Preparation of Peripheral Blood Leucocytes for White Cell Cystine Assay by Dextran Sedimentation

Introduction

Cystinosis is a lysosomal storage disorder that involves a defect in the cystine transporter on the lysosomal membrane (cystinosin) leading to accumulation of cystine within leukocytes. Cysteamine therapy reduces the concentration of cystine within the lysosome leading to a decrease in the symptoms of the disorder.

NOTES

1. The quality of blood collection significantly affects the leucocyte pellet quality. Blood must not contain clots or plastic mixing beads and must be fresh.
2. If a small volume only of blood is obtained (2-4 mL), prepare only a single leucocyte pellet.

Principle

Leucocytes are separated from erythrocytes by differential sedimentation in a dextran/saline solution. The cells are washed briefly in 0.2% sodium chloride to haemolyse the red cells, and then brought back to isotonicity with 1.8% sodium chloride. In order to facilitate subsequent analytical operations, two leucocyte pellets are produced from the original single whole blood sample.

Specimen

Approximately 10 mL (8-12 mL) whole, fresh, EDTA or heparinised blood is required.

Reagents

0.2% Sodium Chloride.

- Dissolve Sodium Chloride ..... 2 g
- In de-ionised water ..... 1 L
- Mix well to dissolve and store at 4°C.

0.9% Sodium Chloride (Saline).

- Dissolve Sodium Chloride ..... 9 g
- In de-ionised water ..... 1 L
- Mix well to dissolve and store at 4°C.

1.8% Sodium Chloride.

- Dissolve Sodium Chloride ..... 1.8 g
- In de-ionised water ..... 1 L
- Mix well to dissolve and store at 4°C.

Dextran/Saline

- Dissolve dextran (MW 200 – 300 kDa) ..... 0.5 g

- In 0.9% Sodium Chloride. ..... 10 mL
- Dissolve well before use. May be stored at 4°C, stable for one week.

Equipment

- 10 mL Centrifuge tubes
- Graduated pipettes
- Disposable pipettes
- Centrifuge

Procedure

1. Keep all reagents refrigerated and ensure that all washing operations are carried out at approximately 10°C.
2. Divide 8-12 mL whole EDTA blood (collected without the use of plastic mixing beads) into two equal volumes in a pair of 10 mL plastic centrifuge tubes; centrifuge both at 3,000 rpm (1550 x g) for 5 min at 10°C.
3. Remove all plasma from both the tubes.
4. To each tube of 'packed cells' add 0.9% sodium chloride to give final volume of approximately 8mL.
5. Add dextran/saline ..... 2-3 mL then mix the tubes well, but gently, by repeated inversion. Remove the lids or caps of the tubes and carefully pipette off any bubbles on the surface of the blood.
6. Allow the blood to stand at ambient room temperature for approximately 30 - 45 min or until the red cells have settled out sufficiently (red cells should occupy about the lowest one-third of the tube).
7. Carefully remove the leucocyte-containing supernatant from both tubes and transfer to a pair of fresh, clean 10 mL centrifuge tubes. Discard the red cells.
8. Centrifuge all tubes at 3,000 rpm (1500 x g) for 2 min at 10°C. Pour off supernatants and retain the leucocyte pellets.
9. To each pellet add 0.2% sodium chloride ..... 4 mL and mix by pipetting gently up and down with a wide bore disposable pipette. Do not leave the cells exposed to the 0.2% saline longer than about 1 min.
10. Add to each tube 1.8% Sodium Chloride ..... 3.2 mL and mix gently by inversion.
11. Centrifuge once more at 3,000 rpm (1550 x g) for 2 min. Pour off and discard the supernatant.
12. Repeat steps 9 to 11 once more to obtain a white cell pellet free of red cells.

NOTE: The pellet at this stage should be white or, at most, just slightly pink in color. If there are still many red blood cells present, repeat steps 9 to 11 one more time.

13. After the final centrifugation, remove all supernatant with a cotton swab and transfer the clearly labelled pellets immediately to a -80°C freezer.
14. Transport the white cell pellet to the Biochemical Genetics Department on dry ice.