Isolation of agranulocytes from peripheral blood

- **Isotonic sucrose solution 1083 gl $^{-1}$** – osmolarity adjusted with sodium chloride to 300 mosm.l -1 , thickened with the addition of 3% gelatin, colored blue with food coloring E-132
- **Isotonic sucrose solution 1077 gl $^{-1}$** – osmolarity adjusted with sodium chloride to 300 mosm.l -1 , thickened with the addition of 3% gelatin, colored yellow with food coloring E-102
- **Physiological solution** – sodium chloride 0.9 gl -1
- **LeucoPHAN**
- H**eparinized blood** diluted 1:1 with saline

Principle

On a discontinuous gradient with media with a density of 1083 and 1077 gl -1 , blood cells are separated during centrifugation in such a way that erythrocytes sediment to the bottom of the test tube, granulocytes fall to the interface of the two media, and agranulocytes with platelets remain above the less dense solution.

We demonstrate the presence of leukocytes in the isolated suspension using the commercial LeukoPHAN test. Leukocyte esterases cleave the indoxyl ester, which then reacts with the diazotized salt to form a colored conjugate.

Procedure

1. 3 ml of a solution with a density of 1083 gl -1 is measured into a polypropylene centrifuge tube . 3 ml of a solution with a density of 1077 gl -1 is carefully layered over it . The two solutions must not be mixed.
2. 6 ml of diluted blood is carefully placed over the gradient.
3. Centrifugation for 20 minutes at 2000 rpm.
4. With a plastic Pasteur pipette, the plasma is aspirated above the gradient, leaving about a 7 mm layer of plasma above the upper edge of the gradient.
5. A part of the column located between 7 mm above and 7 mm below the edge of the gradient is taken into a glass tube with a plastic Pasteur pipette. This fraction contains agranulocytes and platelets.
6. The presence of leukocytes in the fraction is demonstrated using LeukoPHAN - the field turns purple after immersion.
7. The agranulocyte fraction with platelets is kept for further experiments.

Links

Source

- VEJRAŽKA, M.: *Základní techniky práce s tkáňovými kulturami*. Praha, 2004.