

Basic Bi methodology for Laboratory Mice

Introduction:

This training program was developed to assist you in becoming proficient in performing common techniques in the mouse. The information provided illustrates the most common mouse techniques used at the National Institutes of Health. These techniques must be described in your approved Animal Study Proposal before you attempt to perform them. To obtain maximum benefit from this program, we suggest that you review the Definitions and the References available.

Module 1 – General Information:

We all have an ethical responsibility to animals in terms of minimizing pain and distress. This can be accomplished, in part, by using proper animal handling and experimental techniques. If you are unfamiliar with the correct way to perform a particular procedure, you should review the appropriate module and consult your veterinarian for further training. There is a scientific responsibility in terms of performing and reporting good science, but there is also a legal responsibility.

The Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Animal Welfare Act require institutions to ensure that people caring for or using animals are qualified to do so. Institutions base their animal care and use program on the Guide for the Care and Use of Laboratory Animals, also referred to as the Guide. This CD represents one component of the comprehensive training program offered by the NHGRI Office of Laboratory Animal Medicine to meet the Guide specifications on the training of personnel.

The following principles, described in the Guide, apply to basic bi methodology for laboratory mice: personnel caring for animals should be appropriately trained. The institution should provide for formal or on-the-job training to facilitate effective implementation of the program and humane care and use of animals.

Entrance Procedures

Access to most NIH animal facilities is by card-key and/or punch pad access codes. They are for the sole use of the person to whom it was issued. Card keys and access codes are not to be shared. Sharing of personal codes or card keys could result in termination of your access privileges. Animal users are not provided with access to the animal facility until all training requirements are met.

When traveling between multiple animal housing areas, the veterinarian should be consulted for the proper traffic pattern to avoid the possibility of cross-contamination between facilities. A shower and change of clothes may be required before entrance is allowed into a second facility.

Equipment and supplies should not be transported between facilities or from the lab to the vivarium without undergoing proper disinfection. There are several choices of disinfection procedures, which include spraying down with Clidox® or another similar disinfectant, ethylene oxide sterilization, steam autoclaving or cold sterilization. You should consult your veterinarian for the proper selection of disinfection procedures.

Personal Protective Equipment

All animal facilities require some level of protective clothing in order to protect the animals housed within from contaminants that may enter the facility via the personnel and to protect the personnel from exposure to animal allergens or other potential hazards. Examples of protective clothing are lab coats, jumpsuits, shoe covers, hair bonnets, masks and gloves.

In most cases, entry requirements are posted on the facility or animal room doors. If you have questions, you should contact the facility veterinarian.

Microisolator Technique

It is your responsibility to become familiar with facility requirements prior to beginning any animal work. Some facilities require that all animal cages be opened inside a biosafety cabinet. In this case, the cages are sprayed with disinfectant prior to placing them in the cabinet and prior to removing them from the cabinet. Hands are sprayed with disinfectant prior to handling the cage and again prior to handling the cage contents.

Other facilities manipulate the cages on a cart or bench top and use a modified microisolator technique. In this case, the cage and the gloved hands are sprayed with disinfectant. The microisolator lid is removed and placed inverted on the bench or cart. The hands are sprayed with disinfectant again. The wire bar lid is removed and placed on the inverted microisolator top. The mice are transferred. Disinfection of the gloved hands is repeated between each cage. Make sure the disinfectant is allowed to drain from the gloved hands so that excessive amounts of disinfectant do not come into contact with the animal. Disinfectants used for microisolator technique vary between facilities.

Assessing the General Health of Mice

A brief assessment of the health of every animal should be conducted prior to performing any technical procedures. The animal should be observed for signs of illness including ocular or nasal discharge, rough hair coat, abnormal posture, uterine, rectal or penile prolapse, limb abnormalities, abdominal distension, malocclusion, dehydration, dystocia, or abnormal behavior. Any signs of pain or distress should be reported immediately to the veterinarian, using the reporting procedures established by the animal facility Standard Operating Procedures. Any animal welfare concerns should also be reported immediately via the appropriate channels.

Observe the feed and water supplies to ensure that there is evidence that the animal has been eating and drinking.

Mice are social animals and should be housed in compatible groups. However, group-housed males will often fight. They should be observed closely for fight wounds and separated immediately if fighting is noted.

Barbering may also be seen in group-housed mice of both sexes. The muzzle and other areas of the body are shaved by the dominant mouse in the cage. Removing the dominant animal may stop the behavior, but frequently another mouse assumes the role.

It is important that every animal handler be properly trained to distinguish between male and female mice. The anogenital space is almost twice as long in the male as it is in the female. Male mice also lack nipples. It is more difficult to differentiate the sex of neonatal mice. Sometimes it is helpful to compare two animals side by side for a reference point.

Mice are nocturnal. Mice also exhibit strong burrowing and nesting behavior and should be provided with bedding materials, such as Nestlets™, that encourage this activity. Other environmental enrichment devices should also be utilized, as appropriate. Some examples include paper or plastic tubes, igloos, food treats and chew toys. All enrichment devices must be approved by your veterinarian prior to use.

Remember

- You have an ethical and legal responsibility to treat all animals in a humane manner.
- All personnel must be appropriately trained.
- You must adhere to all facility entrance requirements.
- Always use proper microisolator technique.
- Always consult your veterinarian if you need assistance.

Module 2 – Restraint:

When attempting to restrain mice, sudden, jerky moves should be avoided to decrease the likelihood of being bitten. Approaching mice with gentle confidence is best. It is important to select the appropriate method of restraint for the procedure you wish to perform and one that will offer the best access to the area requiring manipulation. If you have questions concerning restraint selection, please consult your veterinarian.

Restraint by the tail or with forceps is only intended for short-term manipulations, such as transferring animals from one cage to another.

Tail Restraint

Mice may be picked up by grasping the base of the tail. Do not grasp the tip of the tail, as this may cause the skin to be stripped off. This method is only used for brief restraint; for example transferring animals from cage to cage. Never suspend the mouse for prolonged periods of time by its tail.

Forceps Restraint

Mice may also be picked up with rubber-tipped forceps by gently grasping the animal by the scruff of the neck or the base of the tail. The forceps should be dipped in disinfectant between cages. This method of restraint should only be used for short-term procedures such as transferring animals to a new cage. Never suspend the animal for a prolonged period of time with the forceps.

Scruff Restraint

Using the scruff or mechanical devices is suggested for procedures requiring more than momentary restraint, such as injections or blood withdrawal. Restraining the mouse by the scruff will allow you to perform many technical procedures such as examination, injection and blood collection. There is a one-hand and a two-hand variation of this technique.

The one-hand method places you at greater risk for being bitten, so beginners should perfect the two-hand restraint method before attempting the one-hand method. For the two-hand technique, restrain the mouse by grasping it near the base of the tail and placing it on a toe-gripping surface, such as a wire bar lid. Pulling back gently on the tail of the mouse causes it to pull forward on the toe-gripping surface. Caution must be used to avoid injuring the tail or toes of the mouse. While grasping the tail with one hand, grasp the nape or scruff of the neck with the other. Position the animal's body firmly across your hand by extending your forefinger and thumb back as far as possible, while maintaining a firm grip on the scruff. Place the tail between the fingers of this same hand to secure the animal. This type of restraint will allow the handler complete access to the ventral side of the mouse. Again, caution must be used. If you do not grasp enough of the scruff, the animal will be able to turn and bite. If you grasp too much skin, the airway will become restricted and the mouse will become cyanotic. Monitor the condition of the animal the entire time it is restrained, being careful to observe the breathing rate and color of the ears, nose and oral cavity. The animal should be released immediately if there are any signs of gasping or change in coloring from pink to blue.

For the one-hand technique, restrain the mouse by grasping it near the base of the tail and placing it on a toe-gripping surface. A good example of an appropriate surface is the wire bar lid. Place the base of the tail between or underneath your last one or two fingers. With the thumb and first finger of the same hand, grasp the nape or scruff of the neck. The same precautions as described for the two-hand technique must be followed.

Mechanical Restrainers

Plexiglass restrainers are available from several different manufacturers in a variety of styles. They allow the user to have both hands free for manipulation. Depending on the type of restraint device, the mouse is placed in the restrainer either tail or head first.

If it is a head-first restrainer, you can use a variety of methods to encourage the mouse to enter the restrainer.

Restrain the mouse by the scruff, as described earlier, and direct its head into the opening of the restrainer. Once you release the scruff, most mice will readily enter the restrainer. If the restrainer has a securing device, affix it firmly to prevent the mouse from exiting the apparatus.

Alternatively, grasp the mouse by the base of the tail with one hand and cover the top of the restrainer with the other to form a darkened tunnel. Most mice, once they are shown the entrance to the tunnel, will readily enter the restrainer. Affix the securing device.

Tail-first restrainers are appropriate for procedures such as tail vein injections and blood collections.

If you are using a tail-first restrainer, grasp the mouse by the base of the tail and slide its hindquarters first into the restrainer, using the slot as a tail guide. Once the mouse is in the restrainer, insert the securing device to prevent the animal from exiting the apparatus. Use caution when placing the securing device to prevent injury or restriction of the animal's breathing.

Monitor the breathing rate and color of the ears and nose for the duration of the restraint. Release the animal immediately should there be any signs of gasping or change in color from pink to blue.

Plastic bag restrainers are another option for short-term restraint of mice. Only use bag restrainers that are specifically designed for use with animals. AIMS® is one example of a commercial vendor that distributes bag restrainers. The bags are available in two sizes for mice, depending on whether the mouse weighs more or less than 20 grams.

Grasp the mouse by the scruff and direct its head first into the large end of the bag. Release the scruff and the animal will move forward into the bag. Ensure that the animal's nose is situated in the opening located in the small end of the bag. Use a twist tie to loosely close the large end of the bag around the tail.

Remember

- Select the appropriate restraint method for the procedure you wish to perform.
- Release the animal immediately should you observe any change in breathing rate or change in coloration.
- Consult your veterinarian should you have any questions concerning animal restraint options.

Module 3 –Identification Methods:

It is important to select the appropriate identification method for your research purposes. The method of identification selected must be described in the Animal Study Proposal. Your choice of identification should be based on the age of the animal you wish to identify, the number of characters you wish to include and the duration of your experiment. It is recommended that you record the identification information on the cage card in the event that clarification of the numbers or characters becomes necessary for any reason.

Indelible markers can be used for short-term identification. Alternatively, ear punches, microchips, and tattooing are all permanent procedures. Ear tags can be long-term, but there is always a chance they can become detached from the ear.

Consult your veterinarian if you have questions on selecting an appropriate identification method for your animals. The NHGRI Guideline 01.3 “Identification Methods for Mice” can also be used as a reference.

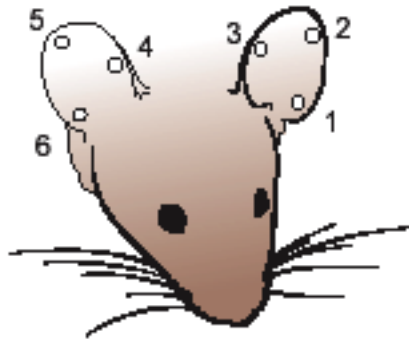
Temporary Identification

Non-toxic, permanent markers can be used to temporarily mark the fur, tail or skin of the animal. This ink, depending on the location, usually lasts 3 - 4 days without the need to remark.

Ear Punches

Different types of ear punches are available. Ear punches should be sterile prior to initial use. Extra ear punches should always be available, as they become dull with repeated use.

NHGRI uses the simplified system shown here, but there are many other numbering systems available that utilize both punches and notches.



To identify the mouse by ear punch, restrain it by the scruff using one of the methods demonstrated in the restraint section of this program. The punch should be placed approximately 3 mm from the edge of the ear pinna. If the punch is placed too close to the edge of the pinna, it is likely to tear and become difficult to read. You should also be careful not to place the punch too far towards the inside of the ear to avoid injuring the animal. The tissue removed with the ear punch can be used for genotyping.

Sanitize the ear punch between each cage of animals with 70% ethanol. Using a chlorinated compound will cause the punch to become corroded. Re-sterilize the instrument after use.

Microchip Transponders

The microchip transponders are implanted subcutaneously between the scapulae for permanent identification of individual animals. Each microchip is encrypted with a unique, non-replicable number. The chips are read with a portable, hand-held scanner.

To implant these chips, the mouse must be briefly anesthetized. The hair is removed from the insertion site by shaving or plucking. The area is prepped with an iodophor, followed by alcohol. The implantation needle, with the syringe attached, is purchased in a sterile package.

Make a tent from the loose skin at the implant site. Insert the needle subcutaneously, with the bevel up, and depress the plunger. Once the needle is removed, the injection site should be observed for bleeding. If bleeding is noted, digital pressure with a sterile gauze pad should be applied. If necessary, a drop of surgical glue can be applied to the needle entry site.

Tattooing

Tattooing can be used on both neonates and adults as a permanent method of identification. Anesthesia is not required, but can be used, if necessary, to immobilize the animal.

There are two options available for tattooing at NHGRI. One is the AIMS® System, which consists of a tattoo machine that can be used to write numbers or other characters on the tails of adult mice. It can also be used to tattoo the footpads of both neonatal and adult mice.

The use of tattoo equipment requires training beyond the scope of this CD. AIMS® provides a course and certification program. Consult your veterinarian or equipment manufacturer for further instructions on performing tattooing.

The Aramis® tattoo system is a mechanical device that can be used to tattoo the footpads of adult or neonatal mice. One example of a numbering scheme is shown below.



It is important to prevent potential cross contamination associated with the use of this equipment. With the AIMS system, needles should be disinfected prior to use. For the Aramis system, needles should be discarded after use. For both systems, excess ink should be discarded after use. The tattoo apparatus and other supplies should be ethylene oxide sterilized, cold sterilized or steam sterilized before use between animal rooms.

Ear Tags

Ear tags are another means for identifying mice. Ear tags can be imprinted by the manufacturer with several digits or letters.

Special attention should be given to the proper placement of the ear tag. Improperly placed ear tags can become easily detached from the ear. The tags can also be torn out when the mice fight or may inadvertently become caught in the wire bar lid.

Restrain the mouse by the one or two-hand method. Place a sterile ear tag into a sanitized ear tag applicator. Locate the proper position for placement. For example, place the numbers in the upward configuration so that they can be easily read without restraining the animal. Apply the tag to the base of the ear, approximately 3 mm from the edge of the ear pinna.

Do not apply the tag too close to the center of the ear. This may cause excessive inflammation, necessitating removal of the tag. Do not apply the tag too close to the outer edge of the pinna. This may cause the tag to become entangled with the foot of the animal or in the wire bar lid, causing it to become detached from the ear.

Toe clipping as a means of identification is not allowed at NHGRI.

Remember

- Select the most appropriate type of identification for your research purposes.
- The method of identification must be described in the Animal Study Proposal.
- Proper placement is essential for the use of ear punches or ear tags.
- Identification procedures should be initiated with sterile instruments. In addition, these instruments should be sanitized between cages of animals.
- Consult your veterinarian if you have questions on the selection or use of identification methods.

Module 4 – Genotyping:

There are various methods available for collecting tissue or blood for genotyping. The technique selected must be described in your approved Animal Study Proposal. Please consult your veterinarian if you need additional assistance in the selection of the technique appropriate for your research.

Tail Snips

Most commonly, genotyping of mice is accomplished by amputating a small portion of the distal tail. This is best performed when the pup is 10 - 15 days old. Prior to attempting this procedure, you should become familiar with the NHGRI Guideline 00.2 “Procedures for Tail Biopsy for DNA Analysis and Genotyping in Mice” and you should receive training from your veterinarian.

Five millimeters, or less, of the distal tail is removed for this procedure. The tail is sprayed with a topical hypothermic prior to collection of tail tissue. As an alternative to the topical hypothermic, the animal may be anesthetized. A scalpel or scissors can be used to remove the tissue. The instruments are sterile at the beginning of the procedure and sanitized with Clidox®, Alcide® or other appropriate disinfectant between animals.

You must assure that adequate hemostasis has been achieved before returning the animal to its cage. Surgical glue, silver nitrate or direct pressure with a sterile gauze pad can be used for this purpose. The tissue removed during ear punching can be used for polymerase chain reaction for rapid screening of mice.

Some transgenic mice are genotyped by blood sampling. Please refer to the blood collection section of this program for the proper technique for obtaining blood samples. Other alternatives to tail snipping for genotyping are also available. These include the analysis of hair, saliva, or feces. This information is available in the reference section.

Remember

- Select the genotyping method appropriate for your research purposes.
- Tail snips should be performed on mice at 10 - 15 days of age.
- A topical hypothermic must be used to numb the tail or the animal must be anesthetized prior to performing a tail snip.
- Do not remove more than 5 mm of the tail.
- Begin with sterile instruments and sanitize between animals.
- Ensure good hemostasis following the procedure.
- The genotyping method must be described in the Animal Study Proposal.

Module 5 – Injections:

Various routes exist for injecting mice. It is important that you discuss the appropriate route, volume, site and needle selection with your veterinarian. All injections must be described in your approved Animal Study Proposal. All injections must be performed using sterile needles and syringes. A new needle and syringe should be used for each cage of mice.

Intramuscular Injections

Regardless of the method used for intramuscular injections, it must be noted that the sciatic nerve runs along the length of the femur. It is very important to avoid injuring this nerve. This is best accomplished by pointing the needle, caudally rather than cranially, into the caudal thigh muscles. It is imperative that the mouse be properly restrained. If the mouse is allowed to kick or struggle, this could cause injury to the muscles or the nerve. It is best to swab the area with 70% ethanol before placing the needle and to aspirate to look for blood before injecting. One of the following methods may be used:

Method #1

Restrain the mouse by the scruff method. Secure the rear foot nearest to you beneath your little finger and lower thumb. Swab the area to be injected with 70% ethanol. Insert the needle, bevel up, into the caudal thigh at a 45° angle. Aspirate to ensure that you have not entered a blood vessel. If no blood is seen, slowly inject the material.

Method #2

Restrain the mouse by the scruff method. Swab the area to be injected with 70% ethanol. Insert the needle into the caudal thigh, bevel up. Aspirate and inject. Since the foot has not been secured, make sure the mouse does not kick.

Method #3

A technique using two people can also be used for IM injections. One person restrains the mouse by the scruff method with one hand and steadies the leg to be injected with the other. The second person identifies the caudal thigh muscles, swabs the area with 70% ethanol, aspirates and injects the material.

Method #4

A tail first restrainer can also be used for IM injections. Gently pull the foot of the leg to be injected through the restrainer and locate the caudal thigh muscle. Hold the foot firmly, swab the site with 70% ethanol, aspirate and inject the material with the needle bevel up.

Subcutaneous Injections

Restrain the mouse by the scruff method. Use your thumb and forefinger to make a tent of skin over the scruff. Prep the area with 70% ethanol. Insert the needle, bevel up, at the base of the tent. The needle should be inserted parallel to the skin and should be directed toward the posterior of the animal. Aspirate to ensure proper placement and inject the material.

Intraperitoneal Injections

Restrain the mouse by the scruff method. Expose the ventral side of the animal, tilting the head down at a slight angle. Prep the site with 70% ethanol. The sterile needle should be placed, bevel up, in the lower right or left quadrant of the animal's abdomen. Insert the needle at a 30° angle. Aspirate to ensure proper placement and inject the material.

Intravenous Injections

Warm the mouse under a heat lamp or other heating device, being sure not to OVERHEAT the animal. The temperature should not exceed 85 - 90° Fahrenheit at the level of the animal. Remove the mouse from the heat source immediately should any change in respiration rate or excessive salivation be observed. Other heating devices, such as disposable handwarmers, may be used in lieu of a heat lamp.

Place the animal in a restraint device and stabilize the tail between the thumb and forefinger of the hand that will not be manipulating the syringe. Prep the tail with 70% ethanol. Attempt the injection starting at the middle or slightly distal part of the tail. With the tail under tension, insert the needle, bevel up, approximately parallel to the vein and insert the needle at least 3 mm into the vein. DO NOT ASPIRATE, as it will cause the vein to collapse. Inject the material in a slow, fluid motion. You should be able to see the vein blanch if the needle is properly positioned. If any swelling at the injection site or resistance to injection occurs, remove the needle and reinsert it slightly above the initial injection site.

Intradermal Injections

In order to perform intradermal injections, the mouse should be anesthetized. Shave or pluck an injection site on the back of the animal to remove the hair. Swab the site with 70% ethanol. Insert the needle into the skin, bevel up, holding the needle nearly parallel to the plane of the skin. Do not aspirate. Inject the material. The volume of the injection should be limited to 50 µl per site to avoid tissue trauma. A properly performed intradermal injection will result in a small, round skin welt.

Oral Gavage

Gavaging is used to dose an animal with a specified volume of material directly into its stomach. Only a specialized, commercially available gavage needle should be used for attempting this procedure. Fill the syringe with the appropriate volume of material and attach the needle.

Restrain the animal by the scruff. Place the tip or ball of the needle into the animal's mouth. Slide the tip gently past the back of the tongue. The needle should slide easily down the esophagus, if properly placed. DO NOT FORCE!!! If any resistance is met, remove the needle and reinsert. Do not aspirate. Once the needle is properly placed, administer the material.

A table of injection routes, sites, volumes and needle sizes is shown below.

INJECTION SITES AND VOLUMES

ROUTE	SITE	MAXIMUM VOLUME	MAXIMUM NEEDLE SIZE
SQ	Scruff	2 ml	22 gauge
IM	Caudal Thigh	0.05 ml	25 gauge
IP	Lower Ventral Quadrants	2 ml	25 gauge
ID	Lateral Abdomen/Thorax	0.05 ml	27 gauge
IV	Lateral Tail Vein	0.5 ml	25 gauge

Remember

- Select the appropriate restraint method, injection route, volume, site and needle gauge for your research purposes.
- Prep the site with 70% ethanol.
- Always inject with the needle bevel up.
- When warming animals, **DO NOT OVERHEAT**.
- All injection procedures must be described in your Animal Study Proposal.
- Consult your veterinarian for further information concerning injections.

Module 6 - Blood Collection:

It is important to select the proper method of blood collection that corresponds to the volume required for your research purposes. Some methods are intended for survival and others are not. Consult your veterinarian for more information.

Retro-orbital Sinus Blood Collection

The retro-orbital sinus is the site located behind the eye at the medial or lateral canthus. This venous sinus is located just underneath the conjunctival membrane. This method is intended for survival blood collection.

No more than 10% of the blood volume should be removed at one sampling. The blood volume of a mouse is approximately 8% of the body weight. For example, a 25 gram mouse has a blood volume of approximately 2 ml, so no more than 200 µl of blood can be removed at a single bleeding without scientific justification and approval of the Animal Care and Use Committee. Mice should not be bled more frequently than every 3 weeks unless smaller volumes are collected.

Restrain the mouse by the scruff method. It is imperative that the mouse be properly restrained. If the mouse is allowed to move its head, severe injury to the eye or surrounding tissues could occur.

A topical ophthalmic anesthetic must be used prior to performing this procedure. Apply one drop of an anesthetic such as proparacaine or tetracaine hydrochloride to the eye. Be careful not to touch the tip of the applicator to any part of the mouse. This will cause contamination of the anesthetic. Wait 5 - 10 seconds after the anesthetic is applied before attempting this procedure. Gently blot away excess anesthetic with a clean gauze pad, being careful not to scratch the cornea. An alternative to topical anesthesia for this procedure is general anesthesia.

With a gentle rotating motion, insert the tube through the sinus membrane. Continue rotating the tube at the back of the orbit until blood flows. Collect the appropriate volume of blood. Upon completion, ensure good hemostasis with a clean gauze pad before returning the animal to its cage. Be careful not to scratch the cornea with the gauze pad.

To become proficient at this technique, additional training outside the scope of this text is required. Please contact your veterinarian for appropriate training. The NHGRI Guideline 00.1 “Procedures for Retro-Orbital Bleeding in Mice” can also be used as a reference.

Blood Collection Via the Lateral Tail Veins

Tail nicking is a survival procedure that can be used to collect up to 200 µl of blood from the lateral tail veins. This method must be used with caution, as when improperly performed, permanent tail injury or amputation may occur.

Warm the animal under a heat source, being careful NOT to overheat. The temperature at the level of the animal should not exceed 85 - 90° Fahrenheit.

Place the mouse in a restrainer. Prep the tail with 70% ethanol. Stabilize the tail with the thumb and forefinger of the hand that will not be used to nick the tail. Using a #11 scalpel blade, gently nick the lateral tail vein in the general area around the midline of the tail. Start at least half way down the tail so that if there is a problem, you can nick the tail above the initial site and still obtain your blood sample. Allow the blood to flow into an appropriate receptacle. Do not squeeze the tail or attempt to milk blood from the tail. This may cause tissue damage and contamination of the blood sample with tissue fluids. When an appropriate volume has been collected, ensure good hemostasis with a dry, sterile gauze pad, surgical glue or silver nitrate.

Intracardiac Puncture

Intracardiac puncture must be performed under deep anesthesia and is considered a nonsurvival procedure.

Once the mouse is deeply anesthetized, prep the ventral chest area with 70% ethanol. Insert the needle at the base of the sternum, bevel up, into the thoracic cavity at a 15 - 20° angle directed just to the left of the midline. Aspirate slowly. If blood starts to flow into the syringe, continue to aspirate with steady, even pressure. If no blood is seen, reposition the needle and attempt aspiration. Once the required blood volume is collected, the mouse is euthanized while still deeply anesthetized. Up to one milliliter or more of blood may be collected from an adult mouse using this method.

Alternatives to the methods described here include collecting blood from the saphenous vein. A description of this method can be found in the reference section.

Remember

- Select the appropriate restraint technique and method of collection that corresponds to the volume of blood required for your research purposes.
- No more than 10% of the blood volume should be removed at one sampling.
- Always use ophthalmic anesthetic prior to retro-orbital blood collection.
- When warming animals, DO NOT OVERHEAT.
- Ensure good hemostasis.
- The method of blood collection must be described in your Animal Study Proposal.
- Consult your veterinarian for further information on blood collection.

Module 7 – Anesthesia/Analgesia:

This module will provide a brief introduction to analgesia and anesthesia in the mouse. Your veterinarian should always be consulted for advice on selection and administration of analgesia or anesthesia. The use of analgesics and/or anesthetics must be described in detail in your approved Animal Study Proposal.

Injectable

The most commonly used analgesics for mice at NHGRI are shown below. For the management of pain in association with surgery or other procedures causing discomfort, injectable analgesics may be used. Refer to the table shown below and your veterinarian for appropriate dosages and frequency of administration.

TABLE OF COMMONLY USED ANESTHETICS AND ANALGESICS FOR MICE

DRUG (GENERIC NAME)	DRUG (BRAND NAME)	ROUTE OF ADMINISTRATION	CONCENTRATION	DOSE	FREQUENCY	USE
tribromoethanol	Avertin®	Injectable - IP	1.25%	0.02 ml/gm	N/A	Anesthetic
bupivacaine	Marcaine®	Painted on incision site	0.50%	1-2 drops	N/A	Analgesic
buprenorphine	Buprenex® CV	Injectable - SQ, IP	0.3 mg/ml	0.2 mg/100 gm	Every 12 hours	Analgesic
butorphanol	Torbutrol® CIV	Injectable - SQ	0.5 mg/ml	2.5-5.0 mg/kg	Every 1-2 hours	Analgesic Dermal
ethyl chloride	Ethyl Chloride®	Topical		3-7 second spray	N/A	Anesthetic
halothane*	Fluothane®	Inhalant		100% O ₂	N/A	Anesthetic
				≤4%HAL-induction		
				~1.5%HAL-maintenance		
isoflurane*	Aerrane®	Inhalant		100% O ₂	N/A	Anesthetic
				≤4%ISO-induction		
				~1.5%ISO-maintenance		
ketamine/xylazine	Ketaset®CIII/ Rompun®	Injectable - IM, IP	100 mg/ml (ketamine) 20-100 mg/ml (xylazine)	100 mg/kg Ketamine# 10 mg/kg Rompun#	N/A	Anesthetic
ketoprofen	Ketofen®	Injectable - SQ	100mg/ml	0.08-0.16 mg/kg	Every 12 hours	Analgesic
pentobarbital	Nembutal® CII	Injectable - IP	50 mg/ml	75-100 mg/kg	N/A	Anesthetic
proparacaine	Alcaine®	Topical Ophthalmic	0.50%	1-2 drops	N/A	Analgesic
ropivacaine	Naropin®	Painted on incision site	0.50%	1-2 drops	N/A	Analgesic
tetracaine	Tetracaine®	Topical Ophthalmic	0.50%	1-2 drops	N/A	Analgesic

NOTE: These are suggested drugs and doseages only. All anesthetic and analgesic doses may vary with strain, sex, or age of the mice. Always check with your veterinarian before administration.

*Requires proper scavenging equipment

#There are many other dose combinations suggested in the literature.

It is important to weigh the mice prior to dosing with injectable anesthetics to avoid over or under dosing the animals.

Topical

As an adjunct to, or in lieu of injectable analgesics, topical anesthetics may also be used. These long-acting agents are painted or dropped into the surgical wound before the skin is closed.

To facilitate retro-orbital sinus blood collection, an ophthalmic anesthetic is used as a topical analgesic. A single drop of the solution is placed on the eye. Be careful not to touch the tip of the applicator to any part of the mouse. This will cause contamination of the anesthetic. After approximately 5 - 10 seconds, gently blot away the excess anesthetic with clean gauze, being careful not to scratch the cornea. Proceed with blood collection. Pay special attention to the storage requirements for the ophthalmic anesthetics, as some require refrigeration.

For mice undergoing tail snips for genotyping, a topical hypothermic agent is sprayed on the tail. This topical analgesic is required for mice greater than 10 days of age. The tail is sprayed continuously for 3 - 7 seconds from a distance of 3 - 9 inches. The tail will blanch showing a proper amount of the agent has been applied. The distal 5 mm or less of the tail is then excised, as described in the genotyping section of this program.

At NHGRI, the most commonly used injectable anesthetics for mice are tribromoethanol, pentobarbital and a ketamine/xylazine mixture.

Since tribromoethanol, known by its European brand name Avertin®, is not commercially available in the U.S., it is prepared in the laboratory. This requires strict aseptic technique and special storage considerations. The solution should be filtered through a 0.22 micron filter. This will remove debris, most bacteria, and some viruses. The concentrated stock solution should be stored at -20° Centigrade or colder. The working solution should be stored at 4° Centigrade in a sterile amber or aluminum foil-wrapped bottle.

When diluting the stock solution of Avertin® to the working solution, it is important to use a *buffered* diluent. Decomposition of the anesthetic can result from improper storage. The pH should be greater than 5. Avertin® is administered as an IP injection. The most common dose is 0.24 to 0.4 micrograms per gram body weight. Refer to NHGRI Guideline 03.2 “The Use of Tribromoethanol in Mice” for additional information on storage and handling requirements.

Pentobarbital, buprenorphine and butorphanol are controlled substances and can only be purchased through your Controlled Substances Officer. Ketamine is also considered a controlled substance and must be purchased through your Controlled Substances Officer.

Detailed records are required for the use of controlled substances. Failure to maintain proper records can result in the loss of your privilege to use these agents. Please note that dosages may vary according to the strain, age, sex, etc. of the animals.

Inhalants

At NHGRI, the most commonly used inhalant anesthesia is isoflurane. There are several types of gas anesthesia machines available for use. Isoflurane is administered in 100% O₂. Induction concentrations of isoflurane are 3 – 4%. Maintenance concentrations are 1.25 – 1.75%. Inhalant anesthetics must be used with scavenging devices.

One acceptable scavenging method is the use of a downdraft table. Chemical fume hoods, charcoal canisters or Type IIB2 biosafety cabinets, which are vented to the outside, can also be used. Note that charcoal canisters must be weighed before, and after, each use. Most must be replaced after an increase in the recommended weight. Depending on the size of the canister and the vendor, the canister should also be weighed during especially long procedures to assure its continued effectiveness.

Contact your veterinarian for further training in the appropriate use of the anesthesia machines available within your facility.

Monitoring

Anesthetized animals must be closely monitored during the procedure to assure that they are maintained in the proper anesthetic plane. If the anesthetic plane is too light, the animals may start to move or struggle. If the anesthetic plane is too deep, the animals may die. Once the anesthesia has been administered and enough time has elapsed for it to take effect, the anesthetic plane can be assessed by pinching the toe, tail or ear of the animal. Any reaction from the animal indicates that the anesthesia is too light and that additional anesthesia should be given.

The respiration and color of the mucous membranes and exposed tissue of the animal should also be closely monitored. The respiration rate should be even. An increased respiration rate is a sign that the anesthesia is too light. A deep, shallow, decreased or irregular respiration rate is indicative of anesthesia that is too deep. The color of the mucous membranes and exposed

tissues should be bright pink to red. Dusky gray or blue color is indicative of an anesthetic plane that is too deep.

Core body temperature can also be monitored in rodents. The most common anesthetic complication is hypothermia. Measures must be taken to control the body temperature before, during and after anesthesia. There are several choices of warming devices. Your veterinarian should be consulted to determine the appropriate equipment to meet your research needs.

Recovery

During recovery, the animal should be placed on clean, dry gauze or paper toweling to avoid contact with the bedding, which may be inadvertently inhaled and result in asphyxiation. Recovery from anesthesia can also be aided by the administration of warmed fluids given subcutaneously or intraperitoneally. Your veterinarian should be consulted for appropriate volumes and routes of administration.

Once the animal has reached sternal recumbency and appears to be making a normal recovery, it may be returned to the animal holding area. Animals should continue to be monitored closely for several days following the procedure. It is important to identify the cages with “Special Watch” cards or other similar methods so that particular attention will be afforded these animals during the daily health checks. A surgical record of some form must also be kept when surgery is performed on rodents. These records must be maintained for at least three years following closure of the protocol.

Depending on the nature of the procedure, it may be beneficial to monitor weight and hydration for several days. A softer, more palatable diet and/or fluids may be necessary.

Remember

- Consult your veterinarian for advice on the selection and administration of anesthesia and analgesia.
- The use of anesthetics and or analgesics must be described in your Animal Study Proposal.
- It is important to weigh animals prior to dosing.
- Use a topical hypothermic prior to performing tail snips.
- Use a topical analgesic prior to performing retro-orbital sinus blood collection.
- Be certain to follow NHGRI Guideline 03.2 when preparing or handling Avertin®.

Module 8 – Euthanasia:

Your veterinarian should always be consulted for advice on selection and administration of euthanasia agents. The euthanasia method selected for use must be described in detail in your approved Animal Study Proposal. Refer to NHGRI Guideline 01.1 “Euthanasia of Rodents” and the AVMA Panel on Euthanasia for additional information.

CO₂

Compressed CO₂ gas is the only recommended source of CO₂ for euthanasia. Carbon dioxide generated from dry ice is unacceptable.

With an animal in a chamber, an optimal flow rate should displace 10 – 20% of the chamber volume per minute until the mouse is unconscious. This flow rate is associated with a rapid loss of consciousness and minimal distress to the animal. Once the mouse is unconscious, the flow rate can be increased. Gas flow should be maintained for at least 1 minute following apparent clinical death. Death should be verified by the absence of the heartbeat, performing cervical dislocation or by perforating the diaphragm prior to proper disposal of the animal.

Injectable

Injectable anesthetics can also be used for euthanasia, when administered at higher doses. Barbiturate anesthetics produce rapid and humane euthanasia when injected intraperitoneally. Barbiturates are controlled substances and must be procured through your Controlled Substances Officer. The user of controlled substances is accountable for strict record-keeping procedures.

Inhalant

Inhalant anesthetics can also be used for euthanasia of rodents. Halothane is the most effective inhalant anesthetic for euthanasia, but isoflurane is also acceptable.

Inhalant anesthetics used for euthanasia are best utilized with the open drop method using a closed receptacle containing cotton or gauze soaked with the liquid. Care must be taken to prevent direct contact of the animal with the liquid anesthetic. The anesthetic can also be introduced through a vaporizer, but this will increase the time required to achieve euthanasia.

Inhalant anesthetics must be used with a down draft table, in a type IIB2 biosafety cabinet vented to the outside or in a chemical fume hood.

Physical

Cervical dislocation, when properly performed, is a humane method of euthanasia. Anesthetics or CO₂ must be used to narcotize mice prior to cervical dislocation or other physical methods of euthanasia. Physical methods of euthanasia, for example cervical dislocation or decapitation, can be performed without prior narcotization only if scientifically justified in the Animal Study Proposal and approved by the Animal Care and Use Committee.

Fetuses and neonates are resistant to many methods of euthanasia. Special considerations must be given to this age group and are addressed in the NIH ARAC “Guidelines for the Euthanasia of Mouse and Rat Fetuses and Neonates”. This document is found in the reference section.

Remember

- Your veterinarian should always be consulted for advice on selection and administration of euthanasia agents.
- Inhalant anesthetics must be used on a downdraft table, in a type IIB2 biosafety cabinet or in a chemical fume hood.
- Narcotization is required prior to the use of cervical dislocation.
- For the proper methods of euthanasia for fetuses and neonates, Refer to NIH ARAC “Guidelines for the Euthanasia of Mouse and Rat Fetuses and Neonates”.

Definitions:

ACUC: Animal Care and Use Committee

Analgesia: A type of drug that alleviates pain without rendering the animal unconscious.

Anesthesia: A type of drug that eliminates pain by rendering the animal unconscious or by blocking nerve sensation in a certain area of the body.

Anogenital: Space between the anus and the genitals.

Anterior: Anatomical term meaning toward the front of the body.

ARAC: Animal Research Advisory Committee

Aseptic: Free from most microorganisms.

Aspirate: Application of negative pressure to a syringe and needle in an attempt to withdraw fluid.

Asphyxiation: Cessation of breathing and loss of consciousness caused by excess CO₂ or lack of O₂.

Barbering: A common, generally harmless, behavior where a dominant mouse chews the fur off of a subordinate mouse.

Barbiturates: General anesthetic agents that produce unconsciousness (e.g.; pentobarbital, thiopental)

Bevel: The sloping edge or surface of a needle, i.e. as in performing an injection “bevel up”.

Blanch: To become pale.

Canthus: The angle at either end of the fissure between the eyelids.

Cervical: Of the neck.

Caudal: Anatomical term meaning toward the rear of the body or tail.

Cranial: Anatomical term meaning toward the head.

Cyanotic: A condition in which the skin and/or mucous membranes appear blue; caused by lack of oxygen in the blood.

Dehydration: Inadequate water consumption or loss of water from the body.

Disinfection: To destroy or inhibit the growth of microorganisms.

Distal: Anatomical term meaning further from a specific point on the body.

Distention: Swollen from pressure from within.

Dorsal: Anatomical term meaning toward the back; opposite of ventral.

Dystocia: Difficult and unproductive labor.

Euthanasia: Intentional induction of a painless death.

Gavage: To deliver a drug directly into the esophagus or stomach using a bulb-end needle or stomach tube.

Genotyping: A method to determine the genetic constitution of an animal.

Hemostasis: To stop bleeding.

Hypothermia: The condition of low body temperature.

Intracardiac (IC): Into the heart.

Intradermal (ID): Into the thick dermal layer of the skin.

Induction (of anesthesia): The initial establishment of a state of anesthesia.

Inhalant: To receive exposure to a substance via breathing.

Intramuscular (IM): Into a muscle.

Intraperitoneal (IP): Into the abdominal cavity.

Intravenous (IV): Into a vein.

Lateral: Anatomical term meaning away from the midline of the body.

Malocclusion: Improper contact of upper and lower teeth when biting.

Medial: Anatomical term meaning situated in the middle.

Microchip: Implantable device used for identification and tracking of animals.

Microisolator: Caging system with a filtered top that limits air exchange between the room and the cage interior.

Microisolator technique: Procedure utilized to maintain the integrity of the microisolator caging system.

a. Full microisolator technique - Everything that enters the hood (including gloved hands) must be sprayed down with disinfectant.

b. Modified microisolator technique - Procedure utilized when microisolator cages are changed on a cart. The outside of the cage and hands are sprayed with disinfectant prior to removing the microisolator top. The hands are sprayed with disinfectant again prior to handling the wire bar lid and other contents of the cage.

Narcotization: To induce a state of semiconsciousness from which it is difficult to arouse the animal.

Neonates: Newborn animals.

Nestlet™: Commercially available shreddable material used for the environmental enrichment of mice.

NHGRI: National Human Genome Research Institute

NIH: National Institutes of Health

Nocturnal: Animals active primarily at night.

OACU: Office of Animal Care and Use

Ocular: Of, for, or by the eyes; visual.

Pinna: The flap of the ear.

Posterior: Anatomical term meaning toward the rear.

Prolapse: When an organ of the body slips forward or down out of its place.

Proximal: Anatomical term meaning closer to a specific point on the body.

Recumbency: Lying down, prone.

Respiration: Ventilation or the exchange of O₂ and CO₂ between the atmosphere and the cells of the body.

Retro-orbital sinus: Area located behind the eye from which blood is collected from mice.

Scavenging: Device or mechanism by which waste anesthetic gases are either actively or passively drawn away from the patient and/or operator to prevent accidental occupational exposure.

Sterilization: A state of sanitation in which there are no living microorganisms present.

Subcutaneous (SC or SQ): Into the space between the skin and the underlying muscle.

Sternal: On the breastbone.

Topical: Substance applied to the skin.

Ventral: Directed toward or situated on the abdominal surface; opposite of dorsal.

REFERENCES:

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2. Blood collection using the saphenous vein: An alternative to retro-orbital collection http://www.uib.no/vivariet/mou_blood/Blood_coll_mice.html
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8. Hofstetter, JR, A. Zhang, *et al.* Genomic DNA from Mice: A Comparison of Recovery Methods and Tissue Sources. *Biochem. & Mol. Med.* 62:197-202. 1997.
9. NHGRI Guideline 01.1 Euthanasia of Rodents
10. ARAC Guidelines for the Euthanasia of Mouse and Rat Fetuses <http://oacu.od.nih.gov/ARAC/euthmous.pdf>
11. NHGRI Guideline 01.3 Identification Methods for Mice

12. Training in Survival Rodent Surgery CD (available from OLAM or from rodentcd@od.nih)
13. AVMA Panel on Euthanasia <http://www.avma.org/resources/euthanasia.pdf>
14. NIH Guide for the Care and Use of Laboratory Animals <http://www.nap.edu/readingroom/books/labrats/>
15. Animal Welfare Act <http://www.aphis.usda.gov/ac/awa.html>
16. Animal Identification and Marking Systems (tattooing) <http://www.animalid.com>
17. Aramis System (tattooing) <http://www.ketchum.ca/aramis.html>
18. Public Health Service Policy <http://grants.nih.gov/grants/olaw/references/phspol.htm>
19. SOP for Reporting Animal Welfare Concerns
20. NHGRI Guideline 03.2 Use of Tribromoethanol (TBE) in Mice
21. Injection Volume Table
22. Dosage Table

Credits:

This CD was developed by the NHGRI Office of Laboratory Animal Medicine as a training tool for animal users to assist in the development of proper mouse handling and technical skills. It illustrates the most common practices used in the NIH intramural research program.

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Disclaimer:

Illustrations of products and materials in this program are not meant as endorsements by the NIH, but as examples of items commonly used in performing rodent techniques. Similar products may work as well.

In some instances, anesthetized mice were used to demonstrate procedures that would ordinarily be performed without anesthesia. This was done in order to provide more humane conditions, as the procedures performed are intended to be momentary and the photography process required more prolonged restraint.