

Lymphoprep™

The golden standard in PBMC isolation



Introduction

A simple and effective method for the isolation of mononuclear cells from human blood was reported by Dr Arne Bøyum in 1968. For more than 45 years a commercial medium known as Lymphoprep™ has been widely used for isolating these cells.

Mononuclear cells (monocytes and lymphocytes) have a lower buoyant density than the erythrocytes and the polymorphonuclear (PMN) leukocytes (granulocytes). The vast majority of mononuclear cells have densities below 1.077 g/ml. These cells can therefore be isolated by centrifugation on an iso-osmotic medium with a density of 1.077 g/ml, which allows the erythrocytes and the PMNs to sediment through the medium while retaining the mononuclear cells at the sample/medium interface.

The described method is rapid, simple and reliable and gives excellent results with blood samples from normal individuals and patients.

To obtain the maximum yield it is important that the blood sample is diluted 1:1 with physiological saline before being applied to the gradient.

The contamination of erythrocytes in the mononuclear cell suspension is usually between 3- 10% of the total cell number.

Some immature PMNs may band with the lymphocytes during intense immunosuppressive therapy.

When heparinised blood is used, it is essential to remove most of the platelets, in order to avoid inhibition in the cytotoxicity test.

Lymphoprep™ is a ready-made, sterile and endotoxin tested solution with the following specifications:

Sodium diatrizoate:	9.1% (w/v)
Polysaccharide:	5.7% (w/v)
Density:	1.077 ± 0.001 g/ml
Osmolality:	290 ± 15 mOsm
Endotoxins:	< 1.0 EU/ml

Each batch of Lymphoprep™ is checked on the level of endotoxins using a specific LAL test. Our goal is to produce batches with an endotoxin level lower or equal to 0.13 EU/ml.

For every batch produced a Certificate of Analysis showing the actual values of density, osmolality and endotoxins is made available at www.axis-shield-density-gradient-media.com. We also claim sterility according to Ph.Eur. The last step in our production is autoclaving the bottles at 121°C to make sure that the product is completely sterile.

Lymphoprep™ is manufactured, packed and released by a GMP compliant and ISO 13485 certified manufacturer.

Lymphoprep™ has the same specifications as the expensive PLUS and PREMIUM media from other manufacturers.

Why-pay more when you can use Lymphoprep™?

Lymphoprep™ can be used for the preparation of pure lymphocyte suspensions for tissue typing, anti lymphocyte sera and immunological research. Thorsby and Bratlie used this technique with only slight modifications in the preparation of pure lymphocyte suspensions for cytotoxicity tests and lymphocyte cultures.

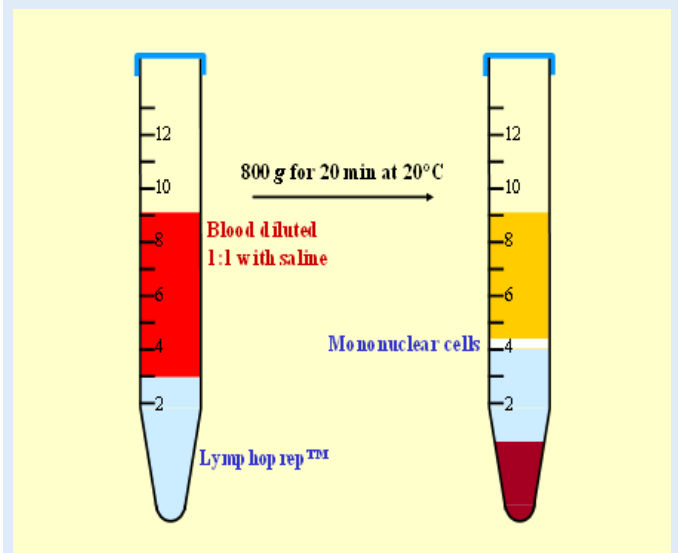
Lymphoprep™ is supplied as a sterile solution in the following package sizes:

Prod. No. 1856	4x250 ml
Prod. No. 1858	6x500 ml

References

Bøyum, A. (1968)

Separation of leucocytes from blood and bone marrow *Scand. J. Clin. Lab. Invest.*, **21**, suppl.97



Lymphoprep™ Tube

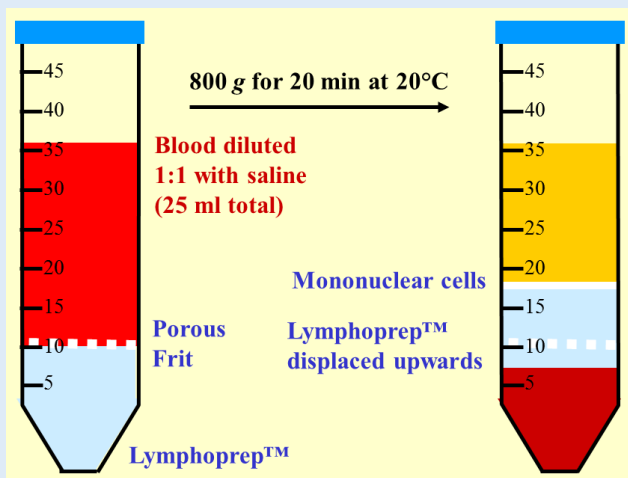
Lymphoprep™ Tube is a sterile tube in which Lymphoprep™ is contained below a plastic filter disc. This allows diluted blood to be poured simply and directly into the tube, the disc preventing any mixing with the separation medium.

Lymphoprep™ Tube can be used for the preparation of pure lymphocyte suspensions for tissue typing, anti-lymphocyte sera and immunological research. Thorsby and Bratlie used this technique with only slight modifications in the preparation of pure lymphocyte suspensions for cytotoxicity tests and lymphocyte cultures.

Lymphoprep™ Tube is supplied as a sterile solution in the following package sizes:

Prod. No. 18002 30 tubes (each filled with 2ml)

Prod. No. 18001 18 tubes (each filled with 10ml)



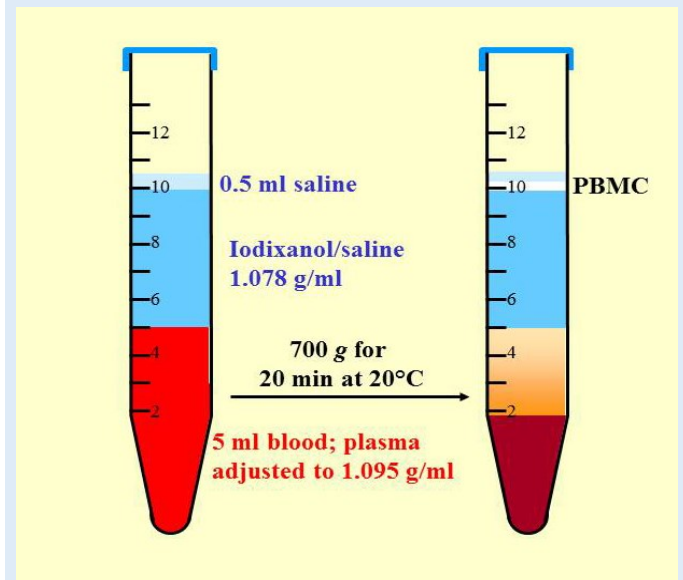
Interested in mononuclear cells free from platelets?

Any sedimentation technique in which a minor population of low density particles (mononuclear cells) is being separated from a much larger population of rapidly sedimenting denser particles (erythrocytes) suffers from the problem of entrapment of the former by the latter.

An alternative flotation strategy is to add OptiPrep™ (or a dense solution prepared from OptiPrep™) to whole blood and thus adjust the density of the plasma to approx. 1.095 g/ml and then layer it under a density barrier of 1.078 g/ml (OptiPrep™ diluted with buffered saline). Mononuclear cells float to the top of the density barrier while the erythrocytes and PMNs stay in the load zone. It is the only simple technique that separates the mononuclear cells completely from the plasma (see figure).

More importantly, it is the only technique that also separates these cells from the platelets. The mononuclear cells are also "washed" free of plasma proteins by the low-density barrier. In all methods involving sedimentation on to a 1.077-1.078 g/ml barrier, all of the platelets lie on the top of the mononuclear cell band. In this strategy the platelet contamination depends on the underlying plasma that is harvested.

The lack of platelets is a huge advantage to any subsequent culturing of monocytes from the mononuclear cells.



For a detailed protocol and references see Application Sheet C05 at www.diagnostic.serumwerk.com

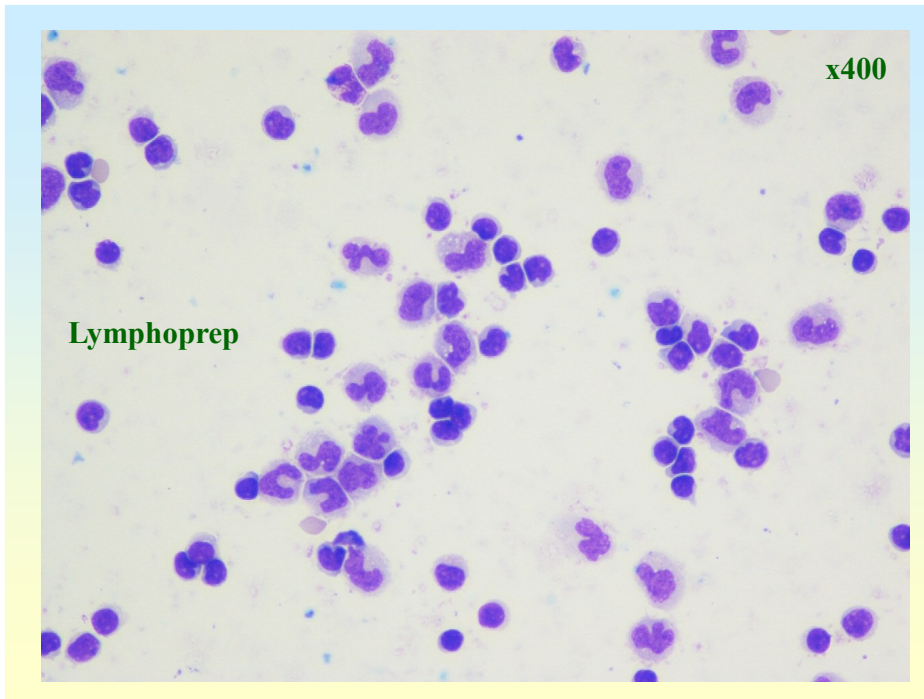
OptiPrep™ is a sterile endotoxin tested solution of 60% iodixanol in water with a density of 1.32 g/ml.

Iodixanol was developed as an X-ray contrast medium and has therefore been subjected to rigorous clinical testing.

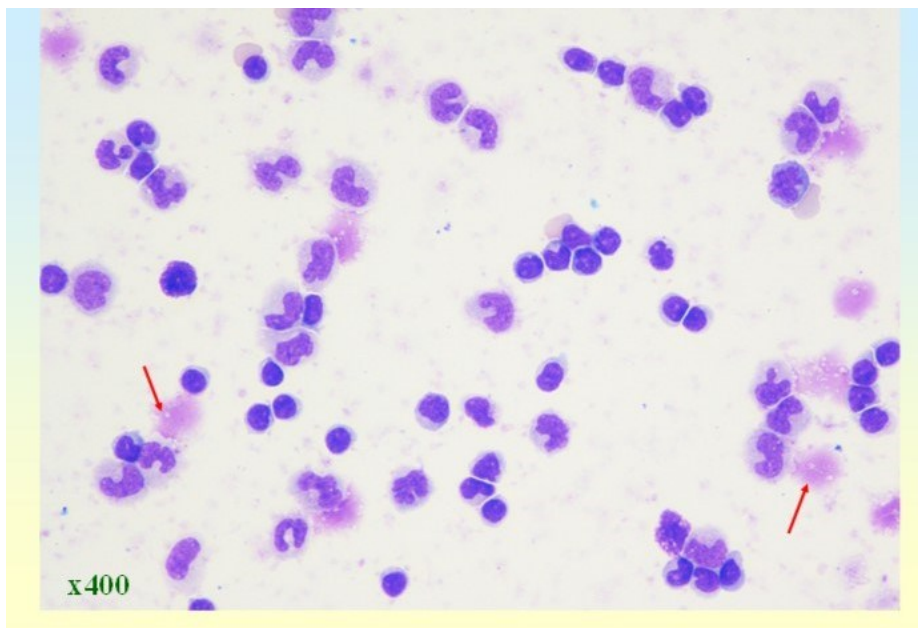
Iodixanol is non-ionic, non-toxic to cells and metabolically inert.

Iodixanol solutions can be made iso-osmotic at all useful densities.

Iodixanol solutions have low viscosity and osmolality.



Isolation of mononuclear cells using Lymphoprep™



Isolation of mononuclear cells using a competing product. Some cells shows evidence of vacuolation (arrowed). This is not observed in the Lymphoprep™ isolated cells.

www.diagnostic.serumwerk.com



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