Microfluid Nanofluid
DOI 10.1007/s10404-013-1273-y

RESEARCH PAPER

Portable bead-based fluorescence detection system for multiplex nucleic acid testing: a case study with *Bacillus anthracis*

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Received: 14 May 2013 / Accepted: 13 October 2013

Abstract This paper describes the design, functioning and use of a portable detection platform for multiplex nucleic acid testing. The system features a bead-supported DNA hybridization assay performed inside a microfluidic cartridge. Polystyrene particles modified with DNA capture probes are confined in the detection area and exposed to a solution of fluorescently labeled target DNA strands. The cartridge, fabricated from inexpensive thermoplastic polymers, allows for conducting up to eight assays in parallel. The detection instrument is equipped with a pneumatic module and a manifold lid serving as an interface to mediate fluid displacement on the cartridge. The fluorescence signal deriving from each assay is recorded by a semi-confocal fluorescence reader embedded in the detection platform. The compact design of the instrument and its level of integration make it possible to obtain an analytical result in less than 15 min, while only few manual steps need to be performed in between. A proof-of-concept demonstration involving Cy3-labeled, PCR-amplified genomic DNA confirms the ability to detect *Bacillus anthracis* in a multiplexed single-assay format using *lef* and *capC* genes. Limits of quantification are on the order of 1×10^9 copies/μL for *lef* targets.

Keywords Biothreat detection · DNA hybridization · Integration · Microfluidic chip · Point-of-care testing

1 Introduction

Rapid detection of pathogenic agents is crucial for public health and life stock management in the event of a bioterrorist attack (Inglesby et al. 1999; Lim et al. 2005). The ability to not only confirm (or disprove) the presence of a biological hazard but also accurately identify its phenotype at a contaminated site would enable authorities to implement adequate countermeasures with minimum delay. Despite significant advances in the development of suitable detection methods, on-site testing remains challenging, however, and first-responder teams are often limited to performing risk-assessment while collected samples are transferred to a secured environment for in-depth investigation (Emanuel et al. 2008). One reason is the diversity of pathogenic agents that can range from toxins to virus and bacteria, each of which requires a different detection approach. Another reason is the incompatibility of many technologies with unfavorable conditions in the field that include humidity, shock and temperature-variations, among others. A third reason is the constitution of the sample which often contains low levels of microbial content in a complex matrix (e.g., air, soil, water or food), demanding for purification and concentration of target analytes—a process that is both time intensive and labor intensive (Lim et al. 2005; Isabel et al. 2012; Geissler et al. 2012).

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Published online: 02 November 2013