

## ADDITIONAL TIPS for Preparation of Autologous Conditioned Serum

PLEASE USE with DIRECTIONS FOR USE: This includes some tips that may help make this process easier.

### Per kit you should need:

5 - 6cc luer lock syringes

Sterile caps for 6cc Syringes or Sterile capped injection needle for IA treatment procedure

5 – 20 gauge needles OR Luer caps

1 - Spinal needle- 18G, 3.5"

1 to 5 - .22 micron millipore filter

1 – Female to female luer lock adapter

1 - 20ml luer lock syringe

1. Use the same preparation techniques as you would when you put in an IV catheter, There is no need to shave the jugular site.
2. ALWAYS wear sterile gloves during this whole process.
3. Take your time and draw SLOWLY to avoid hemolysis. Also fill the syringe a full as possible to maximize the yield of treatment serum.
4. Label syringe with Horse's name & client name and date processed.
5. Be very gentle when with syringe because we want to minimize the amount of cell lysis that will occur to the redblood cells. Because red blood cell particulate can pass through the millipore filters. Maintain syringe at Room temperature until placed in incubator.
6. Make sure to get the kit into the incubator within 2 hours of collection. Incubate for 16-23 hours at 37°C. Place kits on side red cap down, not vertical. [this will make the retrieval of serum much easier because of where the blood clot will form.] DO NOT incubate LONGER than 24 Hours, if so, discard syringe and start over.
7. Make sure the centrifuge is preset at 10 minutes and 4000 rpm. Also when centrifuging, make sure to counter balance. After Centrifugation: ROOM needs to be Free of Dust and Airborne particulate. CLEAN as possible!!! ALWAYS work within a Sterile Field (ie. Sterile gloves field is OK) Helpful the FIRST time to have assistance with this process. Best to harvest the serum right after centrifuging (maximum delay time after centrifuging the syringe would be 60 minutes)
- 8A) Construct needs to be placed vertically in a rack or clear container with white portal open. It is necessary to change gloves after this. Connect the spinal needle to 20cc syringe first.
- 8B) Keep the tip of the spinal needle shallow within the serum. This will help to keep the serum/RBC interface undisturbed when you start to aspirate the serum.

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8C) Try to get as much serum as you can without disturbing the RBC interface, it has been our experience that you can easily get 12cc - 16cc of serum.

DRAW until 3/8" of serum is left in original kit. DO NOT go into the RBC! You can always go back after this remaining serum once you have transferred the current serum to the 6 cc syringes.

If the interface is disturbed and there are a lot of red blood cells clouding up the serum, it is acceptable to centrifuge the kit again. (keep in mind the counterbalance weight will need to be adjusted)

8D) Once you have your unfiltered serum, we can now break it down into aliquots to be used for the IA treatment injections. (4 cc's typically per treatment)

Place the Millipore filter on the 20cc syringe, and the female to female luer lock adapter on the other side of the filter. You can now take your 6cc syringe and directly lock it into the filter. Most veterinarians break the aliquots into 4cc doses. (Treating a stifle will require 8 cc's of serum per treatment; for the tarsus bottom joints 2 cc & tibiotarsal 4cc)

USE IMMEDIATELY for treatment or FREEZE the treatment serum 6 cc syringes. Do NOT microwave to defrost – the serum will thaw in 10 minutes at room temperature, or thaw in hand. Keeps in freezer for up to 7 months at -18°C.

STORAGE: When freezing remaining aliquots to be used at a later date, make sure they are either capped with a luer lock cap, or a sterile needle will suffice. Make sure that each syringe is properly labeled with owner and horse information as well as the date that the blood was processed. They can then be placed in a ziplock bag to keep them all together within the freezer. OR, labelling the bag may also be beneficial for easier retrieval. Frozen treatment serum will be viable up to 7 months at -18°C.

### FREQUENTLY ASKED QUESTIONS:

*Q: How should the syringe be transported after drawing blood from the patient?*

A: Room temperature is best

*Q: After drawing the blood, how long do I have before it needs to be placed in the incubator?*

A: Maximum 2 hours. Blood may be transported in a portable incubator as needed. Transport should be gentle and should be avoided if possible.

*Q: What can be done if there is a fibrin clot in the middle of the serum layer?*

A: Sometimes a fibrin clot forms in the middle of the serum layer and extends to the RBC layer of the syringe. If this occurs, simply bend the needle slightly. As the needle pierces the stopper, you can then bend the needle through the clot and into the serum. Draw the serum normally.

*Q: What happens if you incubate longer than 24 hours?*

A: If you incubate for more than 24 hours, you will need to discard the syringe and start over.

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*Q: How long can the syringe sit AFTER centrifuging if unable to get to it immediately for transfer to the 6cc syringes?*

A: Should be transferred immediately, discard after 60 min

*Q: How soon after thawing should the treatment serum be used?*

A: IMMEDIATELY. It can be re-frozen if absolutely necessary, do not re-freeze if thawed longer than 60 minutes.

*Q: Treatment Protocol requested: recommended to be 1X per week for three weeks initially*

A: Best results have been observed with an initial treatment schedule of 3 weekly IA treatments. Additional treatment(s) at discretion of veterinarian.

*Q: Can HA and/or antibiotics be used during treatment?*

A: Do NOT add anything to the IRAP

*Q: Should the horse receive time off during the initial treatment period or what level of activity/rest is recommended?*

A: At least ONE day of rest (stall rest) after treatment is recommended, ideally a period of 4 days rest AFTER each treatment is administered. The more severe the condition being treated, the longer the period of rest indicated between treatments.

*Q: Can you wrap the leg after injection?*

A: At the discretion of the veterinarian

*Q: When should veterinarians begin to see improvement in the horse treated?*

A: Depends on the case and severity of the OA: response to treatment has been reported to occur the day after the first treatment, and in some cases, improvement wasn't observed until after the third treatment

*Q: Is it possible to treat too often?*

A: No because of what you are injecting, but weigh risk of infection.

*Q: Any recommendation on the 4th treatment timing?*

A: As needed, typically around 4 – 6 months after completing the initial 3 treatment series. Since the frozen serum is viable up to 7 months, you would want to use the treatment serum prior to the end of seven months from harvest.

*Q: Will insurance cover this treatment procedure?*

A: Consult your insurance company for applicable coverage guidelines.