DGM
Density Gradient Media

OptiPrep™

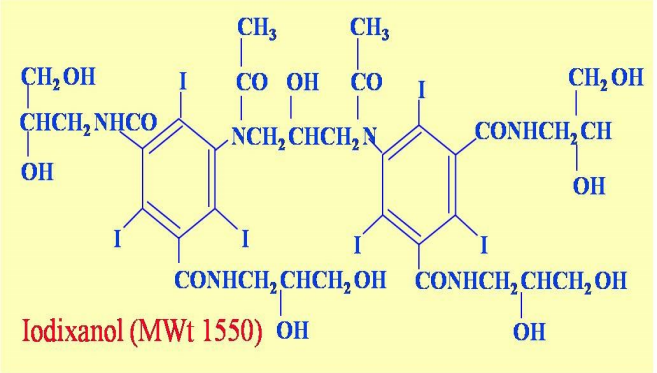
The ideal density gradient medium for isolation of blood cells

serumwerk bernburg

OptiPrep™, non-ionic, iso-osmotic gradients for high resolution

OptiPrep™

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 an 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 osmolarity

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

Actual endotoxin levels in each batch are usually measured at < 0.13 EU/ml.

Unlike manufacturers of other density gradient media, Serumwerk Bernburg do not have to offer special, more expensive, low endotoxin-containing products; all their density gradient media have always been, and continue to be, produced to the same high rigorous standards.

For every batch produced, a Certificate of Analysis is available.

The high density of **OptiPrep™** facilitates the fractionation of cells by flotation from a dense load zone through either a continuous or discontinuous gradient or through a simple density barrier.

Isolation of human mononuclear cells free from platelets

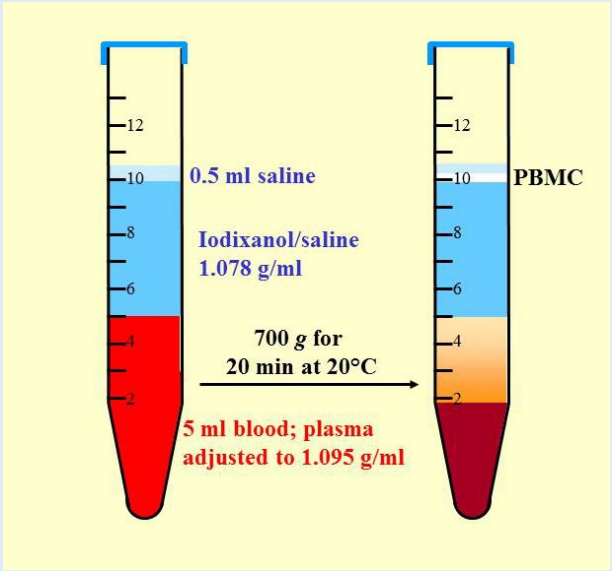
Any sedimentation technique in which a minor population of low density particles (mononuclear cells) is being separated from a much larger population of rapidly sedimenting denser particles (erythrocytes) suffers from the problem of entrapment of the former by the latter.

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).

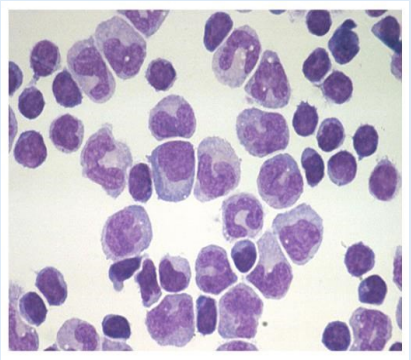
More importantly, it is the only technique that also separates these cells from the platelets. Mononuclear cells are also ”washed” free of plasma proteins by the low-density barrier. In all methods involving sedimentation on to a 1.077-1.078 g/ml barrier, the vast majority of platelets will co-sediment with the mononuclear cells forming a broad band across the interface. In this strategy the platelet contamination depends on the underlying plasma that is harvested.

Careful aspiration of the PBMC layer (top 2-3 ml) to avoid any material from the lower interface is important. The photomicrograph shows the complete absence of platelets in the mononuclear cell suspension.

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 C06 at www.diagnostic.serumwerk.com

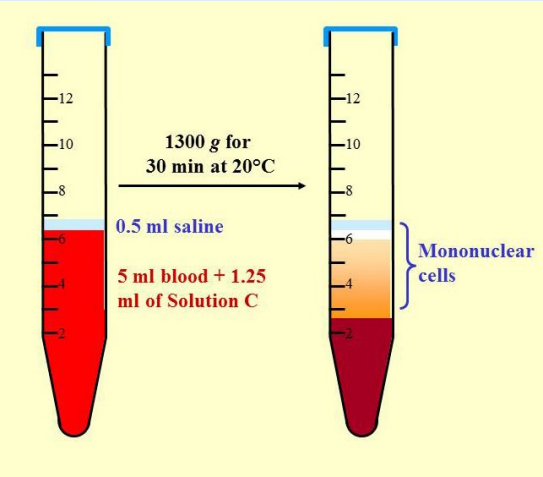


Isolation of mononuclear cells from rat, mouse and bovine blood

Standard human peripheral blood mononuclear cell (PBMC) isolation media such as Lymphoprep™ are less effective for the isolation of these cells from the blood of certain experimental animals. The density of the PBMCs from mice, rats and rabbits is apparently slightly higher than that from humans. Some commercial media simply address this problem by having a correspondingly raised density. This simple solution however fails to address the simultaneous problem that the density of the (PMNs) is the same. Thus although recoveries of PBMCs are satisfactory, contamination from PMNs can be significant.

The alternative strategy solves this problem by maintaining the density at 1.077 g/ml, while reducing the osmolality of the medium from 295 mOsm to 265 mOsm. The density of the osmotically-sensitive PBMCs is thus reduced to a value less than 1.077 g/ml. In this manner, the difference in density between the PBMCs and the PMNs is enhanced and the cells behave essentially the same as those from human blood.

PBMCs may also be isolated by flotation: the method involves adjustment of the density of the plasma of whole blood to approx. 1.078 g/ml by addition of a dense solution, which allows cells with a density lower than 1.078 g/ml to float to the surface during the centrifugation (see figure). This flotation strategy, for reasons

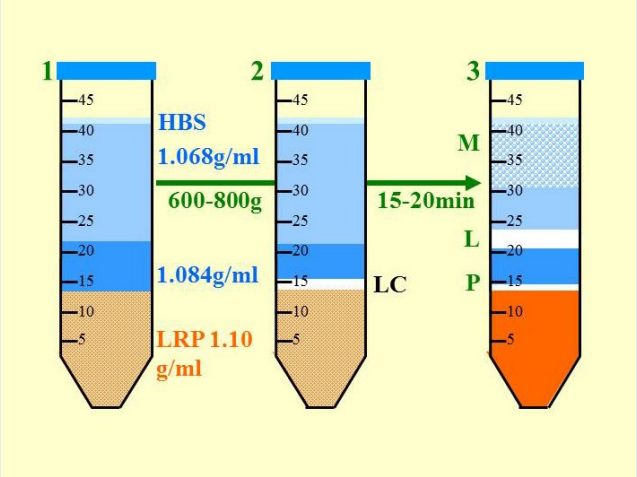


that are not clear, allows satisfactory separation of PBMCs and PMNs from other species without modulation of the osmolality. It seems not to be species-sensitive and has now been successfully applied to mouse, rat and bovine blood using OptiPrep™. The figure relates to Application Sheet C06.

For detailed protocols and references see Application Sheets C07, C08, C09 and C43 at: www.diagnostic.serumwerk.com

Isolation of human monocytes from a leukocyte-rich plasma

The efficacy of the OptiPrep™ flotation method relies on the slightly lower density of monocytes in combination with their larger diameter, which allows them to float more quickly through a density barrier than the lymphocytes. OptiPrep™ is added directly to a leukocyte-rich plasma (LRP) to raise its density to approx. 1.1 g/ml. When this suspension is centrifuged, the leukocytes, will float to the top of the plasma (see figure). In this way the leukocytes, particularly the mononuclear cells rapidly form a narrow band at the interface between the sample and a ρ = 1.084 g/ml solution layered on top. Monocytes float upwards through this layer and through a second low-density barrier (ρ = 1.068 g/ml) to form a broad band which extends downwards from the top of the 1.068 g/ml layer. Because lymphocytes tend to be smaller and more dense than monocytes, they float at a slower rate and in the time of the centrifugation, band predominantly at the bottom of the 1.068 g/ml layer. The separation is therefore more rate-zonal than isopycnic and is very sensitive to small changes in conditions. Whole blood can be used but the yields of cells are reduced and the amount of leukocytes which can be processed in a single tube is restricted by the need to form a sharp band of leukocytes at the top of the sample layer, the volume of this cannot therefore be increased significantly. Starting with an LRP permits the use of a higher concentration of leukocytes than is possible with whole blood. Small variations in run conditions from laboratory to laboratory may influence the success of the method. For example, reducing the density of the middle layer from 1.085 g/ml to 1.078 (or even 1.074)



may provide a more effective separation by arresting the flotation rate of the lymphocytes more than that of the monocytes.

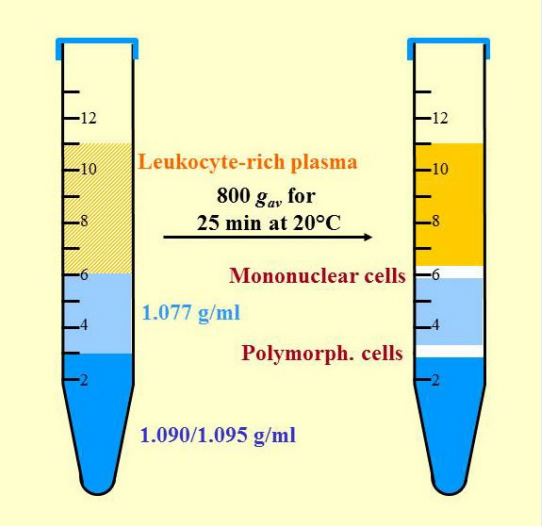
For a detailed protocol and references see Application Sheet C10 at: www.diagnostic.serumwerk.com

Isolation of polymorphonuclear cells from a leukocyte-rich plasma (LRP)

With the exception of basophils, polymorphonuclear leukocytes (PMNs) have a much greater buoyant density (>1.082 g/ml) than mononuclear cells but the denser neutrophils and all the eosinophils have a density that seriously overlaps that of the less dense erythrocytes. Using an LRP it is possible to use a discontinuous gradient to separate the PMNs from the mononuclear cells. This application was originally developed using Nycodenz® and has now been adapted also to OptiPrep™.

The figure shows the isolation of PMNs using two layers of density 1.077 and 1.090 g/ml which can be prepared from either Nycodenz® or OptiPrep™. The mononuclear cells will band at the sample/1.077 g/ml interface; the PMNs at the 1.077/1.090 g/ml interface. Most of the residual erythrocytes in the LRP will pellet. Sometimes the density of the lower layer is increased to 1.095 g/ml.

Using OptiPrep™ this method has been reported for a variety of studies using human blood.



For a detailed protocol and references see Application Sheet C12 at www.diagnostic.serumwerk.com

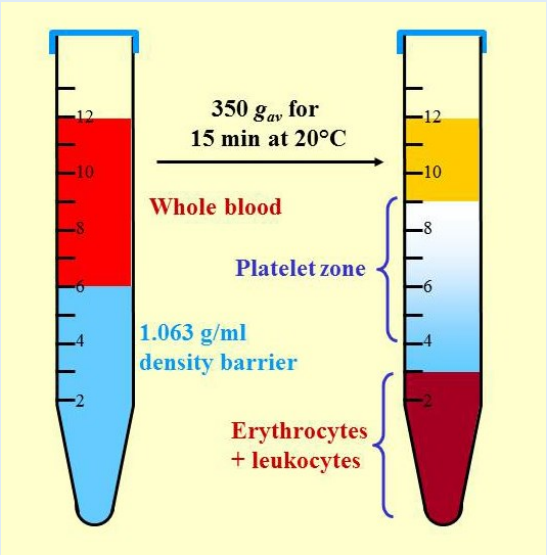
Isolation of platelets from whole human blood

Although platelet-rich plasma (PRP) is relatively easy to produce by centrifugation of whole blood, yields of platelets may be variable, because many of them are trapped within the erythrocyte layer. Although they can be recovered by washing these cells with isotonic saline, it is a general rule that to avoid activation of the platelets, the number of centrifugations and resuspensions should be kept to a minimum. Another problem is that aspiration of the PRP must be performed carefully to avoid contamination from leukocytes in the buffy coat which lies atop the erythrocytes.

To provide a highly purified platelet fraction from human blood, Ford et al layered whole blood over a density barrier of Nycodenz® (ρ = 1.063 g/ml) that allowed the erythrocytes and leukocytes to pellet during centrifugation at 350g. The platelets, because of their small size, sediment much more slowly: they were recovered as a broad band extending into the density barrier from just above the interface (see figure).

The platelets recovered from this density barrier method were used directly in aggregation studies, the Nycodenz® did not interfere with this process. The method has been adapted to OptiPrep™ and it is in this form that the method has been most widely used.

Several papers have confirmed the purity (by flow cytometry) and functional integrity of the platelets.



For a detailed protocol and references see Application Sheet C13 at: www.diagnostic.serumwerk.com

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