Osmolality As A Key Metric Within Cryopreservation

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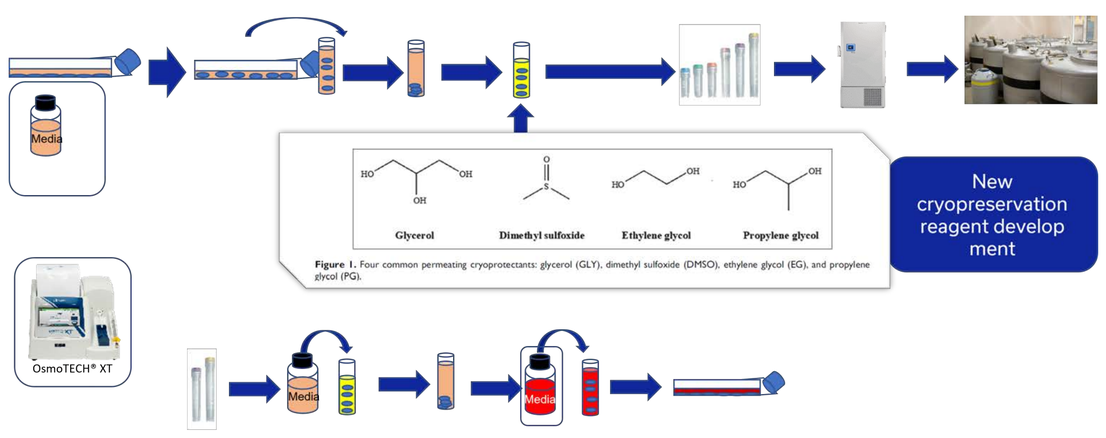
Introduction

Cryopreservation uses unique reagents and very low temperatures to preserve structurally intact living cells, spheroids and tissues for use in multiple bioprocessing applications such as cell banking and long-term storage of cell lines. The technique originates back to the late 1800s and has since kept many of the practical theories, however, there has been significant progression and numerous advancements made within cryopreservation protocol optimization. One important process improvement tool is the use of osmolality, a measurement of moles of solute per kilogram of water - a test that is therefore unaffected by the type of molecule, the degree of ionization, or the pH. This unique feature allows for the use of osmolality testing within the cryopreservation workflow where challenging reagents are utilized, an example being Dimethyl Sulfoxide (DMSO). The following poster will examine the use of osmolality, using Advanced Instrument's freezing point depression technology, in the cryopreservation workflow.

Testing of Osmolality Ensures Optimal Conditions For The Freezing and Thawing Workflow

Every part of this workflow that requires media, ideally needs osmolality testing. This quick and efficient analytical tool can be used as a quality control check to ensure the solution is correct and within specification prior to its addition. This therefore can reduce the potential for errors and prevent any disruptions to the freeze/thaw cycle. Osmolality can also be used as a CQA within new cryopreservation reagent development. These reagents require specific concentrations to prove optimal for the process, with osmolality testing providing capability and ease to do this, ensuring cell health and reducing any potential cell injury or death.



Whaley, D., et al., Cryopreservation: An Overview of Principles and Cell-Specific Considerations 2021, Cell Transplantation (30) pp1-12

Cryopreservative Formulation

Sample	Instrument	N	Mean (mOsm)	StDev	% CoefVar	Minimum (mOsm)	Maximum (mOsm)
Cryostor CS5	XT	180	1416.7	13.9	1.0	1393	1464
Cryostor CS10	XT	180	2749.7	26.6	1.0	2675	2818
PrimeXV FreezIS	XT	180	2111.3	17.9	0.9	2015	2163

Osmolalities of common cryopreservatives in cell and gene therapy. Summarized data are shown for osmolality testing (n=180) on various cryopreservative solutions with a freezing point depression osmometer. Despite high concentrations, osmolality testing showed sound reliability and repeatability.

Can a Freezing Point Depression Osmometer test samples with anti-freezing agents?

The answer, is yes. An in-house study was recently completed to test various commercially available cryopreservatives for osmolality using freezing point depression technology. The figure shows the successful measurement of osmolalities of several cryopreservatives, as determined by a freezing point osmometer – the OsmoTECH® XT.

Improving iPSC Recovery

During the thawing process of iPSCs, there can be a sudden change in the extracellular osmolality. This rapid change in conditions can induce osmotic shock within the cells, which can then, in turn, reduce cell viability after thawing. The scientists at the Centre for Regenerative Medicine at the Clínica Alemana-Universidad del Desarrollo saw a 15% increase in cell survival when using the optimized process detailed below:

Not Optimized Process	Optimized Process	Critical Steps
The medium is poured into a 15mL conical tube, then all thawed cells form the cryovial are added suddenly and all at once to the medium.	Put the thawed cells into a 15mL conical tube first, and the medium is added slowly, in a dropwise manner	A sudden change in the osmolality of the freezing solution around the cells may cause a rapid stream of water across the membranes. This may stress the cells, leading to potential cell death. Avoiding this stress can lead to better survival.

*Improving Cell Recovery: Freezing and Thawing Optimization of Induced Pluripotent Stem Cells. Markus Uhrig *, Fernando Ezquer and Marcelo Ezquer* * European Bank for induced pluripotent stem cells (EBiSC)

Using Osmolality to Calculate Osmotic Tolerance Limits - Literature Evidence

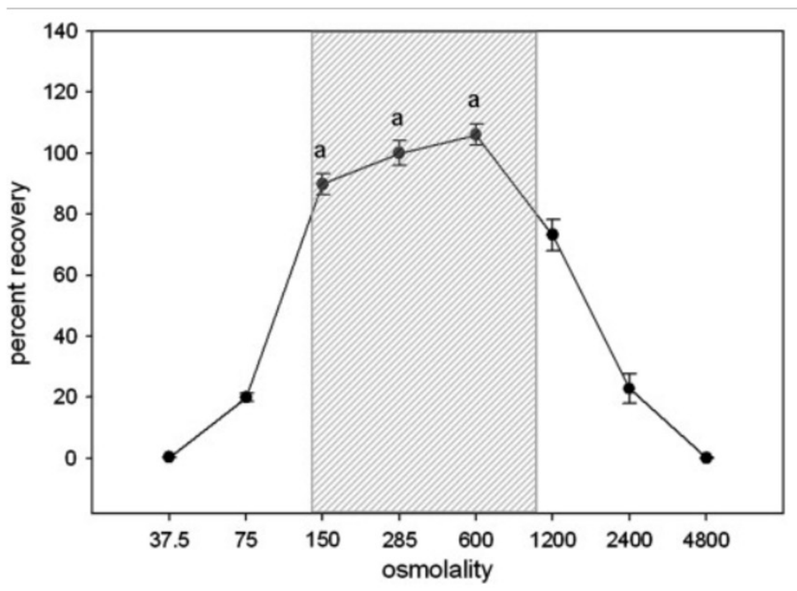


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Fig. 4. Mean (±SEM) osmotic tolerance limits of C57BL/6 mouse ES cells as determined by plasma membrane integrity. Equal numbers of ES cells were exposed to solutions of 38, 75, 150, 600, 1200, 2400, and 4800 mOsm ($n = 6$ for each solution) for 10 min at room temperature, and compared with controls in which the same quantities of cells were exposed to isosmotic solution (285 mOsm) in the same manner. Plasma membrane integrity was assessed by flow cytometry analysis of propidium iodide exclusion. The shaded area of the graph represents the osmotic range in which 80% of cells maintain cell membrane integrity. Data points with similar subscripts are not significantly different as compared to isosmotic solution ($p < .05$).

- The osmotic values were used to predict optimal cooling rates, warming rates, and type of cryoprotectant, which were then verified experimentally.
- Osmotic tolerance limits were established on the retention of membrane integrity in 80% of the cell population following exposure to aniosmotic solutions using NaCl as the impermeable solute.
- The 80% limits for mouse embryonic stem cells were between 139 and 1075mOsm. 1.0M CPA would maintain the osmotic tolerance limits – comparable to the standard freezing protocol which includes 50% FBS in cell culture medium.

An improved cryopreservation method for a mouse embryonic stem cell line q. M. Kashuba Benson, James D. Benson, John K. Critser *Comparative Medicine Center, Research Animal Diagnostic Laboratory, College of Veterinary Medicine, University of Missouri, 1600 East Rollins Street ,Columbia, MO 65211, USA Received 15 May 2007; accepted 3 December 2007

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