

## FLSC Standard Operating Procedure for Cheek Bleeding Methods of Mice

### Purpose:

Retro-orbital blood sampling has been banned in Europe. FLSC has evaluated alternatives to the retro-orbital route to promote the most humane procedures among the animal care staff and researchers.

<b>Equipment:</b> Paper towels	Cotton ball(s)
Eppendorf tube 1.5 ml	Heparinized hematocrit tube
Goldenrod Lancets	22g – 18g sterile needle

### Procedure for Submandibular Vein Sampling:

1. Select a needle depending on the sex and age of the mouse (see chart below).
2. Restrain the mouse by scruffing the skin at the back of the neck.
3. Identify the landmarks for the venipuncture. The submandibular vessel is found where lines drawn from the corner of the eye and the corner of the mouth intersect near the lateral/caudal aspect of the mandible. This coincides with a small hairless dimple found caudal to the corner of the mouth.
4. Insert the tip of the needle perpendicular to the skin to a depth of 1 – 2.5 mm.
5. Upon removal of the needle, blood will begin to flow.

### Procedure for Facial Vein Sampling:

1. Select a lancet depending on the sex and age of the mouse (see chart below).
2. Restrain the mouse by scruffing the skin at the back of the neck.
3. Identify the landmarks for the venipuncture. The facial vein is located at the end of the mandible approximately the length of the eye down and the width of the eye over toward the curve of the jaw.
4. Using the tip of the lancet locate the jaw line and find the point where the bone ends.
5. Hold the lancet so that the tip is angled toward the nose and insert it perpendicular to the skin to a depth of 4 – 5.5 mm at the point just caudal to the bone. This prevents puncture of the ear canal.
6. Upon removal of the lancet, blood will begin to flow.

### Collection of the Sample:

1. Collect the blood in either a heparinized hematocrit tube or allow the blood to drip into an eppendorf tube. A single drop may be placed directly on a slide for blood smears.
2. Release the scruff and bleeding should stop. Ensure hemostasis by blotting with either a cotton ball or a paper towel before returning the mouse to the cage.

Lancet/Needle Size	Age/Sex/Weight	Sample Size
4mm lancet/22g needle	3 wks – 4 wks old mice (under ~15 g) mice over 15g	small single drop
5mm lancet/20g needle	female mice under 10wks (under ~20g) male mice under 6 wks (under ~20g) mice over 20g	large large small
5.5mm lancet/18g needle	female mice over 10 wks (over ~20g) male mice over 6 wks (over ~20g)	large large

### Technical Tips:

1. Alternate sides for repeated blood sampling if sample intervals are less than 5-7 days.
2. With the needle method, do not insert beyond a depth of 3.5mm to avoid penetration of the oral cavity and trauma to the muscles, nerves and vessels causing uncontrolled hemorrhage.
3. Restraint is critical. Too tightly scruffed will restrict blood supply and breathing.
4. To assist blood flow, position the mouse with the head lower than the heart. For better accuracy in puncturing the vessel, position the mouse in lateral recumbency.