HawkZ05-based HIV/HCV real-time RT-PCR assays stabilization with PCRstable® technology

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 the PCRstable® technology to stabilize a real-time HIV/HCV molecular assay with proprietary biostability compounds in a simple, air dried format. Following accelerated aging procedures at elevated temperatures, we perform real-time RT-PCR assays for quantification of HIV and HCV RNA to assess the performance of the stabilized assay. We demonstrate that the PCRstable technology successfully stabilizes the complete assay for at least one year at ambient temperatures, based on accelerated aging calculations.

MATERIALS AND METHODS

Reagent Preparation

Two HIV/HCV duplex real time RT-PCR assays were prepared. One assay mix was prepared with the following components: 2.5U HawkZ05 DNA polymerase (Roche), RT-PCR buffer, 200µM dATP, 200µM dCTP, 200µM dGTP, 300µM dUTP, 30µM dTTP, 1.5mM manganese acetate, 0.4µM of each primer, and 0.1µM of each fluorescent probe. The second assay master mix was prepared with the following components: HawkZ05 Fast One-step RT-PCR Lyo Kit diluted to 1x (Roche), 0.4µM of each primer, and 0.1µM of each fluorescent probe. Mixes were set up containing the enzyme plus one or more of the above listed components, as noted in the figure legends.

PCRstable® Stabilizers A to F were added to separate PCR master mixes at a 1:1 (vol:vol) ratio. Each reaction was set up in triplicate, with a final reaction volume of 10µL.

Drying and Storage

Assay reagents mixed with PCRstable® stabilizers and the Non Protected controls (NP) were dried for 1 hour 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 60 days (2 months) of storage, the assay reagents were rehydrated with 10 µL of HIV/HCV RNA template at 10x LOD, along with any other assay components not included when the assay was stabilized. 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) using the following cycling conditions: 52°C for 5 minutes, 55°C for 5 minutes, 60°C for 10 minutes, 65°C for 5 minutes, and 45 cycles of 94°C for 10 seconds and 60°C for 1 minute. Endpoint fluorescence was measured using the CFX96 following real-time PCR.

RESULTS AND CONCLUSIONS

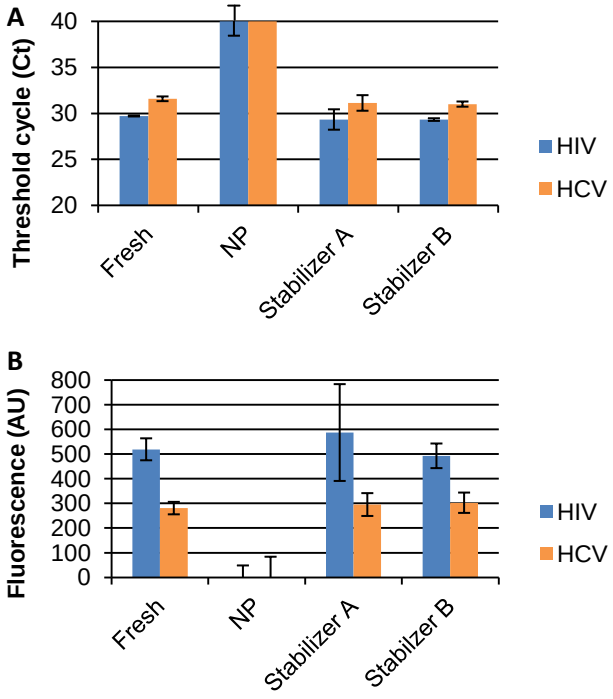


Figure 1: HIV/HCV RT-PCR reactions from dry stabilized HawkZ05 DNA polymerase and dNTPs. *HawkZ05 enzyme and dNTPs were stabilized and stored for 2 months. Following storage, mixes were rehydrated with RNA template, buffer, primers, probes, and water. Fresh represents the positive control, NP represents Non Protected control, and Stabilizer A and B represent stabilized reactions. Error bars represent the standard deviation from triplicate reactions. (A) Cycle times of PCR reactions. Cts of 45 were assigned to any sample that failed to amplify. (B) Fluorescence of PCR reactions following amplification in arbitrary fluorescence units (AU).*

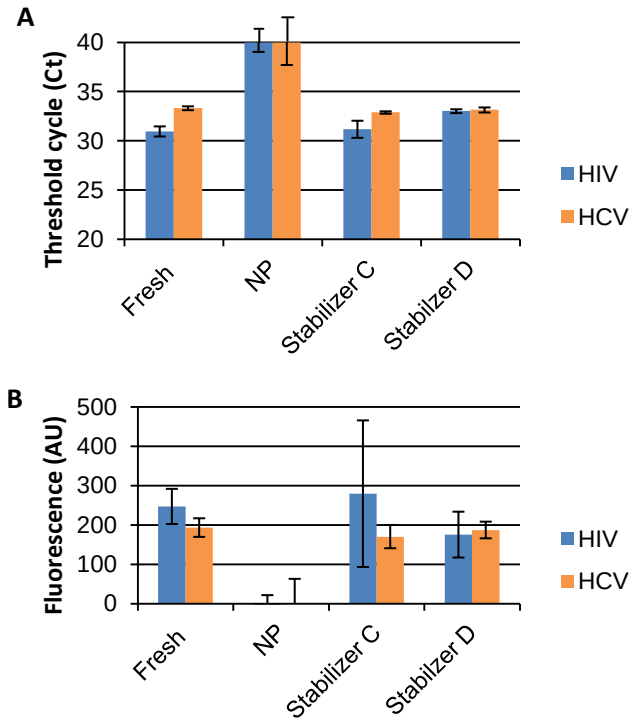
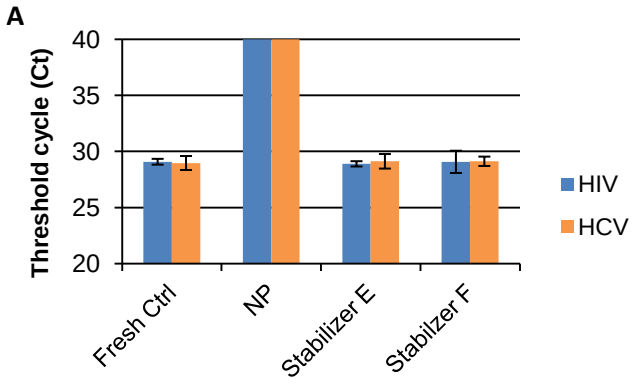


Figure 2: HIV/HCV RT-PCR reactions from dry stabilized HawkZ05 DNA polymerase, dNTPs, and primers & probes. *HawkZ05 enzyme, dNTPs, primers, and probes were stabilized and stored for 2 months. Following storage, mixes were rehydrated with RNA template, buffer, and water. Fresh represents the positive control, NP represents Non Protected control, and Stabilizer C and D represent stabilized reactions. Error bars represent the standard deviation from triplicate reactions. (A) Cycle times of PCR reactions. Cts of 45 were assigned to any sample that failed to amplify. (B) Fluorescence of PCR reactions following amplification in arbitrary fluorescence units (AU).*



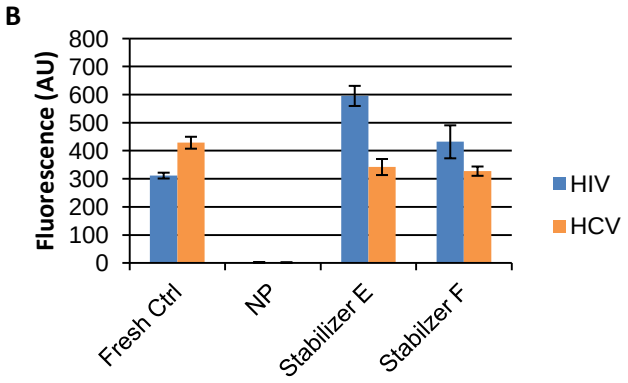


Figure 3: HIV/HCV RT-PCR reactions from dry stabilized HawkZ05 Fast One-step RT-PCR Lyo kit containing enzyme, buffer, dNTPs, and primers & probes. *HawkZ05 Fast One-step RT-PCR Lyo mix with primers and probes was stabilized and stored for 2 months. Following storage, mixes were rehydrated with RNA template and water. Fresh represents the positive control, NP represents Non Protected control, and Stabilizer E and F represent stabilized reactions. Error bars represent the standard deviation from triplicate reactions. (A) Cycle times of PCR reactions. Cts of 45 were assigned to any sample that failed to amplify (B) Fluorescence of PCR reactions following amplification in arbitrary fluorescence units (AU).*

The data presented in Figure 1 shows that real-time RT-PCR reactions using stabilized HawkZ05 DNA Polymerase and nucleotides retain their efficiency to amplify HIV/HCV target RNA after being dried in the presence of PCRstable® stabilizers and stored for 2 months at 45°C (equivalent to 12 months at 25°C). Both the cycle times and endpoint fluorescence are comparable to freshly prepared controls. Figure 2 shows that similar positive performance can be achieved when stabilizing HawkZ05 DNA polymerase in the presence of primers, probes, and nucleotides. Finally, Figure 3 shows that a complete PCR master mix using HawkZ05 Fast One-step RT-PCR Lyo enzyme can also be stabilized.

Taken together, the data presented demonstrates that PCR assay reagents stabilized by PCRstable have similar performance to assay reagents prepared from frozen stocks, providing an all-ambient alternative to frozen storage for molecular diagnostic assays.

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