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Instructions for Processing Blood Samples for Progesterone Testing in Dogs

Thank you for helping with the ovulation timing for this bitch. Please read the following instructions for processing blood samples. Please call if there are any questions about processing or if you are unable to perform any of the necessary steps.

- 1) Bitches should be fasted for at least 6 - 8 hours prior to blood draw to prevent lipemia.
- 2) Blood should be drawn using as clean a stick as possible to prevent hemolysis from occurring. If multiple sticks are required then a clean needle and syringe should be used.
- 3) Preferred venipuncture sites for bitches that are having surgical implants are the jugulars or saphenous veins whenever possible to save the cephalic veins for anesthesia.
- 4) The Vacutainer® tube stopper should be removed along with the needle and the blood placed directly into the tube – no vacuum should be applied to the blood as it is inserted in the Vacutainer® to prevent hemolysis from occurring.
- 5) Clotting time and centrifugation time are critical to accurate progesterone results. Please use timers whenever possible to prevent delays in processing.
- 6) A **plain, glass red top** tube is preferred. The new red top tubes with silicone added to enhance clotting time should not be used as they falsely lower progesterone concentrations. Plain, plastic red top tubes are not preferable to glass but can be used if glass tubes are not available.
 - a. The rubber stopper should be removed and the needle removed from the syringe before expelling the sample into the tube to prevent hemolysis.
 - b. Blood should be allowed to clot for 30 minutes (no longer than 60 minutes).
 - c. The blood clot should be stirred using a wooden applicator stick
 - d. The blood should be centrifuged immediately for 10 minutes at a minimum of 2500 g (standard blood centrifugation speed).
 - e. The serum should be removed immediately and placed in another clean glass red top tube (or non-treated plastic red top tube – no silicone treated tubes should be used). It is *imperative* that there be **NO** RBC transferred with the serum. If RBC are accidentally aspirated with the serum, the sample should be re-centrifuged and the serum drawn off again.
- 7) **Serum separator tubes are NOT recommended.** If for some reason only serum separator tubes are available, they can be used following the protocol below but the timing of clotting and centrifugation and the prompt removal of the serum is even more critical to prevent erroneous results.
 - a. The rubber stopper should be removed and the needle removed from the syringe before expelling the sample into the tube to prevent hemolysis.
 - b. The stopper is replaced and the tube inverted so the blood does not contact the serum separator gel.
 - c. The blood should be allowed to clot for 15 minutes (use a timer).
 - d. The tube should be immediately placed in the centrifuge.
 - e. The blood should be centrifuged for 4 minutes at a minimum of 2500 g.
 - f. The serum should then be immediately removed and placed in a clean, glass red top tube (or non-treated plastic red top tube or plastic serum tube – no silicone treated tubes should be used).
- 8) Under **NO** circumstances should blood be sent to the lab without centrifugation and removal of serum from the RBC or serum separator gel. Both RBC and the SST gel leach progesterone from the serum, altering the results.
- 9) Serum should be refrigerated until processed at the lab. If serum will sit for more than 2 days before processing it should be frozen.
- 10) Samples for LH testing should be handled in a similar manner as described above but should be frozen after removal of serum until needed.
- 11) If you can get a vaginal cytology at the same time as the blood samples, you can either read them yourself if you are comfortable doing that, or mail them to me regular mail (should arrive next day if in Portland area) and I will read them. Please do not send them to the lab for interpretation (they take too long).