Development of a rapid Point-of-Care test system for the detection of toxigenic *Clostridium difficile* infections

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Introduction

Over the past decade *Clostridium difficile* infections (CDI) have been challenging the health care systems world wide due to the continuous increases in incidences as well as severity, both in the hospitals and communities. A pre-requisite for the best possible management of CDI is a sensitive, specific and cost effective medical diagnostics. However, currently available diagnostic tools for the detection of CDI are often time consuming and labor intense, alarmingly insensitive or associated with high costs for equipment and maintenance. Here we present a new point-of-care (POC) test system that is fast and sensitive using a small maintenance-free and affordable appliance.

Objectives

We performed a feasibility study to work out a time-efficient pre-analytics protocol without the need for sophisticated laboratory equipment.

The goal was to obtain PCR-able DNA within minutes, while at the same time keeping PCR inhibition rates under 10 % using a currently optimized and commercially available set of primers and master mix.

With only minimal adaptations of the PCR conditions the preliminary assay ought to reach a sensitivity of 75 % compared to toxigenic culture.

Methods

We have previously developed a generic platform for rapid multi-parameter quantitative molecular diagnostics at the POC. The platform combines a disposable microfluidic test chip with a compact device for chemiluminescence-based optical detection called GENSPEED® Reader.
To obtain PCR-ready DNA from native stool samples we developed a simple, yet effective pre-analytics protocol that requires only 5 minutes hands-on time. PCR was optimized using a proprietary *C. difficile* master mix (Amplex Diagnostics). Test chips were designed to include three controls (PCR-control, hybridization control and negative control), to detect the toxin B gene and distinguish *C. difficile* ribotype 027. PCR products were mixed with a hybridization buffer, denatured at 95 °C and applied onto the designated GENSPEED® Cdiff test chip. The system uses an enzyme complex and a chemiluminescence substrate, to detect specific DNA-binding. Signals were read in the GENSPEED® Reader and quantified by the GENSPEED® software.

Fig. 1

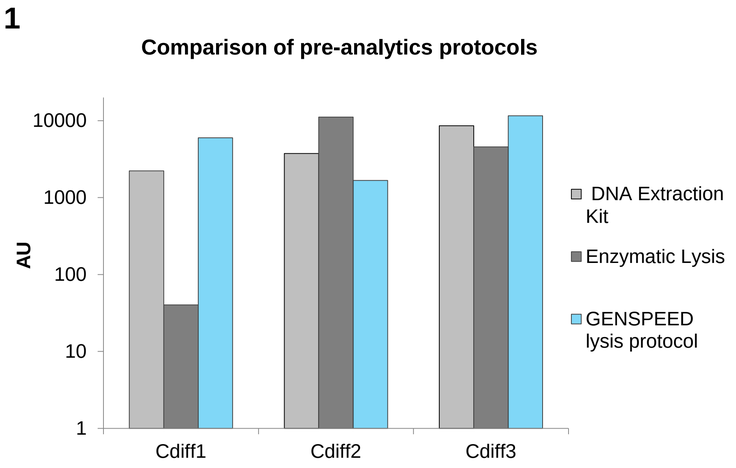


Fig. 2

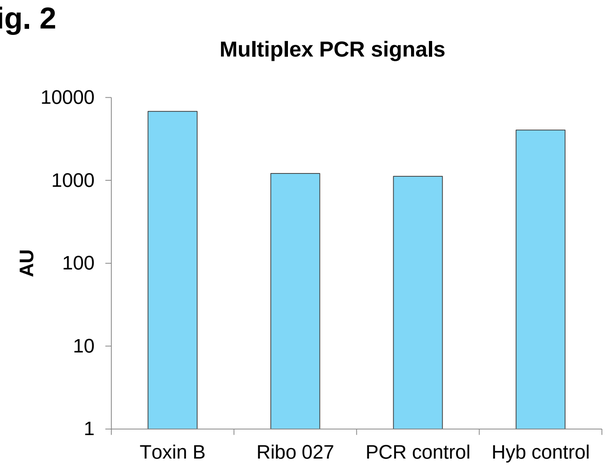
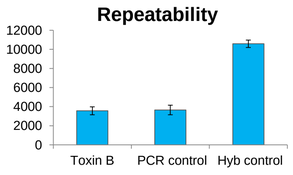


Table1: Summary of analyses

Total number of native stool samples	80		
Assay	positive	negative	invalid
C. difficile culture	42	38	n/a
Toxin assay	38	42	n/a
GENSPEED Cdiff	27	42	2

Inhibition rate: 2.5 %
Current sensitivity: 71 %
Current specificity: 100 %

Fig. 3



Results

We developed a point of care test that comprises three basic steps: (a) pre-analytics, (b) polymerase chain reaction (PCR) and (c) hybridization.

(a) To obtain PCR-ready DNA from native stool samples we developed a simple, yet effective pre-analytics protocol that requires only 5 minutes hands-on time. Stool samples are resuspended in our proprietary lysis solution, mechanically filtered and diluted. These lysates can efficiently amplified with an inhibition rate of 2,5 %, which is comparable with other, more elaborate and time consuming DNA Extraction protocols (Fig.1).

(b) Multiplex PCR was only slightly adapted using a proprietary *C. difficile* master mix (Amplex Diagnostics), to obtain well balanced specific signals compared to control signals (Fig.2)

(c) The optimized microfluidics of the GENSPEED test chip enables a fast hybridization with less than 15 % signal variation (Fig.3).

Test chips were designed to include three controls (PCR-control, hybridization control and negative control), to detect the toxin B gene and distinguish *C. difficile* ribotype 027. However, the test system may be extended to identify up to 8 analytes (including controls). Eighty native stool samples were analyzed and compared to the results obtained by bacterial culture and toxin assay. Our fast and simple pre-analytics method allowed efficient PCR amplification with less than 4 % PCR-inhibition. Forty-two samples were culture positive of which 38 appeared positive in a commercial toxin B assay. Twenty-seven samples (out of 38) were tested positive and 42 (out of 42) samples tested negative using our point-of-care test system, thus reaching a sensitivity of 71 % and a specificity of 100 % (Table 1).

Conclusion

The GENSPEED test system comprised of the GENSPEED test chip, reader and report software (Fig.4) together with the newly developed pre-analytics protocol provide results on the CDI status within at total time of 90 minutes and a hands-on time of less than 15 minutes

Fig. 4

Acknowledgements

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