Freeze-Thawing PRP & ACS

How to: Freeze-thaw PRP (platelet rich plasma) and ACS (autologous conditioned serum) for leukocyte reduction or to use as a treatment for joint tissue regeneration

Equipment required

Tubes containing PRP or ACS
0.2-micron filters (2-4 required)
LL Neg connector
3-inch blunt cannula
20ml syringe
2ml syringe and syring cap
Transfer needle (any guage)
Freezer at -18 degrees

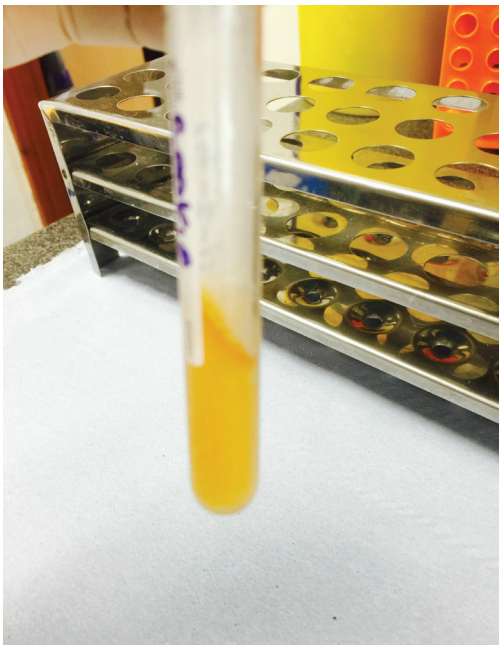

Figure 1
Frozen serum or PRP

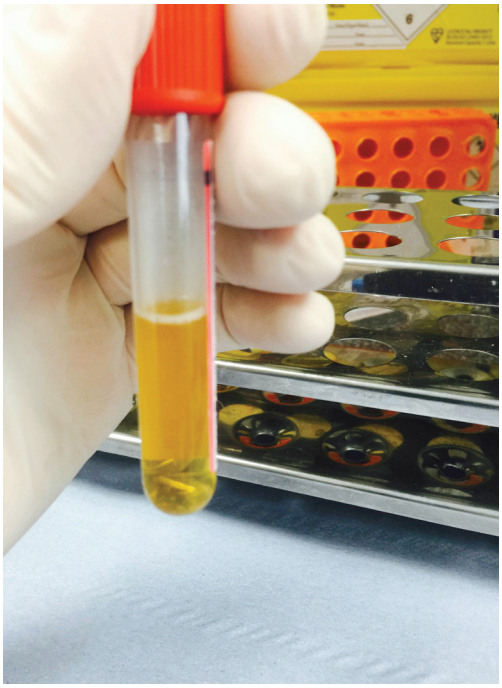

Figure 2
Thawed serum or PRP

Introduction

The heterogeneous clinical outcome reported in the literature on PRP treatment for joint tissue regeneration reflects the lack of guidelines regarding the use of platelet concentrates, starting from their production up to their clinical application. The increasing awareness on the need for PRP standardisation is shown by the numerous biological studies investigating the role of each PRP variable on the healing potential of platelet concentrates. Among these factors, the possibility to store PRP and the effects of freeze/thawing PRP remain a controversial point. Although some researchers avoid freeze/thawing, fearing deleterious effects on platelet function and GF release, others consider it to be a procedure that physically activates PRP and further leukocyte destruction as a result of freezing leads to reduction in Il-1β, pro inflammatory cytokine.

Roffi et al 2014, showed that growth factor release from platelets α-granules in frozen PRP both the immediate and at 7-day release were lower with respect to that of the fresh preparation, but without affecting the ability of PRP to induce proliferation and ECM production in chondrocyte and synoviocyte cultures. The only significant difference was detected for synoviocyte HGF expression, which was higher in the freeze/thawed PRP induced cells, thus suggesting that PRP cryopreservation is a safe procedure, which sufficiently preserves PRP quality and its biological activity. Furthermore, with regard to pro-inflammatory cytokines, the immediate release of IL-1β does not significantly differ in fresh PRP with respect to that of frozen PRP. The absence of an increment of IL-1β detected in frozen PRP might be explained by the fact that freezing and thawing leads to leukocyte destruction (which can produce inflammatory cytokines directly and indirectly by platelet stimulation), resulting in a reduction in WBC number, preventing the "de novo" synthesis of this cytokine.

"PRP or ACS must be thawed thoroughly before processing to avoid clogging the filter"

Warning
This process involves the preparation of a substance intended for administration to a patient. Sterility and aseptic technique is paramount. Nupsala cannot be held responsible for operator error.

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Technique

- Collect PRP or ACS via manufacturer's instruction and collect enough for the treatment. Excess product can remain frozen for future use;
- Transfer the PRP or ACS to a sterile vaccutainer with no added substance (red top) 4ml in each tube;
- Place the tube(s) in a zip lock bag or container to keep the tubes for that patient together;
- The tubes are then placed in the freezer for a minimum of 45 mins or until completely frozen (Figure 1);
- Long term storage of PRP or ACS should not exceed 6 months

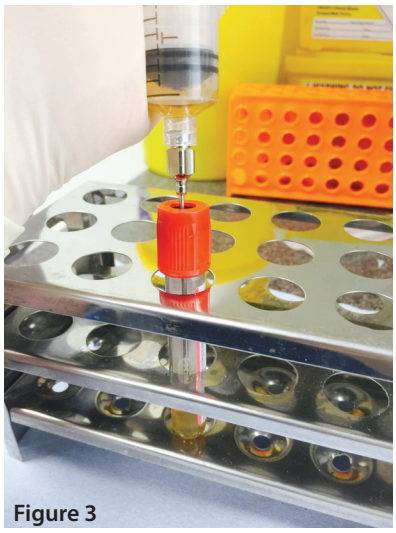
Figure 3


Figure 4

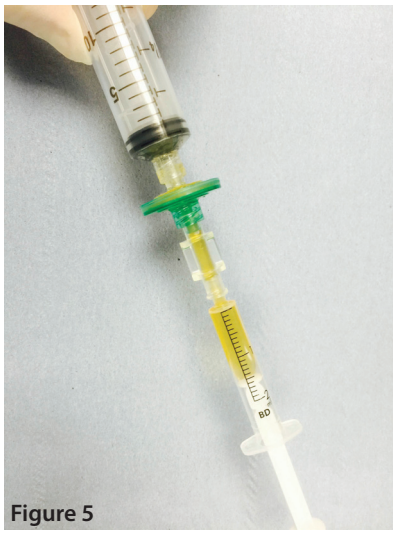
Figure 5

- Remove from the freezer. One tube will give approximately 2ml of final preparation.
- Thaw the frozen PRP completely – holding in your hand for 10mins is normally enough (Figure 2).
- Withdraw the PRP (Figure 3) and pass through a 0.2micron filter (Figure 4 & 5) to give the final preparation. If the filter becomes blocked, replace with new filter. Ice crystals can block the filter so ensure the tube is completely thawed.
- Place a cap or needle on the syringe (Figure 6)
- Once the final preparation is produced it should be used immediately.

Figure 6

References

C. T. FJORDBAKK, G. M. JOHANSEN†, A. C. LØVÅS, K. L. OPPEGÅRD and A. K. STORSET (2014) Surgical stress influences cytokine content in autologous conditioned serum Equine Vet J.

Hraha, T.H., Doremus, K.M., McIlwraith, C.W. and Frisbie, D.D. (2011) Autologous conditioned serum: the comparative cytokine profiles of two commercial methods (IRAP and IRAP II) using equine blood. Equine Vet. J. 43, 516-521.

Nupsala *offers training and advice to vets using ACS treatment for the first time. Please ask for more details.*

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