**Cell Biology and Physiology Lab**

**Cryopreserving Mammalian Cell Lines**

An established cell line is a valuable resource. Therefore, you want to freeze some of them down for long-term storage. This allows you to replace your cell line if you have a contamination in your working cell line.

Materials:

* Culture vessels containing the cells you want to freeze
* Media, PBS, trypsin (for dislodging adherent cells)
* Cryoprotective agent – DMSO (sterile)
* Sterile cryovials (labeled with details)
* Sterile 15ml 0r 50ml centrifuge tubes
* Controlled rate freezing apparatus or isopropanol chamber (Mr. Freeze)
* -80ºC freezer
* Liquid nitrogen

Methods:

* Prepare freeze medium at 2ºC or 8ºC until use (media with 10% DMSO)
* Bring flasks to hood
* For adherent cells, detach cells using same protocol for subculturing
  + Resuspend in media in centrifuge tube
* Do a cell count
* Centrifuge cell suspension a 200g for 5 minutes
* Bring back to hood
* Resuspend cell pellet in cold media at density for that cell type (~3x106)
* Dispense into cryovials
* First freeze cells in controlled freeze apparatus at -80ºC overnight
* Transfer to liquid nitrogen

