SAN DIEGO STATE UNIVERSITY

IACUC Guidelines

Blood Collection – Intracardiac - Rodent

IACUC Approved: October 14, 2019

PERSONS RESPONSIBLE: **Only experienced staff may perform this procedure.**

MATERIALS REQUIRED: 3cc syringe
1" 21-23g needle
Blood collection tube

PROCEDURE:

1.0 Critical Points

1.1 This is a terminal procedure.

1.2 The mouse must be anesthetized prior to performing this procedure.

1.3 The procedure must be followed by an acceptable means of euthanasia such as CO2, anesthetic overdose, or cervical dislocation.

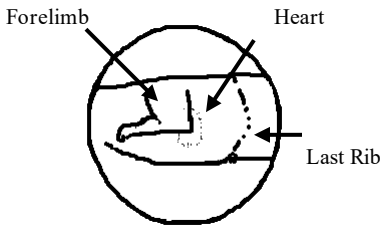
2.0 Anesthesia

2.1 Some typical anesthetic regimens that are used include isoflurane gas anesthesia administered via a precision vaporizer or ketamine(90-120mg/kg) and xylazine (10mg/kg) administered intraperitoneally.

3.0 Positioning of Animal

3.1 Intracardiac blood collection may be performed with the animal in either dorsal or lateral recumbency.

3.2 Orient the animal so that it will be the most comfortable for you to perform the procedure. Most individuals use the dominant hand to hold the syringe/needle and the non-dominant hand to hold the animal. A good landmark for determining the location of the heart is where the point of the elbow falls on the thoracic cavity when the forelimb is in a normal, bent position.



3.3 Dorsal Recumbency

3.3.1 Place the animal on its back. With one hand, place your index finger over the xyphoid process and your thumb and remaining fingers on either side of the thorax to stabilize the chest. You may feel the heart beating under your fingers.



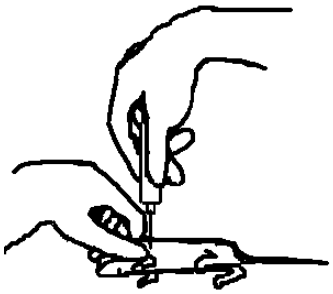
3.3.2 Hold the syringe with needle attached in the other hand and orient the syringe so that the needle will be inserted just under the xyphoid process and into the thoracic cavity at approximately a 45 degree angle, aiming for the heart based on the palpable heatbeat under your fingers as well as the location established using the point of the animal's elbow.

3.3.3 Carefully yet with a firm thrusting motion, insert the needle into the thoracic cavity and apply a small amount of negative pressure by withdrawing the plunger slightly. A successful entry into the heart will be evidenced by blood observed in the syringe. Once blood is observed, stabilize the syringe and slowly and steadily withdraw on the plunger to retrieve the blood sample.

3.3.4 A successful collection may yield anywhere from 0.2-1ml, but will depend on the size of the mouse and the skill of the person performing the procedure. Once the sample is obtained, carefully withdraw the needle, transfer the sample to the appropriate collection vial and discard the syringe/needle in the approved biohazard sharps container. Upon completion of the procedure, euthanize the mouse by an approved method.

3.4 Lateral Recumbency

3.4.1 Position the mouse on its side. Locate the position of the heart by the location of the point of the elbow as described above.



3.4.2 Stabilize the mouse with one hand, taking care to keep your fingers away from the puncture site. With the other hand, orient the syringe/needle so that the needle will enter the thoracic cavity at the location of the heart.

3.4.3 With the same careful, yet firm thrusting motion, insert the needle perpendicularly (straight up and down) into the thorax. Follow the same steps as outlined above with regard to withdrawing the sample (maintain slight negative pressure, stabilize the syringe, slow, steady withdraw of sample). Once the procedure is completed, euthanize the mouse by an approved method.

4.0 Troubleshooting

4.1 Blood is observed initially in the syringe but then flow stops.

4.1.1 Attempting to withdraw the sample too quickly may lead to a large amount of negative pressure applied to the heart which can

collapse the tissue which will effectively occlude the needle. To alleviate this problem, discontinue applying negative pressure for several seconds. This will allow blood to refill the heart chamber which should permit continued sampling. Upon resuming the collection, apply less negative pressure to the syringe and withdraw the sample more slowly to help prevent recurrence of this problem.

4.1.2 If blood flow stops, another reason may be that the needle is no longer within the heart chamber. The needle could have entered the muscle of the outer wall or the inner septum. To alleviate this problem, carefully insert or withdraw the needle by a very small amount and apply a small amount of negative pressure to see if blood flow resumes. Only minimal changes to the needle location should be made. If these changes do not result in the resumption of blood flow, it may be necessary to completely withdraw the needle and begin the process again with a new needle and syringe.

4.2 No blood is obtained on the initial attempt.

4.2.1 It is likely that you have missed the heart on your initial attempt. Withdraw the needle partway, reposition the needle at a slightly different angle and carefully reinsert the needle again. If no blood is obtained on this second attempt, completely withdraw the needle and begin the process again with a new needle and syringe.

4.3 If repeated attempts are unsuccessful it may mean that your technique is inappropriate. It may be necessary to get assistance from another individual proficient in the technique or seek additional training. **Under no circumstances should a mouse that has undergone repeated attempts at sampling be recovered from anesthesia**. Even if the sampling is unsuccessful, the mouse must be euthanized as it is likely there has been significant trauma to the thoracic structures due to the repeated needle insertions.

5.0 Possible Complications

5.1 The most likely complication associated with this technique is premature death of the animal.

5.2 One should avoid repeated insertions of the needle into the thoracic cavity or moving the needle around once it has entered the thoracic cavity as it is possible to lacerate the heart, lungs or great vessels of the heart. Trauma to the heart, vessels and/or lungs can lead to significant hemorrhage and premature death of the animal eliminating the possibility of successful intracardiac blood collection.

6.0 Exceptions

6.1 Rarely, this technique may be approved by the IACUC as a survival procedure in certain species under special circumstances (such as in field studies) with strong justification.

6.2 In those instances, animals must be anesthetized prior to performing the procedure.

6.3 No more than one attempt is made to collect the sample.

6.4 The volume of blood withdrawn is consistent with acceptable survival blood collection volumes which are typically 10-20% of circulating blood volume or 1-2% of body weight for a single sample.

6.5 Animals are monitored closely during recovery from anesthesia for any signs of distress, prolonged or abnormal recovery, abnormal behavior upon recovery, or evidence of thoracic trauma such as pallor, or respiratory distress.

6.6 Any animal that exhibits the signs noted above must be euthanized by an acceptable means consistent with the most recent AVMA Guidelines on Euthanasia.

7.0 Assurances

7.1 Any and all procedures performed on animals must be in an IACUC approved protocol.

7.2 It is the Principle Investigators responsibility to assure that any individual performing this procedure is adequately trained.

7.3 Training on this procedure can be arranged through the Office of Laboratory Animal Care (OLAC) office (x5421).