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

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Abstract: This work aims to translate lab-scale techniques for the detection of diseases towards lab-on-card(LOC) devices. Indeed, these point-of-care(POC) tests allow detecting diseases in resource-limited areas. The necessary functions of the LOC can be split up into four different sections: extraction of the cells from the biological sample, lysis of the cells to harvest DNA, DNA amplification based on loop-mediated isothermal amplification(LAMP) and DNA detection by adding SYBR Green I nucleic acid stain. These functionalities have separately been tested on LOCs. The results of the individual functions show that a fully functional POC device is an achievable goal.

Keywords: Lab-on-card(LOC), Point-of-care, single step loop-mediated isothermal amplification(SS-LAMP), Lysis, cell extraction, SYBR Green I

Introduction

Diagnostic methods are generally speaking expensive, time-consuming and inaccurate in endemic areas. This is often a consequence of resource limitations in these areas. This project concentrates on the development of a rapid molecular diagnostic test for the early diagnosis of Mycobacterium Tuberculosis Complex and is based on the single step loop-mediated isothermal amplification(SS-LAMP) technique [1]. A lab-on-card(LOC) technique can be used to develop such a molecular diagnostic test. Figure 1 shows the concept of the LOC.

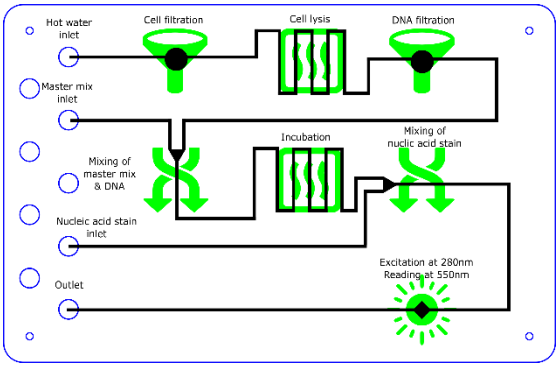

Figure 1: *The concept of the LOC for a molecular diagnostic test.*

Results and Discussion

The working principle of the LOC can be divided into 4 functions: the extraction of cells from the matrix, cell lysis, SS-LAMP and detection with SYBR green I nucleic acid stain. The work has been tackled in these four steps and tested on individual cards. Firstly, TB cells are extracted from viscous sputum by adding hot water. Indeed, sputum is highly viscous and adding hot water liquefies it. The sample is then applied onto filter paper with a pore size of 30µm. A cavity underneath the filter is put on negative pressure, hence cells are extracted from the liquefied sputum and then move on to the lysis step. Currently two options are pursued: lysis based on high electrical potential and lysis based on heat. After cell disruption the lysate passes a 200nm MF-MilliPore^{TM} filter, as a consequence only DNA remains in an aqueous medium. In the third step, the extracted DNA is mixed with a master mix by means of an acoustic mixer [1][2]. Further experiments will show if only a structured channel suffices. The mix is incubated at 65°C for one hour for the LAMP reaction to occur. This is performed by incorporating a flexible heater structure in the card. The last step adds SYBR green I to the amplified DNA. The nucleic acid stain is excited at 280nm with a UV-LED and read at 522nm with photodiode.

Conclusions

All individual functions have been tested successfully on LOC format. However the LAMP reaction takes a long time to perform which is unfit for point-of-care devices. Future experiments aim to decrease the incubation time needed.

References

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