Isolation of a polymorphonuclear (PMN) leukocyte (granulocyte) fraction from human blood

Product Description

- Polymorphprep™ is a ready-made, sterile and endotoxin tested solution for the isolation of pure PMNs from whole blood.
- The solution contains 13.8% (w/v) sodium diatrizoate and 8.0% (w/v) polysaccharide
- Physicochemical characteristics: density, 1.113 ± 0.001 g/ml; osmolality 445 ± 15 mOsm

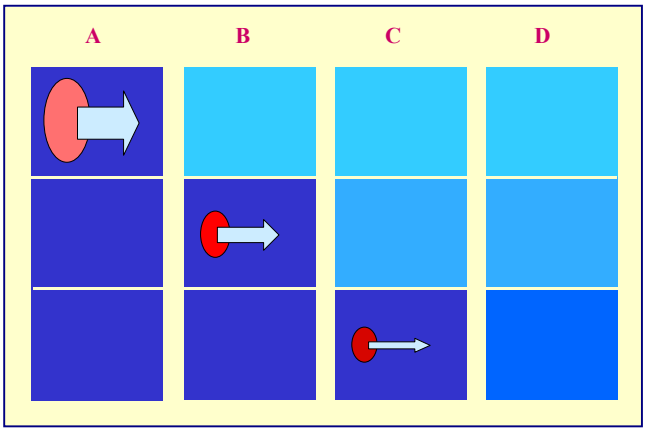

Figure 1: Sedimentation of erythrocytes through Polymorphprep™; for details see text

Principle of the separation procedure

Bøyum [1] devised an isoosmotic density barrier (1.077 g/ml) containing metrizoate and a polysaccharide that separated human peripheral blood mononuclear cells, which banded at the interface, from the PMNs + erythrocytes, which pelleted. The density and osmolality of the barrier was later increased [2] to permit the simultaneous separation of the PMNs and erythrocytes.

The theory of the separation is that the polysaccharide aggregates the erythrocytes and causes them to sediment rapidly through the medium, ahead of the leukocytes. In Figure 1, the column of Polymorphprep™ is divided hypothetically into three zones. When the erythrocytes enter the top zone (A), there is a large difference in osmolality across the erythrocyte membrane, which causes water to move from the erythrocytes into the medium (thereby diluting it significantly), while the osmolality inside the erythrocytes increases. Consequently, when these erythrocytes sediment into the middle zone (B), the difference in osmolality across the erythrocyte membrane is now less pronounced, so the erythrocytes lose less water than they did in the top zone, the medium is consequently diluted less. In the bottom zone the difference in osmolality across the erythrocyte membrane and consequently the loss of water into the medium, are reduced further (C). In this way, the density of the medium is decreased most in the top zone and least in the bottom zone and a gradient is generated (D). In reality, the erythrocyte sedimentation and medium dilution changes occur progressively and smoothly; the gradient is therefore continuous rather than discontinuous. It is within this continuous gradient that the mononuclear and PMNs are resolved (see Figure 2). The standard protocol is described on p2.

Stability and storage

Polymorphprep™ is stable for 5 years provided the solution is kept sterile and protected from light. Prolonged exposure to direct sunlight leads to a release of iodine from the diatrizoate molecule. This effect is negligible when working with the solution on a day-to-day basis. Polymorphprep™ should be stored at room temperature.

Sample requirements

For optimal results the blood must be from a healthy individual; drawn into K-EDTA as anticoagulant and processed within 2 h of drawing.

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Solutions required

A. Polymorphprep™
B. Hepes-buffered saline: 0.85% (w/v) NaCl, 10 mM Hepes-NaOH, pH 7.4 (see Note 1)
C. Ammonium chloride lysis buffer (optional): 0.83% (w/v) NH_4Cl, 10 mM Hepes-NaOH, pH 7.4

Protocol

1. Bring all solutions to room temperature.

2. Collect human venous blood with EDTA (final concentration 1.5-2.0 mM) as anticoagulant (see Notes 2 and 3).

3. Using a Pasteur pipette, or syringe and metal cannula (i.d. 0.8 mm), carefully layer 20 ml of blood over 20 ml of Polymorphprep™ in a 50 ml centrifuge tube, or 5 ml of each in a 15 ml centrifuge tube (see Notes 4 and 5).

4. Centrifuge at $500g_{av}$ for 30-35 min at room temperature (see Notes 6 and 7).

5. Allow the rotor to decelerate without the brake (see Note 8).

6. Remove the plasma and mononuclear cells (upper band of cells) and harvest the lower band of PMNs (see Figure 2).

7. Dilute an aliquot of solution B with an equal volume of water and mix 1 vol. of this half-concentration saline with the PMN suspension (see Note 9).

8. Harvest the PMNs by centrifugation at 400g for 10 min and resuspend in Solution B.

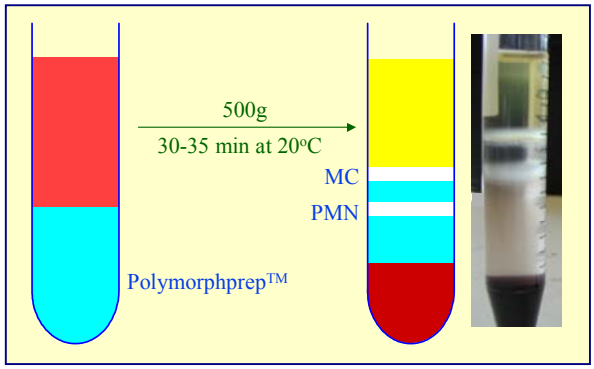

Figure 2: Purification of PMNs using Polymorphprep™ MC = mononuclear cells; PMNs = polymorphonuclear leukocytes. Inset right shows an actual separation in a 15 ml tube

9. To remove any residual erythrocyte contamination of the granulocytes, resuspend the cell pellet in 3 ml of Solution C and incubate at 37°C for 7 min (see Note 10).

10. Harvest by centrifugation and resuspend in a suitable medium.

Notes

1. Any buffered saline, balanced salt solution or culture medium may be used instead of Hepes-buffered saline.
2. EDTA (K salt) will provide the best result; citrate is acceptable as an anticoagulant but inferior to EDTA, and heparin should be avoided.
3. Polymorphprep™ only works optimally with whole, undiluted blood, collected from normal healthy volunteers and used within 2 h of drawing.
4. For non-standard volumes check the height of the meniscus and the blood/Polymorphprep™ interface against the standard format of 5 ml + 5 ml in a 15 ml conical centrifuge tube.
5. The Polymorphprep™ may alternatively be layered underneath the blood.
6. The efficacy of Polymorphprep™ relies on the loss of water from the erythrocytes to the hyperosmotic medium. This only happens effectively at approx 20°C. Make sure that when the refrigeration cuts in during the centrifugation that the temperature does not drop below 17°C.
7. Some blood samples might benefit from an extra 5 min centrifugation if the erythrocytes are poorly packed.
8. Use of the brake to decelerate the rotor will cause cross-contamination between the two bands of cells and swirling of the loosely-packed erythrocytes.

9. Use of half-strength saline returns the PMN suspension to an isoosmotic condition.
10. An alternative erythrocyte lysis strategy is ice-cold water for 30 sec, after which time add an equal volume of 2xHepes-buffered saline.

References

1. Boyum, A. (1968) *Scand. J. Clin. Lab. Invest.*, **21**, Suppl 97
2. Ferrante, A. and Thong, Y. H. (1980) *J. Immunol. Meth.*, **36**, 109-117

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