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# Modular development of a prototype point of care molecular diagnostic platform for sexually transmitted infections

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ABSTRACT

This paper presents the design of a modular point of care test platform that integrates a proprietary sample collection device directly with a microfluidic cartridge. Cell lysis, within the cartridge, is conducted using a chemical method and nucleic acid purification is done on an activated cellulose membrane. The microfluidic device incorporates passive mixing of the lysis-binding buffers and sample using a serpentine channel. Results have shown extraction efficiencies for this new membrane of 69% and 57% compared to the commercial Qiagen extraction method of 85% and 59.4% for 0.1 ng/μL and 100 ng/μL salmon sperm DNA respectively spiked in phosphate buffered solution. Extraction experiments using the serpentine passive mixer cartridges incorporating lysis and nucleic acid purification showed extraction efficiency around 80% of the commercial Qiagen kit. Isothermal amplification was conducted using thermophillic helicase dependant amplification and recombinase polymerase amplification. A low cost benchtop real-time isothermal amplification platform has been developed capable of running six amplifications simultaneously. Results show that the platform is capable of detecting $1.32\times10^6$ of sample DNA through thermophillic helicase dependant amplification and $1\times10^5$ copy numbers *Chlamydia trachomatis* genomic DNA within 10 min through recombinase polymerase nucleic acid amplification tests.



## 1. Introduction

The accurate and rapid identification of pathogens is important in global health to enable immediate and appropriate treatment for vulnerable and hard to reach populations. This is particularly true for sexually transmitted infections (STIs) with the occurrence of extremely drug resistant *Neisseria Gonorrhoea* [1]. Nucleic acid amplification testing (NAAT) has become increasingly used for point of care test (POCT) development due to its potential for high sensitivity and selectivity, but there is a challenge to provide inexpensive, portable and mains-power independent platforms for remote settings that allow for simple sample handling. Sample collection and integration with preparation methods including nucleic acid extraction has inhibited the uptake of commercial POCT devices. A hand-held, battery operated, integrated microengineered platform is under development for sample collection, automated DNA extraction, isothermal amplification [2] and optical detection directly from raw samples such as urine, blood, swabs and saliva (Fig. 1a). Whilst a number of companies have developed point of care devices, for example the Cepheid GeneXpert, Biofire Filmarray and the LIAT analyser, all of the systems are benchtop, require mains power and some hands on sample preparation [3-6]. The cost of the benchtop devices tend to be high, within this paper the development of a prototype, handheld, low cost amplification and detection platform that cost less than GBP150 for parts and labour is described.

In addition to this, a simple, disposable sample collection device (Fig. 1b) that can be used to collect self-taken urine and swab samples is also being developed. The sample collection device is designed to interface directly with a disposable microfluidic cartridge via a Luer fitting in which the assay is conducted. The sample will be delivered into the cartridge through a plunging mechanism. This device and its corresponding interface mechanism are under development and not described herein. The vision of the project is

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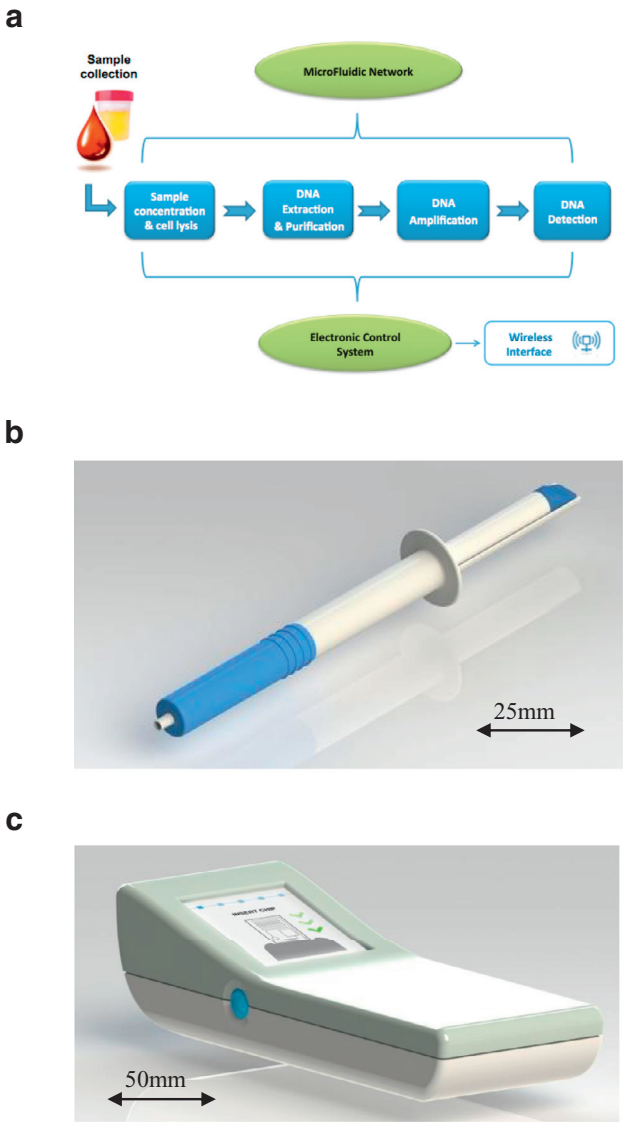


**Fig. 1.** Integrated micro-engineered platform: (a) sample-in-to-answer-out system concept; (b) sample collection device; and (c) envisaged handheld device.

that the prototype amplification and detection platform described herein will be developed into a handheld device (Fig. 1c) integrating the optics and heating elements along with data acquisition, control and communications hardware. This handheld device will automate sample analysis and send results directly to clinicians, via a mobile phone, for rapid diagnosis to expedite time to treatment. We aimed to initially target genital samples for the rapid diagnosis of STIs.

The POCT has been developed using a modular process (Fig. 2) enabling any section to be removed and replaced by an alternative method. For example, communications with the handheld device could be achieved using the state-of-the-art wireless technologies or USB depending on the setting in which the device is employed. Similarly sample preparation, isothermal amplification and detection types can be altered depending on the setting and disease type that is being identified.

Nucleic acid extraction for POCT devices is dominated by solid phase extraction with chaotropic salts using silica membrane, columns [7, 8] and magnetic beads [9]; Drawbacks of these methods for POCT development include the required centrifugation for membranes and an external magnetic field for active mixing of

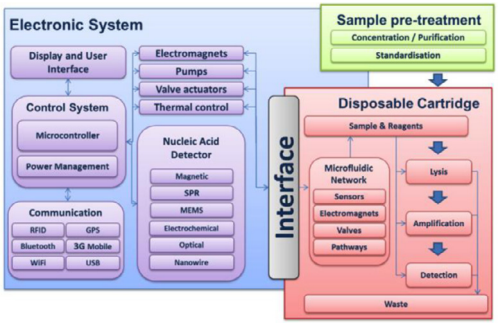


**Fig. 2.** Modular lab on a chip system overview.

magnetic beads, whilst the use of toxic guanidinium thiocyanate can inhibit downstream polymerase chain reaction (PCR) [10]. This paper reports a method of DNA isolation using chitosan impregnated on an organic membrane inserted into polydimethylsiloxane (PDMS)/glass prototyped microfluidic devices. Chitosan was chosen as it simplifies the extraction process and removes the requirement for guanidium thiocyanate. Chitosan is a deacetylated form of chitin. Protonation of amine groups cause chitosan to exist as a polycationic polymer at pH < 6.2, whereas at higher pH, the amine groups are deprotonated. Protonation of the amine groups causes the chitosan to be cationic, thus it adsorbs negatively charged DNA, when deprotonated (at higher pH) the DNA is released into the surrounding solution [11, 12].

Polymerase chain reaction (PCR) was the first method employed for nucleic acid amplification testing (NAAT). More recently isothermal amplification methods have been developed that utilise enzymes for DNA strand separation [1]. Isothermal methods were chosen for this project as they remove the requirement for rapid heating and cooling steps required in PCR, therefore less power is consumed within the handheld device. Optical detection of amplified DNA was chosen as this could be implemented in a low cost manner in a handheld device using off the shelf components, and allows real time visualisation of the NAAT reaction kinetics, the benefit of this over other methods is that the original sample load can be determined [1]. Experiments were conducted using thermophillic Helicase Dependent Amplification (tHDA) and Recombinase Polymerase Amplification (RPA) to show the versatility of the platform.

The following sections describe the fabrication and assembly of the prototype platform, design and optimisation of the heating module and optics. Further, the design of the microfluidic cartridge, on-chip DNA extraction and isothermal amplification are also discussed.

## 2. Platform development

A low cost isothermal amplification and optical detection platform has been developed incorporating a resistive heating element, low cost photodiodes, LEDs and optical filters (Figs. 3 and 4). The control and data acquisition was conducted using two Arduino Uno microcontroller boards.

The platform was produced by assembling the layers of printed circuit boards (PCB) (Fig. 4a–d) with layers of laser cut PMMA as support structures to produce a final device with a height of 32 mm, depth of 54 mm and length of 100 mm. This allows this prototype design to be easily packaged within the envisioned handheld device (Fig. 1c).

### 2.1. Heating element design

A resistive heating element (Fig. 4d) was designed and developed from a two-layer printed circuit board using standard photo-