Clinical Techniques
Rodent restrainers

Cylindrical restrainers:
Broome Restrainers

Pastry bag restrainer:
DecapiCone
Rabbit restrainers
Needles

• Gauge refers to diameter
  – Smaller diameter is the larger number

• Choosing a gauge
  – Smallest that will penetrate (except for IP injections; larger gauge deflects organs)
  – Smallest that will handle the viscosity of the fluid

• Length
  – To desired area of penetration (smaller diameters will only support short lengths)

• Bevel
  – The angled end to produce a cutting edge; usually directed up
Injection sites

- Sites vary in
  - Blood flow/absorption rate
  - Tissue density
    - Volume accepted
    - Potential for damage
- Alcohol prep: Not generally necessary with lab animals unless site is visually dirty
- Aspiration: a technique for determining correct needle placement. Pull back on plunger about 0.05 ml
  - Vein or artery: blood
  - Subcutaneous: vacuum
  - Abdominal: clear
    - Blood—sterile, reposition
    - Green, brown, or yellow—dispose of
Administration Volumes

<table>
<thead>
<tr>
<th>Species</th>
<th>Oral</th>
<th>SQ</th>
<th>IP</th>
<th>IM*</th>
<th>IV (bolus)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>10</td>
<td>10</td>
<td>20</td>
<td>0.05</td>
<td>5</td>
</tr>
<tr>
<td>Rat</td>
<td>10</td>
<td>5</td>
<td>10</td>
<td>0.1</td>
<td>5</td>
</tr>
<tr>
<td>Rabbit</td>
<td>10</td>
<td>1</td>
<td>6</td>
<td>0.26</td>
<td>2</td>
</tr>
</tbody>
</table>

*IM: volumes are ml/site. IM is not recommended for mice or rats

Injection Vehicles

- Vehicles are the solution in which a drug is dissolved. They should be biologically inert and they must not react with the drug or chemical being injected.
  - Sterile water
  - Aqueous isotonic solutions (saline)
  - Oils (corn oil)
  - Buffered solutions (PBS)
Subcutaneous

- SC, SubQ, SQ
- Used in all rodents and rabbit
- Restraint is minimal
- Uptake is relatively slow, but can be used for anesthetics
- Large space between skin and muscle
  - Large volumes
  - Limited damage
SQ--examples
Intraperitoneal (IP)

- Used in all rodents with easy restraint
- Difficult restraint so seldom used in rabbits
- Rapid absorption due to mesenteric capillaries
- Large volume accepted
- Aspiration critical
Examples IP
Intradermal (ID)

• Primarily used for antibody production
  – Antigen plus adjuvant injected ID or SQ
  – Antibodies formed and collected from blood

• ID injections
  – Dorsal-lateral
  – Multiple small doses (0.05 – 0.1 cc/site)

• Watch for infection or abscesses

• Most common in guinea pig and rabbit
Intradermal (ID)

- Pinch skin taut
- Fingers together
- Slide forward into dermis just to end of bevel
- Don’t aspirate
- Drop pinch
- Deliver 0.1 cc
- Look for blanching bleb
Intramuscular (IM)

• Common in guinea pig and rabbit
• Less common in mouse and rat because of small muscle mass
• Dense tissue
  – Rapid absorption
  – More painful and more risk of tissue damage
Examples of IM

**Quadriceps**—
Lateral to midline to avoid sciatic nerve

**Lumbar**—
Lateral to spinal column
Intravenous (IV)

• Fastest uptake
• Volumes vary with size of vein and length of time allowed for injection
  – Bolus: \( \leq 1 \) minute; 5 ml/kg (mouse/rat); 2 ml/kg (rabbit)
  – Slow injection: \( \leq 10 \) minutes; in ml/kg--25 (mouse), 20 (rat), 10 (rabbit)
  – Continuous infusion: \( \sim 10\% \) of CBV over a 2 hour period
• Large volumes should be warmed
• Always inject into veins
• Multiple sticks move distal to proximal
• Rabbits and guinea pigs—marginal ear vein (auricular vein)
• Mice and rats—usually tail vein
• Other veins: jugular, femoral, saphenous, cephalic.
Example IV injection
Oral dosing

• Need to deliver a measured amount of fluid at a specific time.
• Fluid or food replacement or experimental
• Sometimes animals drink from a syringe or eye dropper

Cut and fire polished to deliver food
Oral dosing

• Also stomach tubing or gavage
• Common in mice and rats
• Don’t gavage guinea pigs
• Delivers fluid directly to the stomach
  – Accurate dose and time
  – Unpalatable fluids
  – Fluids degraded by the enzymes in the mouth
  – Biohazards
Palatal Ostium

Don’t gavage guinea pigs
Opening in the soft palate is too small.
Gavage Tubes

Choose the correct size:
Measure from nose to last rib.

Balled tip prevents entry into the larynx
Gavaging
Catheters and Implants

- Alternative to frequent injections
- Catheters are inserted into a large vein
  - End of tube is buried in a jacket or attached to a swivel apparatus to allow freedom of movement.
- Implants are reservoirs buried under the skin to deliver fluid at a constant rate.
Osmotic Pump

Figure 3. Osmotic Pump mechanism

Figure 1. ALZET® Osmotic Pump Models.
*Planned availability in 1998.
Blood collection

• Equipment
  – Choose needles 25 g or larger to prevent hemolysis (RBC lysis)
  – Choose receptacle that will not draw too strong a vacuum (prevent hemolysis and collapsed veins)
    • Microhematocrit tubes
    • Microcollection tubes
    • Syringe
    • Vacutainer tube
Site Preparation

- Vasodilatation
  - Heat: Heating pads or lights
  - Drug-mediated: Acepromazine

- Analgesic
  - Topical: Prilocaine cream
  - Sedatives or anesthesia: Acepromazine

- Clip hair to visualize surface vessels

- May clean site with tape, vacuum, wipe with gauze—dry or with alcohol or water.
  - Some methods cause vasoconstriction.
Lateral Saphenous Vein

- Rodents and rabbit
- No anesthesia required
- Multiple bleeds
Lateral Saphenous Vein
Tail vessels

- Mouse, rat, and gerbil
- Can use vein or artery
  - Vein: lateral, use a tourniquet
  - Artery: ventral, no tourniquet
- No anesthesia required
Tail Vein
Submandibular

- Mouse or rat
- Submandibular or facial vein or junction of the two
- No anesthesia required but restraint must be tight
- Use a 5 mm lancet
Auricular Vessels

- Ear vein (caudal): up to 5 mls, 25 g needle, microhematocrit tube or syringe
- Central ear artery: up to 50 ml in a 5 kg rabbit, use a 21 g needle on a butterfly catheter and collect in a syringe or use vacutainer tube
- Anesthesia is not required but sedation helps with rabbit compliance and vessel dilation
  - Acepromazine- 0.1 ml/kg SQ
- Topical analgesics helps with compliance
  - Prilocaine cream applied 15 - 20 minutes prior to stick
- Move toward source of blood for multiple sticks
Auricular

Vein

Artery
Orbital Sinus

- Mouse, rat, hamster
- Requires anesthesia
- Insert microhematocrit tube between sclera and eyelid.
- Collection comes from puncturing the capillary sinus or plexus behind the eye.
Jugular vein

• Rodents and rabbits
• Usually requires anesthesia
• May be done with or without a surgical incision.
Cardiac Puncture

- Rodents and rabbits
- Requires anesthesia
- Terminal procedure in small animals
- Use xiphoid process as landmark for entry
Hemostasis

• Place a gauze pad over area of insertion and apply pressure before withdrawing the needle.
  – Apply continuous pressure for 30 to 60 seconds
  – Remove tourniquet if one is used
  – Too much pressure occludes blood flow—no clotting factors
  – Wiping removes clot—bleeding will return

• Once bleeding has stopped, observe for 30 seconds to assure that it does not return.
  – Check again in 10 to 15 minutes
Collection Guidelines

• Circulating blood volume
  – Rabbit: 45-70 ml/kg = 5% of bwt
  – Rat: 50-70 ml/kg = 6% of bwt
  – Mouse: 80 ml/kg = 8% of bwt

• Collection volume vs frequency (survival)
  – 1% of body weight (in grams) every 2 weeks
  – 30 g mouse—0.3 ml once every 2 weeks
    • 0.15 ml once a week
    • Serial bleeds: 0.05 ml every hour for 6 hours
  – 300 g rat—3 ml every 2 weeks
  – 3 kg rabbit—30 ml every 2 weeks
Physiological Response to Blood Loss

• 10%: Homeostatic changes such as increased heart rate and vasoconstriction

• 15-20%: Cardiac output and blood pressure decrease. Can be counteracted with replacement fluids.

• 30-40%: Hemorrhagic shock—fast pulse, pale membranes, low body temperature, hyperventilation, restlessness. 50% mortality rate in rats.