LABORATORY PROCEDURE: **FICOLL:** Lymphocyte Collection from heparin tubes

Date: June 18 2020
Authors: Jennifer Albrecht

PURPOSE: To obtain lymphocytes (PBMC) for flow cytometry from Peripheral blood or Bone Marrow (BM) aspirate

SCOPE: This procedure applies to all normal and autoimmune bone marrow processed in the Anolik laboratory.

PRINCIPLE: Blood or Bone marrow (BM) aspirate is collected into green top sodium heparin tubes. The blood or BM is diluted and layered over Lymphocyte separation media (LSM) to obtain lymphocytes.

SAFETY PRECAUTIONS: All work should be performed under the biological safety cabinet observing safety regulations and using sterile technique. Personal protective equipment such as lab coat, gloves and glasses should be used during the procedure. Specimens should be handles as if capable of transmitting infection. All contaminated supplies should be properly disposed of in biohazard or sharps containers and liquid waste should be decontaminated with bleach for 20min before being poured down the drain.

NOTE: Pay particular attention to **"^HOT SPOT**" steps. These are crucial to optimize cell yield and viability.

MATERIALS AND REAGENTS:

Supplies+Equipment

50ml conical (*Falcon 352070)*
15ml conical *(Falcon 352097)*
5 ml pipet *(VWR 89130-896)*
10 ml pipet *(VWR 89130-898)*
25 ml pipet *(VWR 89130-900))*
Pipet aid
Centrifuge
P-20, 200,1000 + Tips
Refrigerator 4°C or Ice/bucket
Hemocytometer/Microscope
Waste container for liquid

Reagents

1X PBS *(Cellgro 21-040-CV)*
0.4% Trypan Blue *(Invitrogen 15250-061)*
Lymphoprep LSM *(Stemcell 07861))*
Trypan/PBS

REAGENT PREPARTION: a. Trypan/PBS: 3 of trypan + 5ml of 1XPBS

REAGENT STORAGE: **Room Temperature:** 1X PBS, Trypan blue, Trypan/PBS, LSM.

SPECIMAN STORAGE: The blood should be at room temperature while doing the procedure. After preparation, cells should in 1X PBS at 4-8°C or on ice.

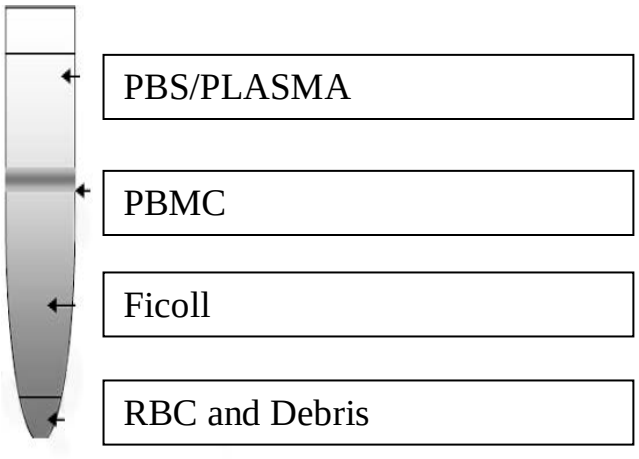
QUALITY CONTROL: Ensure that the LSM is prior to expiration date.

PROCEDURE:

^**HOT SPOT** Before starting ensure that the centrifuge is at room temperature

- Remove the green caps from the vacutainers and discard
- With a 10 ml pipet, add no more than:
  - 20mls of Blood to a 50ml conical
  - 10mls of BM to a 50 ml conical
- Rinse all vacutainers with 10ml of 1X PBS (keep reusing the same 10ml of PBS) and add to 1 of the 50 ml conicols already containing blood or BM
- Add 1X PBS and mix up and down with a 25 ml pipet
  - Blood: add 1:1 1X PBS
  - BM: add 2:1 1X PBS
- ^**HOT SPOT** Slowly add 12ml of LSM underneath the blood with a 10ml pipet
- Centrifuge at room temperature 800 RCF/g for 20 min ^**HOT SPOT NO BRAKE**
- Return the centrifuge temp to 4C and turn BRAKE on
- Remove the lymphocyte layer with a 5ml pipet, being careful not to aspirate any RBC and place lymphocytes in a 50ml conical. (Do not exceed 25ml per tube to allow for PBS)
- Top off the conical with 1X PBS and **invert** 1X to mix
- Centrifuge at 4-8°C 350 RCF/g for 5 minutes
- Invert to discard liquid in waste container
- Resuspend the cells in 1ml of 1X PBS and transfer cells to a 15ml conical
- Add 9 ml of 1X PBS and **invert** 1X to mix
- Centrifuge at 4-8°C 350 RCF/g for 5 minutes
- Invert to discard liquid in waste container
- Resuspend in up to 10 ml of 1X PBS
- Count cells on hemocytometer (see counting SOP)

- Place the tube at 4°C or on ice until use



LIMITATIONS: This SOP is for a minimum of 10ml of Blood and 5 ml of Bone marrow.

CALCULATONS: Calculations can be obtained in the counting SOP

INTERPRETATION: The average cell number from blood is $1X10^6$ cell/ ml of blood. In a lymphopenia patient you might expect as low as $0.5X10^6$ cells/ml and in a robust patient up to $2X10^6$cell/ml. If number deviates from the range, consider error in counting, RBC interference or inadequate harvest of layer. The average cell number from bone marrow varies greatly from $0.5X10^6$ cell/ml in a lymphopenia patient to $200X10^6$ cell/ml in a robust patient.

RESULTS REPORTING: The results are reported with Sample ID, Date, MLS of Blood, and cell#.

TRAINING: Personnel will be trained by staff. Up to one time visual shadowing of staff member AND up to one time hands on training with staff member AND one or more times independent performance with successful completion of SOP.

STATEMENT OF TRAINING: **FICOLL**

I (name)_____ have read the “FICOLL” SOP in its entirety. I have asked questions to the trainer if needed to ensure clear understanding of all the steps involved.

I have viewed the procedure on (date)_____ performed by (name of trainer)_____ and have asked any questions at the time of the procedure.

I have performed hands on training on (date)_____ utilizing real human blood and was observed by (name of trainer)_____ to ensure proper procedure was followed.

I have independently and successfully performed the procedure on (date)_____ . I have verified with (name of trainer)_____ that _____ml of blood, yielding _____total cells are within the expected range of the procedure.

Signature of Trainee

_____ Date_____

Signature of Trainer

_____ Date_____