Capillary-Mediated Vitrification: A Novel Approach for Preparing Thermostable, Ready-to-Use Reference Standards

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ABSTRACT

Biochemical assays require a stable reference standard to produce reliable results. Currently, biological reagents require temperature-controlled storage and distribution, which is both energy and resource intensive and may result in unnecessary material discard. We have developed a bio-preservation method, capillary-mediated vitrification (CMV), that enables storage of reagents at ambient temperatures, without freezing, at concentrations designed to match the assay requirements. In this report, we demonstrate the stability and performance of both a CMV-stabilized IgG used as an ELISA standard and CMV-stabilized cytokine calibrant. The data indicates that the CMV-stabilization process may be a valuable alternative to traditional storage methods.

BACKGROUND

The CMV process leverages ”capillary evaporation” to enable rapid vitrification of formulated solutions. Briefly, biomolecules of interest are mixed with our proprietary Biofix buffer, applied to a porous support, and dried under vacuum. The quantity of material processed can be selected to match the assay requirements and the final, dried product typically exhibits enhanced thermal stability, thus allowing the material to be stored under ambient conditions.

	Frozen Storage	Lyophilization	CMV Stabilization
Aliquot Size	Multi-use vials*	Multi-use vials*	Single-use quantity
Storage Condition	Frozen (-80°C or -20°C)	Frozen or refrigerated	Ambient
Complexity	Complex day-of-use reagent prep procedures	Reagent prep requires specialized equipment/expertise Complex day-of-use prep procedures	Easy to prepare at bench Single step day-of-use prep procedure

*Associated with significant material waste and increased risk of contamination

MATERIALS AND METHODS

CMV samples were prepared as described previously[1-3]. The IgG and TNF-α ELISA kit were obtained from Rockland Immunochemicals and Abcam, respectively. A total of 40 µg of IgG or 400 pg of TNF-α were stabilized per scaffold. The IgG ELISA was performed by coating a 96-well plate with a mouse anti-Human IgG (Jackson Immunochemical) antibody and incubating O/N at 4 °C. The frozen and CMV-stabilized IgG were diluted from 20 µg/mL to 0.3 ng/mL and applied to the plate. The rabbit anti-Human IgG Alkaline Phosphatase (Jackson Immunochemical) conjugate used for detection was diluted to 30 ng/mL and signal generated using 4-MUP substrate (Virolabs). For the precision study, 6 independent preparations of vitrified reagent or 6 independent dilution preparations from a frozen control were analyzed per plate. A total of 3 plates were run per condition (frozen or vitrified) with each plate being analyzed on a separate day. Data were analyzed using a 4-parameter logistic function and parallel-line analysis. The cytokine ELISA assay was run per the vendors instructions.

ANTIBODY CALIBRANT RESULTS

Performance of CMV-Stabilized IgG Calibrant

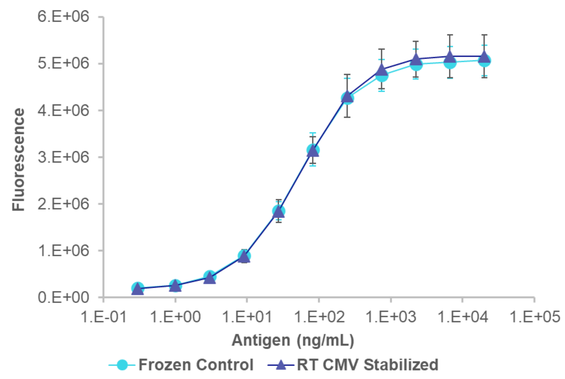


Figure 1: Comparison of ELISA curves generated using freshly thawed or CMV-Stabilized IgG antibody standards. CMV calibrants performed similarly to frozen controls with a mean relative potency of 1.02 ± 0.17 (n=6 ± SD).

Stability of CMV-Stabilized IgG Calibrant

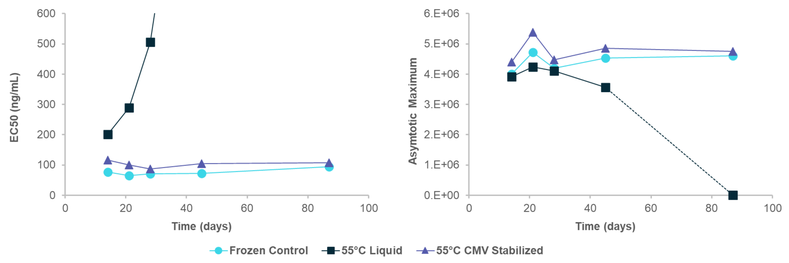


Figure 2. Change in EC_{50} and asymptotic maximum (maximum signal) over time for IgG stored frozen, as a liquid at 55 °C, or CMV-stabilized at 55 °C over time. CMV reagents performed similarly to frozen controls and were more stable than stressed controls. Liquid stored at 55 °C dried out after 45 days.

Preparation Consistency of CMV-Stabilized IgG Calibrant

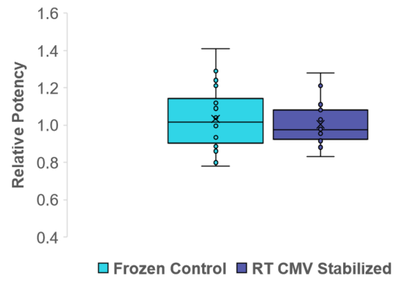


Figure 3. Comparison of precision obtained using multiple preparations from a frozen IgG stock or from CMV-stabilized scaffolds. Relative potency was calculated from repeated preparation of standard within a plate on multiple days, following freezing of liquid or CMV storage at room temperature. CMV calibrants had lower variability than frozen control. Mean relative potency frozen 1.03 ± 0.17; CMV stabilized 1.00 ± 0.11 (n=18 ± SD).

CYTOKINE CALIBRANT RESULTS

Performance and Stability of CMV-Stabilized TNF-α Calibrant

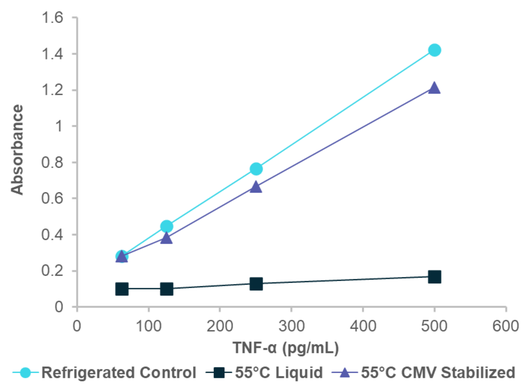


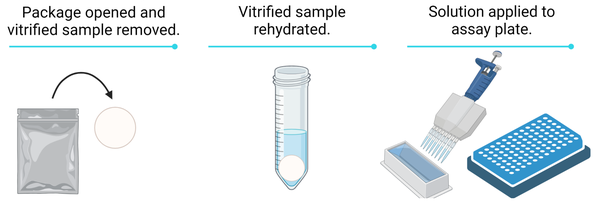
Figure 4: Comparison of ELISA signal response for TNF-α stored refrigerated, as a liquid at 55°C, or CMV stabilized at 55 °C for 7 days. A minor decrease in signal (20%) was observed immediately after vitrification (data not shown) but no change was observed following storage at 55 °C.

CONCLUSIONS

CMV-Stabilized IgG performs as well as, or better than, traditionally stored frozen IgG. CMV reagents maintain activity with extended storage at elevated temperatures, while liquid reagents quickly lose potency. CMV stabilization allows the analyst to follow simple steps for use, empowering greater consistency between repeated preparations.

Capillary-mediated vitrification is a novel, simple and easily-optimized process that enables ambient shipping and storage of a variety of biomolecule standards. Application of this technology can reduce operational costs, expand market access, and improve an organization’s environmental sustainability practices.

Upkara CMV Day-of-Use



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4. Mohanty P, Chakraborty N. Capillary Assisted Vitrification Processes and Devices. United States Patent and Trademark Office; 2020. U.S. Patent U.S. 20200068875March 5.
5. Figures created with BioRender.com

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