Tumor Processing from Frozen Cell Suspension

Procedure:

1. Remove tube from -80 freezer or liquid nitrogen and place on dry ice
2. Thaw tube in water bath over 30sec-1min
3. Transfer to 15ml conical tube
4. Add warmed RPMI slowly to the 15ml tube to the top and gently swirl to mix
5. Spin for 10min at 750 RPM
6. Aspirate and discard supernatant
7. Resuspend in matrigel (which was thawed slowly on ice) using a cold pipette tip (see below)
8. Place cells in matrigel on ice

Resuspension in Matrigel (Step 7 above):

- For Rhabdomyosarcoma: 1 x 10^6 cells/0.1 ml Matrigel per injection.
  - 1 cryo vial of cells→ resuspend in 0.5 ml Matrigel (enough for 5 injections)
  - Injection 0.1 ml per IM injection (using no hub TB syringe). If using hubbed syringe, account for ~ 60 uL dead space so must charge syringe with blank matrigel.

- For Ewing's sarcoma/Osteosarcoma: 1 x 10^6 cells/10 microliters Matrigel per injection.
  - 1 cryo vial of cells→ resuspend in 50 microliters Matrigel (enough for 5 injections)
  - Injection 10 microliters per bone marrow injection (using Hamilton syringe). Account for ~ 60 uL dead space to charge syringe with blank matrigel.

- Neuroblastoma: 200,000 cells/10 microliters Matrigel per injection.
  - 1 cryo vial of cells→ resuspend in 200 microliters Matrigel (enough for 20 injections)
  - Injection 10 microliters per para-adrenal injection (using Hamilton syringe). Account for ~ 60 uL dead space to charge syringe with blank matrigel.