A DISPOSABLE DNA AMPLIFICATION PLATFORM FOR THE DETECTION OF *CLOSTRIDIUM DIFFICILE* INFECTED STOOL SPECIMENS

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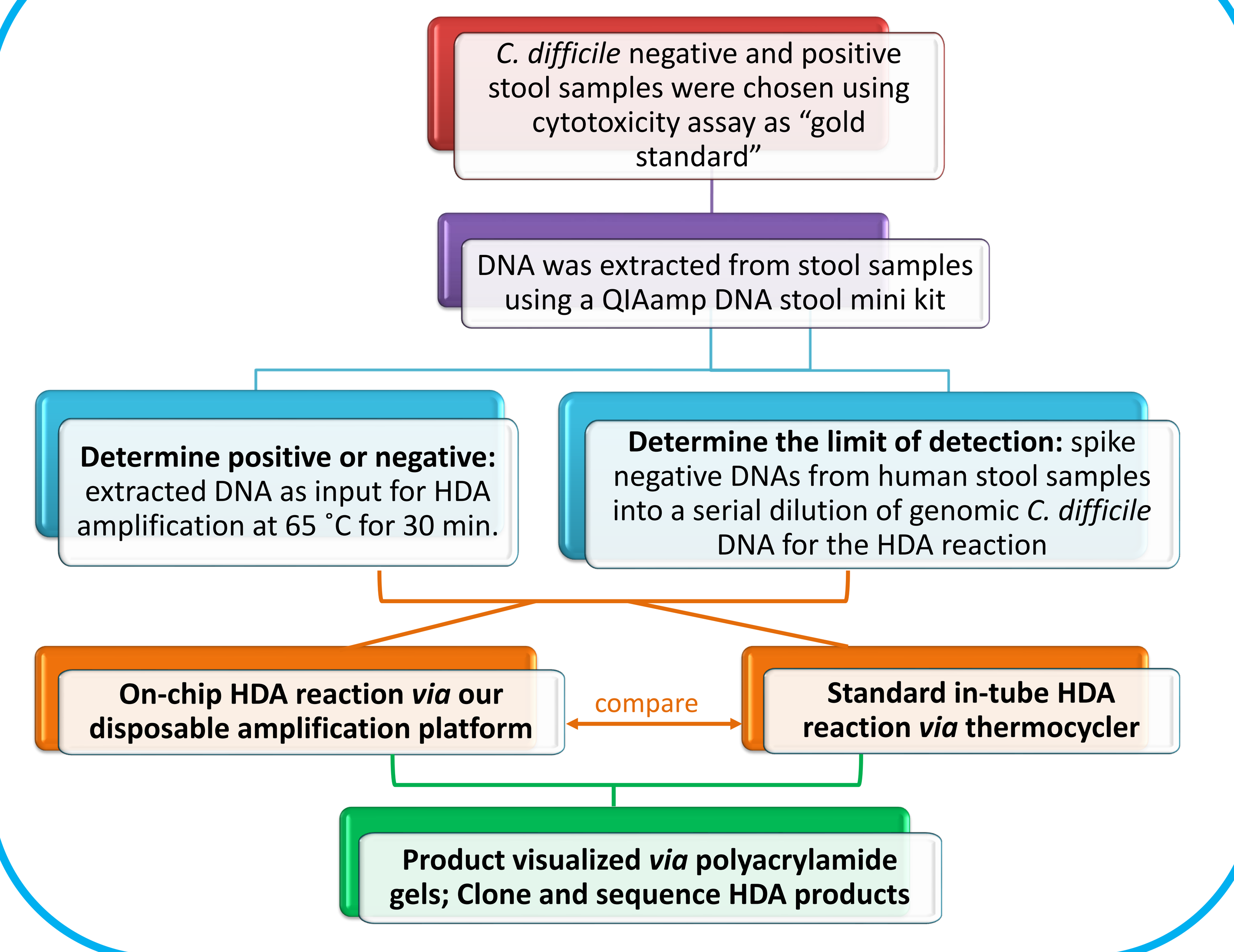
Background and Purpose

•Each year, over 9.5 million deaths are caused by infectious diseases, **nearly all occurring in developing nations**.
•**Appropriate, easy-to-adapt diagnostic technologies** for accurately identifying pathogens in a timely manner are needed.
•Nucleic acid-based assays, especially PCR have the advantage of rapid, accurate analysis. However, the need for accurate temperature control and skilled personnel for operation make it challenging to implement PCR in developing areas.

•In this work, **we sought to develop a disposable DNA amplification platform that is composed of a low-cost polymer-based microfluidic chip as a reaction chamber, a pair of toe warmers, and styrofoam cups as a passive temperature control system to conduct an isothermal helicase-dependent amplification (HDA) assay.**
•**This work is proof of concept of a rapid, inexpensive, disposable point of service test for *Clostridium difficile* toxin A (*tcdA*).**

Methods and Procedures



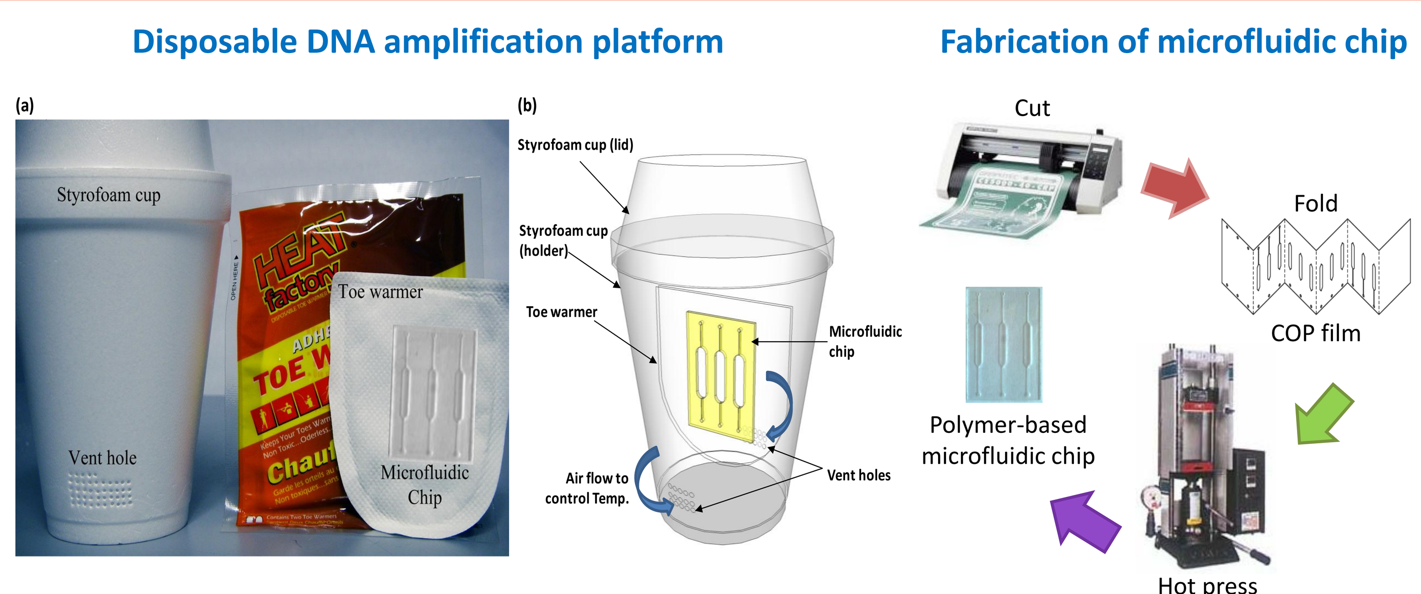


Figure 1. (a) Components of the disposable DNA amplification platform: a cyclic olefin polymer (COP)-based microfluidic chip with multi-reaction chambers (0.75 x 2.5 x 15 mm) was sandwiched between two toe warmers, and the entire assembly was then placed inside the pre-punched styrofoam cups. Finally the existing styrofoam cup was capped with another cup to complete the overall isothermal chamber; (b) The number of vent holes (1mm diameter) on both sides of the styrofoam cup supply air which initiates and maintains the oxidation reaction in the toe warmer, and hence, control the temperature of the reaction chamber.

Results

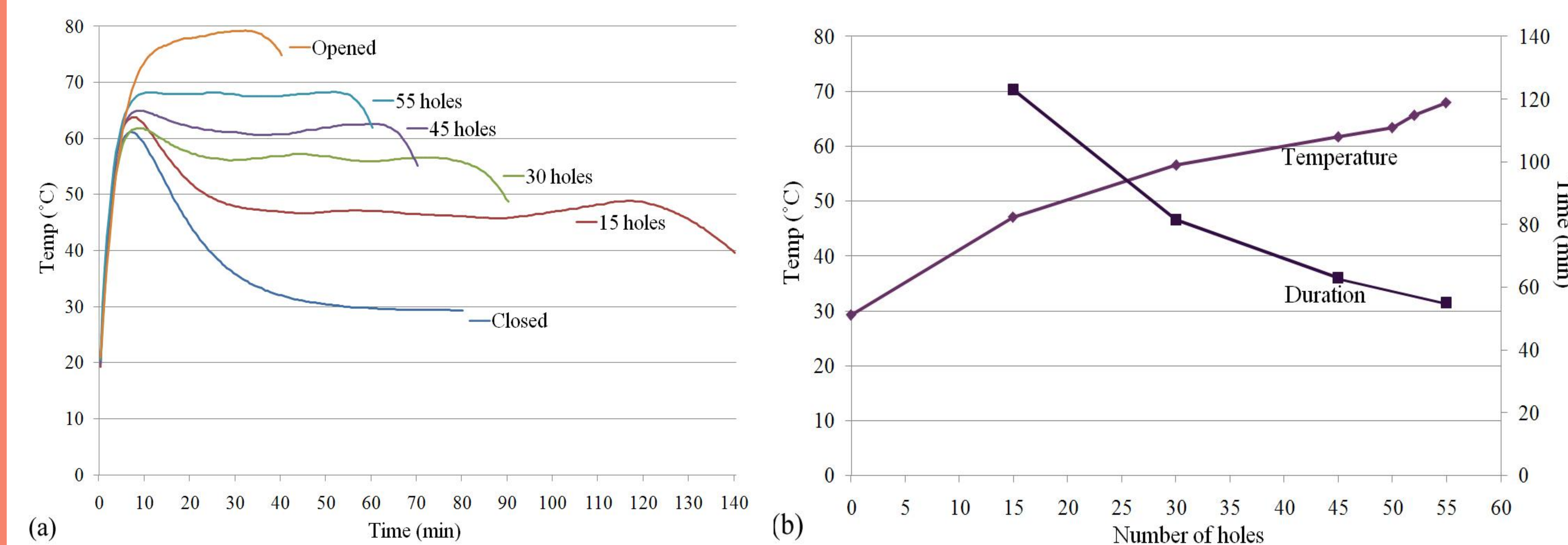


Figure 2. Temperature stability of oxidation reactions of toe warmer contained within styrofoam cups with 15, 30, 45, and 55 holes on both sides. The intra-cup temperature was measured and recorded by a thermocouple attached to the microfluidic reaction chamber. The platform is able to maintain the temperature at 65˚C (optimum temperature of HDA reaction) for more than 55 min.

- **HDA amplification (on-chip *via* our platform & in-tube *via* thermocycler) to determine whether the patients are infected by *Clostridium difficile tcdA***
- **Also standard PCR for amplifying human GAPDH gene was performed to determine the efficiency of human specific stool DNA extraction by QIAamp DNA stool mini kit.**

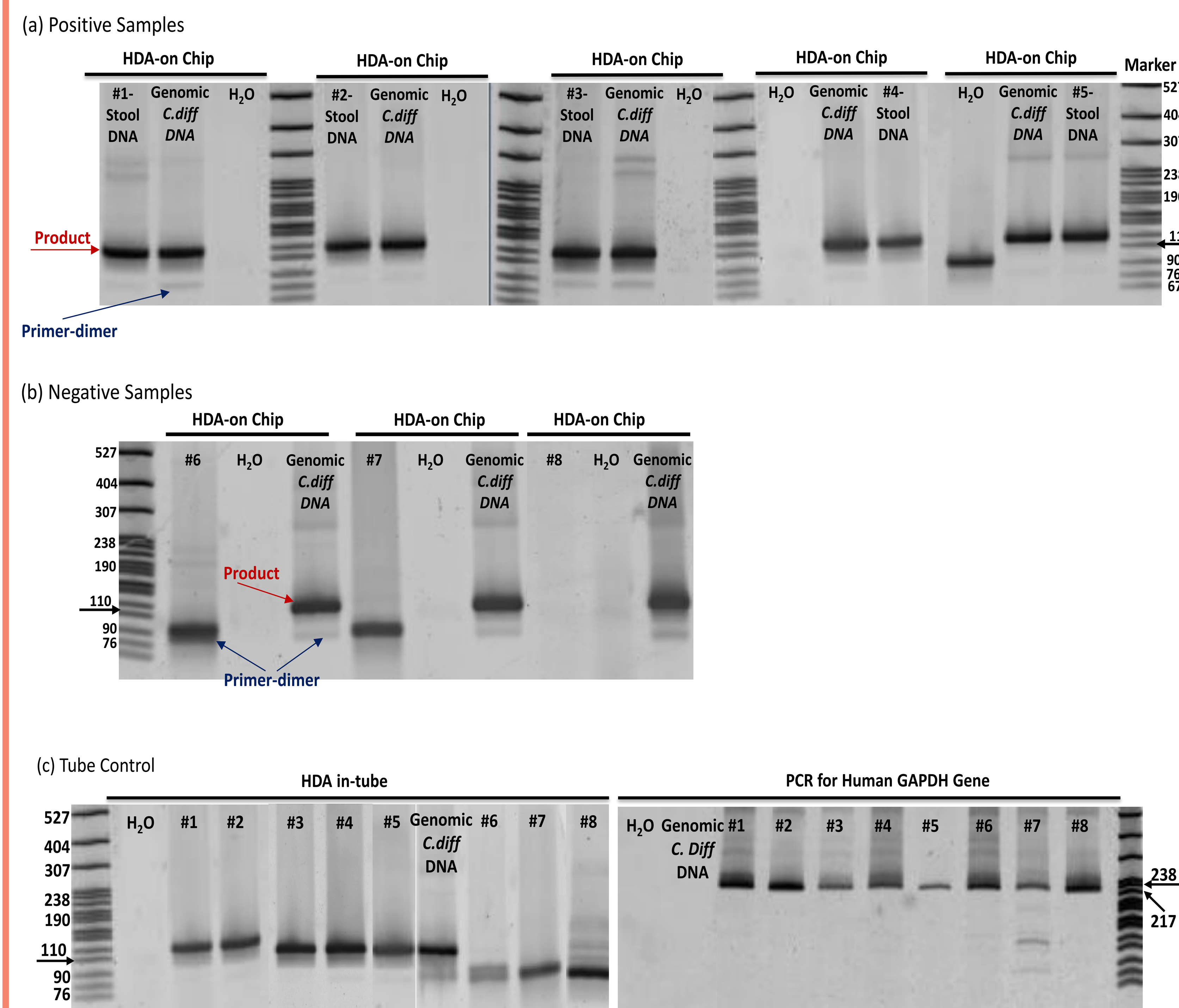


Figure 3. Gel electrophoresis analysis of the HDA on-chip amplicons using 12% polyacrylamide gel with MspI digested pBR322 as marker: (a) five positive human stool DNA samples that are infected by *C. difficile*; (b) 3 negative human stool DNA samples that are not infected by *C. difficile*; (c) HDA in-tube as a control, and PCR reaction to determine human GAPDH gene.

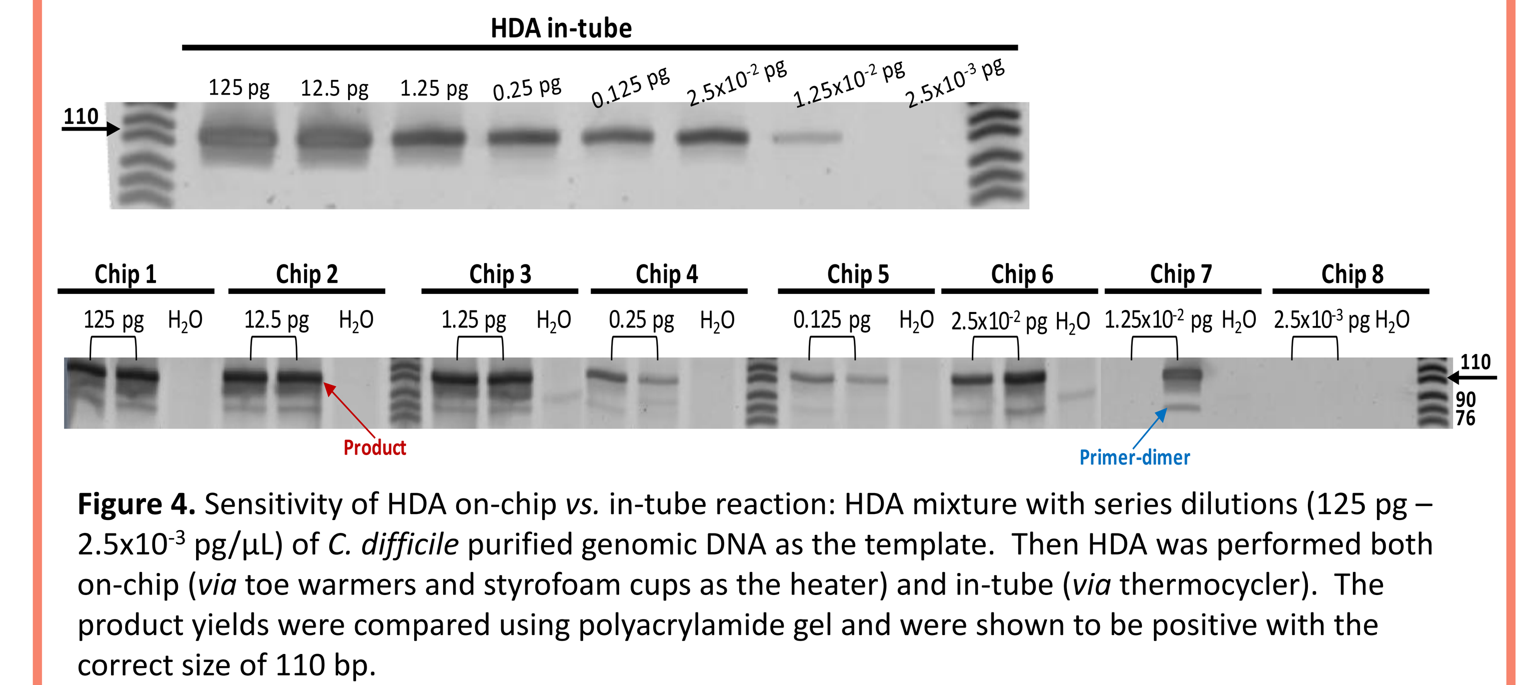


Figure 4. Sensitivity of HDA on-chip *vs.* in-tube reaction: HDA mixture with series dilutions (125 pg – 2.5×10^{-3} pg/μL) of *C. difficile* purified genomic DNA as the template. Then HDA was performed both on-chip (*via* toe warmers and styrofoam cups as the heater) and in-tube (*via* thermocycler). The product yields were compared using polyacrylamide gel and were shown to be positive with the correct size of 110 bp.

Results

•We developed a disposable DNA amplification platform (Fig.1) for isothermal DNA amplification.

•Heating is achieved by using two commercially available toe warmers and two Styrofoam cups, capable of maintaining the optimum temperature for the HDA reaction at 65 ˚C ± 2 ˚C for more than 55 min (Fig.2).

•To document that lysis occurred in all samples, we performed a PCR to identify the presence of the human GAPDH gene (226 bp) in all extracted stool samples (Fig. 3(c)).

•Using this platform we demonstrated that an HDA product of appropriate size (100 bp) is generated only from positive stool DNA samples that was infected by *Clostridium difficile*, but not from negative stool DNA samples (Fig. 3(a)&(b)).

•When compared to a tube based reaction, the toe-warmer platform has approximately the same DNA detection limit (10^{-2} pg) (Fig. 4).

Conclusions

•We have performed isothermal amplification of DNA extracted from human stool sprecimens a disposable DNA amplification platform, and specific identification of *Clostridium difficile* based on HDA for toxin A. This result is consistent with the cytotoxicity assay for *Clostridium difficile* in stool.

•This is a major step toward the fabrication of an inexpensive, handheld, point-of-service disposable diagnostic assay. Continuing work includes integration of on-chip stool extraction to make a completely handheld device.

•Such a device is ideal for deployment in resource-poor settings, and could be extended to other infectious disease diagnostics.

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