TITLE: Blood collection from the retro-orbital sinus

OBJECTIVE: To collect specific volumes of whole blood from the mouse via the Retro-Orbital Sinus.

BACKGROUND: The mouse has a large peri-orbital venous sinus that fills the bony orbit of the eye.

Blood can be collected from either the medial or lateral canthus approach.

The following must also be noted prior to collecting blood using this method:

- Mice must be at least 6 weeks of age and their body weight should ideally be 20gms or above
Before and after you perform the procedure record any abnormalities regarding the health of the mouse or its eyes on the cage card and on any relevant data base.

- The interval between bleeds must be a minimum of 2 weeks
- Each mouse can only be bled for a maximum of six (6) times
- Only 1% of the blood volume of the mouse can be collected (e.g. If the mouse is between 22 and 25 grams then 220 µl is the maximum amount of blood to be collected)
- If a mouse has one damaged eye, then it is preferable to use this eye for bleeding to minimize the risk of damaging the healthy eye
- Unless passed by the facility veterinarian, mice displaying the following conditions must not be bled from:
  - Heavily pregnant
  - Females with young pups
  - Sick, runted, or injured animals

### RISK STATEMENT:

<table>
<thead>
<tr>
<th>Use of glass capillary tubes</th>
<th>Puncture from broken tubes</th>
<th>Sharps safety training</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working within the animal facility</td>
<td>Injury from slips, trips and falls</td>
<td>Training Signage Procedures Supervision Removable mouse barriers</td>
</tr>
<tr>
<td>Restraint of small animals</td>
<td>Bite/scratch Repetitive Strain Injury</td>
<td>Training Use of chemical or mechanical restraints Alternate routines</td>
</tr>
<tr>
<td>Blood collection from larger quantity of animals</td>
<td>Repetitive Strain Injury</td>
<td>Training Minimising numbers of bleeds. <em>(Suggested numbers for a fully trained tech: Max of 80 animals in one session. Max of 3 sessions per week.)</em> Alternate routines</td>
</tr>
<tr>
<td>Chemicals Irritants</td>
<td>Reaction/sensitivity to irritants eg. Skin rash, eye inflammation and irritation to air passages</td>
<td>Correct labelling/storage/handling of chemicals; training; use of appropriate PPE and review if necessary</td>
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</tbody>
</table>

For the full Risk Assessment for this procedure contact administration.heb@anu.edu.au

9/19/2013

UNCONTROLLED IF PRINTED
**PPE:**
The following personal protective equipment MUST be used during this procedure:

- Gloves
- Mask
- Gown
- Safety glasses

**PREREQUISITES:**
This procedure requires animal ethics approval under an approved protocol before it can be undertaken.

Signed off in the basic core competencies of:
- mouse identification,
- handling
- restraint
- euthanasia
- Trained in this procedure

**EQUIPMENT REQUIRED:**

- 75 µl haematocrit (capillary) tubes, either heparinised or non-heparinised – depending on purpose
  - **Heparinised** – do not want the blood to clot, blood is used for assay work (FAC’s)
  - **Non – heparinised** – want the blood to clot to be able to collect the sera (ELISA’s, health screening for antibody responses in the white blood cells)
- Collection tube (eg: cluster or Eppendorf tube)
- Tube rack/holder
- Paper towel
- Sharps bin
- Gloves
- Gown
- Face mask
- Disinfectant
- Ethanol
- Sample collection grid (as backup)
- Tissues

**PROCEDURE:**
9/19/2013
PREPARATION – MUSTERER

1. Log in to Musterer using your university ID and HORUS password.
2. From the menu bar shown in Figure 1 press the button.

3. Select the type of bleed requested, as stated on the bleed template, in the ‘Category’ drop-down list shown in Figure 2 below. E.g. ABA-CGG
4. Select the room number from the Room(s) list shown in Figure 2. Note: more than one room can be selected by holding down the Ctrl button on your keyboard.

5. Select ‘Across columns (blood)’ from the Plate direction list shown in Figure 2.

6. Press Create – a template will appear on the screen as in Figure 3.

7. Scan each cage prior to collecting blood and check that you are placing the cluster tubes into the right slot.

8. Although the APF Plate Maker is set to save automatically at regular intervals whilst the plate is open on Musterer, upon completion of bleeding you must ensure you press the ‘Save Plate’ button shown in Figure 3.

9. Press the ‘Close Plate’ button shown in Figure 3 when the bleed is completed.

10. A barcode must be attached to the bleed plate once finished.

11. To print the plate barcode, press the button shown in Figure 1. The screen shown in Figure 4 will appear.

12. Enter the plate number or select the plate from ‘The 20 most recent plates…’ list shown in Figure 4.
13. Click on the barcode shown in Figure 5 and follow the prompts to print two barcodes.

Figure 5 – Print Barcode screen

14. Attach the printed barcodes to the zip lock bag the bleed plate will be placed in and stick into room diary on the appropriate day.

COLLECTION - LATERAL CANTHUS

1. Ensure you have the relevant documentation indicating:
   a) Animals to be collected from
   b) Reason for collection (Sera, FACS etc)
   c) Amount required from each animal

2. Locate mice to be bled and double check details

3. Set up relevant computer based programs if required

4. Take first cage to the hood and check that all mice are healthy.

5. Cross check the identity of the mice within the cage card and relevant paperwork

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6. Place mouse on wire and restrain with your non-dominant hand in a secure grip ensuring that the middle or index finer are restricting movement of the head. The eyes should slightly bulge.

7. Pick up the haematocrit and cluster tubes together with your spare hand.

8. Place the haematocrit tube parallel (outer side of the eye away from the nose) to the bulging eye, applying gentle pressure. The eye should now bulge further.

9. Gently tilt the tube at an angle until it slides into the outer edge of the bulging eye. The tube should not go any deeper than the coloured marking.

10. Slowly rotate the haematocrit tube until the orbital blood vessel is nicked and blood freely flows down the haematocrit tube into the cluster tube.

11. Allow it to flow until the required amount of blood is collected.

12. Remove haematocrit tube from eye and immediately release restraint.

13. If relevant, tap collection tube gently onto a level surface before replacing in rack. This mixes the blood with the anticoagulant in the tube to prevent clotting.

14. Discard used haematocrit tube into designated sharps container.

15. Place mice that have already been bled into a clean cage or the lid of their cage to make identification of remaining mice simpler. The mouse will stop bleeding when it is released (no longer restrained, lower stress, lower blood pressure). The mouse is able to be placed back into cage with other mice, normal behavior is that the mouse or its cage mates will clean its eye and the bleed should stop within seconds.

16. Cross off mouse number on mouse request document. If cannot be bled add reason to blood request form and comment section on blood program.

17. Select the next mouse to be bled if applicable and repeat steps 8 to 17.

18. When all relevant mice in the cage have been bled, ensure all information is recorded on the cage card. Level 2 HEB has designated stickers available for recording bleeds on cage cards. Contact the area supervisor to request. As a minimum the cage should be marked with e.g: “ebYX 245/11” (i.e. eye bled by person of initials YX on day 245 of 2011).

19. Return the cage to its rack.

20. When finished bleed session wipe down work station thoroughly, with an appropriate disinfectant e.g. Virkon and then 80% ethanol, ensuring that no blood is left on any surface. Check under the hood surface as blood often drips into the tray.
21. Secure the tube holder to ensure tubes cannot be dislodged.

22. Place plate inside sealed zip lock bag before leaving mouse-holding room.

23. Ensure all relevant documents and barcodes stay with the samples.

24. Contact relevant personnel to notify that the blood is ready for collection.

**COLLECTION - MEDIAL CANTHUS**

Place the ANAESTHETISED mouse on a table or cage lid in lateral recumbancy. The body of the mouse is restrained against the table with the palm of the hand. The thumb and forefingers of the same hand restrain the head and gently open the eyelids to expose the eye. Insert the tube into medial canthus and hold it at a 30 degree angle to the nose.

**POTENTIAL COMPLICATIONS:**

****Should you notice any of these complications please record it immediately and seek veterinary attention if required

Injuries to the eye caused by retro-orbital bleeding can be seen in the following ways

- The mouse squinting when the procedure has finished
- The eye looks bulged and swollen
- The area around the eye is swollen
- Opacification of the eye, (clouding over), usually happens the following day
- Orbital discharge after the procedure
- Clean fluid entering the capillary tube during the procedure may mean that you have nicked the eye ball and ocular fluid is leaking

**HANDY HINTS:**

- High blood pressure occurs when mice are restrained; therefore always punch mice before bleeding to avoid haemorrhaging.
- Sometimes the nictitating membrane (third eyelid) of the mouse protrudes following this procedure, the mouse will rectify this problem itself when grooming.
- Check blood plate program at regular intervals to insure that samples are aligned in sequence on the physical plate and the plate program.
- If plate program is unavailable keep copy of written template to rescan at the earliest possible chance. It is the responsibility of the bleeder to ensure all plates are entered on the plate program.
Standard Operating Procedure
045 - Blood collection from the retro-orbital sinus
Version 2.2

VERSION HISTORY

<table>
<thead>
<tr>
<th>Version Number</th>
<th>Date Initiated</th>
<th>Changes Made</th>
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<tbody>
<tr>
<td>1.0</td>
<td></td>
<td>Original</td>
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<tr>
<td>2.0</td>
<td>25 Nov 2011</td>
<td>Addition of cage card recording and Musterer preparation. Addition of ANAESTHETISED for medial canthus collection</td>
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<tr>
<td>2.1</td>
<td>21 Nov 2012</td>
<td>ABRF to APF</td>
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<tr>
<td>2.2</td>
<td>20 August 2013</td>
<td>AEEC review and approval</td>
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SUMMARY: Complete a brief dot point summary of the procedure including warnings and important points. Summary page to have the following signature box placed at the end of it.

I have read the full version of the above summarised SOP and recognise the risks associated with this procedure.

<table>
<thead>
<tr>
<th>Name</th>
<th>Date</th>
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<tbody>
<tr>
<td>Signature</td>
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<tr>
<td>Supervisor</td>
<td>Date</td>
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<tr>
<td>Signature</td>
<td></td>
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</tbody>
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A copy of this summary page must be kept in each employee’s handbook.