Separation of lymphocytes from whole blood

Note: The following procedure is one of many variants of the procedure originally described by Boyum. This procedure was developed for use with heparin-treated blood from mice; alterations may be necessary for use with blood from other species or with other tissues.

1. Thoroughly mix the LSM by inverting the bottle gently.
2. Aseptically transfer 3 ml of LSM to a 15 ml centrifuge tube.
3. Draw peripheral blood into syringe containing 10 U/ml heparin.

4. Mix 2 ml of defibrinated, heparinized blood with 2 ml of physiological saline.
5. Carefully layer the diluted blood over 3 ml of LSM (room temperature) in a 15 ml centrifuge tube, creating a sharp blood-LSM interface. DO NOT MIX DILUTED BLOOD INTO THE LSM.
6. Centrifuge the tube at 400 x g (~1500 rpm) at room temperature for 20-30 minutes. Centrifugation should sediment erythrocytes and polynuclear leukocytes and band mononuclear lymphocytes above LSM (Bands will be Plasma layer -----> Mononuclear cell layer -----> LSM layer -----> RBC pellet).
7. Aspirate the top layer of clear plasma to within 2-3 mm above the lymphocyte layer.
8. Aspirate the lymphocyte layer plus about half of the LSM layer below it and transfer it to a centrifuge tube. Add an equal volume of buffered balanced salt solution to the lymphocyte layer in the centrifuge tube and centrifuge for 10 minutes at room temperature (18-25^{o} C) at a speed sufficient to sediment the cells without damage. i.e. 160-260 x g . (Washing removes LSM and reduces the percentage of platelets).
9. Wash the cells again with buffered balanced salt solution and resuspend in appropriate medium for your applications.
10. Count cells with a hemocytometer if necessary.

Note: Store at room temperature (18-25^{o} C) and protect from light.