Improving the Stability and Performance of Bioassay Reagents using Capillary Mediated Vitrification

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ABSTRACT

The performance of a bioassay is dependent on the storage, handling, and stability of the reagents. Novel technologies that can improve both the stability and consistency of critical reagents are needed to enhance method performance and robustness. We have developed a novel bio-preservation method called capillary-mediated vitrification (CMV). The CMV process can be performed at the bench in under an hour, does not require reagent specific optimization, eliminates the need for freezing, and presents minimal risk of matrix interference. In this report, we used the CMV process to prepare a variety of reagents in a pre-diluted, single-use format including: antibodies and antibody conjugates, luciferin, luciferase, and mRNA. Linearity and precision data generated with an alkaline-phosphatase conjugate indicates that CMV is a promising alternative to traditional storage practices and could significantly improve analytical workflows. Stability data collected at temperatures ranging from 25-55°C demonstrates that our CMV technology could reduce the need for cold storage for biological reagents.

BACKGROUND

- ✓ Capillary-mediated vitrification (CMV) is a novel method that leverages the naturally-occurring process of capillary evaporation to rapidly remove moisture from an aqueous matrix without freezing or boiling, transitioning biological reagents into a stable, glassy state.
- ✓ The pores within the scaffold act as capillaries, increasing the surface area and surface tension. The increase in surface tension prevents boiling, allowing the material to be dried under vacuum without a freezing step (1-4).
- ✓ The quantity of material processed can be selected to match the assay requirements and the final, dried product typically exhibits enhanced thermal stability and performance, allowing the material to be stored under ambient conditions.

Current storage and distribution issues reduced by the CMV process

Waste
- ✗ Concentrated formats
- ✗ Dilution waste
- ✗ Limited shelf life

Efficiency
- ✗ Time intensive
- ✗ Risk of error
- ✗ Reagent defrosting
- ✗ Documentation

Storage
- ✗ Expensive
- ✗ Unsustainable
- ✗ Deviation risk
- ✗ Material loss risk

METHODS

Preparation and usage of CMV samples

Step 1 Solution of BioFix™ buffer and reagent is mixed

Step 2 Mixture is dispensed

Step 3 Inserts are stabilized using a chamber

Step 4 Distribution and storage at ambient

Step 5 Reagent is eluted from scaffold

Step 6 Reagents are used in assays

RESULTS

Thermal Stability of CMV-Stabilized Antibody-Conjugates

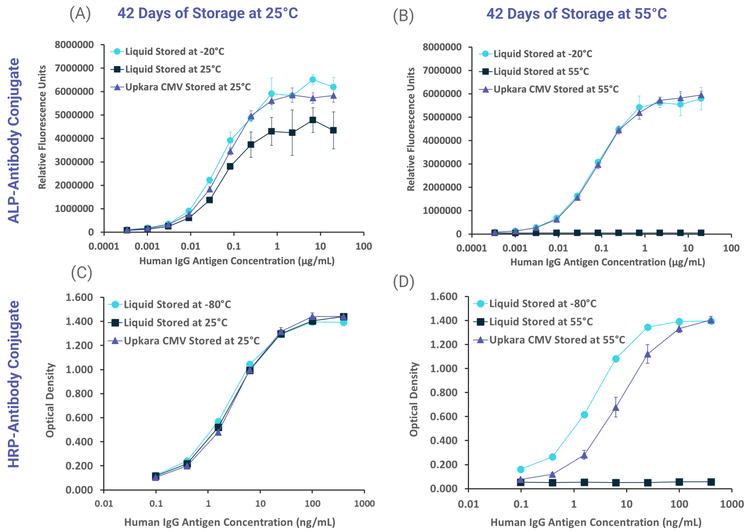

Figure 1. CMV preserved enzyme-antibody conjugates. Binding curve of stabilized and control ALP (A, B) and HRP (C, D) antibody conjugates was evaluated via ELISA. Activity was measured following 42-days of storage at 25°C and 55°C. Reagents performed similarly to frozen controls. At 55°C a small shift in ED50 was seen for the HRP conjugate, however a full dose response was obtained (n=3 ± SD).

Assay Performance of CMV-Stabilized ALP Antibody-Conjugates

(A)

Parameter	Antigen Low Dose (6.7 µg/mL)		Antigen High Dose (60 µg/mL)	
	Liquid stored at -20°C	**Upkara CMV stored at 25°C**	Liquid stored at -20°C	**Upkara CMV stored at 25°C**
Number of runs	36	36	36	36
Overall Average (µg/mL)	5.8	5.5	61.7	61.9
CV (%)	23	16	26	16
Bias (%)	-5.5		0.3%	
Significance (p-value, 0.05)	0.24		0.95	

(B)

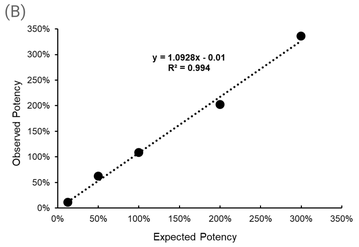

Table 1 and Figure 2. CMV preserved ALP-antibody conjugate. (A) Relative potency results obtained by testing IgG samples diluted to 6.7 or 60 µg/mL in 6 assays each using the frozen control and the Upkara CMV-stabilized ALP conjugate. The six runs were divided between two analysts and each analyst executed the testing on three separate occasions. (B) Observed potency for IgG samples formulated at concentrations ranging from 2.5-60 µg/mL. The CMV processed samples observed good linearity and a comparable/lesser assay variability than the frozen control samples.

Thermal Stability of CMV-Stabilized Luciferin

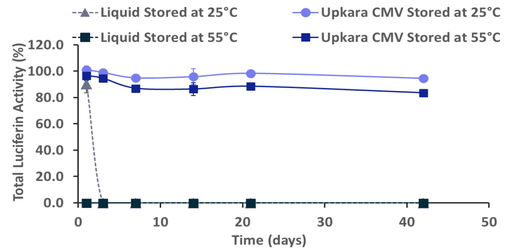

Figure 3. CMV preserved luciferin. Total luciferin activity following 42 days of storage at 25°C and 55°C. Percentage of total activity relative to the frozen controls was measured via microplate reader (n=3 ± SD).

Thermal Stability of CMV-Stabilized Luciferase

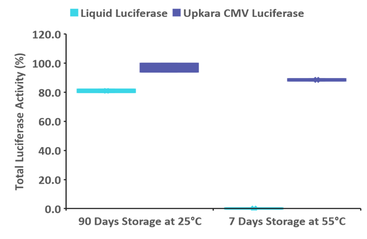

Figure 4. CMV preserved luciferase. Total luciferase activity following 90 days of storage at 25°C and 7 days of storage at 55°C was measured via microplate reader (n=3 ± SD).

Thermal Stability of CMV-Stabilized GFP-mRNA

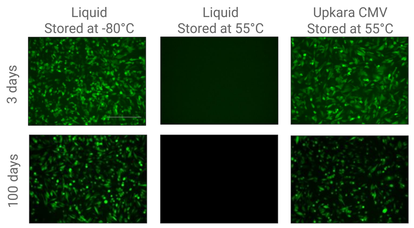

Figure 5. CMV preserved green fluorescent protein encoding mRNA (GFP-mRNA). CHO-K1 cells were transfected using CMV preserved and liquid control GFP-mRNA following storage at -80°C or 55°C (n=3). The CMV sample had comparable transfection efficiency of frozen controls.

CONCLUSIONS

- ✓ Capillary-mediated vitrification (CMV) is an innovative and simple process for preserving different types of biomolecules.
- ✓ Using the CMV process, we have efficiently preserved and improved the performances of antibody conjugates, a small molecule, an enzyme, and mRNA.
- ✓ The CMV process is expected to be easily scalable, fit into current workflows, adaptable to wide range of biomolecules and may enable storage, distribution, and deployment of reagents, clinical samples, and therapeutics which require cold chain.

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