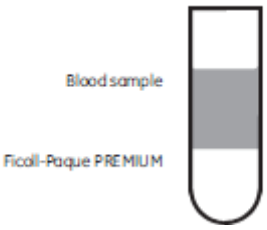
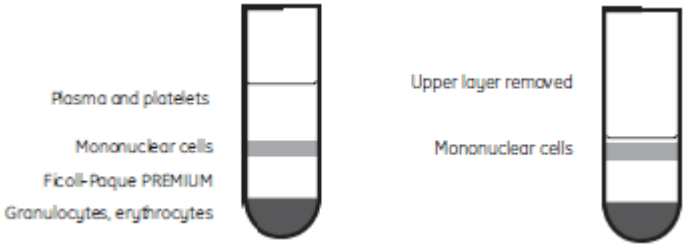
Procedure for isolation of canine blood lymphocytes

1. Homogenize 2 ml of blood, collected with anti-clotting reagent (heparin or EDTA), with another 2 ml of PBS at 20-22 °C;
2. In a 15 ml conical tube add 3 ml of Ficoll-Paque (density 1077g/dl) at 21-22 °C and transfer carefully the diluted blood to this tube, pipetting to wall sides very slowly and avoid mixing with Ficoll, as illustrated below:



3. Centrifuge at 900g / 30 min at 21-22 °C, no brakes;
4. After centrifuging, take care not to disturb the layers of the gradient. Discard the upmost layer which contains plasma and platelets. Following, pipette in another tube carefully the mononuclear cell layer, called the buffy coat.



5. Resuspend mononuclear cells in 7 ml of PBS and centrifuge at 350g / 10 min a 18 °C; count cells and continued for CFSE labeling……..