INTRODUCTION
The following information is designed to help the investigator understand the necessary equipment and skills to perform the commonly used procedures used in animal research. In many cases, especially anesthesia, the state of the art is continually changing as new information is generated. Improvements in these methods are always possible. We would be grateful to receive comments and recommendations from any and all who use this information.

I. ANESTHESIA OF MICE, RATS AND GUINEA PIGS

A. INHALATION ANESTHESIA

1. Equipment
   a. 1 screw top, wide mouth, glass jar 16 or 32 oz capacity.
   b. Cotton pledget
   c. Screening or paper towel
   d. Metofane (methoxyflurane) [SAFETY NOTE: personal hazard]

2. Method
   a. Handle all volatile aesthetics, including metofane in a properly ventilated space. Close containers holding the anesthetic as quickly as possible after task is completed to avoid breathing the material.

   b. Place cotton pledget in bottom of glass jar. Add 3-5 ml of metofane to cotton close jar quickly to avoid breathing anesthetic fumes.

   c. After liquid is absorbed onto the cotton, cut a paper towel to size and add to top of pledget to provide a barrier to prevent direct cutaneous absorption of the anesthetic to the animal.

   d. Grasp mouse by tail and quickly drop into jar replace lid and quickly screw top down after shaking to make certain various appendages are not in the threads of the lid.

   e. Wait until the mouse passes through the excitement phase of anesthesia, approximately 30 seconds to 1 minute. This time will vary with the adrenal output (by operator and operatee severally and together) occasioned by the effort to secure the animal in the jar and the concentration of anesthetic remaining in the jar.

   f. When the animal has passed through the excitement phase it lies still, breathing rapidly with an occasional twitch with the forelimb against the eyes and nares.
g. Remove the animal from the anesthetic chamber and perform procedure immediately.

h. The animal should recover from retro-orbital bleeding in one to several minutes. In order to maintain anesthesia the animal must be breathing the anesthetic gas. Once it is breathing 'clean' air the anesthetic leaves the nervous system and the animal wakes up. Prolonged or repeated anesthetic procedures may result in death of the animal.

B. INJECTABLE ANESTHESIA/ANALGESIA

1. Short procedures such as laparotomy, embryo transfer, vasectomy, tail amputation: Tribromoethanol [SAFETY NOTE: harmful, light sensitive, handle and store under nitrogen - please refer to attached Material Safety Data Sheet] (0.2 ml/10g body weight of a 1.2% solution injected intraperitoneally in the caudal abdomen). See: Lab An Sci 43:189, 1993.

2. Other injectable methods of sedation and anesthesia are listed below:

a. Sedation:
   (1) Diazepam, 5 mg/kg i.p. [SAFETY NOTE: toxic, addictive, possible mutagen, possible teratogen - please refer to attached Material Safety Data Sheet]

   (2) Chlorpromazine (Thorazine) 10 - 30 (50) mg/kg [SAFETY NOTE: highly toxic, sensitizer, reproductive effects - please refer to attached Material Safety Data Sheet]

b. Analgesia:
   (1) Butorphanol, 0.01 - 0.06 mg/kg s.c., i.m.

   (2) Meperidine, 0.7 - 3.0 mg/kg, s.c., i.m. [SAFETY NOTE: toxic - please refer to attached Material Safety Data Sheet]

c. Anesthesia:
   (1) Halothane-Inhalation to effect [SAFETY NOTE: possible carcinogen and mutagen, severe eye irritant - please refer to attached Material Safety Data Sheet]

   (2) Hexobarbital, 60-100 mg/kg i.p. or 6-10 mg/kg i.v. [SAFETY NOTE: toxic, addictive - please refer to attached Material Safety Data Sheet]

   (3) Thiopental, 25-50 mg/kg, i.p. or i.v. [SAFETY NOTE: sensitizer, skin absorber, addictive - please refer to attached Material Safety Data Sheet]

   (4) Pentobarbital [6mg/ml], 90 mg/kg i.p. [SAFETY NOTE: toxic, addictive, reproductive disorders - please refer to attached Material Safety Data Sheet] Avoid using concentrations above 6 mg/ml to prevent excessive irritation of
the peritoneal membranes. The effect of pentobarbital differs in different strains and age animals.

(5) Ketamine + Xylazine + Atropine; K:35-35 mg/kg + X: 5 mg/kg + A:0.05 mg/kg) i.m. or i.p. [SAFETY NOTE: atropine is highly toxic, may be fatal if inhaled, swallowed or absorbed through skin - please refer to attached Material Safety Data Sheet] The effect of this combination is also variable depending on the strain and age of the animals.

C. TOPICAL ANESTHESIA
Tail amputation: Ethyl chloride [SAFETY NOTE: highly flammable, absorbed through intact skin, carcinogen - please refer to Material Safety Data Sheet] spray for local anesthesia.

II. ASCITES PRODUCTION IN MICE

METHOD
2. Prime the abdomen using 0.3 ml of pristane
3. Use minimal Freund’s Complete Adjuvant [SAFETY NOTE: avoid contact and inhalation - please refer to attached Material Safety Data Sheet]
4. Check for ascites daily
5. If animal is in respiratory distress tap abdomen to withdraw ascites using an 18 ga. needle
6. Tap abdomen not more than 2 to 3 times before killing the animal

Please be advised that there is evidence that the mouse ascites method of monoclonal antibody production causes discomfort, distress and/or pain. Practical in vitro methods exist which can replace the ascites method in many experimental applications without compromising the aims of the study. If you believe that you must continue to use the ascites method, you must convince the HMA Standing Committee in your Animal Experimentation Protocol that: (1) the proposed use is scientifically justified (provide references) and (2) the methods which avoid or minimize discomfort, distress, and pain such as in vitro methods have been considered and have been found unsuitable. Cost is not considered a suitable reason for exclusion.

III. TAIL AMPUTATIONS

C. ANESTHESIA - using any of the above mentioned methods and local analgesia using ethyl chloride spray.

D. METHOD - Cut the terminal 1.3 cm of the tail using scissors.

E. POST OPERATIVE HEMOSTASIS
1. Cautery.
2. Topical application of a stptic compound (e.g. Kwik-Stop, available from Farmers Exchange, order #: 532 363, 2 oz, 1 2 oz. Phone: (508) 872-3508.
3. Mouse tail biopsy. The tissue bond adhesives should be applied to the cut tissue with as little intervening blood as possible. (Shake blood off or wipe with gauze and then apply a drop of
the tissue glue.)

Vet bond adhesives:
Henry Schein, cat. #: 700-3449; 1 800 872 4346
Webster, cat. #: 480200; 1 800 225 7911
Provet, cat. #: 48056; 1 800 435 6902

These preparations are procoagulant substances. A reasonable method of application is to
decant some of the powder into an eppendorf tube for use. Place the cut end of the tail in the
powder to promote clotting.

Blood stop powder
Webster, cat. #: 570006; 1 800 435 6902

Kwik stop
Farmer-s Exchange, cat. # 532 363; 1 508 872 3508

IV. DRAWING BLOOD AND INTRAVENOUS INJECTIONS

A. MOUSE, HAMSTER, GUINEA PIG: See retro-orbital bleed.

B. RATS:
Rats may be anesthetized using methoxyflurane [SAFETY NOTE: personal hazard] as for mice. A
nose cone containing anesthetic on a cotton pledget may be used to maintain anesthesia. Use a
warming lamp to dilate the blood vessels of the tail. Use a 25 ga needle to obtain blood from one of
the lateral tail veins.

C. VOLUME OF BLOOD: See Retro-orbital bleed.

D. INTRAVENOUS INJECTIONS: Use the above method described in B in order to locate the vein.
Use slight negative (aspiration) pressure on the syringe to see that blood enters the syringe. Then
reverse direction with plunger of syringe to slowly inject liquid substance into the vein. For animals
other than rats, any easily identifiable superficial vein may be used.

V. EUTHANASIA FOR MICE, RATS, GUINEA PIGS

A. ASPHYXIATION WITH CARBON DIOXIDE

1. Equipment needed:
   a. Desiccator with a top equipped with a valve to admit gas.
   b. Source of carbon dioxide. If using dry ice care should be taken that the animal to be
euthanized is not frozen but is asphyxiated. That is to say a grid which itself does not
get cold separates the animal from the dry ice.

2. Method
   a. Pre-fill the chamber with carbon dioxide. (CO₂ is heavy and stays in the bottom of a
b. Place animal in the uncrowded chamber and turn on gas to fill the chamber.
c. Wait until animal stops breathing and wait 1 to 2 more minutes more.
d. The operator may open the pleural cavity to collapse the lungs or perform cervical dislocation on the unconscious animal if he/she cannot wait for anoxic death of the animal.

CAUTION: Neonatal animals are somewhat resistant to anoxia in part because their RBC’s contain fetal hemoglobin which has a higher affinity for oxygen. Therefore the operator must wait longer to ensure the death of neonates than adults.

B. ANESTHETIC OVERDOSE

1. Method:
   a. Place animal as for anesthesia using methoxyflurane [SAFETY NOTE: personal hazard]
   b. Wait until respiration ceases.

VI. IMMUNIZATION

A. ANTIGEN
   Antigen emulsification in the adjuvant if it is in a aqueous solution may be accomplished by using two 3cc syringes and a plastic 3 way stopcock and pushing the solution back and forth between the two syringes. Use Monoject or Air-tite syringes in preference to BD syringes whose gaskets seem to dissolve in the adjuvant. Start emulsification with cold (4°C) Freund’s.

B. IMMUNIZATION OF RATS, MICE AND GUINEA PIGS
   Immunization of rats, mice, and guinea pigs should follow standard sterile practice. The material to be used as an antigen should be sterile. Avoid using injection sites which are habitually traumatized by handling (between the shoulders of rabbits, tail base of other animals) or during normal activities such as feeding and ambulation (foot-pads).

C. INJECTION SITES AND VOLUME
   Injection sites should be prepared by clipping the hair and cleaning of the injection site using Betadine followed by alcohol. This is important to avoid formation of abscesses particularly when injecting irritating substances such as Complete Freund’s Adjuvant [SAFETY NOTE: avoid contact and inhalation]. If Freund’s Complete Adjuvant is used in an animal it should be used only with the initial immunization and any booster immunizations should be performed using Incomplete Freund’s Adjuvant. Dilutions with antigen should be 1:1.

The volume to be injected depends on the size of the animal and the site to be used.

<table>
<thead>
<tr>
<th>Location of Injection</th>
<th>Volume</th>
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<tbody>
<tr>
<td>1. Intra dermal (ID)</td>
<td>0.05-0.1 ml/site</td>
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<tr>
<td>2. Subcutaneous (SC)</td>
<td>0.5 ml/site</td>
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VII. RETRO-ORBITAL BLEED
NOTE: Blood collection should not exceed 10% of the blood volume of the animal every 2 weeks. Blood volume is calculated by taking the mass in grams and multiplying by 0.06. i.e., 30 g x 0.06 = 1.8 ml x 10% = 180 to 200 microliters.

A. EQUIPMENT
2. Anesthetized mouse or hamster (see above).
3. Separator 'red top' blood collection vials.

B. METHOD
1. Arrange separator tubes in a holder and remove rubber stopper so that the mouse may be inverted over the tube for collection of blood.
2. Grasp anesthetized mouse in left hand (gloved) so that it is held firmly but without choking. To do this the mouse’s head should protrude from the hand between thumb and forefinger. The thumb may be used to apply pressure laterally, on the mouse’s right jaw using the animal’s right forelimb as a cushion. Pressure should not be applied to the airway which is ventral. Gentle pressure applied in this way will cause mild proptosis of the right eye (unilateral bug-eye). The smooth end of a broken micro-hematocrit tube approximately 2.0 cm long is applied using the thumb and forefinger of the right hand to the medial canthus of the eye and directed into the retro-orbital sinus by rotating the tube on its axis using the same sort of motion as that applied to smooth the frayed ends of a thread in order to pass it through the eye of a sewing needle. The tube should be directed medially and slightly dorsally. Rotation will ensure that the tube is well seated. Blood should flow from the micro-hematocrit tube and should be directed into the vial. Ten drops should suffice for serology needs.

VIII. CARDIOCENTESIS

A. EQUIPMENT
1. 1 ml tuberculin syringe.
2. 22 to 25 ga. needle.
3. Carbon dioxide chamber (plastic desiccating chamber with CO₂ supplied by line. Dry ice should not be used.

B. METHOD
1. Note: This is a terminal procedure.
2. Place mouse in the CO₂ chamber and add gas until the animal has just ceased breathing. At this point the animal’s heart is still beating and will provide cardiac return necessary for blood collection.
3. Place animal in dorsal recumbency and insert needle 0.2 cm to the right of the...
xiphoid and direct the needle along the long axis of the animal toward the base of the animal’s skull. The angle formed by the needle and syringe and the ventral surface of the animal should be approximately 25 to 30 degrees. Gentle aspiration of the plunger should draw blood. If blood flow stops wait for cardiac return to fill the right ventricle. If no luck, reposition needle by rotating syringe without moving the end backwards or forwards and aspirate. If no luck, try repositioning, try new needle, try new mouse. With practice, 1 ml of blood may be reliably obtained routinely by this method.

IX. INJECTIONS

A. SUBCUTANEOUS INJECTIONS

1. Most animals, unlike humans, have mobile skin which permits relatively painless subcutaneous injections. Obviously the pH and chemical constitution of the material injected will bear on the actual level of comfort experienced by the animal after an injection.

2. Method: With the rat, rabbit, mouse, or guinea pig restrained by mechanical, manual or anesthetic methods, prepare the injection site by using an alcohol swab to wet the fur, pick up a fold of skin along the flank (rabbit), the dorsum or abdomen (hamster, mouse, rat, gerbil) and insert the needle along an axis roughly parallel to the underlying body, insert the needle behind the fold, draw back on the syringe and look for blood in the syringe to ascertain the location of the needle tip and if satisfied depress the plunger. The drawing back on the syringe permits the operator to assess whether the needle has exited the other side of the skin tent or has entered a blood vessel; both conditions would be undesirable if the operator intends to inject into the subcutaneous space.

B. INTRAMUSCULAR INJECTIONS

The volume of intramuscular injections should be tailored to the size of the animal. Large volumes injected into the muscles of a small animal are painful and cause muscle necrosis. Intramuscular injections should be performed into a large muscle mass such as the thigh muscles of all laboratory animals and the lumbar muscles of the rabbit. The method of injection is as follows: wipe the area with an alcohol swab to flatten the fur and help delineate the anatomy of the region, tense the skin over the area using thumb and forefinger of the hand opposite the one holding the syringe and insert the needle quickly into the muscle using an angle of attack roughly perpendicular to the underlying muscle. Draw back on the syringe and look for blood in the syringe to ensure that you are not placed in a vein or artery and if satisfied complete the injection.
C. **INTRAPERITONEAL INJECTIONS**

Intraperitoneal injections are most commonly performed on rats and mice. Possible adverse sequelae of this procedure include perforation of bowel and subsequent peritonitis, puncture of the spleen and liver, injection into the bowel with consequent diminution of pharmacologic effect. The majority of these adverse consequences may be avoided by injecting into the caudal abdomen while the animal is held in a head down position so that the viscera descend toward the diaphragm.

1. **Method**
   Sanitize the injection site using Betadiene or other antiseptic soap followed by alcohol. Both mice and rats may be restrained manually or after anesthesia. The rat may be handled by its tail and permitted to grasp a wire cage top with its forelimbs. The animal thus exposes its caudal abdomen to the operator and the injection may be performed. For the mouse the animal is best restrained in the left hand holding the skin of the scruff of the neck between the thumb and forefinger and the tail is then pinioned to the palm of the hand by the ring and little fingers. Before injection the animal should be positioned with its head down and the injection should be performed in the caudal abdomen.

D. **INTRADERMAL INJECTIONS**

Intradermal injections are performed largely for purposes of immunization. Immunization injections are often associated with substantial inflammation and should be positioned so that this inflammation is not irritated by human handling or during walking and eating. Avoid the area around the shoulder blades, base of the tail and foot pads for these injections. Restraint should consist of heavy sedation, tranquilization or light anesthesia.

1. **Method**
   Areas to be injected should be clipped and surgically prepared with Betadiene followed by alcohol swabs. The skin of the animal should be tensed by separation of thumb and forefinger or thumb and middle finger. Using a tuberculin syringe and a 25 ga or 26 ga needle with the bevel up the needle is inserted into the skin using an angle of attack virtually parallel to the plane of the skin. Once the needle is in place the material is injected. If properly placed in the dermis, the injected material should result in a bleb or vesicle. If the needle is too deeply placed the no vesicle is apparent on injection and the injection has been subcutaneous. The maximum volume of an intradermal injection should not exceed 0.1 ml and probably should be half that volume.
X. **GAVAGE**
Successful placement of a tube in the esophagus requires heavy sedation or light anesthesia to prevent tissue trauma or inadvertent placement of the tube in the airway. Light halothane or methoxyflurane anesthesia will suffice and recovery is rapid. Injectable agents for anesthesia are adequate, but recovery times are prolonged (see section on Anesthesia for appropriate species).

A. **EQUIPMENT**
1. Polyethylene tubing and tubing adapter to allow syringe attachment (mice) or gavage needle (blunt end or ball-tipped) (rat or guinea pig)
2. Appropriate size syringe barrel cut to a short cylinder to use as an oral speculum to prevent chewing on the tube or a needle.
3. Empty the syringe.

B. **METHOD**
Place anesthetized or sedated animal in lateral recumbency and extend the head. Place the syringe barrel "speculum" into the mouth and hold it open. Introduce the tubing or gavage needle through the syringe barrel and gently to the end of the mouth. Push gently with the head slightly extended to allow swallowing. When the tubing or needle passes a point of resistance and advance, introduce it far enough to reach the distal esophagus. At this point stop and be certain the tube or needle is not in the airway. Hold some light material (i.e., few strands of fur work well) at the opening of the tube adaptor or gavage needle to be sure there is no air current through the opening during breathing. Then attach the syringe and administer the drug slowly, but readily. There may be swallow movement during the procedure. Now, remove the syringe and attach the empty one filled with air and administer the air and clear the tubing or needle of drug before pulling the tubing or needle out past the larynx.

XI. **OTHER RESTRAINT AND SURGICAL EQUIPMENT**

A. Rodent restrainer equipment vendors:
   - **Allentown Caging Equipment, Inc.** 800-762-2243;
   - **Ancare Corp.** Braintree Scientific; Harvard Apparatus; 800-645-6379;
   - **Lomir Biomedical Inc.** 518-483-7697;
   - **Lab Products, Inc.** [http://www.labproductsinc.com](http://www.labproductsinc.com); 800-526-0469;
   - **Plas-Labs, Inc.** 800-866-7527; plaslabs@aol.com;
   - [http://www.plas-labs.com](http://www.plas-labs.com)