Purification of *Toxoplasma* from cells

Needles - 23G, 25G and 27G

10 ml syringe

Toxo filter

50 ml tube -1

15 ml tube -1

1. Remove all the adhered cells from thee flask to the bottom of the flask (Why not trypsin? cell scrappers kills lot of cells, we are not going to maintain these cells!!)
2. Place all the cell suspension into the 50ml tube.
3. Draw cell/parasite suspension through syringe and 23G, 25G and 27G needles move forward and backward three times with all the needles and lyse the contents mechanically.
   Note: 1) if necessary you can add 3-5ml of media or DPBS to increase the volume of your cell/parasite suspension. It makes it easier to filter. 2) Make sure you expel cell/parasite suspension against the wall of the tubes to avoid forming bubbles.
4. Fix the Toxo filter in 15 ml tube. Pass the contents through the filter.
5. You can remove the syringe from the filter, depress the plunger and force air into the filter; or take 5 ml of DPBS or media to push the rest of the contents through the filter.
6. At this step you can either count the parasites in a hemocytometer for infection or
7. Centrifuge 3000 rpm for 8 minutes
8. Remove the supernatant.
9. Freeze parasites by adding freezing media.
   Note: At this step you can decide how many tubes you want to freeze by adding the appropriate volume (1 tube ~ 0.75ml of freezing media.
10. Label you tube appropriately and store in -20^{o} C for 1 hour and move to them to -80° C freezer. 24
    hours later, move to Liquid Nitrogen cryo storage and mark locations in the logbook provided.