Techniques for canine platelet purification

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Abstract
Several emerging techniques for the characterization of platelet function and thrombopoiesis, including proteomic and transcriptomic analysis, require samples with very low levels of leukocyte and erythrocyte contamination. However, to date, there are few protocols for canine platelet isolation in the current literature which address issues of contamination. We compared three methods for isolating platelets from whole blood. Ten and a half milliters of blood was obtained from healthy adult Walker hounds and each 3.5 mL sample was processed by one of three preparation protocols: washed platelet rich plasma (PRP), PRP/Optiprep™ gradient separation, and Histopaque®-1077/Optiprep™ combined gradient separation. In the first protocol, PRP was obtained by centrifugation of whole blood and collection of the third part of the plasma layer. The platelets in this fraction were then washed and resuspended in PBS. In the second protocol, the entire plasma fraction was collected following centrifugation and platelets were pelleted, washed, and resuspended in HEPES-NaCl. This suspension was then layered on Optiprep™, centrifuged, and platelets from the upper fraction were harvested and washed. In the third protocol, whole blood was layered onto Histopaque®-1077, centrifuged, and the top, erythrocyte-free fraction was washed and layered onto Optiprep™. After centrifugation, platelets were collected from the upper fraction and washed. Manual hemacytometer counts and flow cytometry (CD61 and CD45 labels) were used to quantify sample purity. Mean platelet percentage by manual count and mean percentage of CD61 positive cells were, respectively, 99.20±0.3% and 98.61±0.67% for washed PRP (n=4); 99.60±0.61% and 90.17±16% for PRP/Optiprep™ (n=3); and 99.98±0.02% and 99.47±0.21% for Histopaque®-1077/Optiprep™ (n=9). There was no significant difference in mean platelet yield among the three techniques. The Histopaque®-1077/Optiprep™ protocol had a significantly higher purity of platelets (P<0.05) and is thus likely more suitable for platelet purification from small samples.

Methods

<table>
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<tr>
<th>Protocol</th>
<th>Top fraction washed in HEPES-NaCl</th>
<th>Top fraction washed in HEPES-NaCl</th>
<th>Put on top of Optiprep™ gradient (10%, 13%, and 17% from top to bottom)</th>
<th>Layered on top of Histopaque®-1077 (HP)</th>
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<tbody>
<tr>
<td>PRP/Optiprep™</td>
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<tr>
<td>HP/Optiprep™</td>
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<td>Washed platelet rich plasma (PRP)</td>
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Results

There was no significant difference in mean platelet yield among the three techniques. The HP/Optiprep™ protocol had a significantly higher purity of platelets (P<0.05).

Conclusions
The results show that of the three compared techniques, the Histopaque®-1077/Optiprep™ protocol is more efficient in isolation of pure canine platelets from a small amount of whole blood. Thus, this technique is likely more suitable for platelet purification from small samples obtained in a clinical setting.

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References

Introduction
Several emerging assays for the characterization of platelet function and thrombopoiesis, including proteomic and transcriptomic analysis, require samples with very low levels of leukocyte and erythrocyte contamination. Furthermore, the isolation protocol has to permit minimal platelet activation and maintenance of functionality. ¹, ² Purification of human platelets using density gradient fractionation is well established and shown to yield pure and functional platelets. ³ These purification techniques are facilitated by the fact that in human medicine high numbers of platelets from concentrates and platelepheresis are easily accessible. However, in veterinary medicine these platelet products are usually not readily available and, because of the smaller size of the patient, it is not always possible to collect large volumes of blood, even in research animals. Additionally, in clinical settings, it is important to develop a technique that will allow isolation of platelets from small blood volumes (3-20 mL). Unfortunately, protocols describing canine platelet isolation addressing contamination issues are scarce in the current literature.

Here we describe and compare three methods of purifying canine platelets from 3.5 mL of whole blood.

Objectives
The objective was to compare techniques of isolation of platelets from canine whole blood and identify the technique that optimizes platelet recovery and purity.