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Stability and performance comparison of capillary-assisted vitrification and lyophilization for probe qPCR master mixes

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Abstract

Recent advancements in freeze-drying (lyophilization) have led to the creation of lyophilized PCR mixes. The lyophilized cake or bead offers benefits compared to liquid formulations including extended shelf lives and room-temperature storage, but they come with a number of challenges. Lyophilization is incompatible with certain chemical compounds and biological materials limiting the range of components that can be co-formulated. Formulation optimization and long drying cycles are required, resulting in increased costs. To address these challenges, we developed capillary-assisted vitrification (CAV) (formerly called capillary mediated vitrification (CMV)) and reported its capability to rapidly stabilize different biomolecules without freezing, in a short period (1–3 h cycle time) and with improved thermal stability. In this study, we sought to evaluate the efficiency of CAV to stabilize probe qPCR mixes and to compare the performance of CAV-stabilized PCR mixes with lyophilized mixes using Monkeypox (Mpox) virus DNA as a template. Both CAV-stabilized and lyophilized samples observed similar coefficients of determination ($R^2 = 0.99$), with amplification efficiencies of 99% for the lyophilized and 105% for the CAV samples. The analytical limit of detection was 5 copies/reaction for the CAV sample and 10 copies/reaction for the lyophilized sample. Both stabilization reagents showed acceptable variability, with coefficients of inter-assay precision under 2.5%. As expected, CAV-stabilized and lyophilized reagents showed undetermined cycle threshold (Ct) values when no template control was used. Additionally, comparable amplification and Ct values were observed for both CAV-stabilized and lyophilized PCR mix when stored at 37 °C for 26 weeks and 50 °C for 4 weeks. In conclusion, CAV- stabilized PCR mix without freezing, in a shorter cycle, and its performance and stability were comparable to that of lyophilized reagent. The study recommends the CAV process could be an alternative to complex lyophilization for stabilizing PCR reagents.

Keywords QPCR mix, Capillary-assisted vitrification, Lyophilization, Stability

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Introduction

Polymerase chain reaction (PCR) methodologies, along with their specifically formulated reagent mixtures, are optimized to achieve high specificity, sensitivity, and reproducibility, enabling reliable detection in diagnostic and other molecular biology applications (Zheng et al. 2019; Broeders et al. 2014). However, traditional frozen master mixes have limitations, particularly the need for temperature-controlled transportation, storage, and handling (Lopez et al. 2024). Advances in lyophilization technology have enabled the development of PCR reagents in a stabilized, freeze-dried (lyophilized) format, such as cakes or beads (Yang & Wen 2021). This process stabilizes reagents by freezing and subsequently removing water through sublimation and secondary drying, thereby preserving enzyme activity and maintaining reagent integrity, extending shelf life, allowing storage and transport at ambient temperatures, reducing contamination risk, and offering ready-to-use convenience (Nagaraj et al. 2018; Karunnanithy et al. 2024). Despite these benefits, lyophilization is a complex and costly process that requires optimization, the use of glycerol-free enzymes, and extended drying times to ensure a successful and stable PCR product (Cheng et al. 2025; Bircher et al. 2018). To address the limitations associated with traditional lyophilization, we developed a novel stabilization technique termed capillary-assisted vitrification (CAV), previously referred to as capillary-mediated vitrification (CMV). This process utilizes capillary-driven evaporation under vacuum conditions to transform liquid formulations directly into a vitrified, glassy state, thereby eliminating the need for freezing steps (Mohanty 2020). The CAV method significantly reduces processing time, achieving complete drying in less than three hours, and yields product with enhanced thermal stability. Using the CAV process, we successfully stabilized and enhanced the thermal stability of various biomolecules, including luciferase enzyme, mRNA, antibodies, and antibody conjugates (Amle et al. 2023; Renu et al. 2022; Shank-Retzlaff et al. 2022, 2024).

Details of the CAV process-its mechanism, applications, limitations, and advantages over other drying technologies have been presented in our earlier reports (Mohanty 2020; Shank-Retzlaff et al. 2022). The CAV process utilizes two key components—the BioFix™ buffer and a porous scaffold that function synergistically to prevent boiling during drying by regulating the surface tension of the formulated liquid. Boiling can damage biological molecules, resulting in denaturation, aggregation, and shear stress. In the CAV process, the BioFix™ buffer contains saccharides that stabilize biomolecular structures by replacing hydrogen bonds lost during drying, while also increasing the viscosity of the formulation. During processing, the formulation is applied to a porous scaffold that distributes the liquid into a thin layer, enhancing surface tension and minimizing the volume that requires drying. The liquid also penetrates the scaffold pores, generating a capillary effect that further stabilizes surface tension. This mechanism ensures that the vapor pressure of the liquid remains below the vacuum pressure used in the CAV process, effectively preventing boiling (Mohanty 2020).

In contrast, lyophilization prevents boiling by freezing the formulation and maintaining very low temperatures throughout the primary drying phase. However, this introduces several challenges, including the instability of freeze-sensitive molecules, phase separation, pH shifts, and precipitation of poorly soluble components, which can limit co-formulation possibilities. Unlike lyophilization, drying in the CAV process occurs directly from the liquid state and can be completed within 3–10 min under moderate vacuum conditions (Shank-Retzlaff et al. 2022). The rapid and mild nature of the process eliminates the need for ultralow pressures, thereby reducing both equipment costs and maintenance requirements.

Monkeypox (Mpox) is an enveloped DNA virus that causes Mpox disease. The virus poses a zoonotic threat, with transmission to humans primarily occurring from rodents or nonhuman primates, leading to rash and flu-like symptoms (Altindis et al. 2022). Since the global Mpox virus outbreak began in 2022, over 100,000 human infections have been reported across 122 countries (Altindis et al. 2022; Fischer et al. 2022). During outbreaks, accurate diagnosis and continuous monitoring are essential, with PCR being the most commonly used method (Fischer et al. 2022; Li et al. 2010). To improve accessibility and reduce costs, it is critical to enable ambient-temperature storage and transport of PCR reagents, along with a single-use assay format.

In this study, we aimed to evaluate the performance and thermal stability of both lyophilized and CAV-processed TaqMan probe-based PCR mixes for the detection of Mpox virus nucleic acids. The CAV-stabilized PCR mixes and a comparable commercially available lyophilized reagent were assessed by analyzing their coefficients of determination, PCR efficiency, analytical sensitivity, and precision. Additionally, both formulations were stored at elevated temperatures-37 °C for 26 weeks and 50 °C for 4 weeks, and their ability to detect viral DNA after storage was compared. We observed that the CAV and lyophilized PCR mixes exhibited similar performance and comparable thermal stability, despite the presence of glycerol in the CAV formulation (derived from the starting materials), which is absent in the lyophilized sample.

Materials and methods

Materials

Lyophilized PrimePath™ Probe qPCR Mix, provided in 8-tube strips, and its catalog counterpart, the 5X PrimePath™ Probe qPCR Mix, were obtained from Takara Bio (San Jose, CA) and were stored and used following the manufacturer's instructions. Quantitative synthetic Monkeypox virus DNA (2.8×10^5 copies/μL) was purchased from ATCC (Manassas, VA), aliquoted, and stored at − 80 °C according to the recommended guidelines. The synthetic Mpox virus–specific forward primer (CATCTATTATAGCATCAGCATCAGA), reverse primer (GATACTCCTCCTCGTTGGTCTAC), and probe (56-FAM/TGTAGGCCG/ZEN/TGTATCAGCATCCATT/3IABkFQ) were sourced from Integrated DNA Technologies (Coralville, IA).

Methods

Preparation of CAV-stabilized samples

The 5X PrimePath™ Probe qPCR Mix was mixed with BioFix+™ buffer (Ambient Biosciences, Ann Arbor, MI) and applied to circular BioFix™ scaffolds measuring 7.5 mm in diameter (Ambient Biosciences, Ann Arbor, MI) (Mohanty 2020). The formulation loaded scaffolds were then placed in a stabilizer (Ambient Biosciences, Ann Arbor, MI) and desiccated for 30 min. The resulting CAV-stabilized samples were either used directly or sealed in Mylar pouches (Impak, Los Angeles, CA) for long-term storage (Amle et al. 2023; Renu et al. 2022).

Karl-fischer titration

Residual moisture content in the CAV-stabilized PCR mixes was measured using a Metrohm 917 Coulometer coupled with an 885 Autosampling Oven (Metrohm AG, Switzerland), according to the manufacturer's instructions. Three independent samples were analyzed, and results were expressed as the mean ± standard deviation (SD) of the residual moisture content (%).

Reconstitution and qPCR usage of stabilized CAV and lyophilized samples

The CAV samples were placed in a Spin-X centrifuge column tube (Corning, Glendale, AZ), DNase/RNase-free water was added, and the samples were centrifuged for 15–30 s to collect the reagent. The required reconstitution time for the CAV samples was less than 1 min. The lyophilized cake in the striped PCR tube was reconstituted with DNase/RNase-free water, vortexed until fully dissolved, centrifuged, incubated for 5 min, vortexed again, and spun down to collect the reagent.

The reconstituted CAV and lyophilized mix were transferred to a MicroAmp™ optical 384-well reaction plate (Thermo Fisher Scientific, CA). Forward primer (400 nM), reverse primer (400 nM), and probe (400 nM) were added to each well. Mpox DNA or DNase/RNase-free water (for the no-template control) was added to the appropriate wells. The 20 μL reactions were then subjected to PCR amplification using the QuantStudio 5 Real-Time PCR System (Thermo Fisher Scientific, CA). The cycle threshold (Ct) value was used for the analysis.

Linearity and PCR efficiency

CAV and lyophilized reagents were evaluated using a five-fold serial dilution of Mpox virus DNA at concentrations of 2.8×10^5, 5.6×10^4, 1.1×10^4, 2.2×10^3, 4.5×10^2, and 9.0×10^1 copies per reaction. Performance was assessed through three independent qPCR assays. Ct values from each dilution, collected over three consecutive days, were averaged and plotted against the logarithm of the DNA copy number. Linear regression analysis was used to calculate the slope and coefficient of determination (R^2). PCR efficiency was then determined from the slope using the equation Efficiency (%) = [10^(−1/slope) − 1] × 100 (Hays et al. 2022; de Almeida-de-Oliveira et al. 2019).

Analytical sensitivity

The analytical sensitivity of the CAV and lyophilized mixes was assessed using Mpox virus DNA templates at 10, 5, and 1 copy per reaction. Eleven qPCR reactions were performed across multiple days, testing two to three stabilized mixes daily. The limit of detection (LOD) was determined as the lowest DNA copy number at which Ct values were consistently detected with 95% confidence (Hays et al. 2022; de Almeida-de-Oliveira et al. 2019).

Precision and no template control

The precision of the CAV and lyophilized qPCR mixes was calculated over three independent days, with each day comprising three replicates. Each qPCR reaction was carried out using 2.8×10^5 copies of Mpox virus DNA as the template. Ct values from all runs were averaged, and the standard deviation (SD) and coefficient of variation (%CV) were calculated using Microsoft Excel to assess inter-day variability (Hays et al. 2022; de Almeida-de-Oliveira et al. 2019).

To assess specificity, no-template control (NTC) reactions were prepared using the CAV and lyophilized mixes without the addition of viral DNA. NTC reactions were run in triplicate across three independent qPCR runs.

Long-term stability

Triplicate samples of both CAV and lyophilized mixes were prepared for each time point and stored in incubators maintained at 37–50 °C (Xu et al. 2020). Samples stored at 37 °C were retrieved on day 0 and at weeks 1, 2, 4, 9, 13, and 26, while those stored at 50 °C were retrieved on day 0 and at weeks 1, 2, and 4. Each sample was tested

by qPCR using 2.8×10^5 copies of Mpox virus DNA as the template. Ct values from the three replicates were averaged, and the SD was calculated using Microsoft Excel.

Results

Physical characterization of CAV stabilized samples

After completion of the stabilization process, the CAV samples were evaluated for residual moisture using Karl-Fischer titration, which showed a moisture content of 4.35 ± 0.11%. The CAV-stabilized samples retained a visual appearance comparable to the original scaffold, with no observable discoloration. Following drying, the scaffolds exhibited slightly reduced pliability. Upon rehydration, both the lyophilized and CAV-stabilized samples produced clear, colorless solutions without any visible cloudiness or particulate matter.

Stabilization of PCR mix using CAV process

The liquid Probe qPCR mix, which contains glycerol according to the vendor, was formulated with BioFix+™ buffer. This preparation was added to a BioFix™ scaffold designed to provide a high surface area and surface tension. Increased surface tension allows for direct vacuum drying of the material without inducing boiling and without the necessity of a pre-freezing step. When placed in the vacuum-based stabilizer, the scaffold's porous structure enabled capillary action, facilitating rapid water evaporation and resulting in a desiccated, vitrified, glass-like stable product. A schematic illustrating the preparation of CAV samples was reported previously (Amle et al. 2023; Renu et al. 2022).

CAV and lyophilized PCR mix performance

The performance of both the CAV and lyophilized PCR mixes was evaluated by analyzing the coefficients of determination and PCR efficiencies derived from standard curves generated using serial dilutions of the Mpox virus DNA template. A 5-fold serial dilution of DNA (10^5 to 10^1 copies) produced nearly identical curve plots for both mixes, each with a coefficient of determination of R^2 = 0.99 (Fig. 1). Amplification efficiency was calculated using linear regression analysis, yielding slopes of −3.2053 for the CAV mix and − 3.3442 for the lyophilized mix, corresponding to efficiencies of 105% and 99%, respectively (Fig. 1).

Sensitivity and precision of stabilized reagent

To assess analytical sensitivity (limit of detection, LOD), qPCR reactions were performed using the CAV-stabilized and lyophilized PCR mixes with 10, 5, and 1 copies of viral DNA. Sensitivity was confirmed for both mixes in an independent set of 11 PCR reactions. All reactions detected 10 copies of DNA in both formulations. For the 5-copy template, all 11 reactions were determined with the CAV-stabilized mix, while 9 out of 11 were positive with the lyophilized mix. At the 1-copy level, detection was achieved in 6 reactions with the CAV-stabilized mix and in 4 reactions with the lyophilized mix (Table 1).

Precision was assessed for both the CAV-stabilized and lyophilized PCR mixes over three separate days. Consistent results were observed, with acceptable standard deviations and coefficients of variation across inter-day replicates. The CAV-stabilized mix showed a mean ± SD of 21.22 ± 0.24 and a %CV of 1.15, while the lyophilized

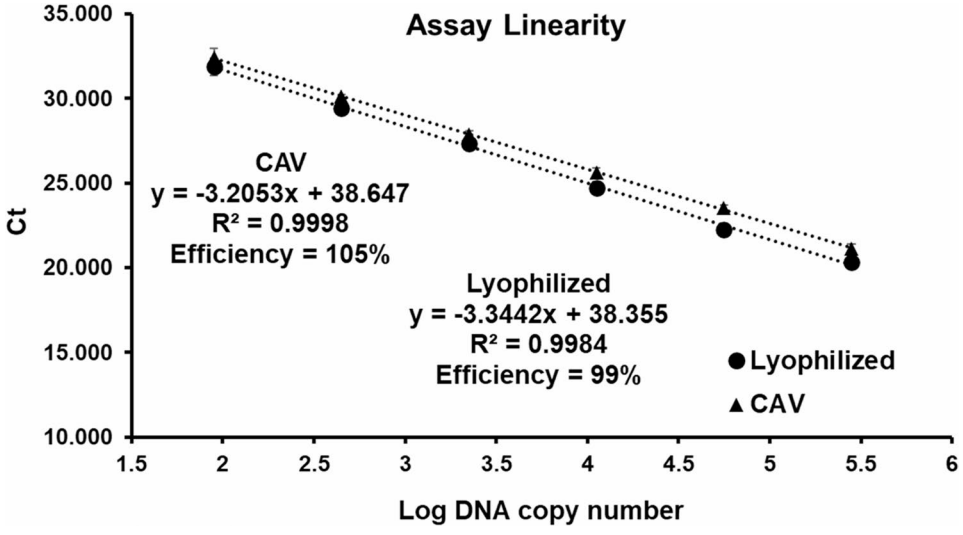


Fig. 1 Performance evaluation of CAV and lyophilized probe qPCR mixes. CAV and lyophilized reagents were tested using a five-fold serial dilution of Monkeypox virus DNA ranging from 2.8×10^5 to 9.0×10^1 copies per reaction. Primers and probe were included in all reactions. Ct values from three independent qPCR assays were averaged and plotted against the logarithm of the DNA copy number. Linear regression analysis was used to calculate the coefficient of determination (R^2) and PCR efficiency. Results are presented as mean ± standard deviation (SD)

Table 1 Sensitivity of CAV and Lyophilized Probe qPCR Mixes. A total of eleven qPCR reactions were performed using CAV and lyophilized mixes with primers, probe, and Monkeypox virus DNA at concentrations of 10, 5, and 1 copy per reaction. Ct values were analyzed to determine the limit of detection (LOD) of each stabilized reagent

Groups	10 copies	5 copies	1 copy
CAV	11/11	11/11	6/11
Lyophilized	11/11	9/11	4/11

mix showed a mean ± SD of 20 ± 0.42 and a %CV of 2.08 (Table 2). The CAV-stabilized samples exhibited an approximate increase of one Ct compared to the lyophilized mix. In addition, both stabilized reagents yielded undetermined Ct values in the no-template control reactions (Fig. 2).

Longterm stability

To evaluate thermal stability, CAV-stabilized and lyophilized PCR mixes were stored at 37 °C for 26 weeks and 50 °C for 4 weeks. At day 0, the Ct values were 20.43 ± 0.38 for the CAV-stabilized mix and 19.88 ± 0.48 for the lyophilized mix (Fig. 3). Consistent with the precision study, the CAV-stabilized samples exhibited an increase in Ct value compared to the lyophilized samples. However, the Ct values then remained stable at 37 °C over time: 21.22 ± 0.25 (1 week), 21.53 ± 0.33 (2 weeks), 21.55 ± 0.29 (4 weeks), 21.36 ± 0.69 (9 weeks), 21.19 ± 0.45 (13 weeks), and 21.42 ± 0.33 (26 weeks) (Fig. 4). Similarly,

Table 2 Reproducibility of CAV and Lyophilized Probe qPCR Mixes. Triplicate reactions of CAV and lyophilized mixes were run on three independent days using 2.8×10^5 copies of Monkeypox virus DNA along with primers and probe. Ct values from all replicates were averaged, and the standard deviation (SD) and coefficient of variation (%CV) were calculated using Microsoft Excel

Groups	Ct value Day 1	Ct value Day 2	Ct value Day 3	Average	Standard deviations (SD)	Coefficients of variation (%CV)
CAV	21.47	21.34	21.26	21.22	0.24	1.15
	21.26	21.40	20.97			
	21.22	21.35	20.69			
Lyophilized	20.32	19.77	20.02	20.00	0.42	2.08
	19.34	20.40	20.30			
	19.96	20.45	19.42			

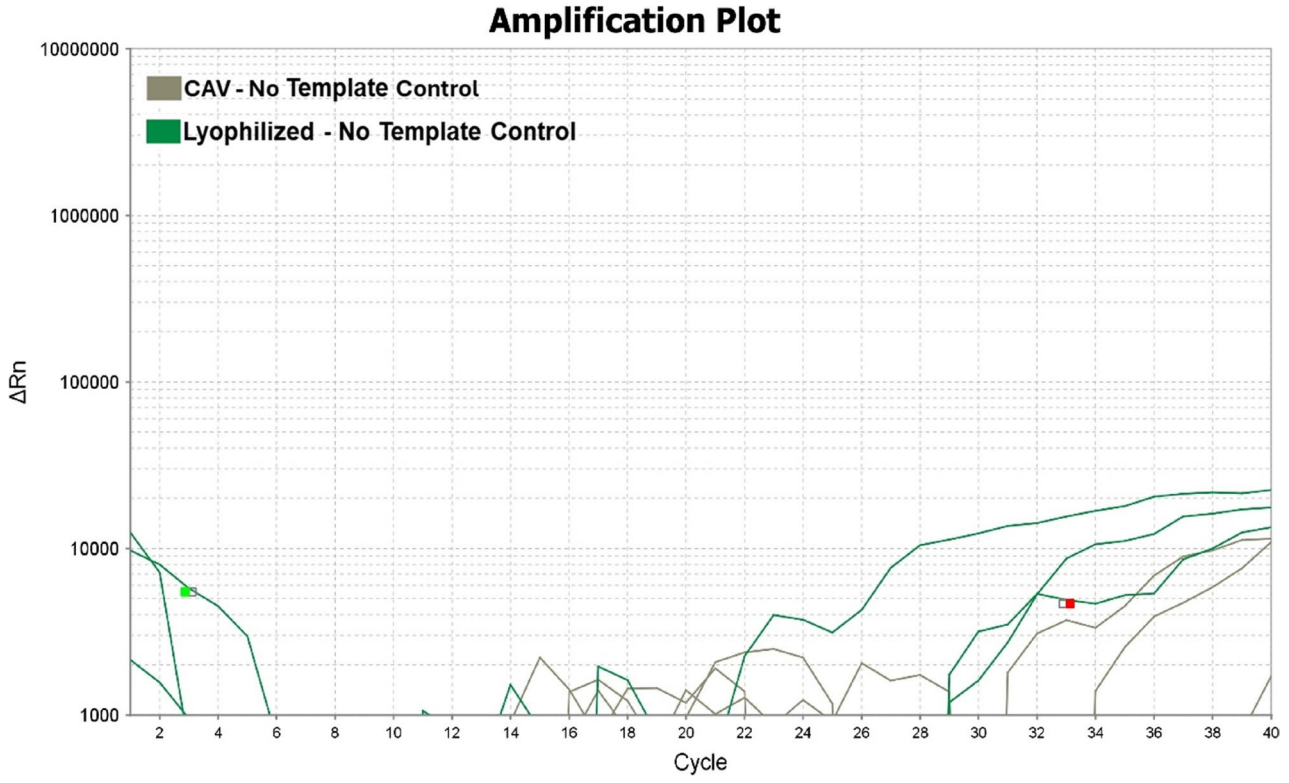


Fig. 2 Amplification curves of CAV and lyophilized mixes in no-template control reactions. Triplicate reactions containing CAV or lyophilized mixes with primers and probe, but without DNA template, were run to evaluate non-specific amplification

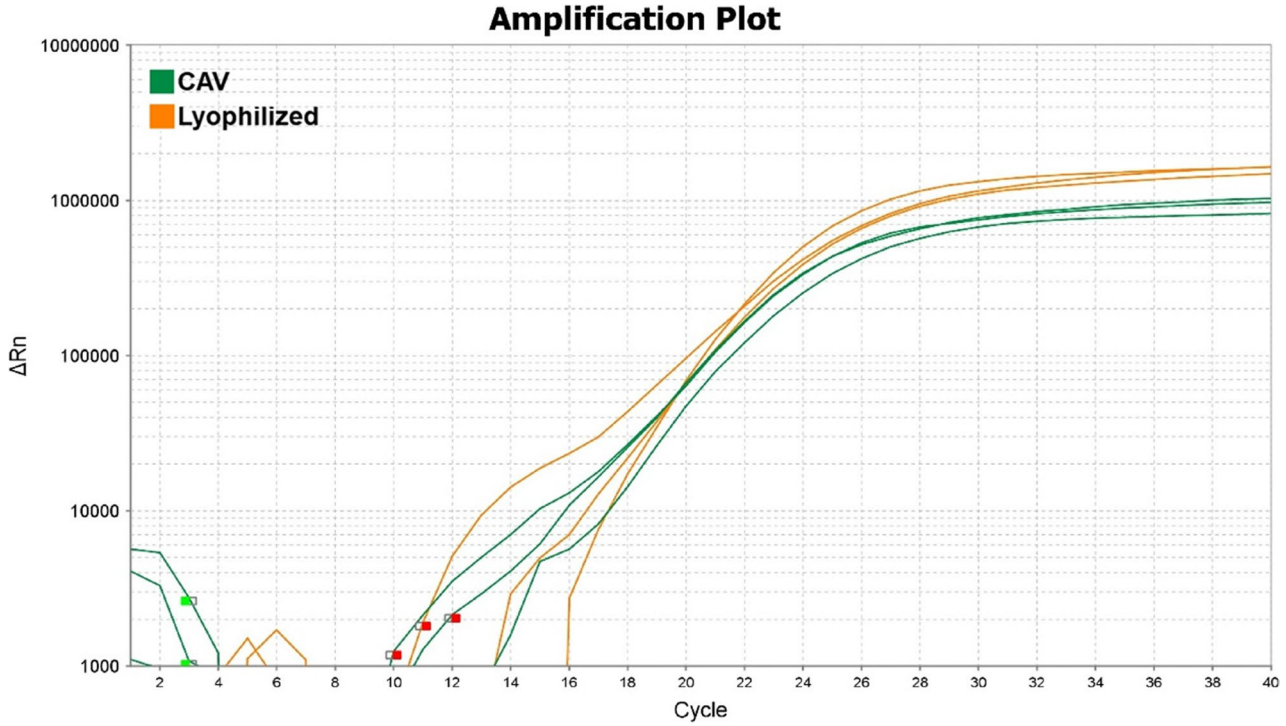


Fig. 3 Amplification performance of post-processed CAV and lyophilized qPCR mixes. Three independent samples of each mix were tested by qPCR using 2.8×10^5 copies of Monkeypox virus DNA, primers, and probe. Amplification curves demonstrate the performance of the stabilized reagents following processing

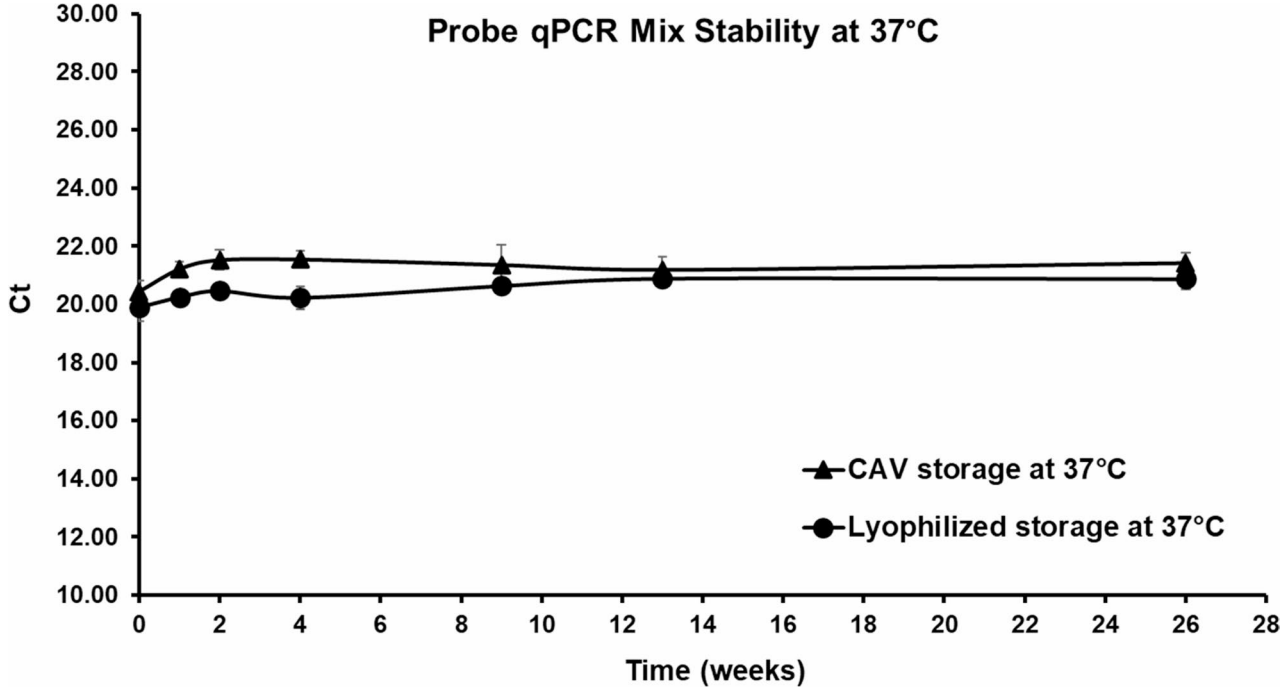


Fig. 4 Stability of CAV and lyophilized qPCR mixes stored at 37 °C. Triplicate samples were stored at 37 °C and tested on day 0 and at weeks 1, 2, 4, 9, 13, and 26. On each test day, samples were eluted and combined with 2.8×10^5 copies of Monkeypox virus DNA, primers, and probe for qPCR analysis. Ct values were plotted, and results are reported as mean ± standard deviation (SD)

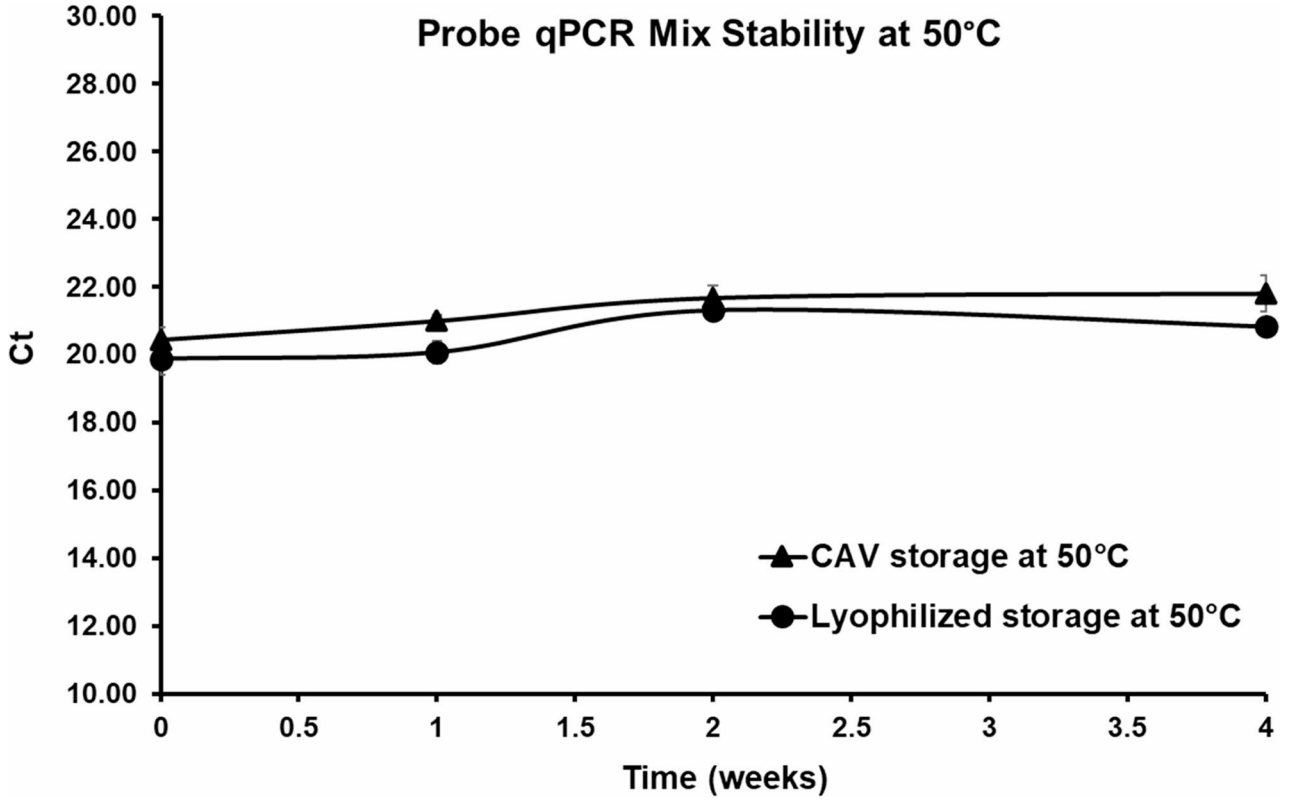


Fig. 5 Stability of CAV and lyophilized qPCR mixes stored at 55 °C. Triplicate samples were stored at 55 °C and tested on day 0 and at weeks 1, 2, and 4. On each testing day, samples were eluted and combined with 2.8×10^5 copies of Monkeypox virus DNA, primers, and probe for qPCR analysis. Ct values are shown as mean ± standard deviation (SD)

at 50 °C, the CAV-stabilized mix maintained consistent Ct values: 20.99 ± 0.19 (1 week), 21.68 ± 0.35 (2 weeks), and 21.81 ± 0.54 (4 weeks) (Fig. 5).

The lyophilized samples showed minimal Ct variation compared to day 0 values after storage at 37 °C for 1 week (20.24 ± 0.19), 2 weeks (20.46 ± 0.27), 4 weeks (20.22 ± 0.40), 9 weeks (20.63 ± 0.26), 13 weeks (20.89 ± 0.14), and 26 weeks (20.87 ± 0.37) (Fig. 4). At 50 °C, they also retained stable Ct values: 20.08 ± 0.34 (1 week), 21.31 ± 0.21 (2 weeks), and 20.83 ± 0.20 (4 weeks) (Fig. 5).

Discussion

The PCR mix plays a vital role in real-time PCR by enabling accurate, rapid, and sensitive quantification of DNA and RNA. Its precise formulation is critical to the effectiveness of numerous molecular biology applications, ranging from clinical diagnostics to environmental analysis, making it an essential tool in contemporary life sciences (Yang & Rothman 2004). The emergence of new infectious diseases and the need for reliable diagnostics and environmental monitoring in both urban and rural settings underscore the importance of developing PCR assay reagents that are temperature-stable, enabling storage and use at ambient temperatures without compromising sensitivity (Xu et al. 2020). While lyophilization improves the thermostability and shelf life of PCR reagents, it remains a time-consuming, complex, and expensive process (Patel & Pikal 2011). Thus, we developed the CAV stabilization method, designed to produce cost-effective, thermostable PCR reagents that support ambient storage and transport with a faster turnaround time. This study compares the assay performance and long-term thermal stability of a commercially available lyophilized PCR mix with a liquid PCR mix stabilized using the CAV process. Given the recent outbreak of the Mpox virus and the global focus on developing PCR-based diagnostics, we selected Mpox virus DNA as the template to assess the performance of the stabilized reagent.

This study utilized a rapid 30-minute desiccation step in the CAV process to stabilize the PCR mix. The physicochemical properties of CAV-stabilized samples have been previously characterized (Shank-Retzlaff et al. 2022). In the CAV process, the qPCR mix is assumed to contain glycerol, while the lyophilized reagent is glycerol-free, as glycerol is generally incompatible with lyophilization (Nagaraj et al. 2018; Xu et al. 2020; Bodzen et al. 2021). Consequently, there is a slight compositional difference between the lyophilized and CAV-stabilized

samples. Although the CAV formulations contain glycerol and exhibit a slightly higher residual moisture content than the typical lyophilized product, stability studies demonstrate that the CAV-stabilized materials remain stable over extended storage periods.

We observed that both the CAV-stabilized and lyophilized PCR mixes demonstrated comparable and acceptable performance, with coefficients of determination (R^2 = 0.99) and amplification efficiencies of 105% and 99%, respectively. These results indicate that the stabilized reagents are capable of accurately quantifying the target template. PCR efficiencies within the accepted range of 90–110% reflect how effectively each cycle doubles the DNA product, serving as a key measure of assay reliability and performance (Mukherjee et al. 2023; Smith et al. 2021). An R^2 value of ≥ 0.98 is generally considered acceptable, as it demonstrates strong linearity and a good fit of the experimental data to the regression curve (Mukherjee et al. 2023; Smith et al. 2021). Additionally, no Ct values were observed in the no-template controls for either reagent formulation, confirming that the stabilization process did not introduce contamination (Hays et al. 2022).

The LOD was determined to be 5 copies of DNA per reaction for the CAV-stabilized sample and 10 copies for the lyophilized sample, based on the lowest template concentration consistently detected in ≥ 95% of replicates (Smith et al. 2021; Arnaout et al. 2021). Although the CAV reagent detected lower DNA copy numbers than the lyophilized formulation, additional studies with a larger sample size are needed to better understand and confirm this observation.

The lower SD and CV observed in both the formulations, suggested that the stabilized reagent has minimal variability when tested in inter days assay. Notably, though small sample size, the precision study confirms that the CAV samples variability is slightly lower than the lyophilized product. The PCR method validation studies were accepted as the variability of inter-assay precision of ≤ 10% (Zheng et al. 2019; Karlen et al. 2007).

To evaluate thermal stability, stabilized samples were stored at accelerated temperatures of 37 °C and 50 °C. This approach helps predict long-term shelf life under ambient conditions and assesses the reagent resilience to environmental fluctuations during transport and storage (Xu et al. 2020). Both the CAV-stabilized and lyophilized formulations demonstrated stability under elevated temperatures, as shown by consistent amplification of the target DNA and Ct values similar to those recorded at the baseline. Precision and stability analyses showed that CAV-processed samples exhibited a modest increase of approximately one Ct value after stabilization, indicating a minor initial change of reagent performance. However, no further decline was observed with extended storage, confirming sustained stability. Furthermore, assay linearity and sensitivity data demonstrated that the slight Ct shift observed in CAV samples did not affect DNA quantification accuracy.

As a proof of concept in this study, a single-assay PCR reagent was stabilized within the scaffold, and before testing, the sample was placed into column tubes, and the eluted material was transferred into PCR tubes for use in the assay. For commercialization, we engineered form factors that allow direct vitrification of reagents onto a 96-well plate format. During testing, the vitrified reagents were eluted directly from the plate into PCR tubes, enabling a robust, single-use workflow comparable to PCR strip–based lyophilized products.

In conclusion, the CAV process successfully stabilized a glycerol-containing PCR mix- capable of amplifying Mpox virus DNA. Unlike traditional lyophilization, the CAV method transitions the reagent directly from a liquid to a dry state, eliminating the need for a pre-freezing step. The process requires only 30 min of desiccation, and the resulting product demonstrated performance and long-term thermal stability comparable to lyophilized products. Additionally, CAV supports faster cycle times for high-throughput manufacturing, and reduces capital investment. These results indicate that the CAV process offers a practical and efficient alternative to lyophilization for stabilizing PCR reagents.

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Authors' contributions
SR, MC, MSR, LB, and PM conceptualized and designed the study. SR drafted the original manuscript and performed subsequent revisions. YPT, SR, and JS carried out the experiments and analyzed the data. All authors reviewed and approved the final manuscript for publication.

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Declarations

Competing interests
Ambient Biosciences, Inc. holds the license for the capillary-assisted vitrification (CAV) patent and plans to commercialize the technology. All authors, with the exception of Pravansu Mohanty, are employees of Ambient Biosciences, Inc. Pravansu Mohanty holds a financial interest in the company.

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