego, 998-990, (2008).
2. "DNA detection using recombinant protein”, O. Piepenburg, N. Armes, PLoS Biology, **4**, Issue 7, 1115-1121, (2006).