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# Preparation of Platelet Rich Plasma in Dogs – Standardisation of Double Centrifugation Method

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**ABSTRACT:** Several studies demonstrate Platelet Rich Plasma (PRP) is an effective promoter of tissue healing due to the high concentration of growth factors in regenerative therapy. The aim of the study was to determine the optimal relative centrifugal force and spin time for preparing PRP from canine blood using the double centrifugation method and to standardise easy to adopt and cost-effective protocol for maximum and consistent yield of platelets from whole blood in canines. The whole blood samples from six healthy dogs were collected in 3.8% w/v sodium citrate solution at the rate 1ml/10ml whole blood. The collected blood was subjected to double centrifugation in various RCF- Time combinations. After first centrifugation, a portion of the top supernatant plasma fraction adjacent to buffy coat that contained platelets was again subjected to a second centrifugation at various RCF- Time combination. The platelets in whole blood and concentrated fractions were counted and compared to identify the most suitable method that can be used to prepare PRP with maximum platelet count. The optimal RCF-Time combination which yields PRP with highest platelet count was 1000g, 5 min for first spin followed by 1500g, 10 min for second spin in dogs.

**KEYWORDS:** whole blood, dogs, 3.8% w/v sodium citrate, double centrifugation, RCF- Time combinations, Platelet Rich Plasma (PRP)

Running Title: Preparation of platelet rich plasma in canines

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## I. INTRODUCTION

Platelets - also called thrombocytes, with approximately 2µm diameter, are nucleated cytoplasmic fragments of megakaryocytes, formed in the bone marrow. Platelets perform a variety of important functions, including repairing tissue damage, preventing blood loss through coagulation, secreting proteins, cytokines, and other mediators, inducing tissue regeneration through cell migration, proliferation, and angiogenesis, and preventing infections through antibiotic activity. Anti-inflammatory, immunomodulatory and analgesic properties are also present. More than 30 bioactive proteins are found in platelets, several of which play an essential role in tissue healing or hemostasis (Dhurat, R. and M.S. Suresh, 2014).

PRP is blood plasma with a high concentration of platelets, often two to four times higher than blood plasma. PRP provides higher concentration of essential growth factors (GF) including Vascular endothelial GF (VEGF), platelet-derived GF (PDGF), transforming GF (TGF), fibroblastic GF (FGF), epidermal GF (EGF) and insulin-like GF (IGF), and hence has a major role in wound healing (Carr *et al.*, 2016). The use of non-transfusional haemo-components for tissue healing has gained increasing popularity for the treatment of soft tissue and musculoskeletal lesions in human and veterinary medicine. Among the haemo-components, PRP is a good adjunctive therapy for the treatment of orthopedic



and soft tissue conditions (Faillace *et al.*, 2017). PRP, sometimes referred to as platelet concentrate, platelet rich fibrin matrix, and platelet rich growth factors, is a portion of plasma with a platelet concentration above the baseline. But the PRP is being prepared by kits which are imported and very costly. Blood component separators can be used for separating platelets in large volumes, but the establishment cost of the same is extremely costly. Commercial products of platelet rich plasma are available abroad, for treatment of corneal wound healing. But not available in India. A search of published literature reveals an interesting fact that small amounts of platelet rich plasma are economically prepared from whole blood of humans, in ordinary laboratories with basic minimum infrastructure. But there is lack of cost-effective procedures that could be adopted in all basic clinical settings, for in-house preparation of PRP from whole blood of animals, as that described in human literature. The aim of this study was to identify optimal relative centrifugal force and spin time for the preparation of PRP from blood of dogs using a double-centrifugation method and to standardize easy to adopt and cost-effective protocol for maximum and consistent yield of platelets from whole blood of dogs.

## II. MATERIALS AND METHODS

Six clinically healthy dogs aged between 2-4 years of age having normal blood picture presented at TVCC, Pookode were selected for this study. Their body weight ranging from 10-30kg. Three syringes of 12.5 ml size - each loaded with 1ml of sodium citrate (3.8% w/v) were taken. 10-ml of whole blood was drawn from cephalic vein into each of these syringes to collect 30 ml of whole blood for the preparation of PRP. The blood was then mixed and 5-ml each of the blood samples were transferred to five centrifuge tubes of 15-ml size. A double centrifugation method was adopted, at room temperature for all the samples collected. After the first centrifugation, the whole plasma fraction along with buffy coat (PRP1) was transferred to an empty tube, and its volume was measured. After mixing this fraction well, a 1-ml aliquot was taken for CBC. The PRP1 was subjected to second centrifugation step using their respective relative centrifugation force (RCF)-time combinations (Table 1). For first spin different RCFs 100, 250, 500, 1000 and 1500 g for 5 min were tried. For the second centrifugation PRP-1 fraction obtained from each first spins were divided into 1- ml aliquots, which were again centrifuged in eppendorf tubes at room temperature at 250, 500, 1000, 1500 and 2000 g for 10 min (Shin *et al.*, 2017). The platelet and leucocyte counts were measured before and after each centrifugation using an auto haematology analyser (Mindray BC-2800Vet) of all samples. The results were statistically analysed using paired t test.

**Table 1.** Various RCF-time combination used for the preparation of PRP

Run	First centrifugation		Second centrifugation	
	RCF (×g)	Centrifugation time (minutes)	RCF (×g)	Centrifugation time (minutes)
R1	100	5	250	10
R2	250	5	500	10
R3	500	5	1000	10
R4	1000	5	1500	10
R5	1500	5	2000	10

## III. RESULTS AND DISCUSSION

The platelet count following first and second centrifugation was significantly high when compared to the base value. Observations obtained from our study on whole blood samples from dogs is noted in table below (Table 2). The findings clearly show PRP with the highest platelet count was obtained after the first centrifugation at 1000g for 5

minutes and the second centrifugation at 1500g for 10 minutes. The findings in this study were in accordance with Shin *et al.* (2017). The sampled blood had an average platelet count of  $169 \times 10^3/\mu\text{l}$ . After first centrifugation, the count increased to an average of  $294.5 \times 10^3/\mu\text{l}$  which is 1.7 times the base value. Second centrifugation from PRP1 fraction showed a platelet count of  $385 \times 10^3/\mu\text{l}$ , which was 2.2 times increase from base value. PRP products must have at least  $300 \times 10^3$  platelets/ $\mu\text{l}$  to be therapeutically effective, which is 2 to 3-fold higher concentration than whole blood. The findings in the current study were similar to those reported by Shin *et al.* (2017).

Regarding leukocyte count, there was a significant difference between whole blood and second centrifugation while there was only negligible variation between base value and first centrifugation. WBC concentration in the final PRP was 2-fold lower than the normal range.

**Table 2.** Platelet concentration in whole blood, first and second centrifugation step (values expressed in mean  $\pm$  standard deviation)

Whole blood – platelet concentration Base Value (Mean $\pm$ SE) ( $10^3/\mu\text{l}$ )	RCF-Time Combinations(Run)	First Centrifuge platelet concentration (Mean $\pm$ SE) ( $10^3/\mu\text{l}$ )	Second Centrifuge platelet concentration (Mean $\pm$ SE) ( $10^3/\mu\text{l}$ )
169 $\pm$ 17	R1	203 $\pm$ 19	280 $\pm$ 58
	R2	185 $\pm$ 71	315 $\pm$ 17
	R3	179 $\pm$ 17	343 $\pm$ 23
	R4	294.5 $\pm$ 8.5	385 $\pm$ 10
	R5	189 $\pm$ 13	289.5 $\pm$ 8.5
Total 169 $\pm$ 56		210.1 $\pm$ 18	322.5 $\pm$ 16

Paired t test showed there was significant increase in platelet count following first centrifugation ( $t=-2.437$ ;  $df=9$ ;  $p < 0.05$ ) and second centrifugation ( $t=-2.437$ ;  $df=9$ ;  $p < 0.001$ ) when compared to whole blood. Platelet counts were maximum in the RCF (g) – Time (min) combination 1000 g for 5 min (first spin) followed by 1500 g for 10 min (second spin).

#### IV. CONCLUSION

Our aim to obtain PRP with highest platelet concentration and to standardize this optimal RCF-Time combination for preparing PRP in canines were accomplished. The standardized RCF(g)– Time(min) combinations with maximum platelet yield in case of dog was 1000g, 5 min for first spin followed by 1500g, 10 min for second spin. This optimised method can readily be carried out in any veterinary clinical setting, as it does not require any expensive equipment or high technical skill and it can be explored in regenerative therapy.

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#### Conflict of interest

The authors declare that they have no conflict of interest.

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