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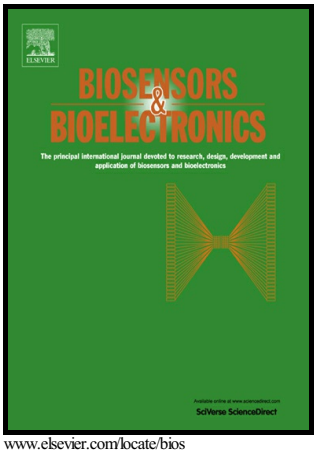

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Integrated LAMP and immunoassay platform for diarrheal disease detection

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Abstract

The challenges of diagnosing infectious disease, especially in the developing world, and the shortcomings of available instrumentation have exposed the need for portable, easy-to-use diagnostic tools capable of detecting the wide range of causative microbes while operating in low resource settings. We present a centrifugal microfluidic platform that combines ultrasensitive immunoassay and isothermal amplification-based screening for the orthogonal detection of both protein and nucleic acid targets at the point-of-care. A disposable disc with automatic aliquoting inlets is paired with a non-contact heating system and precise rotary control system to yield an easy-to-use, field-deployable platform with versatile screening capabilities. The detection of three enterotoxins (cholera toxin, Staphylococcal enterotoxin B, and Shiga-like toxin 1) and three enteric bacteria (*C. jejuni*, *E. coli*, and *S. typhimurium*) were performed independently and shown to be highly sensitive (limit of detection = 1.35-5.50 ng/mL for immunoassays and 1-30 cells for isothermal amplification), highly exclusive in the presence of non-specific targets, and capable of handling a complex sample matrix like stool. The full panel of toxins and bacteria were reliably detected simultaneously on a single disc at clinically relevant sample concentrations in less than an hour. The ability of our technology to detect multiple analyte types in parallel at the point-of-care can serve a variety of needs, from routine patient care to outbreak triage, in a variety of settings to reduce disease impact and expedite effective treatment.

Keywords

microfluidics; centrifugal; point-of-care; immunoassay; isothermal amplification; LAMP; enteric pathogens

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1. Introduction

Diagnostic methods must evolve to meet the need for accurate, timely, and comprehensive screening of infectious diseases. In addition, delivering these capabilities at the point-of-care is critical in settings that lack access to laboratory infrastructure. Despite the many innovations in molecular biology and instrumentation, the versatility of state-of-the-art devices remains limited. Since symptoms can often be traced to a variety of potential etiologies, including viruses, parasites, bacteria, and the toxins they produce, a comprehensive diagnostic tool should be able to detect targets in more than one of these categories. While methods of multiplexing have been developed for more efficient panel-based testing of a single class of target, the flexibility to probe multiple target classes has eluded the single-purpose devices of the past and present.

Traditionally, three techniques have been the gold standard methods of identifying pathogens: culture and microscopy-based techniques (Zhao et al., 2014) , nucleic acid amplification tests (NAATs), and immunoassays. Time from sample to answer from these processes can vary from several hours, in the case of NAATs and immunoassays, to several days , in the case of culture and microscopy-based detection, and all require a well-equipped laboratory staffed with experienced technicians. Although follow-up patient evaluations often still require conventional culture methods for test-of-cure purposes, NAATs and immunoassays occupy an important role in medical diagnostics.

Chief among NAATs is the polymerase chain reaction (PCR), which can provide highly sensitive and specific detection in hours . Variants of this technique include quantitative PCR, which provides real-time detection and quantification of a genetic target, and multiplex PCR, which simultaneously amplifies multiple distinct targets for efficient screening of a sample. Another type of NAAT is isothermal amplificationwhich is performed at a single temperature, allowing for simpler, more energy-efficient instrumentation. Of the isothermal methods available, loop-mediated isothermal amplification (LAMP) has established itself as a robust diagnostic tool, providing performance comparable to PCR methods while tolerating relatively high amounts of contaminants and therefore requiring less sample preparation (Mori and Notomi, 2009; Song et al., 2005; Yamazaki et al., 2009).

While most of the recent advances in enteric pathogen screening have been nucleic acid-based, immunoassays can be extremely sensitive and allow for the direct identification of various toxins. This can be critical to differentiate between toxigenic and non-toxigenic strains of bacteria in endemic areas where incidence is low or in the early stages of an outbreak (NCID and PAHO, 1994). Methods such as enzyme-linked immunosorbent assay (ELISA) are commonplace in sophisticated laboratories, while the more

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