

OptiPrep™ Mini-Review MC03

Purification of platelets from whole blood and their removal from blood leukocyte preparations

- ◆ OptiPrep™ is a sterile 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml
- ◆ This Mini-Review principally provides (in Section 2) a bibliography of all those papers reporting the use of OptiPrep™ in the purification of platelets from both human and rodent blood and for their removal from leukocyte preparations. Section 1 briefly summarizes the advantages of using the OptiPrep™ methodology.

1. The OptiPrep™ technology

The routine method for the purification of platelets from whole blood is to centrifuge the blood at a g -force that will sediment the erythrocytes and leukocytes, while leaving the smaller platelets suspended in the plasma supernatant. While this is a simple concept it is technically very difficult; if the g -force is high enough to sediment all of the erythrocytes and leukocytes then many of the platelets will also be in the cell pellet; if it is sufficiently low to prevent the majority of platelets from pelleting then many of less dense leukocytes will also remain in the plasma supernatant. As a result the procedure needs to be repeated several times to recover more of the platelets from the pellet and to remove more leukocytes from the supernatant. It becomes a very tedious process.

In the one-step OptiPrep™ method, OptiPrep™ is diluted with a buffered saline to produce a solution of density 1.063 g/ml (lower than all of the leukocytes); an equal volume of whole blood is layered over it and centrifuged at 350 g for 15 min. The procedure is summarized in Figure 1. Typically 5 ml each of blood and the density barrier are used in a standard 15 ml tube. The separation is based on the much lower sedimentation rate of the platelets. The yields are approx. 90-92%.

The method is equally effective for removing platelets from previously purified leukocyte fractions, particularly human peripheral blood mononuclear cells (PBMCs) produced by centrifugation of blood over a density barrier of approx. 1.077 g/ml (e.g. Lymphoprep™ or Nycoprep™ 1.077). A common method of removing the platelets is to dilute the cell harvest with saline and to use the lowest feasible g -force to pellet the cells selectively (usually about 300-350 g for 5-10 min). This is then repeated at least twice more – like the preparation of platelets by differential centrifugation (see above) this is inefficient, tedious and detrimental to the cells. Instead the platelets can be removed in a single step by layering the diluted harvest over the 1.063 g/ml barrier.

The method is described in OptiPrep™ Application Sheet (C12); it may be accessed from the Index of the “Mammalian and non-mammalian cells” file on the OptiPrep™ Applications flash-drive or from the following following website: www.axis-shield-density-gradient-media.com (click on “Methodology”).

2. Bibliography

- ◆ The references are listed according to source of platelets and research topic. Within each section references are listed alphabetically by first author.

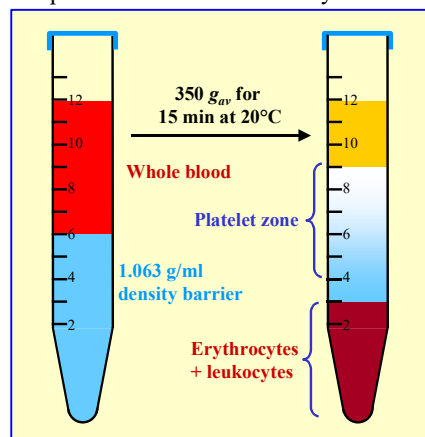


Figure 1: Isolation of platelets from whole human blood. (1) Equal volumes of blood and 1.063 g/ml density barrier layered in tube. (2) After centrifugation at 350 g for 15 min, platelets are harvested from the broad turbid band below the interface

Canine platelets

Isolation and purification

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Human platelets

Acetylcholinesterase expression

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Activation

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Natal, C., Restituto, P., Inigo, C., Colina, I., Diez, J. and Varo, N. (2008) *The proinflammatory mediator CD40 ligand is increased in the metabolic syndrome and modulated by adiponectin* J. Clin. Endocrinol. Metab., **93**, 2319-2327

α_2 -Adrenoreceptors

Piletz, J., Baker, R. and Halaris, A. (2008) *Platelet imidazoline receptors as state markers of depressive symptomatology* J. Psychiatr. Res., **42**, 41-49

Aggregation

Onselaer, M-B., Eeckhoudt, S., de Meester, C., Hue, L., Vanoverschelde, J-L., Bertrand, L., Oury, C., Beauloye, C., Horman, S. (2010) *Calcium and calmodulin-dependent kinase kinase beta signalling controls human platelet aggregation* Circulation, **122**, Abstract 17868

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AMP-activated protein kinase

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Antioxidative protection

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Calumenin - thrombospondin-1 binding

Hansen, G.A.W., Vorum, H., Jacobsen, C. and Honore, B. (2009) *Calumenin but not reticulocalbin forms a Ca^{2+} -dependent complex with thrombospondin-1. A potential role in haemostasis and thrombosis* Mol. Cell Biochem., **320**, 25–33

Carbon monoxide

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Carcinomas

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Serine residue, differential phosphorylation of

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α -Synuclein

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- Hansen, G.A.W.,** Vorum, H., Jacobsen, C. and Honore, B. (2009) *Calumenin but not reticulocalbin forms a Ca²⁺-dependent complex with thrombospondin-1. A potential role in haemostasis and thrombosis* *Mol. Cell Biochem.*, **320**, 25–33

Thrombocytopenia

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Primate bone marrow platelets

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Rodent platelets

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Platelet survival

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Alere Technologies AS

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