Ficoll gradient to isolate peripheral blood mononuclear cells

Reagents

- Ficoll (density 1,077 for humans) – from Sigma it is called Histopaque
- 1X PBS
- FBS-supplemented culture medium
- blood/buffy coat

Materials

- Falcons (best if done in 15-mL tubes)
- Pipette-boy
- Pipettes
- Sterile flasks if you have >15 mL blood

Protective measures

When working with human blood or tissue, use lab coat and gloves and work in a laminar flow cabinet.

Before trashing the material, disinfect everything with a 10% bleach solution for at least 2 hours.

Procedures

Note: before the gradient is finished, keep all solutions at room temperature.

1. Dilute the blood 1:1 in 1X PBS that must be at room temperature. If you have more than 15 mL blood, use plastic disposable flasks (such as culture flasks).

    - ex: for 5 mL blood, add 5 mL PBS

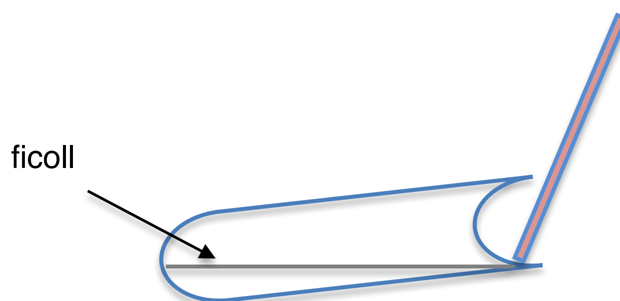
2. Mix gently by inversion or up-and-down pipetting.

3. Pipette the same original blood volume of Ficoll (since it is kept at 4°C, warm it up in the water bath up to room temperature) and distribute them by an appropriate number of new tubes.

    - ex. for 5 mL blood, take 5 mL Ficoll

4. With a serological pipette, aspirate the diluted blood (twice the volume of the original blood sample) and carefully and slowly overlay it on the top of the Ficoll. Try to avoid "turbulence" on the interface between Ficoll and the diluted blood during the process. The easiest way to

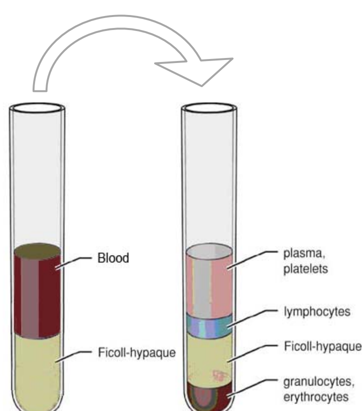
do it is to set the pipette-boy to the lowest power for the "pipette-out" function, and keep the pipette almost vertical while pipetting into the top of the tube, that you should lay down into an almost complete horizontal position:



As the volume is being dispensed, start bringing the tube back to the vertical position slowly. Pay attention to the interface to avoid mixing the blood with Ficoll.

Therefore, in each tube one should have 1:1:1 blood:PBS:Ficoll.

5. Centrifuge for 30 minutes at 2000 rpm, without break and at room temperature (22-30 °C). At the end, you should obtain something like this:



6. In the next steps, you should be very careful while aspirating the different phases. Set the pipette-boy to the lowest power for the "pipette-in" function. Aspirate the top layer (which is yellow) containing the blood serum to an empty tube. Stop aspirating when you get close to the 2nd layer, leaving some serum in the tube. If you plan to use this serum to supplement culture media, filter it first.

7. With a 2-mL serological pipette or a Pasteur pipette aspirate the 2nd layer (which is white) containing the PBMCs. If the gradient went well, this layer should seem like a very well defined white ring. Try to aspirate from the top of the layer and not from the inside of the ring. It is normal that some ficoll (underneath the ring) is also taken during aspiration. Transfer the PBMCs into a tube containing FBS-supplemented medium. In the end, you can clearly see the top of the ficoll layer without PBMCs.

8. Trash the tubes containing the ficoll and granulocytes + red blood cells.

9. Wash PBMCs at least 3 times before culture to ensure that ficoll is completely removed. Centrifuge 1200-1300 rpm for 5 minutes at 4ºC.

10. When counting the PBMCs under the microscope, if there are many cells extremely small, it is because your donor had a high % of platelets. Low centrifugation speed (1200 rpm) should help you to get rid of them. In addition, cells seeming to have a wrinkled membrane and/or a more brownish color than the others are erythrocytes or their precursors. Do not count them as PBMCs.