ORPHAN EUROPE

WBC Cystine Group

GUIDELINE No. 2

Prepared by :

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POLYMORPHONUCLEAR LEUCOCYTE PREPARATION

Polymorphonuclear leucocytes should be the biological material of choice for measurement of cystine because cystine in cystinotic leucocytes is located primarily in phagocytic blood cells (polynuclear and monocytes) rather than in lymphocytes.

- Blood (10ml) was collected drawn via venipuncture and collected in ACD (citric acid, citrate, dextrose) tubes.
- Cell separation should be performed within 24 hours after taking the sample
- Whole blood is conserved at room temperature

Procedure

- Transfer 10ml of whole blood to a 15ml conical plastic centrifuge tube containing 500µl 0.2mM EDTA.
- Mix gently
- Centrifuge 1200g for 15 min
- Discard the plasma and collect the middle fraction containing total leucocytes (figure 1)
- Transfer this fraction to a plastic tube containing 6ml phosphate buffer saline (PBS)
- Mix gently
- Layer this solution gently on top of a discontinuous gradient consisting of 3ml histopaque 1119 and 3ml of histopaque 1077 (use a 15ml conical plastic centrifuge tube)
- Centrifuge 500g for 30min
- The resulting bands are illustrated in figure 2
- Discard the plasma, the fraction 1 containing the lymphocytes, monocytes and platelets, and the middle layer until fraction 2.

- Collect the fraction 2 containing PMN leucocytes without including the red blood cell pellet.
- Transfer this fraction 2 to a 15ml conical plastic centrifuge tube
- Add 12ml 0.9% NaCl containing 5mM EDTA
- Centrifuge 600g for 7min
- Remove the supernatant by aspiration
- Resuspend the pellet in 1.6ml 0.9% NaCl
- Mix gently
- Add 4.8 ml H2O to lyse red blood cells
- After 90sec, solutions were made isotonic by addition of 1.6ml 3.6% NaCl
- Centrifuge 600g for 10min
- Remove the supernatant
- Repeat the lyse 1 or 2 times if necessary
- Centrifuge 600g for 10min
- Wash one more time with 4 ml 0.9% NaCl with 5mM EDTA
- Centrifuge 600g for 10min
- Remove all the supernatant and resuspend the pellet in 150µl 5.2mM NEM
- Add 50µl 12% sulfosalicylic acid and freeze until assay

Figure 1

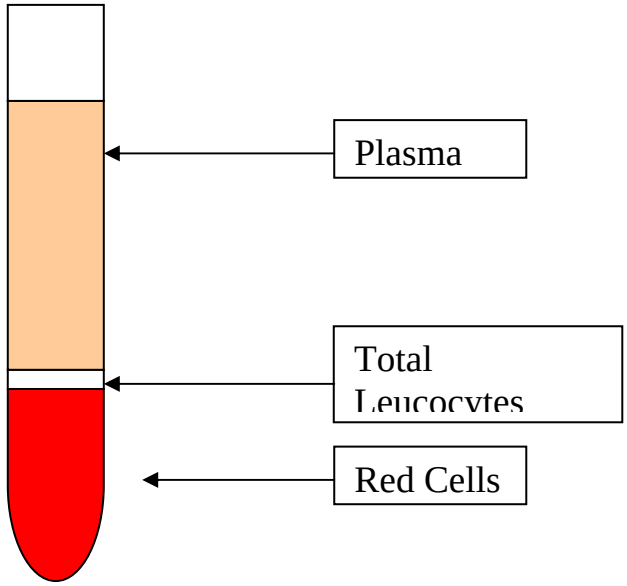

Figure 2