NUPSALA
Veterinary Services

Your partner in regenerative medicine

Processing ACS Using Z Serum Tubes

Equipment required

5 x sterile Z serum clot tubes (gas sterilised)
4 x red top plain vaccutainer (gas sterilised)
0.2-micron filters (2-4 required)
LL Neg connector
3-inch blunt cannula
20ml syringe
Transfer needle (any guage)
Bench top incubator (37 °C)

Figure 1
Sterile Z serum tubes

How to: Use Z serum tubes to process ACS (autologous conditioned serum)

Introduction

In a recent study by the Norwegian School of Veterinary Science (Fjordbakk *et al.* 2014) it was shown that serum from blood incubated in plastic vacutainer tubes containing Z Serum Clot Activator contained similar levels of the cytokines and growth factors investigated as serum incubated in two specialised ACS containers. These results corroborate those of a previous study (Hraha et al 2011) and indicate that cytokine production may be a consequence of whole blood incubation rather than an effect of specialised ACS containers. Z Serum clot activated tubes were capable of producing the same output as specialised ACS containers if not more.

Technique

- Using the Z serum tubes (Figure 1) collect 7.5ml of blood in each of the four tubes.
- Write the name of the patient and time of blood collection on each of the tubes and place in the incubator as soon as possible.
- Keep the tubes in an upright position while transporting and in the incubator (Figure 2).
- Incubation starts once the clot is formed. ACS preparation should be performed no later than 8hrs after the time of blood collection.
- After 8hrs remove the 5 tubes from the incubator keeping them in the tube rack (Figure 3). Place the rack on a clean, sterile surface.

Figure 2
Z serum tubes in the incubator

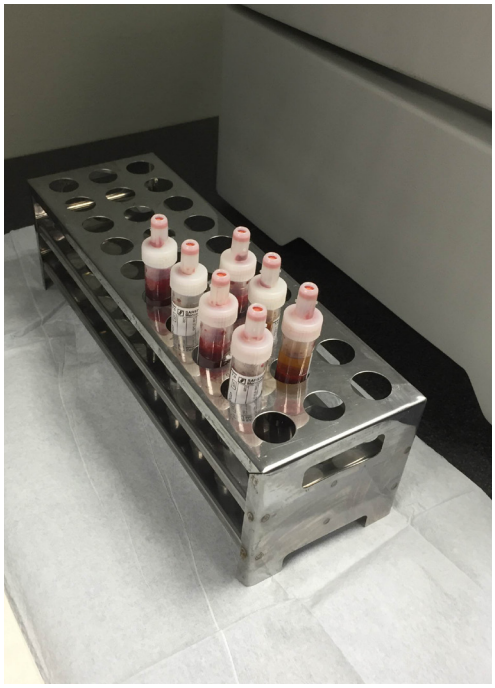

Figure 3
Z serum tubes in a tube rack

"Preparation of ACS must be performed no later than 8 hours after the time of blood collection"

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.

Processing ACS Using Z Serum Tubes

- Using the Harvest SmartPReP 2 centrifuge, use two counter weights of equal size. Place a serum tube into each of the center tube retaining ports (Figure 4). Close the lid and spin on the PRP cycle. If not using the Harvest centrifuge the g-rating will need to be calculated to give RPM. (2500g for 10min). Call the Nupsala office for help with g/RPM calculations (+44 (0)1865 922227). Once spun, remove the serum tube and place back into the rack (Figure 5).
- Using the 3-inch cannula attached to a 20ml syringe, penetrate the red rubber bung at the top of the tube, keeping the tube in an upright position (Figure 6). Extract all available serum (c2.5ml per tube – 4 tubes giving a total volume of c10ml).

Figure 4

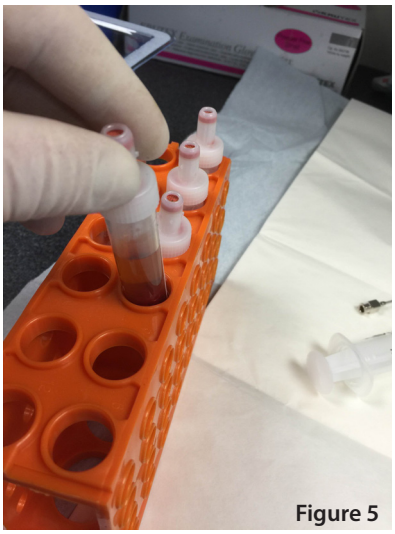
Figure 5


Figure 6

- Place a 0.2-micron filter on the 20 ml syringe containing the serum. Place the needle on the end of the filter (Figure 7). Penetrate the top of a red plain vaccutainer and transfer 2.5ml (or equal quantities /4) of the plasma, having passed through the filter, into the vaccutainer (Figure 8). Do this for all four vaccutainers. Label each vaccutainer and place in a clear zip-locked bag with the patient's ID clearly labelled on the bag. Place the bag containing the tubes in the freezer.
- ACS should be used 10 days apart. Remove a vaccutainer containing serum from the freezer and thaw thoroughly (30 mins at room temp). Using a 3-inch cannula and a 5ml syringe remove the thawed serum from the red vaccutainer (Figure 9).
- Remove the cannula and place a 0.2-micron filter onto the syringe. Place a L-L connector on the remaining filter port and connect a 5ml syringe to the L-L connector. Transfer the serum from one syringe to the other via the filter (Figure 10). Remove the syringe now containing the serum and place a stopper or needle onto the syringe. The serum is now ready for administering to the patient.

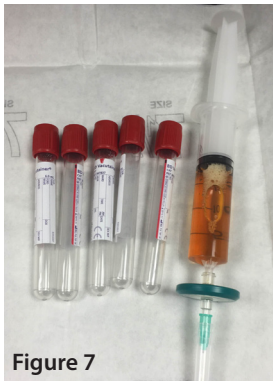
Figure 7


Figure 8


Figure 9


Figure 10

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.***