

RABBIT BLOOD COLLECTION PROCEDURE

This procedure may be used to collect large quantities (10-30 cc) of blood from rabbits, using aseptic technique. In rabbits, a guide to safe blood volume depletion is to collect no more than 5 ml of whole blood per kilogram of body weight every 3 weeks (larger volumes or shorter collection interval need to be discussed with a DLAR veterinarian). DLAR veterinarians are available for training in or consultation regarding any aspect of blood collection in rabbits.

1. **ASSEMBLE EQUIPMENT** - using the "Rabbit Blood Collection Equipment" list as a guide, gather the equipment.

2. **SEDATE THE RABBIT**

The rabbit is more comfortable, and the procedure is easier to perform, when the rabbit is sedated with Torbugesic (butorphanol)(1 mg/kg) and acepromazine maleate (1 mg/kg). Vial tops must be thoroughly disinfected before inserting a needle for withdrawal. Both drugs can be drawn up in the same syringe, and then given subcutaneously. Allow approximately 10 minutes for the drugs to take effect before proceeding. This combination of drugs will cause dilatation of the auricular artery, therefore the use of xylene should be unnecessary.

3. **PLACE THE RABBIT IN THE RESTRAINER**

4. **PREPARE THE EAR**

Choose the ear you want to bleed; if this is a repeat procedure, alternate ears. Next, to disinfect the sampling site, wipe the central artery area thoroughly with an alcohol swab.

5. **CANNULATE THE ARTERY**

It is best to insert the needle into the artery as distally (toward the tip of the ear) as possible. If another needle insertion is required, the needle can be moved proximally about 1" for the insertion site.

A. Connect a 21 ga. E-Z infusion set to a 10 or 20 cc syringe (the syringe and the infusion set tubing must be filled with anticoagulant if plasma is to be harvested). About 2/3 the way up the central artery, insert 1/2 the length of the needle into the artery, with the tip of the needle pointing toward the base of the ear. When the needle is in place, tape the infusion set to the ear.

OR

B. Insert a 20 ga. needle into the artery (about 2/3 the way up the central artery, with the tip of the needle pointing toward the base of the ear). Collect blood into a vacutainer, or open tube/bottle. The vacutainer or open container is used if blood is being collected for serum harvest.

6. **COLLECT THE BLOOD**

A. The blood should begin to flow immediately through the tubing. Gently draw on the syringe to collect the blood. Once the syringe is full, change syringes by disconnecting from the infusion set or needle hub.

OR

B. If a vacutainer is used, excess suction (a normal occurrence with vacutainers) may cause a problem (collapse of the blood vessel). Repositioning the needle, or gently massaging the artery, pushing blood toward the tube, may help. Massaging may also increase blood flow.

C. Note that rough handling of blood, such as excessive suction or the forcing of clotted blood from a syringe, will cause hemolysis. This can interfere with the serum or plasma fractions needed.

7. **TROUBLE SHOOTING**

Once you have inserted a needle into the artery, you must establish a flow of blood into the collection tubing or syringe with 15-30 seconds. If blood flow has not been established within 30 seconds, most likely blood has clotted in the needle tip and you need to try a new site using a new needle.

Excessive suction on the syringe will cause the artery to “fade out,” and blood will cease flowing. Changing the position of the ear, inserting the needle further, or withdrawing the needle partially, may aid in restoring blood flow through the needle. If blanching of the artery occurs, decreasing the vacuum or the suction and/or repositioning the ear should solve the problem.

When a clot forms at the tip of the needle, it is best to either insert the needle further or withdraw the needle partially. If a clot forms in the tube or needle that cannot be remedied, you will probably need to remove and discard that infusion set, and start over, at a lower position on the artery (or the other ear) with a new infusion set and syringe.

8. **FINISHING UP**

Once the blood has been collected, remove the infusion set or needle from the artery, and hold off the artery with gauze until bleeding has stopped (this may take 2-5 minutes). Then clean drops or smears of blood from the ear with hydrogen peroxide. If the circulation in the ear seems restored and there is no bleeding, return the rabbit to the cage. Check the rabbit later in the day to make sure there is no further bleeding.

9. **TERMINAL EXSANGUINATION AND EUTHANASIA**

DLAR technicians are available to perform this service. Contact a DLAR veterinarian for information. Training is available, through DLAR veterinarians, for researchers and qualified research technicians. Appropriate anesthetics and techniques for this procedure differ significantly from survival ear bleeding methods.

10. **DRUG ACQUISITION**

You will need to submit a protocol modification to purchase and use the specified drugs, unless they were specified in the original animal care and use protocol. Controlled substances (e.g., Torbugesic), must be obtained by coming to DLAR and signing a controlled substances issue form. The PI or other designated researcher (not graduate students) must sign the form in person. Proper record keeping for controlled substances is mandated by law.

RABBIT BLOOD COLLECTION PROTOCOL

RECOMMENDED BLOOD VOLUMES

Per information extracted from McGuill, M.W. and Rowan, A.N., "Biological Effects of Blood Loss: Implications for Sampling Volumes and Techniques," *ILAR News*, Vol. 31(4), Fall 1989, pp 5-20, the following is provided:

Recommendations for a Single Blood Sample:

- a. Limit a single sampling to 15 % of the total blood volume and allow a 30-day recovery period. Up to 20 % of the blood volume may be feasible in an animal in prime health that is given replacement fluids (4 ml/kg IV isotonic saline). In general, larger animals have a lower blood volume than smaller animals. If the total blood volume of an animal is unknown, a rough "rule of thumb" is that 6 % of the body weight is blood volume. Rabbit blood volume is normally 56 ml/kg body weight - assuming the animal is mature, healthy, and on an adequate plane of nutrition.
- b. Blood loss of 30 % of blood volume, or greater, is life threatening.
- c. The common 10 percent-10 percent rule (this estimates a safe volume as 10% of the total blood volume, and that the total blood volume is approximately 10% of the body weight) may result in an excessive blood loss.

Recommendations for Multiple Samplings:

- a. Acceptable limits are a 7.5 % collection of the rabbits total blood volume weekly.
- b. Certain bleeding regimens required by a protocol, such as needing to bleed the rabbits weekly at volumes greater than the 7.5 % limit, or single collections of large volumes may be overly stressful for them. Close observation of the animals will help keep them from becoming anemic. DLAR is willing to perform periodic packed cell volumes (PCVs ,or, hematocrits) on the rabbits and might be able to do so each time you draw blood. One of the DLAR veterinarians or the clinical laboratory technician can show you how to obtain the proper sample and where to take the sample for analysis. The PCV of a rabbit should be maintained above 29 %. The health status of any rabbit whose PCV falls below 30 % should be evaluated by a DLAR veterinarian.

RABBIT BLOOD COLLECTION EQUIPMENT

1. **Acepromazine** (1 mg/kg body weight)
2. **Butorphanol** (1 mg/kg body weight)
3. **Syringes**--for injection - 3 cc; for blood collection - two 20 cc
4. **Needles** for injections (20G)
5. **Exam gloves**
6. **Detergent** or **disinfectant** to clean countertops.
7. **Paper towels**
8. **Alcohol swabs** to clean the ear and multi-use vial tops.
9. **Rabbit restrainer**
10. **A. E-Z Set infusion sets** (Becton Dickinson cat no. 3853741) 21 gauge, 3/4 inch needle, 3 inch tubing

OR

- B. Sterile needles**, 22, 21, or 20 gauge, 3/4 to 1 inch long.
11. **Container** for blood collection; syringe, vacutainer or tube/bottle of desired volume.
12. **Anticoagulant**, if plasma is needed
13. **Tape** to secure E-Z set to ear.
14. **3 Sterile 30 cc tubes** for the blood (that's 1 extra for possible clotted blood)
15. **Marker** to mark tubes
16. **Gauze pads** to hold off artery and clean the ear
17. **Hydrogen Peroxide and water** to clean blood from ear
18. **Sharps Container**
19. **Trash container**