Selected TaqMan® Copy Number Reference Assays and Endogenous Controls shipped at ambient temperature reduce environmental impact and retain quality and stability

Abstract

To minimize the adverse environmental impact of packaging and shipping products on gel or dry ice, Thermo Fisher Scientific investigated the feasibility of shipping selected TaqMan® Copy Number Reference Assays and Endogenous Controls at ambient temperatures. This report describes functional and stability testing of TaqMan® Copy Number Reference Assays, VIC®/TAMRA™ Endogenous Controls, and QSY® Assays after subjecting them to simulated summer shipping conditions. Analytical and functional testing demonstrated that assays subjected to summer ambient shipping conditions maintained the same quality and performed as well as assay kits kept at the recommended storage conditions. By shipping at ambient temperatures, the need for expanded polystyrene (EPS) coolers and added refrigerant is eliminated and the fuel consumption and greenhouse gas emissions required to transport the product are significantly reduced.

Introduction

The adverse environmental impact of shipping refrigerated or frozen products is tremendous. The annual carbon footprint to manufacture EPS and convert it into coolers for our TaqMan® Copy Number Reference Assays, Endogenous Controls, and QSY® Assays is approximately 2.2 tons CO_2 equivalents (CO_2e) [1]. Factoring in the number of shipments, the average distance traveled per package, and the fact that most packages are shipped via air, the annual total carbon footprint for transporting TaqMan® Assays and Endogenous Controls is 11.8 tons CO_2e [2].

There are other factors to consider beyond the greenhouse gas emissions. When a cooler arrives at the laboratory, the researcher is often put in the untenable position of deciding whether to burn additional fossil fuels to transport the empty cooler across country for reuse/recycling or to dispose of the cooler in a landfill. The best way to address the total environmental impact of "cold-chain" transport is to follow the hierarchy of "reduce, reuse, recycle": 1) Design the product for stability to ensure it can withstand the rigors of ambient shipping conditions without added refrigerant or insulation; 2) Design the packaging to be reusable, without increasing source material consumption; and 3) Recycle locally. We have opted to reduce whenever possible, reuse when it is an environmentally preferable option, and to encourage our customers to recycle locally.

Thermo Fisher Scientific has been systematically evaluating novel ways to minimize the impact of shipping Life Technologies™ products on gel or dry ice, and the CO_2 footprint left by these products during distribution. Here we demonstrate that selected TaqMan® Assay products are stable at ambient temperatures during shipping. By avoiding the cooler and refrigerant, the product can be shipped in a smaller, corrugated cardboard box, which improves the carrier's freight density (less fuel and emissions per box) and reduces the amount of packaging materials requiring disposal or recycling. By eliminating the cooler and gel or dry ice for these products, Thermo Fisher Scientific is helping to divert an annual total of nearly 675 kg (2,235 cu ft) of EPS from landfills and incinerators by replacing it with recyclable corrugated paper packaging, and to reduce the annual total carbon footprint by 14 tons CO_2e [1,2].

life technologies

In 2009, we investigated the stability of five TaqMan® Assays: TaqMan® Gene Expression, Custom TaqMan® Gene Expression, TaqMan® MicroRNA, TaqMan® Drug Metabolism Genotyping, and TaqMan® SNP Genotyping Assays [3]. These assays comprise a preformulated set of unlabeled locus-specific oligonucleotide primers and minor groove binder–nonfluorescent quencher (MGB-NFQ) probes labeled with a fluorescent dye (VIC® or FAM™ dye), and are supplied in liquid form. A total of 42 different TaqMan® Assays were selected to represent the widest range of performance as well as chemical, sequence, and structural motifs. Assays were subjected to simulated summer ambient shipping conditions and subsequently analyzed for physical integrity and functional performance. Stressed samples were compared to controls in analytical HPLC and functional real-time qPCR assays. In all cases, simulated ambient shipping of the assays had no effect on their quality, integrity, or functional performance. This study provided ample evidence for the stability of a wide range of structural motifs and oligonucleotide sequences under ambient shipping conditions and also demonstrated the stability of the VIC® and FAM™ dyes and the MGB moiety at the concentrations found in the assays.

For many years, TaqMan® Copy Number Reference Assays and Endogenous Controls have been shipped refrigerated on gel or dry ice (with storage after shipping recommended at –20°C). Building on our 2009 study, this paper describes results from stability testing carried out after selected TaqMan® Assays and Endogenous Controls were exposed to established summer shipping temperature profiles. These experiments demonstrate that by shipping selected TaqMan® Assays and Endogenous Controls under ambient conditions, not only can we supply researchers with the same superior-quality product they are used to receiving, but we can also reduce our environmental footprint in the process. This is a win for our customers (eliminating packaging waste and extra costs associated with refrigerated shipments), a win for our planet (reducing resource consumption and total carbon footprint), and a win for our company (eliminating the need to manage cold-chain transport).

Materials and methods

Products tested. The TaqMan® Copy Number Reference Assays and Endogenous Controls investigated here are gene-specific assays consisting of two unlabeled primers and a VIC®/TAMRA™ probe at set concentrations. TaqMan® Copy Number Reference Assays are designed with mouse (Tfrc and Tert) and human (RNase P and TERT) genes as endogenous references, and the TaqMan® Endogenous Controls are designed for a variety of human genes (Table 1). Because our previous investigation of the TaqMan® Assays established that oligonucleotide sequence does not impact product stability, we chose one TaqMan® Copy Number Reference Assay and one TaqMan® Endogenous Control to represent these two product groups: TaqMan® Copy Number Reference Assay RNase P (Cat. No. 4403326) and Human PPIA Endogenous Control (Cat. No. 4310883E). The TaqMan® QSY® Assays are a new Life Technologies™ product group, and we tested both the primer-limited and primer-unlimited RNase P Assays with the ABY® reporter dye (Cat. Nos. 4485715 and 4485714, respectively). For this study, a single lot of each assay was selected from inventory for testing.

Simulated shipping conditions. To simulate temperatures experienced during shipping, samples were placed in a cycling environmental chamber (Thermotron® S-16) programmed to reproduce a "worst-case" 288-hour (12-day) summer temperature profile (Figure 1). This profile is adopted from one developed and validated by Amgen, Inc. to simulate global ambient shipping conditions and mimics product temperature extremes encountered during transit of over 2,500 shipments during summer months between the latitudes of 59.9° N and 37.8° S [4]. Testing of winter ambient conditions was not considered because of the low risk of exposing the TaqMan® Assays to cold conditions.

Stability/integrity testing. Structural integrity changes in stressed samples compared to controls were measured by reverse-phase HPLC (RP-HPLC). RP-HPLC samples were analyzed using an Agilent® 1200 HPLC. The HPLC column used was a Waters® XBridge® C18 column, 2.5 µm, 2.1 mm ID x 50 mm. Mobile phases used were 0.1 M TEAA (triethylamine acetate) in water and 0.1 M TEAA in 50% acetonitrile/50% water.

Table 1. TaqMan® TAMRA™ and QSY® Assays represented in this study.

Product	Probe/reporter dye	Cat. No.
TaqMan® Copy Number Reference Assays		
TaqMan® Copy Number Reference Assay RNase P, 750 reactions, 3,000 reactions	**VIC®/TAMRA™**	**4403326,** 4403328
TaqMan® Copy Number Reference Assay TERT, 750 reactions, 3,000 reactions	VIC®/TAMRA™	4403316, 4403315
TaqMan® Copy Number Reference Assay Mouse Tfrc, 750 reactions, 3,000 reactions	VIC®/TAMRA™	4458366, 4458367
TaqMan® Copy Number Reference Assay Mouse Tert, 750 reactions, 3,000 reactions	VIC®/TAMRA™	4458368, 4458369
TaqMan® Endogenous Controls		
Human PPIA Endogenous Control, 2,500 reactions	**VIC®/TAMRA™**	**4310883E**
Human RPLP0 (Large Ribosomal Protein), 2,500 reactions	VIC®/TAMRA™	4310879E
Human ACTB (Beta Actin), 2,500 reactions	VIC®/TAMRA™	4310881E
Human GAPD (GAPDH), 2,500 reactions	VIC®/TAMRA™	4310884E
Human PGK1 (Phosphoglycerate Kinase 1), 2,500 reactions	VIC®/TAMRA™	4310885E
Human B2M (Beta-2-Microglobulin), 2,500 reactions	VIC®/TAMRA™	4310886E
Human GUSB (Beta Glucuronidase), 2,500 reactions	VIC®/TAMRA™	4310888E
Human HPRT1 (HGPRT), 2,500 reactions	VIC®/TAMRA™	4310890E
Human TBP (TATA-Box Binding Protein), 2,500 reactions	VIC®/TAMRA™	4310891E
Human TFRC (CD71) (Transferrin Receptor), 2,500 reactions	VIC®/TAMRA™	4310892E
Eukaryotic 18S rRNA, 2,500 reactions	VIC®/TAMRA™	4310893E
TaqMan® QSY® Assays		
TaqMan® RNase P Assay, 250 reactions	**ABY®/QSY®**	**4485714**
TaqMan® RNase P Assay, primer-limited, 250 reactions	**ABY®/QSY®**	**4485715**
TaqMan® GAPDH Assay, 250 reactions	JUN®/QSY®	4485712
TaqMan® GAPDH Assay, primer-limited, 250 reactions	JUN®/QSY®	4485713

Products tested are in **bold**.

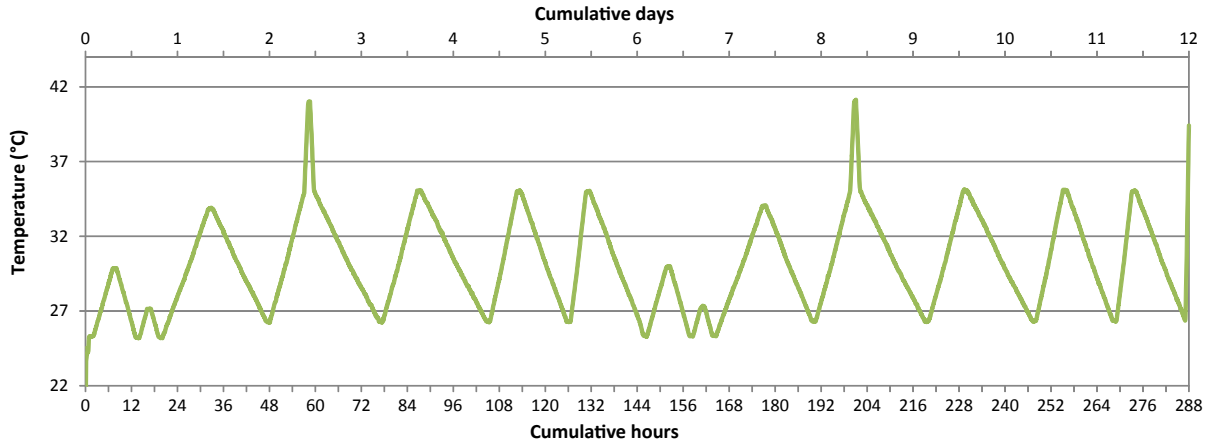

Figure 1. Summer temperature profile used to simulate shipping conditions for 288 hours (12 days). The summer temperature profile was used to mimic average high temperature extremes between the latitudes of 59.9° N and 37.8° S. Profile derived from the Amgen protocol [4].

Functional performance. Twelve matched test and control tubes from a single lot of each representative assay were functionally tested. Reactions were set up following the standard protocol for a 20 µL reaction volume; 2.5 ng of cDNA were used as the template for all reactions. PCR reactions were conducted with TaqMan® Universal PCR Master Mix II (Cat. No. 4440098) for the Copy Number Reference Assay and Endogenous Control, and MUSTANG PURPLE® Master Mix for the QSY® Assays. The QSY® Assays were run on an Applied Biosystems® PRISM® 7500 Real-Time PCR System, and the C_t values were analyzed using the 7500 v2.0.6 software (autobaseline; threshold set at 0.2). The Copy Number Reference Assay and Endogenous Control were run on an Applied Biosystems® 7900 Real-Time PCR System, and the C_t values were analyzed using the SDS 2.4 software (autobaseline; threshold set at 0.2). Real-time PCR was performed using universal cycling conditions (95°C, 10 min; 95°C, 15 sec; 60°C, 1 min, for 40 cycles). C_t variability was calculated using JMP11 statistical software.

Results

Stability/integrity testing. RP-HPLC was used to create peak profiles of the oligonucleotide assay components (VIC®/TAMRA™ or ABY®/QSY® probe oligonucleotides, and unlabeled oligonucleotide primers) using UV/Vis absorbance detection. Matched test and control tubes from each assay were analyzed. An example of the data is shown in Figure 2. Test and control peak profiles were compared. Test samples were judged as identical to matched controls (no degradation), confirming that the simulated shipping stress did not affect product integrity.

Functional performance. Functional performance of the TaqMan® Copy Number Reference Assay was assessed by calculating the C_t and C_t variability. A no-template control (NTC) test was also done. Results are shown in Figure 3. The data show that functional performance is equivalent between simulated ambient-shipped and control samples for the tested Copy Number Reference Assay, as measured by the C_t. The mean difference between test and control C_t was 0.27, and the NTC consistently gave a C_t >38.

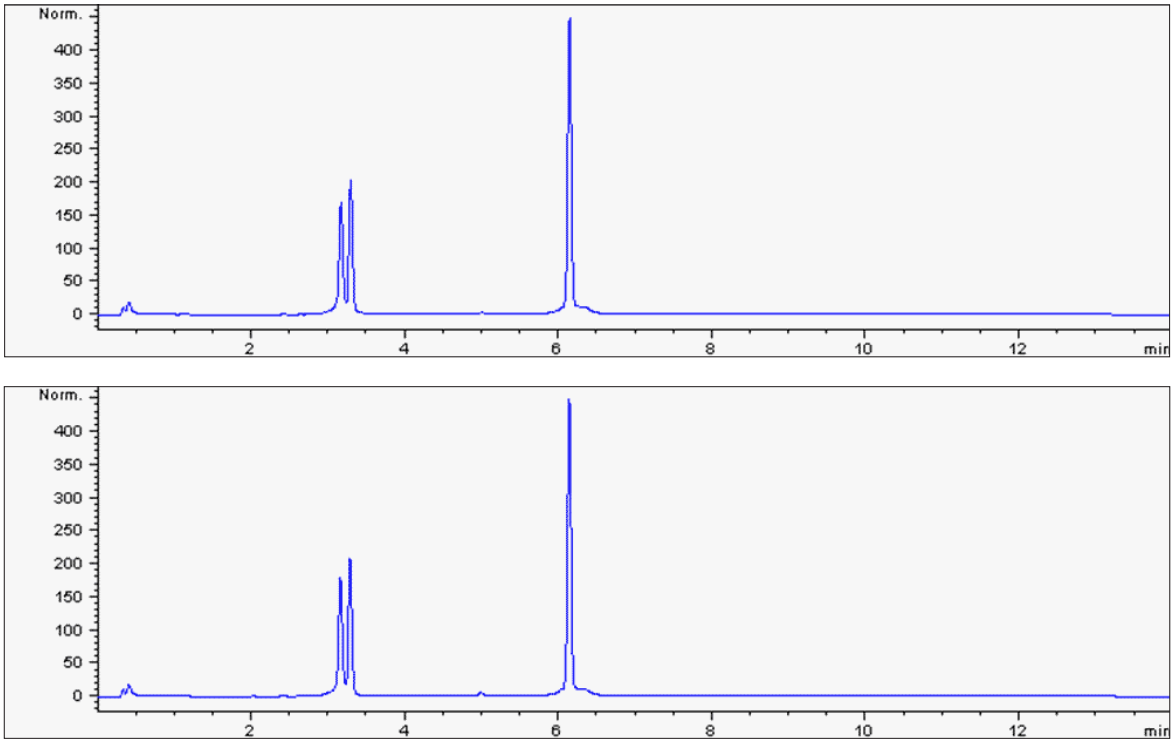

Figure 2. Simulated summer ambient shipping does not affect oligonucleotide stability—representative HPLC data. The effect of simulated summer ambient shipping on oligonucleotide integrity was measured by comparing RP-HPLC profiles of matched test and control samples. The HPLC chromatogram profiles of the test samples are comparable to the profiles of the control samples. There was no indication of probe or primer degradation in the simulated ambient-shipped Human PPIA Endogenous Control Assay (bottom) compared to the matched control (top).

A

B

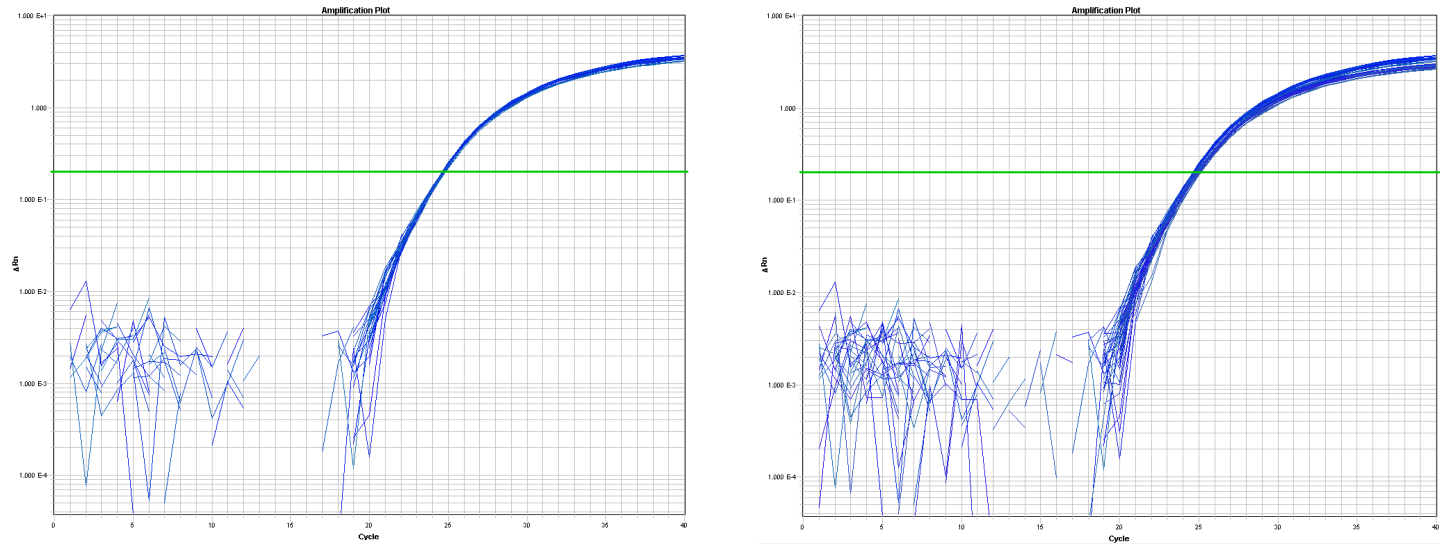

Figure 3. TaqMan® Copy Number Reference Assay. The effect of simulated summer ambient shipping on assay functional performance was evaluated by real-time qPCR of paired test and control samples. **(A)** Amplification plot for control sample stored at –20˚C (n = 6).**(B)** Amplification plot for ambient test sample (n = 6) overlaid with control sample (n = 6).

A

B

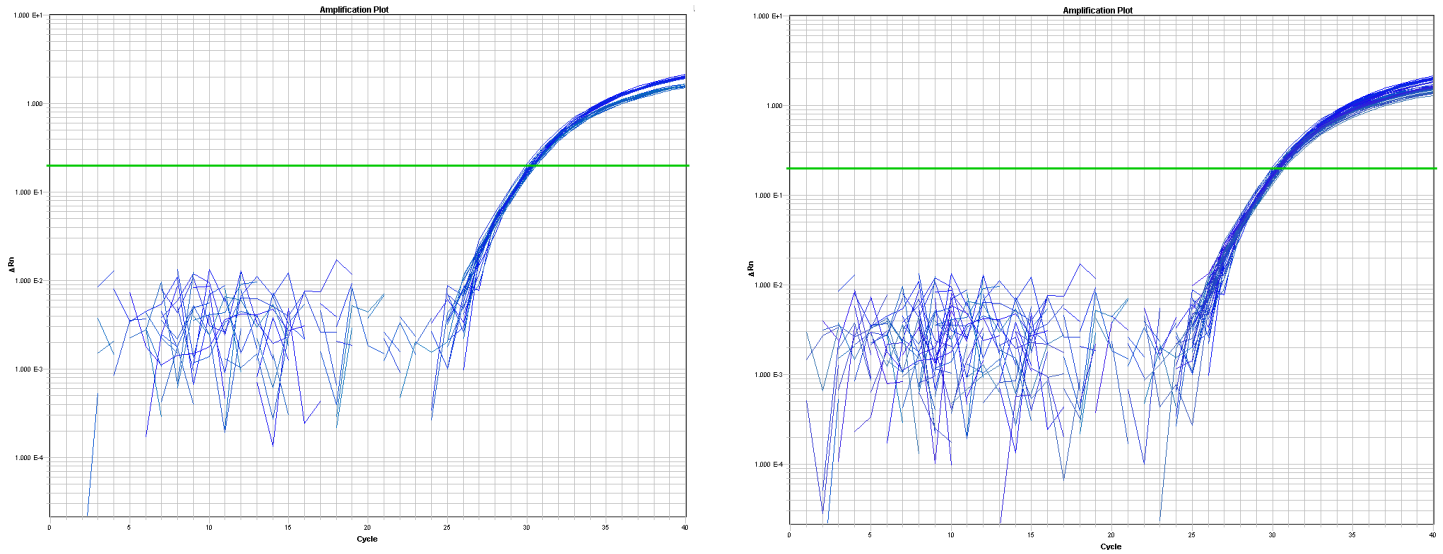

Figure 4. TaqMan® Endogenous Control. The effect of simulated summer ambient shipping on assay functional performance was evaluated by real-time qPCR of paired test and control samples. **(A)** Amplification plot for control sample stored at –20˚C (n = 6). **(B)** Amplification plot for ambient test sample (n = 6) overlaid with control sample (n = 6).

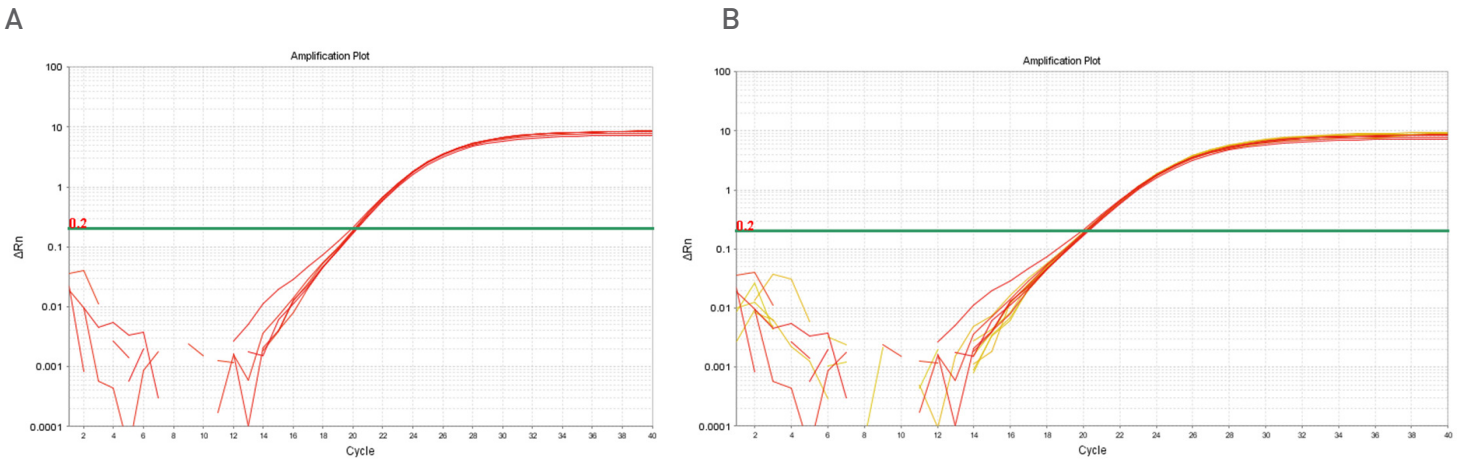

Figure 5. **TaqMan® QSY® RNase P Assay.** The effect of simulated summer ambient shipping on assay functional performance was evaluated by real-time qPCR of paired test and control samples. **(A)** Amplification plot for control sample stored at –20˚C (n = 6). **(B)** Amplification plot for ambient test sample (n = 6) overlaid with control sample (n = 6).

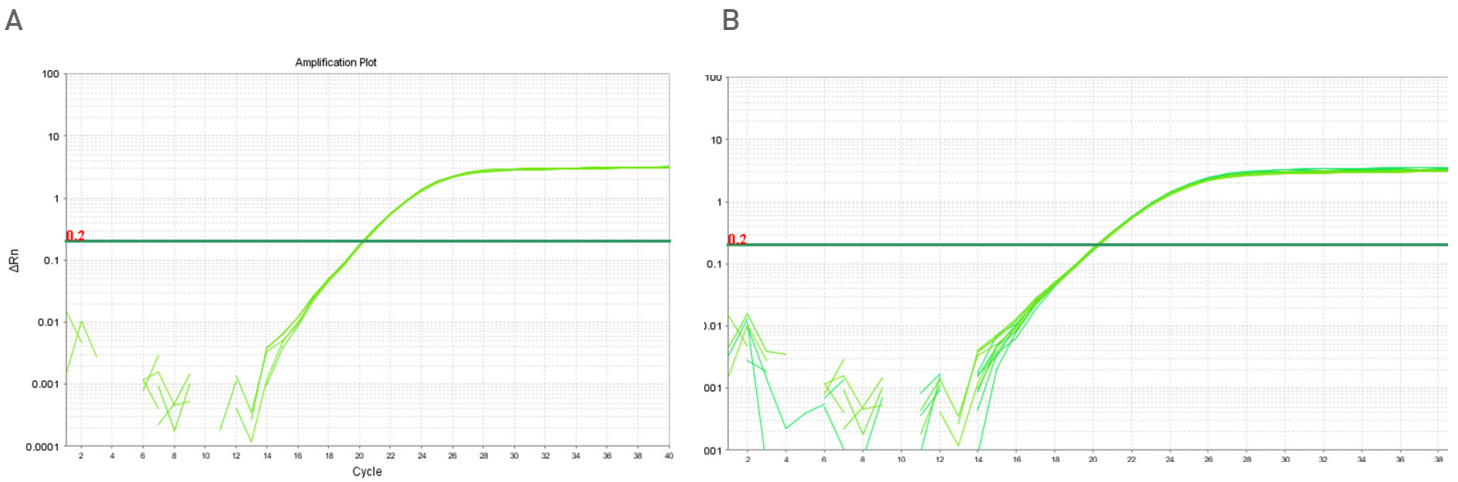

Figure 6. TaqMan® QSY® Primer-Limited RNase P Assay. The effect of simulated summer ambient shipping on assay functional performance was evaluated by real-time qPCR of paired test and control samples. **(A)** Amplification plot for control sample stored at –20˚C (n = 6). **(B)** Amplification plot for ambient test sample (n = 6) overlaid with control sample (n = 6).

Functional performance of the TaqMan® Endogenous Control was assessed by calculating the C_t and C_t variability. An NTC test was also done. Results are shown in Figure 4. The data show that functional performance is equivalent between simulated ambient-shipped and control samples for the tested Endogenous Control, as measured by the C_t. The mean difference between test and control C_t was 0.11, and the NTC consistently gave a C_t >39.

Functional performance of the TaqMan® QSY® Assays was assessed by calculating the C_t and C_t variability. An NTC test was also done. Results are shown in Figures 5 and 6. The data show that functional performance is equivalent between simulated ambient-shipped and control samples for the tested QSY® Assays, as measured by the C_t. The mean differences between test and control C_t were 0.06 and 0.01 for the RNase P and primer-limited RNase P assays, respectively, and the NTC consistently gave a C_t >38.

Conclusions

The data described in this paper demonstrate that simulated ambient temperature shipping conditions have no effect on the quality and stability of VIC®/TAMRA™ TaqMan® Copy Number Reference Assays, Endogenous Controls, or QSY® Assays. For each assay tested, we were able to clearly demonstrate that simulated ambient temperature shipping does not affect the product quality or performance.

These results substantiate the change to ambient shipping conditions, and provide the researcher with confidence that when shipped under ambient conditions, their TaqMan® Copy Number Reference Assays, Endogenous Controls, and QSY® Assays will exhibit no difference in function or stability compared to gel ice– or dry ice–shipped products. In addition to ensuring our customers will continue to receive the highest quality possible, this study enables us to reduce the impact of transporting these products by 11.8 tons CO_2 equivalents. Our customers will see a reduction of 675 kg of EPS waste. Our planet will see CO_2 emissions reduced by 14 tons every year.

References

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