Economically Preserving Mammalian Cell Cultures at the Institute for Antiviral Research

Brennan McEwan, Undergraduate
Utah State University

Dr. Craig Day, Faculty Advisor
Utah State University

Introduction
As part of an internship at the Institute for Antiviral Research, I was tasked to discover the most effective way to prepare cell cultures for cryogenic preservation in liquid nitrogen.

What cell cultures are used for:
- Testing new virus-fighting drugs
- Growing and amplifying stocks of viruses
- Creating experimental vaccines for animal use.

Research has shown that cell cultures survive the freezing process best when cooled at a rate of 1 °C per minute to -80 °C before long-term storage in liquid nitrogen. There are benchtop machines to do this, but they cost thousands of dollars. I was assigned to find a less expensive way to do this using the materials available in our lab.

Methods
I grew a culture of Huh7 cells, a human liver tumor cell line, and prepared them for cryogenic preservation by adding DMSO. I compared three simple methods of freezing the cells:
- Direct immersion in liquid nitrogen
- 24 hours in -80 °C freezer, then liquid nitrogen.
- 24 hours in -80 °C with Styrofoam insulation, then liquid nitrogen.

After freezing the cells and leaving them in liquid nitrogen for 2-3 days, I thawed them out and allowed the cells to grow overnight. I then counted the living cells the next day to estimate survival.

Results
I performed three rounds of testing. In the first and third rounds, the results exactly matched my prediction: Both groups of cells frozen at -80 °C had significantly better survival than those put directly in liquid nitrogen. The Styrofoam insulation also improved survival. (P=.054, first replicate; P=.037, third replicate)

The second round of testing failed because the of a problem with the liquid nitrogen tank. All cells had poor survival regardless of what method was used to freeze them.

Figure 1: A sample of the tools used for this experiment. Hemocytometers are a special microscope slide that are laser engraved to count the number of cells in a specific volume.

Conclusions
Based on my results, the Institute for Antiviral Research will begin training technicians to freeze cells at -80 °C first increases cell survival. The third replicate showed a significant increase in survival with insulation.

Brennan McEwan
Utah State University
Department of Biology
brennan.mcewan@gmail.com