LABORATORY PROCEDURE: MSC isolation from heparin tubes

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PURPOSE: To obtain Mesenchymal stromal cells (MSCs) for flow cytometry from Bone Marrow (BM) aspirate

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

PRINCIPLE: Bone marrow (BM) aspirate is collected into green top sodium heparin tubes. The BM is diluted and layered over MSC separation media to obtain MSCs.

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)*
Ficoll *(GE 17-5446-52)*
Trypan/PBS

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

REAGENT STORAGE: **Room Temperature:** 1X PBS, Trypan blue, Trypan/PBS, Ficoll.
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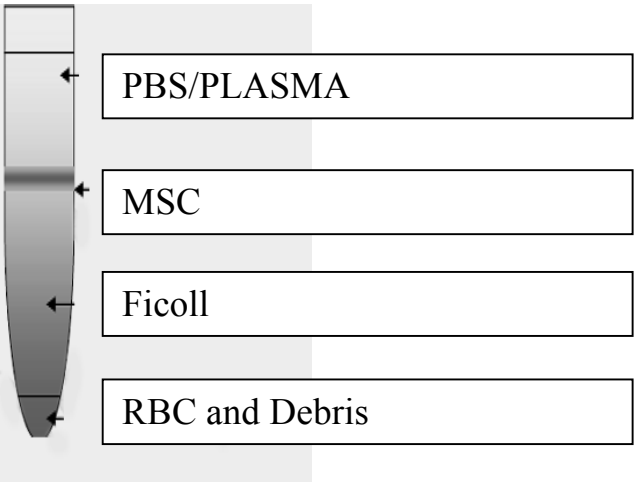
SPECIMAN STORAGE: The BM should be at room temperature while doing the procedure. After preparation, cells should in 1X PBS at 4-8°C or on ice.

QUALITY CONTROL: Ensure that the Ficoll 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:
  - 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 conicals already containing BM
- Add 1X PBS and mix up and down with a 25 ml pipet
  - BM: add 2:1 1X PBS
- ^**HOT SPOT** Slowly add 12ml of Ficoll underneath the BM with a 10ml pipet
- Centrifuge at room temperature 400 RCF/g for 30 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 MSCs 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



CALCULATONS: Calculations can be obtained in the counting SOP