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 sediment-ation rate of the platelets. The yields are approx. 90-92%.

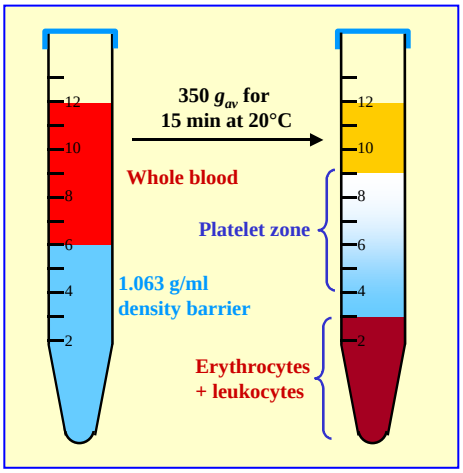

Figure 1: **Isolation of platelets from whole human blood**. (1) Equal volumes of blood and 1.063 g/ml density barrier layered in tube. (2) After centrifugation at 350 *g* for 15 min, platelets are harvested from the broad turbid band below the interface

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 in Section 2a alphabetically by first author; multiple first author papers are listed chronologically.
- The index in Section 2b lists alphabetically the species and principal area of analysis reported in each paper; the numbers against each entry indicate the relevant reference numbers from Section 2a.

2a. Reference list

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2b. Reference index

Canine platelets

Human platelets

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