Ambient temperature stabilization of a HBV real-time PCR assay using PCRstable® technology

INTRODUCTION

As PCR technologies segue into the global diagnostics market, PCR-based assay reagents will require on-board storage and stabilization at room temperature. PCRstable is a service for developing chemical stabilizer alternatives to lyophilization. Drying with the stabilizers produces ambient-stable reagents ready for storage or shipment worldwide. In this study, we use PCRstable® technology to stabilize a real-time HBV molecular assay with proprietary biostability compounds in a simple, air dried format. Following accelerated aging procedures at elevated temperatures, we perform real-time PCR (RT-PCR) assays for quantification of HBV DNA to assess the performance of the stabilized assay. We demonstrate that the PCRstable® technology successfully stabilizes all HBV RT-PCR assay reagents for at least one year at ambient temperatures, based on accelerated aging calculations.

MATERIALS AND METHODS

Reagent Preparation

Duplex HBV DNA RT-PCR master mixes were assembled with standard RT-PCR components: buffer, dNTPs, primers, probes, and GoTaq® glycerol free enzyme (0.25U, Promega). PCRstable® Stabilizers A, B, and C 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 10 µL.

Drying and Storage

Assay reagents stabilized with PCRstable technology and non protected controls (NP) were dried in 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

At days 6, 14 (2 weeks), and 60 (2 months) of storage, the assay reagents were rehydrated with 10 µL of HBV DNA template at 10x LOD. Fresh positive control reactions were set up using the same reagent and template concentrations as the dried reactions. All samples were amplified on a CFX96 Real-Time PCR Instrument (Bio-Rad). Endpoint fluorescence was measured using the CFX96 following RT-PCR.

RESULTS AND CONCLUSIONS

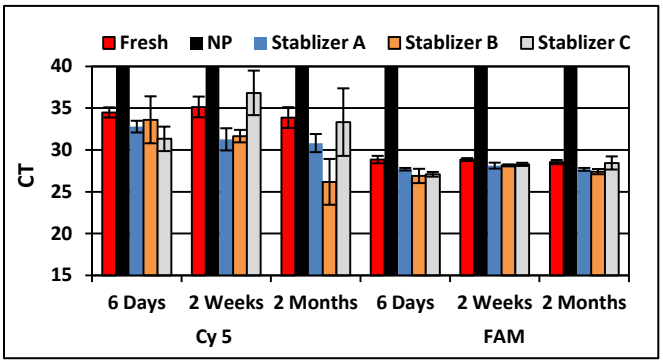

Figure 1: Quantification of cycles for duplex HBV PCR reactions. *Fresh represents the positive control, NP represents Non Protected control, and Stabilizer A, B, C represent stabilized reactions. Error bars represent the standard deviation from triplicate reactions. Cts of 40 were assigned to NP due to no amplification.*

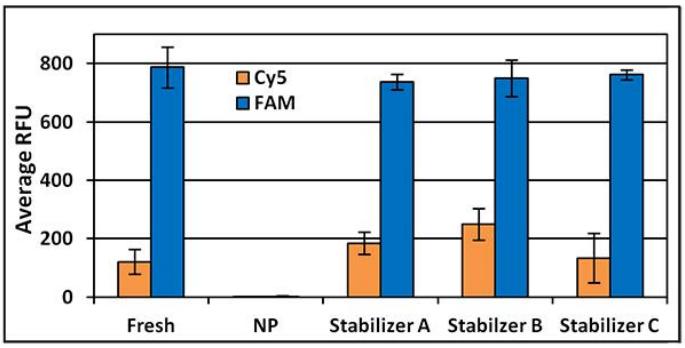

Figure 2: Endpoint florescence of stabilized duplex HBV RT-PCR reaction products following storage for 2 months at 45°C in a dried format. *Fresh represents the fresh positive control, NP represents Non Protected control, and Stabilizer A, B, C represent stabilized reactions. Error bars represent the standard deviation from triplicate reactions.*

The data presented in Figure 1 shows that all the RT-PCR reactions retain their efficiency to amplify HBV DNA after being vacuum-dried in the presence of PCRstable® stabilizers compared to a fresh positive control. Figure 2 shows the endpoint florescence of these reactions after 2 months of storage at 45°C, another indicator of the performance of the probes and enzymes in the reaction. The data presented demonstrates that PCR assay reagents stabilized by PCRstable have similar performance to assay reagents stored frozen, providing an all-ambient alternative to frozen storage for molecular diagnostic assays.

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