PCRstable®: Chemical Stabilization Technology for Ambient PCR-Based Testing

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Abstract

The need for cold storage and shipping of nucleic acid test reagents makes their use expensive and burdensome in locations where access to freezers and dry ice is either inconvenient, non-existent or undependable. PCRstable® is a novel technology developed to preserve PCR-based testing components at ambient temperatures. In this study, we tested the stability and functionality of PCR and RT-PCR reagents preserved with PCRstable technology for molecular diagnostic testing. Specifically, we combined PCR test reagents, such as enzymes, dNTP's, primers, probes, buffers, and master mixes, with proprietary PCRstable formulations and applied a simple air drying procedure for stabilization. Following accelerated aging studies of the stabilized reagents at elevated temperatures, we performed PCR and RT-PCR reactions to assess the effectiveness of the PCRstable formulations. In both PCR and RT-PCR assays, we found excellent reagent functionality for end-point and quantitative PCR.

Additionally, we provide case studies of Focus Diagnostics' Simplexa® Direct RT-qPCR kits containing reagents stabilized by PCRstable chemistry. These case studies demonstrate effective room temperature stabilization and long term functionality of Anthrax and Ebola/Marburg assay reagents stabilized by PCRstable technology.

We conclude that PCRstable technology could be applied to PCR-based diagnostic test reagents to eliminate cold chain requirements.

Introduction

Diagnostic testing in locations where there is unreliable, inconvenient or non-existent access to cold storage makes the use of these tests difficult. A reliable and cost effective alternative to cold chain storage would therefore greatly improve implementation of diagnostic tests in these locations, as well as significantly decrease overall testing costs. Biomatrica is using its core biostabilizing technology to develop ambient temperature stabilizers for PCR-based diagnostic test components. These stabilizers eliminate cold chain requirements and the associated high cost. We have developed and tested the stability and functionality of dried-down, stabilized PCR and RT-PCR reagents described in this study.

Materials and Methods

We have evaluated several different PCR and RT-PCR reagents (enzymes, dNTP's, primers, probes, buffers, and master mixes) both in generic PCR and RT-PCR reactions and in specific molecular diagnostic assays. We performed testing with varying combinations of dried down reagents with increasing complexity. The specified PCR reagents were combined with PCRstable preservatives, incubated in dry format at high temperatures, and tested at defined time points. The dried reaction mixtures were rehydrated with the appropriate components to complete the specified PCR or RT-PCR reactions, and the results were analyzed. Specifically, Ct and melt curve analysis were performed to assess the ability of the dried reagents to perform their indicated functions. Each condition was run in triplicate and compared to a frozen positive control. For stabilization of the Focus Diagnostics' Simplexa® assays presented as case studies, assay reagents were obtained from Focus Diagnostics and stabilized at Biomatrica. Stabilized assays were shipped back to Focus Diagnostics for testing at the indicated storage time points and temperatures, using the 3M™ Integrated Cycler and the Direct Amplification Disc.

Results

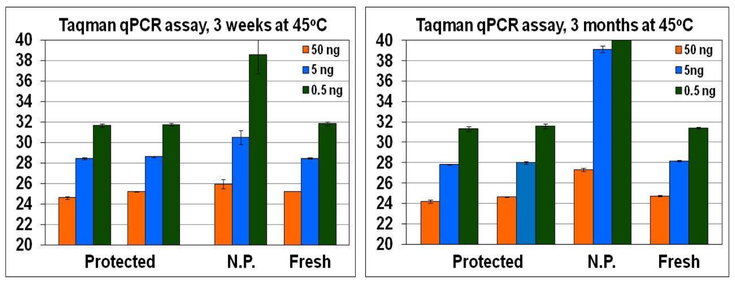

Figure 1. Stabilization of Full TaqMan qPCR Assay. A complete TaqMan probe-based qPCR assay, including reaction buffer, and TaqMan probes, was dried down in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected, N.P.), and stored at room temperature or under stress conditions at 45°C. After the specified storage times, the assay was rehydrated and its stability was assessed by qPCR amplification of a human RNase P amplicon. Reagents stored frozen at -20°C (Fresh) were used as positive controls for the assay. Results show assay stabilization for >1 year at ambient temperatures (based on accelerated aging studies).

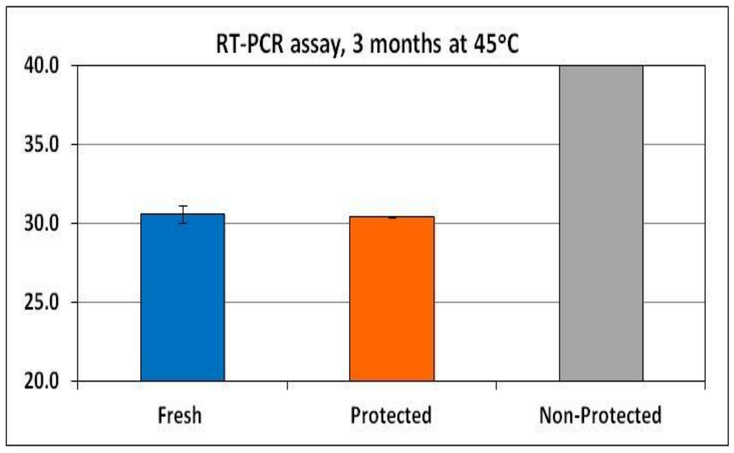

Figure 2. Stabilization of Complete RT-PCR Assay. A TaqMan probe-based RT-qPCR assay, including limiting enzyme amounts, dNTPs and assay buffer, was dried down in the presence of Biomatrica's stabilizing formulations (Protected) or their absence (Non-Protected), and stored under stress conditions at 45°C. After 3 months of storage at 45°C, samples were rehydrated with water, primers and TaqMan probes, and a known input of RNA template was added. Reverse transcription and real time PCR amplification were performed with equivalent amounts of template RNA samples, using RT-qPCR reagents stored at -20°C (Fresh) as a positive control. Results show assay stabilization for >1 year at ambient temperatures (based on accelerated aging studies).

Figure 3. Biomatrica's Easy PCRstable Workflow for Dry Ambient Temperature Assay Stabilization. Biomatrica's robust PCRstable technology allows for an easy workflow for ambient assay stabilization. It eliminates complex and expensive infrastructures (A), and allows for easy manufacturing scale-up. Assay stabilization can be achieved in most formats, including multi-well plates, tubes and microfluidic chips (B).

A

Stabilized PC and Assay Reagents	Storage Time (Days)	Storage Temp. (°C)	Target 1 (chromosome)		Target 2 (pXO1)		Target 3 (pXO2)	
			Av. C(t)	Δ C(t) to Day 0	Av. C(t)	Δ C(t) to Day 0	Av. C(t)	Δ C(t) to Day 0
Simplexa® Anthrax Environmental	50	45	27.5	-2.0	26.2	-3.3	28.6	-1.2
	100	37	27.5	-2.7	26.4	-3.8	28.6	-1.9
	130	30	27.2	-2.3	26.2	-3.3	28.2	-1.6
Simplexa® Anthrax IVD	44	45	27.2	-3.0	25.7	-3.5	28.3	-2.0
	100	37	26.7	-3.6	25.5	-3.7	27.7	-2.6
	162	30	27.3	-2.9	26.0	-3.1	28.1	-2.2

Note: A negative change in C(t) value indicates an improvement from Day 0.

B

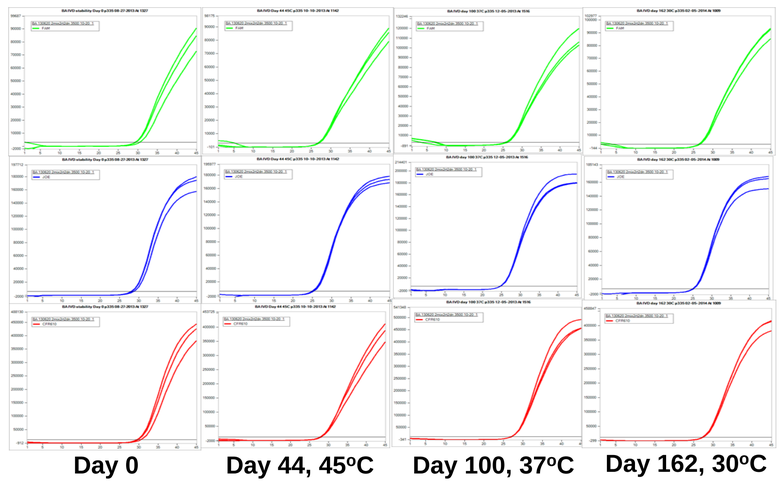

Figure 4: Long term stabilization of Simplexa® Anthrax Environmental and IVD assay reagents at ambient and accelerated temperatures. (A) Real time PCR amplification cycles for 3 different targets at different storage conditions and comparison to frozen controls (Day 0). **(B)** Real time PCR amplification curve examples for the 3 separate targets (Green: FAM, Target 1; Blue: Joe, Target 2; Red: CFR610, Target 3), for the indicated storage times and temperatures, using the Biomatrica-stabilized Simplexa® Anthrax IVD assay.

A

Sample	Storage Time (Days)	Storage Temp (°C)	Average C(t)					
			Target 1: chromosome	# detected/ # tested	Target 2: pX01	# detected/ # tested	Target 3: pX02	# detected/ # tested
1000cfu/mL RA3 spores in soil	41	45	38.7	7/8	37.7	8/8	38.2	8/8
	92	37	39.3	7/8	36.8	8/8	38.9	8/8
	130	30	39.4	7/8	37.7	8/8	38.7	8/8
150cfu/mL Ames strain in blood	41	45	39.2	8/8	38.9	8/8	37.8	8/8
	92	37	38.8	8/8	37.5	8/8	36.5	8/8
	130	30	38.9	6/8	39.4	8/8	38.3	8/8

B

Sample: Spiked blood		Average C(t)					
		Day 14			Day 21		
Reaction Mix:	Storage (°C)	Target 1: FAM channel	Target 2: Joe channel	Target 3: CFR610 channel	Target 1: FAM channel	Target 2: Joe channel	Target 3: CFR610 channel
Control	-20	30.6	30.4	31.5	30.8	30.9	32.0
Biomatrica Stabilized	23	31.1	30.6	31.5	30.9	31.1	32.0
	45	32.5	31.4	32.7	33.1	32.4	35.4

Figure 5: Performance of the Biomatrica-stabilized Simplexa® Anthrax and Simplexa® Ebola/Marburg assays using blood or soils samples. (A) Average C(t) values and detection of *B. anthracis* RA3 and *B. anthracis* Ames targets using Biomatrica stabilized Simplexa® Anthrax Environmental and Simplexa® Anthrax IVD assay reagents, respectively. **(B)** Average Ct values for Biomatrica stabilized reaction mix for Simplexa ® Ebola/Marburg assays.

Conclusions

- **Biomatrica's PCRstable technology effectively stabilizes PCR and RT-PCR reagents (enzymes, dNTP's, primers, probes, buffers, and master mixes) at ambient temperatures for extended periods of time.**
- **qPCR and RT-PCR assay reagents are stabilized at ambient temperatures, and show excellent performance with real diagnostic assays and samples.**
- **Case studies show implementation of Biomatrica's technology into real molecular diagnostic assays**
- **Biomatrica's PCRstable technology allows for a simple and inexpensive workflow that can be applied to most PCR-based assay formats.**