

CLL Sample Receipt and CPT Tube Processing (archiving only)

****Note:** this is a BSL-2 level protocol. Be sure you have completed training and certification prior to starting. Observe all standard precautions for working with these primary human samples.

1. Samples are delivered by the courier, Uniship, to the Health Office usually between 12-12:30 pm. The Health Office staff will call the lab when they arrive. (If you have a cooler from a previous delivery to go back to the collaborator, drop it off with the Health Office staff sometime in the morning before delivery.)
2. Prepare hood with all supplies including pipettes, tips, tip/sharps box, bleach for disposal of liquids, hemocytometer, Trypan Blue, markers, conical tubes, 1.5 mL tubes, cryovials, media, dPBS, DMSO, Mr. Frosty, and racks.
3. Remove sample CBC reports from front pouch of cooler, save to add to binder.
4. Spray exterior of cooler with 70% EtOH and wipe with paper towels. Place in hood.
5. Remove rack containing CPT tubes. Remove cooler from hood, spray again with 70% EtOH and set aside.
6. Label 15 mL conical tubes with sample ID and aliquot numbers corresponding to collected CPT tubes.
7. Gently invert each CPT tube 5-10 times to resuspend cells in the plasma.
8. Carefully remove stopper, and using a p1000 transfer the cell suspension to the corresponding conical tube, be sure to include any suspension stuck in the underside of the stopper. Dispose of CPT tube in sharps box.
9. Bring conical tubes up to 15 mLs with dPBS (add approximately 12 mLs to each tube).
10. Cap tightly and gently invert 5 times to mix.
11. Pellet PBMCs by centrifuging 15 minutes at 300 RCF (approximately 1000 rpm on our clinical centrifuge, speed setting 2.5-2.8)
 - a. While spinning, label cryovials (4 for each patient) with ID number, date, and your name; label Eppi tubes (2 for each patient) with ID number, date, and "DNA" or "RNA")
12. Gently remove supernatant and dispose in bleach. Flick tube to break up pellet.
13. Add 3 mLs 9% FBS RPMI media to resuspend. Gently invert or pipet to mix.
14. Make small dilutions of each suspension either 1:2 or 1:10 in Trypan Blue. Load aliquot into hemocytometer and count on microscope. Calculate the cells/mL and total number of cells recovered for each tube. Record in notebook.
15. Transfer 4-8 million cells per patient sample to 1.5 mL Eppi tube for RNA extraction. Also transfer 2-4 million cells per patient sample to 1.5 mL Eppi tube for genomic DNA prep. Set aside for now.
16. Collect remaining suspensions to a single tube, and centrifuge 5 min at approximately 1000 rpm to pellet.
17. Make 10% DMSO freezing media by adding 500 μ l DMSO to 4.5 mLs 50% FBS freezing media for each patient collected.
18. Remove supernatant from pelleted cells and dispose in bleach. Flick to disperse pellet.

19. Resuspend each pellet in 4 mLs freezing media, gently mix, and transfer 1 mL to each pre-labeled cryovials. Tightly cap and place in Mr. Frosty (be sure it is already filled to line with isopropanol).
20. Place Mr. Frosty in a labeled biohazard specimen bag, and place in a Styrofoam cooler to transport to freezer. Set aside for now.
21. Clean up hood, being sure to wash off plates, pipets, etc. in bleach as necessary. Wipe down instruments with 70% EtOH and place in BSL-2 storage bin. Wipe down hood, close sash, and turn on UV light for 10-30 mins.
22. Pellet cells for RNA/DNA extraction 15 mins at 3000 rcf in tabletop centrifuge.
23. While those are spinning, take cooler with Mr. Frosty to NRB Revco Farm. Place in our freezer on the bottom shelf.
24. Remove supernatant from pelleted cells. Flick tubes to break up pellet.
25. Add 500 μ l Ambion RNAlater reagent to tube for RNA prep. Place at 4 $^{\circ}$ C until use.
26. Add 500 μ l Tail Lysis Buffer and 5 μ l Proteinase K to cells for DNA prep. Incubate o/n at 56 $^{\circ}$ C. (Follow genomic DNA prep protocol the next day).
27. Be sure lab notebook has all details regarding the samples and receipt (including the ambient temperature for shipping, just in case). Enter new labeled dividers in CLL sample binder for each patient received and place CBC report, packing slip, and any other associated information with it.