Chemically Stabilized PCR and RT-PCR Reagents for All-Ambient Assays.

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Abstract

The need for cold or frozen storage of reagents for diagnostic testing makes the use of these tests difficult in regions where access to freezers is either non-existent or undependable. A reliable and cost effective alternative has been developed to stabilize diagnostic test components dry at ambient temperatures. We have developed and tested the stability and functionality of dried-down, stabilized PCR and RT-PCR reagents, which are described in this study. We combined the test reagents with proprietary biostability compounds and applied a simple air drying procedure. Following accelerated aging studies at elevated temperatures, we performed both PCR and RT-PCR reactions to assess the ability of the dried down reagents to perform the desired reactions. For both PCR and RT-PCR assays, we have shown excellent stability at ambient temperatures for both end-point and qPCR. We demonstrate that the dry down of PCR and RT-PCR reagents with biostability compounds leads to high retention of both stability and activity at ambient and elevated temperatures. These procedures can be applied to PCR-based diagnostic assays to eliminate cold chain requirements and simplify testing in places with lack of access to reliable cold storage, as well as in places that may benefit from cost effective shelf life and shipping approaches.

Introduction

Diagnostic testing in regions where there is unreliable 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 bio-stabilizing technology to develop ambient temperature stabilization of diagnostic test components, thereby eliminating 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 assays using similar components, testing varying combinations of dried down reagents with increasing complexity. The reagents specified in each figure were combined with proprietary stabilizers and dried down in single step reactions. The dried reagents were incubated at 45°C and tested at the time points specified in each figure. At each time point, the dried reaction mixtures were rehydrated with the appropriate components to complete the specified PCR or RT-PCR assay and the reactions were tested. Depending on the assay, either gel electrophoresis (for end-point PCR) or Ct and melt curve analysis (for qPCR) 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.

Results

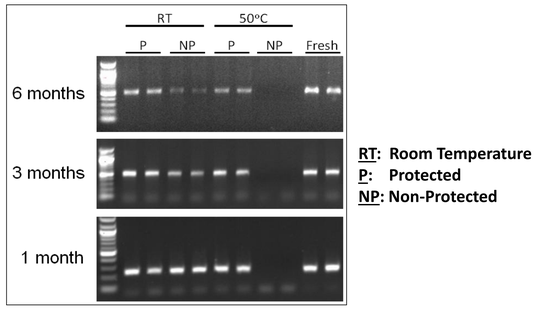

Figure 1. Stabilization of Taq and dNTPs for End Point PCR Assay. Taq polymerase and dNTPs were dried down in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected), and were stored at room temperature or under stress conditions of 50°C. After the specified storage time, the reagents were rehydrated and their stability was assessed by end point PCR amplification of a human RNase P amplicon. Reagents stored frozen at -20°C were used as positive controls for the assay. Results show reagent stabilization for more than 3 years at room temperature (based on accelerated studies).

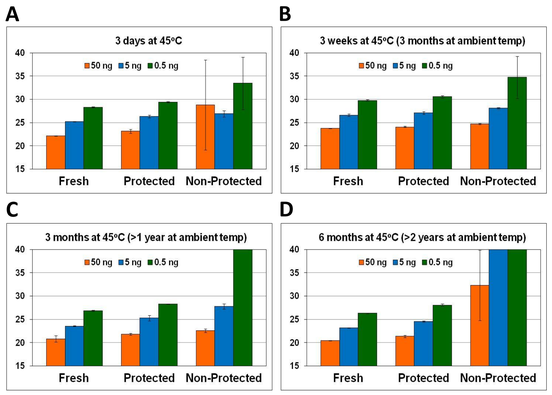

Figure 2. Stabilization of Hot-Start Taq, dNTPs, Primers and SYBR Green for qPCR Assay. Taq polymerase, dNTPs, primers and SYBR green dye were dried down in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected), and stored at room temperature or under stress conditions of 45°C. After the specified storage time, the reagents were rehydrated and their stability was assessed by qPCR amplification of a human RNase P amplicon. Reagents stored frozen at -20°C were used as positive controls for the assay. Results show reagent stabilization for more than 2 years at room temperature (based on accelerated aging studies).

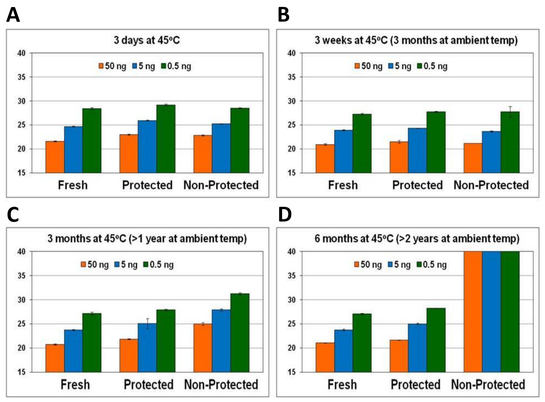

Figure 3. Stabilization of Full qPCR Assay. A SYBR green-based qPCR assay, including reaction buffer, was dried down in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected), and stored at room temperature or under stress conditions of 45°C. After the specified storage time, the assay was rehydrated and its stability was assessed by qPCR amplification of a human RNase P amplicon. Reagents stored frozen at -20°C were used as positive controls for the assay. Results show assay stabilization for more than 2 years at room temperature (based on accelerated aging studies).

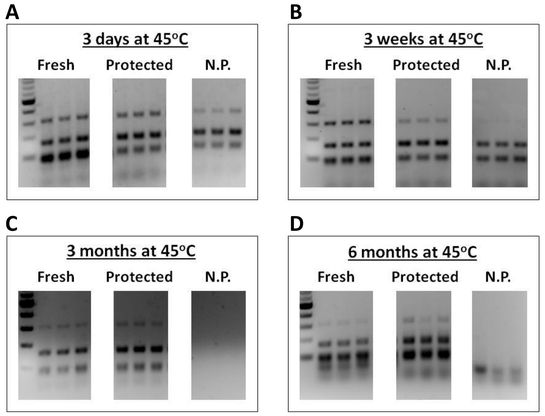

Figure 4. Stabilization of Reverse Transcriptase, Primers and dNTPs for Multiplex RT-PCR Assay. Reverse transcriptase, 3 sets of primers and dNTPs were dried down in the presence of Biomatrica's stabilizing formulations (Protected), or their absence (Non-Protected), and stored under stress conditions of 45°C. After the specified storage time, the reagents were rehydrated and their stability was assessed by reverse transcription followed by multiplex PCR amplification of 3 human TATA binding protein amplicons, using human RNA as template. Reagents stored frozen at -20°C were used as positive controls for the assay. Results show reagent stabilization for more than 2 years at room temperature (based on accelerated aging studies).

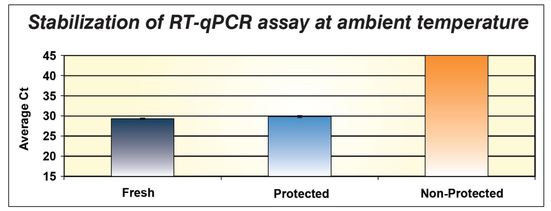

Figure 5. Stabilization of a Full RT-PCR Assay at Room Temperature. All components of a RT-qPCR assay, including Taq DNA polymerase with reverse transcriptase activity, dNTPs, aptamer and PCR buffer including metal co-factor were dried down in the presence of Biomatrica's stabilizing formulation (Protected) or their absence (Non-Protected), and stored under stress conditions of 45°C. At determined time points, 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 RNA samples, and using RT-qPCR reagents stored at -20°C as a positive control. Results show assay stabilization for more than 3 months at room temperature (based on accelerated aging studies).

Figure 6. Biomatrica's Easy Workflow for Room Temperature Assay Stabilization. Biomatrica's robust chemical stabilization technology allows for an easy workflow for room temperature assay stabilization. It eliminates the need of 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).

Conclusions

- **Biomatrica's chemical stabilization technology stabilizes PCR and RT-PCR reagents at room temperature and under stress conditions for extended periods of time (Fig. 1, 4).**
- **qPCR reagents and complete assays are stabilized for at least 2 years at room temperature (Fig. 2, 3).**
- **Complete RT-qPCR assays are completely stabilized at room temperature (Fig. 5).**
- **Biomatrica's chemical stabilization technology allows for a simple and inexpensive workflow that can be applied to most assay formats (Fig.6).**