Freeze-Dried, Worry Thawed: Armored RNA new Lyophilized Controls Offer Potential to Advance Molecular Testing in Resource-Limited Settings

ID046

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Summary

- Lyophilizing molecular control material offers benefits of enhanced stability, simplified transportation and storage, reduced contamination risk, and cost-effectiveness
- Armored RNA Quant is able to be successfully lyophilized with acceptable consistent pellet stability based on accelerated and real time studies.
- Ideal conditions for excipient and vial use have been identified.
- Even after reconstitution, samples are stable at -20C demonstrating the ability to further extend shelf life.

Introduction

Lyophilization is a process where frozen solvents are removed from material through sublimation under heat and high vacuum and then dried. Lyophilizing molecular control material offers many benefits, particularly for diagnostic testing, including enhanced stability, extended shelf-life, simplified transportation and storage, and reduced contamination risk. Collectively this enhances the accessibility and efficiency of molecular diagnostics, particularly in laboratory settings with limited resources and infrastructure.

Armored RNA Quant (ARQ) controls are synthetic nucleic acids encapsulated to create virus-like particles useful as customized full-process QC material. To date, these controls are offered as liquid-stable material with extended shelf life at -20ºC.

Here we tested feasibility and optimization of lyophilizing ARQ samples using multiple vial types and excipient conditions at various concentrations.

Materials and Methods

ARQ Internal Process Control (IPC) was manufactured by encapsulating a 1000nt sequence within a MS2 bacteriophage capsid protein coat. It was formulated to 1E+08 to 1E+06 copies/mL in TSMIII and split into multiple vials. Some were spiked with 3 different excipients (.04g/mL BSA + 6% sucrose, .04g/mL HSA + 6%sucrose, and 250mM trehalose) in 8 vial combinations (borosilicate, polypropylene, amber, and clear glass) while others were frozen to serve as controls.

The lyophilization process was carried out using either an Edwards (custom model) or Hull Lyophilizer (Model Number: 132 FXS 200) following the basic workflow shown in Figure 1.

After lyophilization, 10 tubes of resulting dry material (cake) was assessed for moisture retention using gravimetric methods at intervals after drying the cake at 105ºC. To assess the impact of loading times on the lyophilizers, samples were staged on the machine to be run immediately, and up to 3.5 hours on deck.

Stability testing was performed as a surrogate measure of success for feasibility. Vials were placed at 37ºC for accelerated stability and 25ºC or 4ºC for defined times for real time stability. At each timepoint and for each experiment, samples were reconstituted in 500µL water and heat lysed at 75ºC for 3 minutes. 2-step RT-qPCR was performed using ThermoFisher High Capacity cDNA Synthesis kit (P/N 4374966) and custom TaqMan primers and probes designed against the IPC target. qPCR was carried out using 2X Fast Universal Master Mix (P/N 4366072) on a 7500 Fast Instrument run to 40 cycles. Digital PCR was performed using the Absolute Q 1-step RT-dPCR Master Mix (P/N A55146) and tested on the QuantStudio Absolute Q System using the same primers and probes.

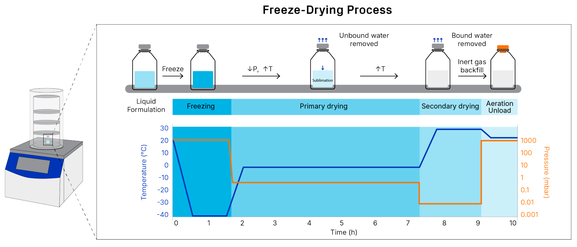

Figure 1. Typical workflow of Lyophilization. Lyophilization involves freezing and drying under high vacuum to remove liquid from the source material.

Results

Table 1. Lyophilization was successful. Two representative lots show that each cake after drying had <3% moisture retained that fell to <0.25% moisture retained at the next interval.

Dry Time	Lot D 1.5E+07 cp/mL		Lot E 1.7E+07 cp/mL	
	Weight (g)	D %Moisture	Weight (g)	D %Moisture
0 hr	1.323 ± 0.006		1.374 ± 0.015	
5 hr	1.318 ± 0.006	0.36%	1.369 ± 0.015	0.37%
6 hr	1.318 ± 0.006	-0.02%	1.370 ± 0.015	-0.01%

Table 2. Loading Time does not impact product lyophilization. Lot C at 1.1E+07 copies/mL was loaded onto lyophilizer at different times and assayed via dPCR to determine % recovery. Initial recovery post lyophilization was 91.9%.

Loading Timepoint	Ave Assayed Conc c/uL	%CV (N=2)	%Recovery vs Time 0 Hours
0.0 Hours	9194	6.72%	
0.5 Hours	8558	0.68%	93%
1.0 Hours	9099	7.72%	99%
2.5 Hours	8713	1.56%	95%
3.5 Hours	8929	2.35%	97%

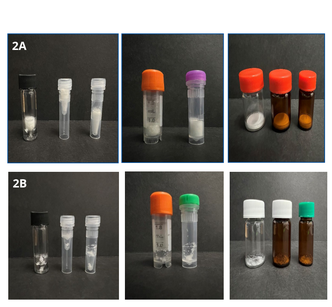

Figure 2. Assessment of 'cake' formation. The uniformity and compactness of the lyo cake is essential to optimal product performance. Based on a matrix of testing performed with multiple excipients and vial types, it was determined that using 250mM trehalose (2A) as opposed to sucrose alone (2B) was preferred. Additionally, all vial types exhibited similar cake formation, so 2mL polypropylene tubes were chosen for ease of use in general laboratory settings.

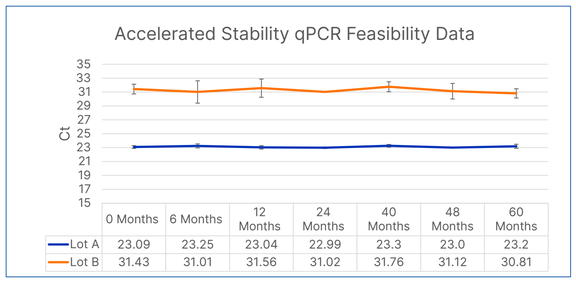

Figure 3. Accelerated Stability of two formulations of ARQ. Lots A and B were formulated to 1.0E+08 cp/mL and 1.0E+06 cp/mL. Seven vials underwent accelerated testing at 37ºC to mirror storage at 4ºC. All data on RT-qPCR show no appreciable decrease in detection out to 60 months, suggesting pellet stability at 4ºC.

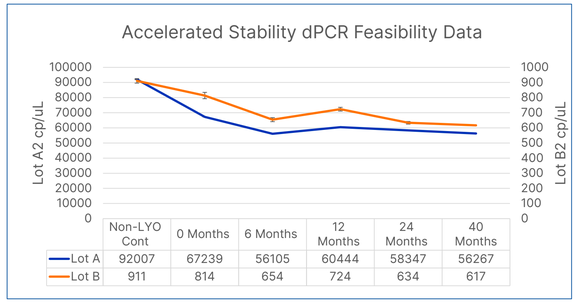

Figure 4. Preliminary testing on digital PCR of Lots A and B. Lots A2 and B2 were tested using RT-dPCR after having been subjected to accelerated stability out to 40 months. Non lyophilized controls showed that formulations were within 92.0% for Lot A and 91.1% for Lot B of expected target concentrations. The lyophilization process, however, showed a loss of material with a drop of 27% for Lot A and to a lesser degree of 11.7% for Lot B. Both lots continue to decrease before leveling off after the 6 month timepoint.

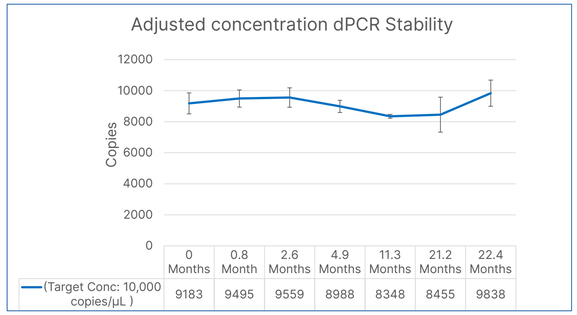

Figure 5. Adjusting concentration of target improves recovery. To compensate for the loss due to lyophilization, material was formulated 50% higher, then run through the lyo process and tested on digital RT-PCR. This time the material showed 91.8% recovery after lyophilization and remained stable and within 80% of expected target concentration through the timepoints tested.

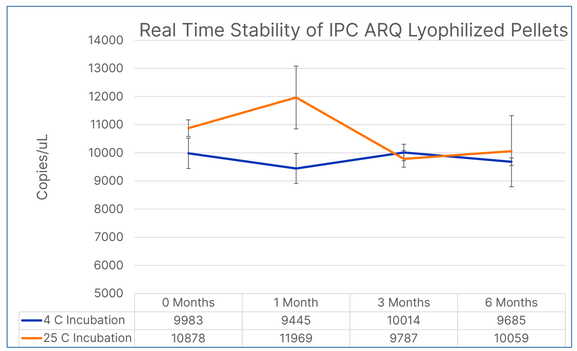

Figure 6. Real Time Stability of Lyophilized Pellets shows promise. Lot E aliquots have been set aside for ongoing real time stability testing. Thus far at 6 months, pellets stored both at 4ºC and 25ºC show detection within 20% of expected target concentration (1.0E+04 cp/uL).

Table 3. Reconstituted pellet stability. In order to assess stability of reconstituted material, the samples that had been reconstituted from Lot E pellet accelerated stability study were kept at -20ºC for 34 weeks. Samples were pulled, thawed, and re-tested on RT-dPCR.

Lot E Accelerated Timepoints	Original Results (cp/uL)	Reconstituted pellets tested at 34 Weeks	%Concentration D (cp/uL)	%CV for Re-assayed Samples
0 Months	9495	9187	3%	1.25%
2.6 Months	9559	9707	2%	5.03%
4.9 Months	8988	9401	5%	0.80%
11.3 Months	8348	9123	9%	4.41%
22.4 Months	9839	8626	12%	2.54%

Conclusions

- We have demonstrated feasibility, recovery, and stability of lyophilizing Armored nucleic acids using qPCR and dPCR.
- Optimal excipient and fill volume have been determined and data show the material is stable lyophilized and reconstituted.
- The ability to lyophilize molecular controls is crucial for accessibility to diagnostics and to improve disease detection, management, and public health outcomes in resource-limited settings.
- Armored RNA once lyophilized can be stable at accelerated temperatures for the equivalent of 25.8 months of storage at 4°C and stable under real time conditions for at least 6 months at both 4 and 25°C.
- Reconstituted pellets stored at -20°C were shown to exhibit similar stability to that of the standard frozen Armored RNA Quant.

Scan code to learn more

1. Tucak-Smajić, A. (2023) BioRender.
2. Boehme, C., Hannay, E. & Pai, M. Promoting diagnostics as a global good. *Nat Med* **27**, 367–368 (2021). https://doi.org/10.1038/s41591-020-01215-3
3. United States Pharmacopeia (2024). *General Chapter, ⟨921⟩ Water Determination.* USP-NF. Rockville, MD: United States Pharmacopeia

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