Implementation of DNA technology using encoded microparticles in an innovative microfluidic platform towards improved target detection

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In this work we implement for the first time DNA technology to improve the analytical sensitivity of the innovative microfluidic platform Evalution™. Rolling circle amplification (RCA) was (1) optimized on encoded microparticles in the microfluidic environment intrinsic to the platform and (2) implemented in a total IgE model immunoassay, resulting in a 10-times increase in fluorescence signal.

The Evalution™ is an integrated system based on three major components: barcoded disc-shaped microparticles to capture target molecules, a disposable microfluidic cartridge and an instrument that integrates all assay steps. The flow is precisely controlled by applying a differential pressure over the in- and outlet of the 16 independent microfluidic channels. The channel-based microfluidic technology together with the barcoded microparticles create a high throughput multiplex environment compatible with a wide range of target molecules (Figure 1)[1,2]. Although the uniquely barcoded microparticles provide a higher encoding capacity than the conventional spectral methods, the potential of detecting low amount (pg/mL) of specific target molecules is currently unexplored.

Here, rolling circle amplification (RCA) is applied to increase the amount of binding sites for the labelling component per target molecule. In order to maintain the multiplexing capacity of the Evalution™, RCA was performed attached to the surface of the microparticles. The double helix formed after hybridization of the circular DNA template to the primer, allows polymerase to attach and start the primer extension which results in a linear chain with repeated sections complementary to the circular DNA template. In contrast to PCR there is no thermal cycling required and immuno activities are retained[3]. The amplification process was optimized for different elongation times and flowrates in the microfluidic environment (Figure 2). The best signal-to-noise ratio, corresponding to a value of 24, was obtained for a flowrate of 600 mBar

and an amplification time of 10 min. The integration of control particles, depicted as red bars, in the same channel indicates the multiplexing potential of the assay maintaining its specificity. Afterwards, this condition was combined with a well-characterized model sandwich assay against IgE (Figure 1D). When compared to the reference signal, the implementation of RCA in the assay results in a 9 x increase in signal while maintaining reproducibility (5,4% CV). The selected assay conditions resulted in a linear calibration curve that reaches a LOD of 0.58 ng/mL, which is a 10 x improvement compared to the reference assay and which could be further improved using an even brighter fluorescent tracer while suppressing non-specific interaction (Figure 3).

The reported amplification strategy can be directly transferred for the detection of various target molecules, which moreover can be detected in multiplex due to the inherent coding of the microparticles. The Evalution^{TM} demonstrates immense potential to be used as a diagnostic platform thanks to its high throughput multiplexing capacity together with a significantly reduced reagent consumption and automation. The importance of this work should be recognized as the combination of these intrinsic advantages together with a highly sensitive and specific detection would greatly benefit diagnostic and biomarker discovery purposes.

References

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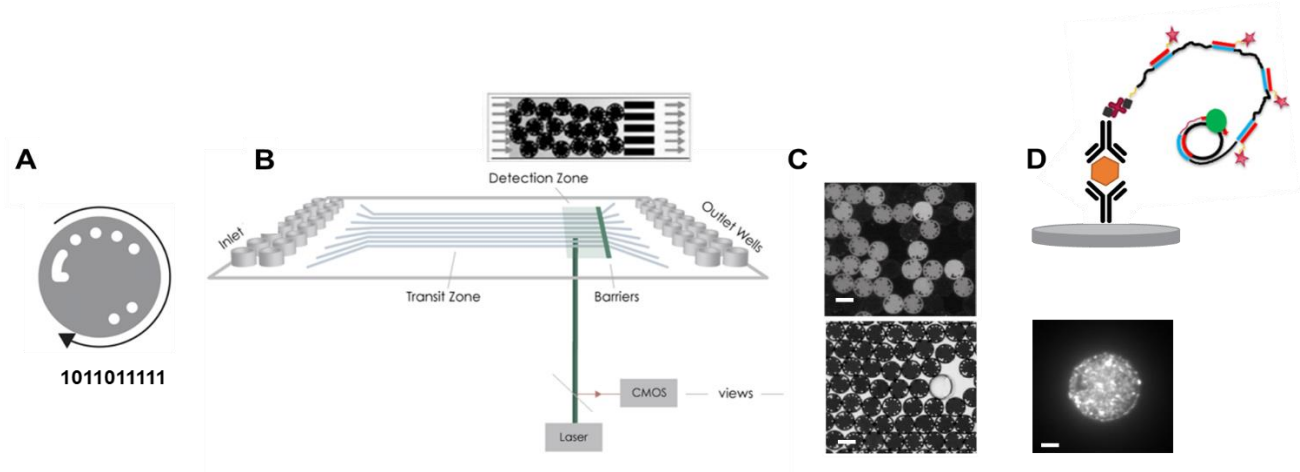

Figure 1. A) Overview of a coded microparticle and the 10-binary identification code used for multiplexing purposes. B) Overview of the cartridge consisting of 16 microchannels including a close up of the detection zone. C) Fluorescence (top) and brightfield (bottom) image of microparticles in the detection zone. D) Schematic representation of the assay (top) and fluorescent (bottom); (i) IgE target is captured by the anti-IgE antibodies immobilized on the microparticle surface, (ii) the biotinylated secondary antibody is added, (iii) the DNA primer is attached through streptavidin and (iv) RCA is initiated. The resulting DNA polymer comprises a plurality of hybridization sites for a complementary short DNA oligo labeled with a fluorescent tag. The scale bars represent 40 µm.

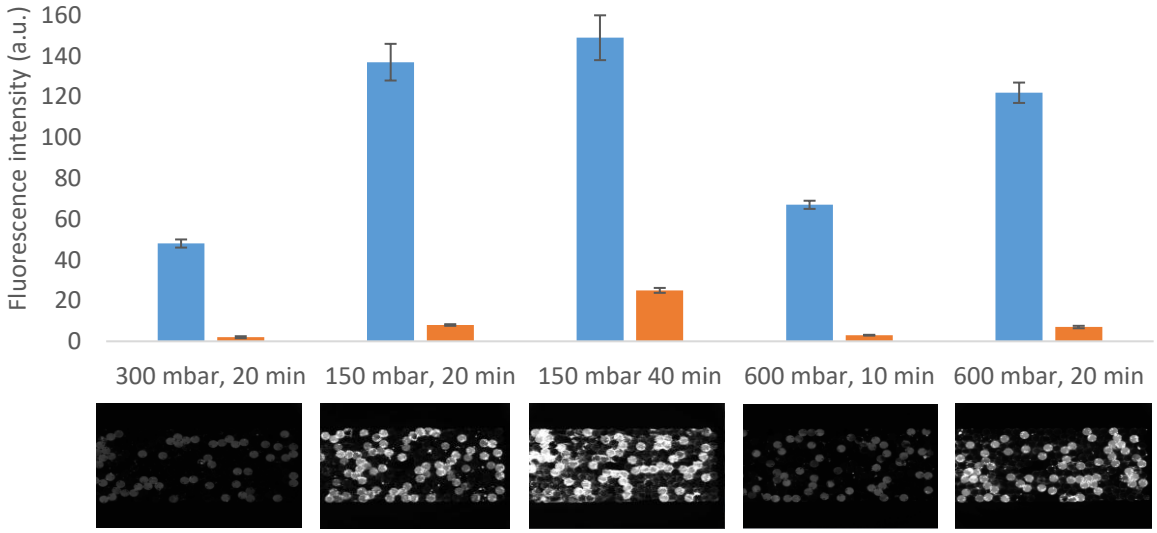

Figure 2. Rolling circle amplification for different flow rates (expressed as the applied pressure over the channel) and different amplification times. The blue bars represent signal obtained performing RCA on top of microparticles functionalized with the biotinylated secondary antibody. The orange bars represent the control particles. The error bars represent one standard deviation (n=-4).

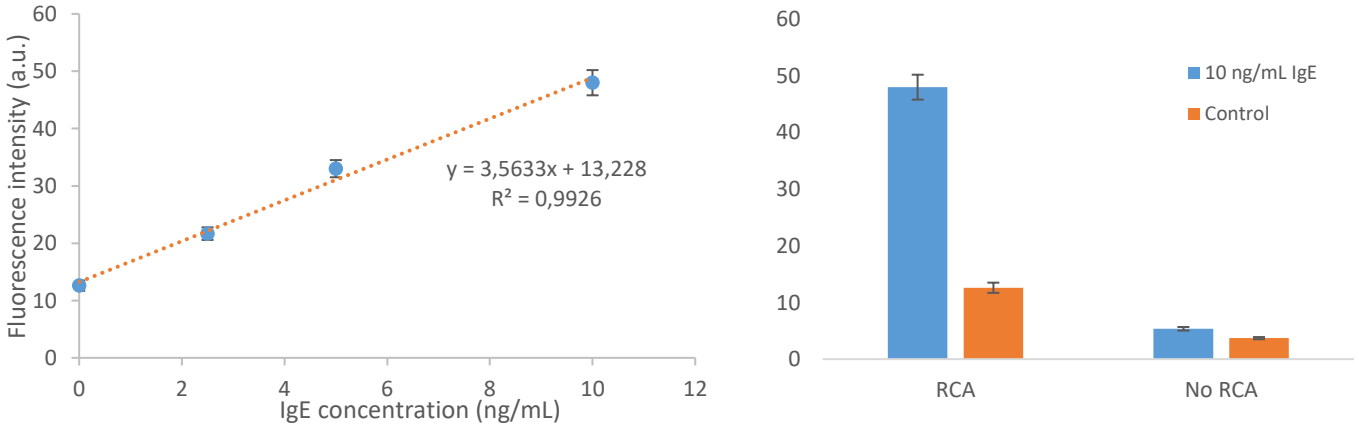

Figure 3. Calibration curve obtained after performing RCA creating multiple binding sites for the oligo label (left). Comparison of fluorescent signal obtained labelling with a single DNA oligo (reference) and using RCA (right). The reference signal was established using the same model assay except labelling with a single DNA oligo label. The error bars represent one standard deviation (n=4).