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# Sample-to-answer on molecular diagnosis of bacterial infection using integrated lab-on-a-disc

J.F.C. Loo[a,b,1], H.C. Kwok[b,1], C.C.H. Leung[c,1], S.Y. Wu[b], I.L.G. Law[a], Y.K. Cheung[d], Y.Y. Cheung[e], M.L. Chin[e], P. Kwan[d], M. Hui[e], S.K. Kong[a], H.P. Ho[b,*]

[a] Biochemistry Programme, School of Life Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong
[b] Department of Electronic Engineering, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong
[c] Department of Anaesthesia and Intensive Care, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong
[d] Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong
[e] Department of Microbiology, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong

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ABSTRACT

Sepsis by bacterial infection causes high mortality in patients in intensive care unit (ICU). Rapid identification of bacterial infection is essential to ensure early appropriate administration of antibiotics to save lives of patients, yet the present benchtop molecular diagnosis is timeconsuming and laborintensive, which limits the treatment efficiency especially when the number of samples to be tested is extensive. Therefore, we hereby report a microfluidic platform labonadisc (LOAD) to provide a sample-to-answer solution. Our LOAD customized design of microfluidic channels allows automation to mimic sequential analytical steps in benchtop environment. It relies on a simple but controllable centrifugation force for the actuation of samples and reagents. Our LOAD system performs three major functions, namely DNA extraction, isothermal DNA amplification and real-time signal detection, in a predefined sequence. The disc is self-contained for conducting sample heating with chemical lysis buffer and silica microbeads are employed for DNA extraction from clinical specimens. Molecular diagnosis of specific target bacteria DNA sequences is then performed using a realtime loopmediated isothermal amplification (RTLAMP) with SYTO-9 as the signal reporter. Our LOAD system capable of bacterial identification of *Mycobacterium tuberculosis* (TB) and *Acinetobacter baumanii* (Ab) with the detection limits $10^3$ cfu/mL TB in sputum and $10^2$ cfu/mL Ab in blood within 2 h after sample loading. The reported LOAD based on an integrated approach should address the growing needs for rapid pointofcare medical diagnosis in ICU.

## 1. Introduction

Severe sepsis is one of the most common causes of death in intensive care units (ICU), and the mortality rate is approximately one in four patients (Dellinger et al., 2013). Early administration of appropriate *anti*biotics is one of the pillars of sepsis management and is key to patient survival (Kumar et al., 2006). Gram negative bacteria such as *Acinetobacter baumannii* (Ab) are key pathogens causing hospital acquired infections, often requiring broadening of the spectrum of *anti*biotics selected (Maragakis et al., 2008). Besides, *Mycobacterium tuberculosis* (TB) infected 9.6 million patients worldwide and caused mortality in 1.5 millions in 2014 according to the WHO (World Health Organization, 2015). Tuberculosis incidence rate is high in Hong Kong (74 per 100,000 population), which is a more common compared to other developed regions such as Sweden and United Kingdom 7.5–12 per 100,000 population) (World Health Organization, 2015). Identification of tuberculosis infection through blood, respiratory secretions and other body fluids is key to diagnosis of tuberculosis infection and initiation of anti-tuberculosis therapy (Dellinger et al., 2013). Current practice on conventional pathogen identification relies on culture method, which usually takes 3–5 days from sample to answer. Nucleic acid amplification has been evaluated substantially these ten years as a rapid and reliable strategy for identification of TB (Aryan et al., 2010; Iwamoto et al., 2003; World Health Organization, 2013). Loop-mediated isothermal amplification (LAMP) is an isothermal nucleic acid amplification method where

*Abbreviations:* Ab, *Acinetobacter baumanii*; CFU, colony-forming unit; ICU, intensive care unit; LOAD, Labona-disc; MDR, multi-drug resistance; PCB, Printed circuit board; PDMS, Polydimethylsiloxane; RT-LAMP, Realtime loopmediated isothermal amplification, TB, *Mycobacterium tuberculosis*

* Corresponding author.
*E-mail address:* hpho@ee.cuhk.edu.hk (H.P. Ho).
[1] Co-first authors.

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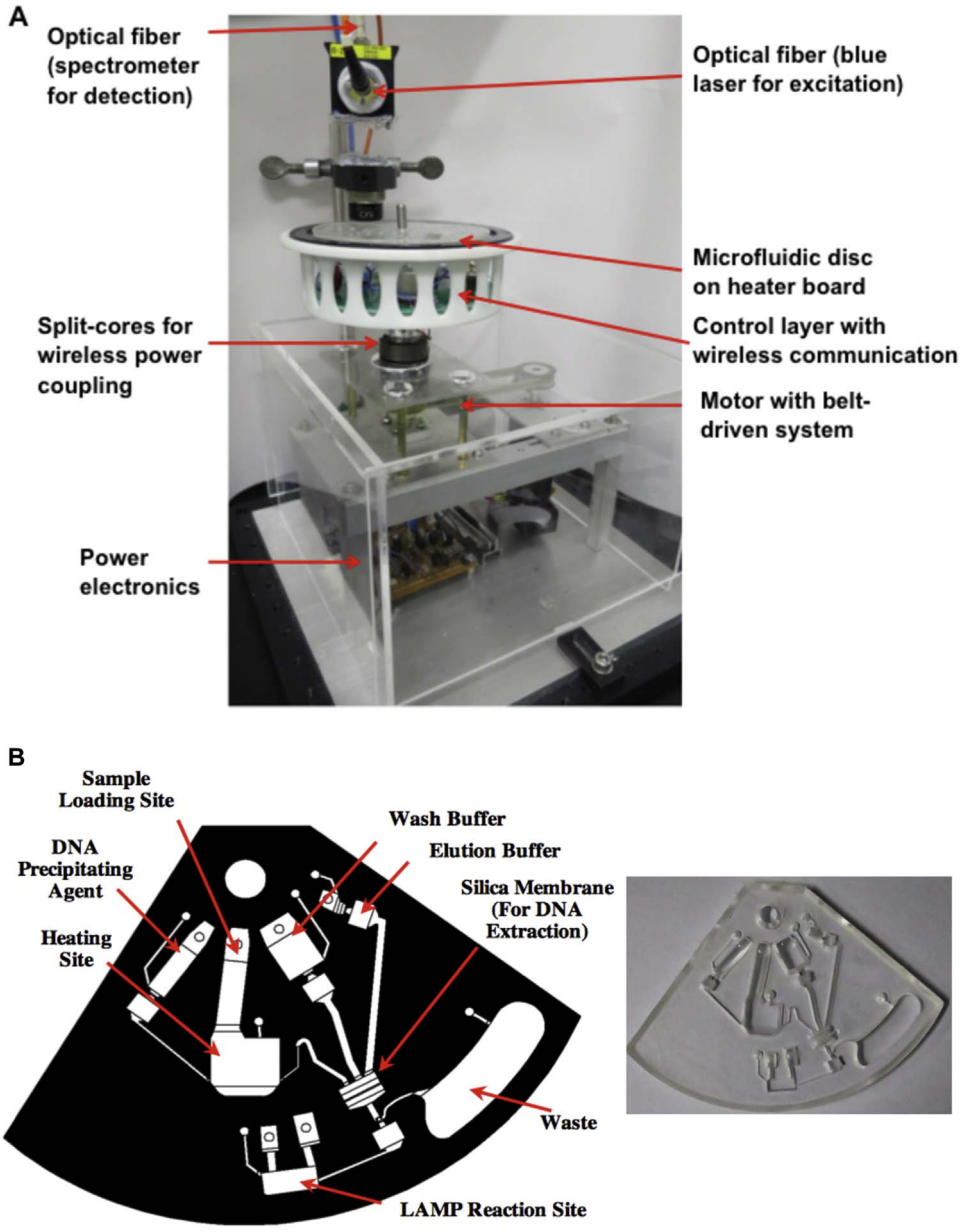


**Fig. 1.** LOAD platform set-up and schematic diagram of microfluidic disc. A. The present platform has capability on using electronic circuit board (PCB) accommodating the heating elements for microfluidic disc to mount on for accurate control of temperature. The PCB allows relevant electronics such as wireless data communication to attach and the electrical power is coupled to the PCB through a split-core transformer; B. Schematic diagram of microfluidic disc (one-quarter of a full disc area). The disc was self-contained to perform DNA extraction from clinical samples using simple heating with chemical lysis buffer and silica microbeads. Molecular diagnosis of specific target bacteria DNA sequences was performed using realtime loopmediated isothermal amplification (RTLAMP) with fluorescence indicator as the signal reporter at LAMP reaction site.

reaction occurs under constant temperature ranged from 60 to 70 °C with high specificity and sensitivity within an hour (Oh et al., 2016 Uddin, et al., 2015). For the core detection of molecular diagnosis based on specific DNA sequence by LAMP, it uses a pair of designed primers (Forward and Backward Inner Primers) to generate a template with loops on both ends. This both-end looped template is displaced from the target DNA sequence with aid of Forward and Backward Outer Primer. It is then used for downstream amplification, by using Forward and Backward Inner Primers replicate and elongate the both-end looped template. Since the replication and the elongation can take place simultaneously, the speed of DNA amplification is increased. Since the temperature for primer annealing, polymerase for both strand displacement and strand construction are at a single temperature, thermal cycler commonly used in PCR for DNA amplification is not needed. Using LAMP for detection of TB complex directly from sputum specimens has been reported and the turnaround times is much shorter than that from traditional culture method (Iwamoto et al., 2003). However, lack of conveniently accessible diagnostic technique at the point of care in the ICU hinders timely identification of infection and underlying pathogens in time in the ICU. Therefore, a sample-to-answer detection has been a main trend for rapid disease detection.

There have been reports on rapid sample-to-answer detection using LOAD with LAMP (LOAD-LAMP) on a microfluidic compact disc platform using pure DNA sample for the diagnosis of pathogenic bacteria such as foodborne pathogen (Oh et al., 2016; Santiago-Felipe et al., 2016; Uddin et al., 2015). However, benchtop DNA extraction using commercial kits was needed, which hinders the practical use when time and availability of trained laboratory staff are limited for diagnosis. Although there are reports using clinical specimen for direct diagnosis based on genetic signature, the optimization of reagents and detection conditions takes effort, since inhibitors