Performance of a PCRstable®-stabilized real-time PCR *C. diff* DNA assay

INTRODUCTION

PCRstable® is a service for developing chemical stabilizer alternatives to lyophilization, reducing assay development time and improving assay workflows. Drying with the stabilizers produces ambient-stable assays ready for testing, shipment and storage worldwide. In this study, we use PCRstable® technology to stabilize a real-time PCR *C. diff* DNA assay with proprietary biostability compounds in a simple air dried format. Following accelerated aging procedures at elevated temperatures, we performed a real-time PCR assay to detect *C. diff* DNA in order to assess the performance of the assay. We demonstrate that the PCRstable technology successfully stabilizes the complete *C. diff* DNA assay at ambient temperatures while maintaining the assay's efficiency.

MATERIALS AND METHODS

Reagent Preparation

Duplex real-time *C. diff* DNA assays with an internal control were assembled with the following standard qPCR components: buffer, 200µM dNTPs, 0.45 - 0.7µM of each primer, 0.3µM of each probe, and 2U GoTaq glycerol free enzyme. PCRstable® stabilizers were added to separate PCR master mixes at a 1:1 (vol:vol) ratio. Each reaction was set up in triplicate, with final reaction volume of 10µL.

Drying and Storage

PCRstable® stabilized assays (Stabilizer A and Stabilizer B) and Non Protected controls (NP) were dried onto 96-well PCR plates using a Vacufuge® vacuum concentrator (Eppendorf). Dried reactions were stored with desiccants in moisture barrier bags at 45°C.

Rehydration and Analysis

After 60 days (2 months), reactions were rehydrated with 10 µL of *C. diff* DNA template at 10x LOD. Fresh control reactions were set up at the same reagent and template concentrations as the dried reactions. All samples were amplified on a CFX96 Real-Time PCR Instrument (Bio-Rad) using 40 cycles of the following conditions: 95°C for 30 seconds and 60°C for 1 minute. Endpoint fluorescence was measured using the CFX96 following real-time PCR.

RESULTS

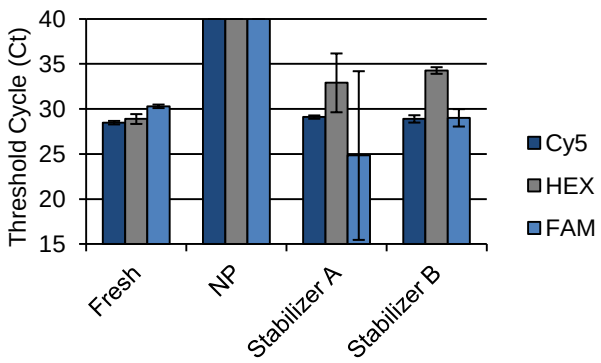

Figure 1: *Quantification cycles of stabilized duplex C. diff DNA reactions plus an internal control after 2 months stored at 45°C in dried format. Fresh represents the positive control, NP represents Non Protected control, and Stabilizer A and B represent stabilized reactions. Error bars represent the standard deviation from triplicate reactions. Cts of 40 were assigned to NP that failed to amplify.*

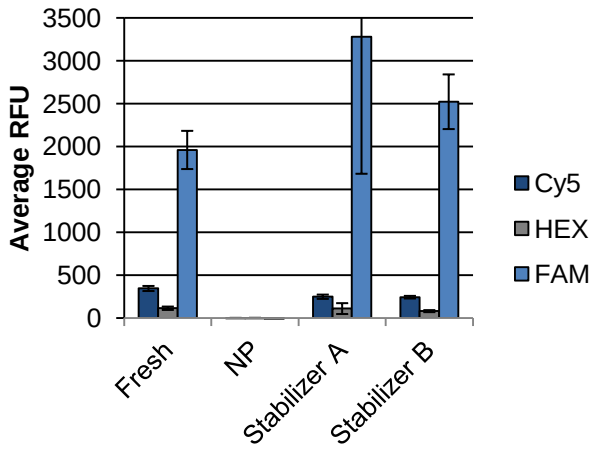

Figure 2: *Endpoint florescence of stabilized duplex C. diff DNA reactions plus an internal control after 2 months stored at 45°C in dried format. Fresh represents the fresh positive control, NP represents Non Protected control, and Stabilizer A and B represent stabilized reactions. Error bars represent the standard deviation from triplicate reactions.*

SUMMARY

The data presented in Figure 1 shows that all the real-time DNA reactions retain their efficiency to amplify *C. diff* DNA after being vacuum-dried in the presence of a PCRstable® stabilizers compared to fresh positive control. Figure 2 shows the endpoint florescence of these reactions after 2 months of storage at 45°C, which is an indication of the performance of the probes and enzymes in the reaction. This demonstrates PCRstable®-stabilized assays retain amplification efficiency, while the probes and enzymes in the assays also maintain their performance.

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