Isolation of Mononuclear Cells from Tonsillar Tissue

Reagent	Vendor	Catalogue #	Location
Pen/Strep (5000 IU Pen/ 5000ug/mL Strep)	Mediatech	30-001-Cl	-20°C, 2.046
DPBS	Life Technologies	14190-136	Cabinet, 2.046
Scalpel (Razor blade)	Klinische Lager	330375	Cabinet, 2.046
Scalpel holder	Klinische Lager	330372	Cabinet, 2.046
Gibco™ Amphotericin B	Thermo	15290018	-20°C, 2.046
Gentamicin solution	Sigma	G1397-10ML	4°C, 2.054
20mL Syringe	BD	302830	Cabinet
0.40um Cell Strainer	Fischer	352340	Shelf
ACK Lysis Buffer	Lonza	10-548E	4°C, 2.054
Hemostat	Klinische Lager		

Preparation of Antibiotic/Antimycotic Solution

Since these samples are very precious, we will prepare a PBS solution that contains a variety of antibiotics in order to prevent any possibility of contamination.
Prepare ~500ml Antibiotic/Antimycotic Tonsil Buffer:

Reagent	Stock Conc.	Volume	Final Conc.
DPBS	-	500ml	-
Penicillin/Streptomycin	5000 IU/mL Penicillin 5mg/mL Streptomycin	5.5mL	50 IU/mL Pen 50ug/mL Strep
Amphotericin B	250ug/mL	5ml	2.5ug/mL
Gentamicin	50mg/ml	1mL	100ug/mL

Mix and label the solution with date and contents. Keep at 4°C for a maximum of 3 months.

Sample Processing

Tonsils will be brought to the lab immediately after surgery. These must be processed as soon as possible and should not be left overnight. All work is performed in a sterile hood with gloves and lab coat. Since sharp objects should not be in the B3 lab, all work will be done in the cell culture lab.

1. Retrieve a scalpel, a handle, and surgical scissors from the drawer and spray down the items before placing them in the hood
2. Spray the tonsil sample bag with 70% EtOH and place in the hood

3. Carefully remove the tonsil specimen from its bag and place it in a petri dish filled with 5mL Tonsil Buffer prepared above
4. If necessary, remove the mucosal membrane by trimming the edge off using the scissors
    a. All waste should be discarded in the black bins or into autoclaveable bags
5. Cut the specimen into 2-3mm^3 blocks with the scalpel
    a. When the sample is very large, hold the specimen with the tweezers (Pinzetten) and cut the specimen with the scissors several times
6. Place a 0.40µm cell strainer in the petri dish.
7. Soak the cell strainer with the PBS tonsil solution
8. Open a sterile syringe and remove the plunger
9. Use a 5/25mL pipette to aspirate the solution in the petri dish (that has several floating pieces of tonsil floating around) and dispense the solution into the strainer
    a. The solution should filter out leaving the small pieces of tonsil in the strainer
10. Using the plunger from the syringe, force the tonsil pieces through the strainer by pressing the plunger firmly down, all the while keep the strainer in the solution
    a. If there is insufficient PBS solution in the dish, add more
11. Repeat steps 9-10 until the only parts left in the strainer are the bright, white mucosal membrane/connective tissue
    a. When processing a large sample, the strainer may become clogged. Use a new one if necessary
12. Remove the strainer from the petri dish and transfer the tonsil cell-containing PBS solution into a 50mL Falcon tube using a 10ml or 25ml pipette
13. Spin cells at 250xg, 5min and aspirate the supernatant
14. If the pellet is red, then the sample contains red blood cells. Use the RBC (ACK) Lysis Buffer to lyse them
    a. Add ~5-10mL ACK Lysis Buffer and pipette mix
    b. Wait 30-60 seconds then dilute with PBS tonsil solution
    c. Spin 300xg, 5 min and aspirate the supernatant
15. Wash the cells with 30mL tonsil solution and spin at 250xg, 5min. Aspirate the supernatant
    a. If cell clumps are still present, pass them through a new strainer again as in step 9
16. Resuspend cells in 30mL tonsil solution and count cells using the Eve counter
    a. Add 10ul Trypan Blue to a 1.7mL Eppy tube.
    b. Add 10ul of resuspended cell solution to the Trypan Blue in the Eppy and pipette mix.
    c. Aspirate 10ul of this new mixture and slowly pipette into one side chamber of a counting slide.
    d. Count. Divide the total number of cells by 10 x 10^6 (round down) to determine how many cryo tubes to prepare
17. Prepare cryolabels for the sample using the computer in the B3 lab. Assign the patient an ID number using the list from the HIV+ log. On the cryolabel write:

Patient: ######
20(or 100) x 10^6
Tonsil
Today's Date

18. Freeze cells in the same manner as done for PBMCs (SOP #08-01, beginning at step 3).
    1.5ml per cryotube at 20 x 10^6 cells/1.5mL