From lab-scale to lab-on-card: Loop-mediated isothermal amplification of biological samples

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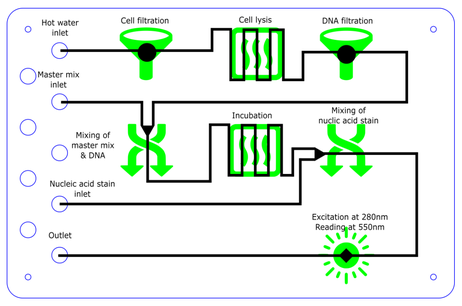
Introduction

The aim of this project is to develop a point-of-care(POC) device for the detection of infectious deceases. In this case the focus is on the detection of mycobacterium tuberculosis complex (MTBC) in biological samples. *A schematic overview* of these steps can be seen on the right. The first step is to perform **cell extraction** from sputum, a viscous excretion. Next, **cell disruption** based on heatshock is carried out in order to harvest the DNA. The later is then amplified via loop mediated isothermal amplification (LAMP). By **mixing** the lysate with a LAMP mastermix, DNA is amplified when incubated at 65°C. The amplified **DNA is quantified** by staining it with fluorescent dye and measuring the emission intensity. In pursuit of developing the final POC device, each required function is tested separately on **a lab-on-card.**

Schematic overview:



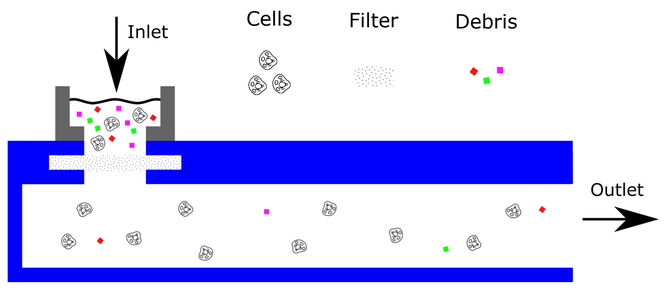
Cell extraction

Materials & Methods:

- Sputum: created artificially contains corn syrup, water, gelatin and yeast cells (S. cerevisiae)
- Sputum liquefying: hot tap water or 4%wt NaOH
- Filter: Whatman nr.113, 30µm pore size
- Quantification: Outlet fluid optically inspected

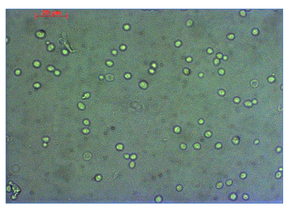
Example:

Schematic:



Results & Discussion:

- Yeast cells extracted from viscous matrix
- Yeast has roughly the same size as MTBC



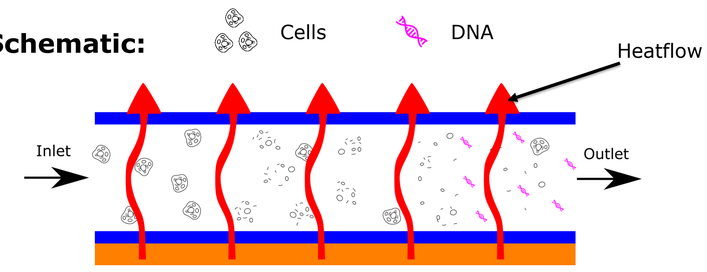
Cell disruption

Example:

Materials & Methods:

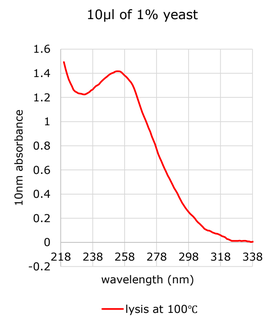
- Technique: thermoshock at 100°C
- Reagent: 10µl PBS + 1%wt yeast (S. cerevisiae)
- Heater: resistive track on polyimide film
- Quantification: absorption of 260nm light

Schematic:



Results & Discussion:

- DNA concentration: 542.4 (ng/ml)
- Absorbance ratio: 260/280=1.94, 260/230=1.10
- Phenol contamination: plastic layer, adhesive layer



Lab-on-card

A lab-on-card is a microfluidic POC device developed with rapid-prototyping techniques. It is constructed out of multiple carrier layers and adhesive layers. The microfluidic channels are crafted by removing material from the adhesive layers. Vias in the carrier layers can connect different flow planes. The strength of lab-on-card is the possibility to incorporate different materials and sensors into it with ease.

In this work:

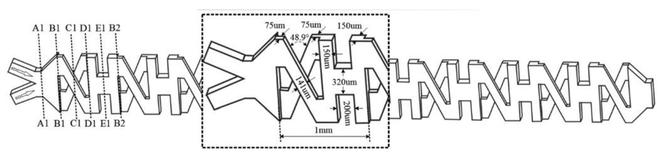
- Rapid-prototyping technique: laser cutting
- Carrier layers: poly(ethylene terephthalate), 100µm thick
- Adhesive layers: double sided adhesive, 50µm thick
- Other materials: filter paper, flexible electronics

Mixing

Materials & Methods:

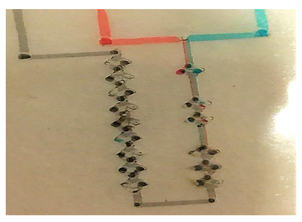
- Technique: splitting-and-recombination [1]
- Coloured solutions: food colouring
- Quantification: visual inspection of colours

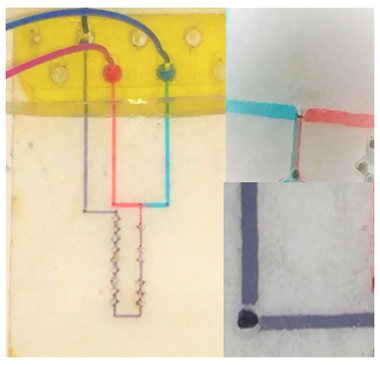
Schematic [1]:



Results & Discussion:

- Perfect mixing occurs
- 6 units are sufficient





Example:

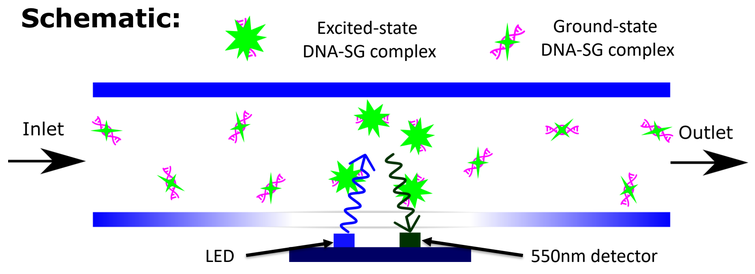
Example:

DNA quantification

Materials & Methods:

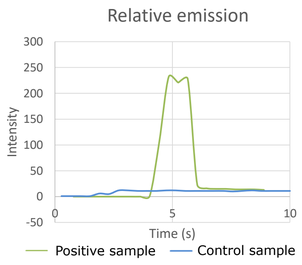
- Technique: staining with SYBR Green I (SG)
- Samples: 5µl, prepared as described in [2]
- Excitation: 480nm (white LED)
- Emission: 550nm (colour sensor)
- Quantification: Intensity increase of 550nm

Schematic:



Results & Discussion:

- Clear distinction between positive and control sample
- No need for expensive optical filters



[1] X. Feng, "An effective splitting-and-recombination micromixer with self-rotated contact surface for wide Reynolds number range applications" *Biomicrofluidics*, vol. 7, 054121, 2013.

[2] E.M. Bentaleb et al. " Development and evaluation of an in-house single step loop-mediated isothermal amplification (SS-LAMP) assay for the detection of Mycobacterium tuberculosis complex in sputum samples from Moroccan patients ", *BMC Infectious Diseases, vol. 16, p. 517*, 2016

Conclusion

The four functions tested on a lab-on-card microfluidic device have yielded very promising results. Cells were extracted from a viscous medium and disrupted by applying heat. An efficient and easy to develop mixing technique has been demonstrated. Finally, the quantification of DNA with SYBR Green I as a nucleic acid stain has been shown with a clear difference between DNA presence and absence. Furthermore, DNA can be quantified without the need of expensive materials or equipment. The ease of development and the results of the separate functions prove that the lab-on-card technique is widely applicably, but more importantly a suiting point-of-care device for the detection of diseases in resource limited areas.

Acknowledgment

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