The Laboratory Mouse

Rodent Users Wetlab

Administered by
Laboratory Animals Centre
National University of Singapore
LAC Veterinarians

- Dr. Patrick Sharp - ext 7164  sharp@nus.edu.sg
- Dr. Leslie Retnam - ext 3051  ahurl@nus.edu.sg
- Dr. Shannon Heo - ext 7870  ahuhsys@nus.edu.sg
- Dr. Jonnathan Peneyra - ext 3790  ahupjl@nus.edu.sg

LAC Laboratory Officers @ Kent Ridge

Animal Holding Unit (AHU, MD1): ext 3291

Mr. Roger Loh (Principal Laboratory Officer) ext 3321  aholohak@nus.edu.sg

<table>
<thead>
<tr>
<th>James Low</th>
<th><a href="mailto:aholowj@nus.edu.sg">aholowj@nus.edu.sg</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jeremy Loo</td>
<td><a href="mailto:ahuley@nus.edu.sg">ahuley@nus.edu.sg</a></td>
</tr>
<tr>
<td>Shawn Tay</td>
<td><a href="mailto:ahutyq@nus.edu.sg">ahutyq@nus.edu.sg</a></td>
</tr>
<tr>
<td>Jonathan Ang</td>
<td><a href="mailto:ahuajsj@nus.edu.sg">ahuajsj@nus.edu.sg</a></td>
</tr>
<tr>
<td>Cecilia Chang</td>
<td><a href="mailto:ahuuccrc@nus.edu.sg">ahuuccrc@nus.edu.sg</a></td>
</tr>
</tbody>
</table>

Satellite Animal Holding Unit (sAHU, MD4): ext 6997

<table>
<thead>
<tr>
<th>Don Heng</th>
<th><a href="mailto:ahuhs@nus.edu.sg">ahuhs@nus.edu.sg</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>Magdalene Koo</td>
<td><a href="mailto:ahuwkml@nus.edu.sg">ahuwkml@nus.edu.sg</a></td>
</tr>
<tr>
<td>Tjou Yanqiu</td>
<td><a href="mailto:ahuaty@nus.edu.sg">ahuaty@nus.edu.sg</a></td>
</tr>
<tr>
<td>S Muhammad Abdul Malik</td>
<td><a href="mailto:ahuusmm@nus.edu.sg">ahuusmm@nus.edu.sg</a></td>
</tr>
</tbody>
</table>
THE LABORATORY MOUSE
Rebecca Schwiebert, DVM, PhD, DACLAM

Taxonomy:

Kingdom       Animal
Phylum          Chordata
Class              Mammalia
Order              Rodentia
Family            Muridae
Subfamily       Murinae
Genus          Mus
Species          musculus

Introduction:

The laboratory mouse was derived from the common house mouse, Mus musculus. Development of the laboratory mouse began with pet fanciers who bred mice for their unique coat colors. These fancy mice then became subjects for research due to interest in the mechanisms of inheritance of these coat colors, and W. E. Castle started studying the genetics of coat color in mice in the early 1900’s. Clarence Cook Little later developed inbred strains of mice in 1909.

As mice became more widely used in research, some individuals began breeding them for sale. Today commercial breeders such as Charles River and Harlan provide most of these animals for the research community. The Jackson Laboratory (Jax), a nonprofit research and training institution, is one of the major suppliers of mice for research in the United States and throughout the world. There are approximately 1750 strains, including inbred strains, hybrids, spontaneous mutants, induced mutants, chromosomal aberrations, and wild derived strains currently available from Jax. Because of the constant discovery of new mutations and the production of knockout and transgenic mice, the number of mouse stocks and strains currently available continues to increase. To give some idea of the importance of rodents in research, during 1998 17.2 million mice and 5.5 million rats were used at 1200 U.S. research institutions, compared to a total of 1.2 million other species. Mice and rats together constitute approximately 90% of the total animals used for all research purposes.

The advantages of mice as research animals are many. Their genetic characterization, the large number of strains available, and the large list of catalogued mutant genes provide animals suited for a number of different areas of research. Mice are easy to care for and handle, and are relatively inexpensive compared to other species. A high reproductive performance with a large litter size and a short gestation means that many generations can be produced in a relatively short period of time (one million descendants after 425 days). The disadvantages of mice as research animals include their small size, which limits the procedures that may be performed as well as the sample volume size that can be obtained from an individual animal. To overcome the latter limitation, samples from several animals may be pooled for research analysis and statistical significance.

The use of the mouse as a research animal has resulted in many scientific advancements. Much of our early understanding of the immune system was derived from studying the mouse. The use of the mouse continues to be an important part of various research endeavors including aging, embryology, cancer
induction, pharmacological and toxicological testing, and infectious diseases research. Transgenic and knockout mice have become important tools for investigating the relationship of genetic make-up to disease states as well as elucidating pathways of normal mammalian development.

Behavior

Mice are timid but social animals. Contact with con specifics (others of their species) is important, and a mouse housed alone may become more aggressive. Although wild mice are nocturnal, laboratory mice have active periods during both the day and night. Categories of common behaviors of mice include: (1) maintenance behaviors (grooming, eating, drinking, nesting); (2) investigative/exploratory behaviors (climbing, digging, chewing, sniffing); and (3) social interactions (huddling together, grooming one another, scent/territorial marking, aggression, defense, sexual behavior). Mice spend a great deal of time manipulating their bedding material, and if the material allows they will build tunnels and nests. Providing appropriate nesting materials to pregnant mice is important, as the nesting behavior is very pronounced in mice.

Behavior may be strain specific (e.g., aggression in C57BL/6 mice compared to C3H), and variations in mouse behavior are becoming increasingly common with the advent of knockout and transgenic mouse strains. In some strains, female mice will attack other females, a behavior that is normally uncommon. Other strains may demonstrate poor nesting behavior, which results in decreased survival of the pups. These behavior changes can affect the reproductive fitness of an individual strain and make production of additional genetically-modified animals difficult.

“Barbering” is a common practice among mice caged together, especially males. The socially dominant animal in the cage will selectively chew off the hair of its subordinates. The missing hair is generally noticed on the head, neck and muzzle, but may also be missing from other parts of the body. The skin of the barbered areas usually appears normal. Barbering patterns may be strain related.

Adult male mice housed together may be very aggressive towards one another. Fighting among cage mates can result in bite wounds over the rump and back of the animal, and are generally more severe in the lowest ranking animals. Separation of fighting animals is required and should be done immediately if a problem is suspected. If male mice are to be housed together, they should be introduced to one another at the time of weaning, and not as mature adults.

Mice that are startled or roughly handled may bite or pinch the handler’s finger with their teeth, but in general mice are easy to handle (with some strain exceptions!).

Housing:

Cage space requirements for mice as listed in the most recent “Guidelines on the Care and Use of Animals for Scientific Purposes” (NACLAR, 2004) are shown in Table 1.

<table>
<thead>
<tr>
<th>Body weight / grams</th>
<th>Floor area per animal / sq. cm.</th>
<th>Cage height / cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>38</td>
<td>12</td>
</tr>
<tr>
<td>10-15</td>
<td>51</td>
<td>12</td>
</tr>
<tr>
<td>15-25</td>
<td>77</td>
<td>12</td>
</tr>
<tr>
<td>&gt; 25</td>
<td>&gt; 96</td>
<td>12</td>
</tr>
</tbody>
</table>

*a Larger mice might require more space to meet performance standards
The practical translation of the cage space requirements for mice are listed in Table 2. The solid-bottomed shoebox cages are the most commonly used method of housing mice.

Table 2.

<table>
<thead>
<tr>
<th>Body weight in grams</th>
<th>Number of mice / NKP cage (372 cm²)</th>
<th>Number of mice / medium cage (406 cm²)</th>
<th>Number of mice / Techniplast and Allentown cages (530 cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10</td>
<td>9</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>10-15</td>
<td>7</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>15-25</td>
<td>5</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>&gt; 25</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Bedding material provides thermal insulation, absorbs fecal and urinary wastes, and in some instances is used for nest construction. The material chosen should be absorbent, not readily eaten, free of infectious agents and injurious substances, and comfortable for the animals. Bedding may consist of paper, hardwood chips, or corn cob materials. The use of aromatic wood shavings such as pine and cedar shavings should be avoided in the laboratory setting as it induces activation of hepatic microsomal enzymes, and this may interfere with experimental results. Dusty bedding should not be used for housing nude mice, as they have no eyelashes to filter dust from the eyes. In addition, use of extremely dusty bedding may result in preputial or respiratory problems in all mice. Only autoclaved bedding should be used for immunodeficient animals to prevent the introduction of opportunistic infectious agents.

The amount of bedding material provided is important. Provision of too much material combined with the digging and piling activities of the mice can result in contact with the water source and result in a flooded cage. Avoid the use of materials like cotton or shredded paper in breeding cages because the pups can become entangled in the fibers, and may suffocate or lose appendages.

The temperature in the mouse room should range from 18°C to 26°C with an average temperature of 22°C. Nude mice or mice that are singly housed may require slightly increased temperatures for comfort. The relative humidity in mouse rooms should be between 40% and 70%. The temperature and humidity conditions of the mouse room are important as low humidity (less than 40%) and high temperature (greater than 26°C) can result in a condition known as ringtail. Ringtail is characterized by the appearance of concentric rings around the tail and frequently results in sloughing of all or part of the tail. The feet may also be swollen and reddened in affected mice. A combination of low humidity and cold temperature can result in tail gangrene in nude mice.

To ensure proper ventilation and removal of ammonia and odors, the minimum number of air changes per hour should be 10-15. In some situations a greater number of air changes per hour may be required to maintain good air quality. The constant presence of the odor of ammonia indicates that rooms are overcrowded or that a greater number of air changes are needed. Keep in mind that the air in cages (the microenvironment), especially those with filter tops, will be 1°-2°C warmer, 5%-10% more humid, and have a greater concentration of ammonia than the surrounding room air (the macroenvironment).

Animal rooms should be regulated by automatic timers to provide cycles with 12-14 hours of light and 10-12 hours of dark. The recommended intensity of light in the mouse room is 100 foot-candles (325 lux) at the working level (about 1 metre above floor level); however, this is for the worker and not for the mouse. Light levels of 30 foot-candles are recommended for albino animals to avoid retinal damage. Keep in mind that mice on the lower racks will receive less light than those housed on the top racks.
Mice are capable of detecting a wide range of auditory frequencies, and can hear both audible sounds and ultrasound frequencies. Ultrasound frequencies are used by mice to communicate during sexual activities, and are also the frequencies of the distress calls of the infant mouse. Audible sound is used in aggressive and defensive behaviors. Noise in the animal facility is a consideration for the management of mice, as audiogenic seizures may occur in some strains of mice exposed to sudden, loud noise stimuli.

**Diet and Nutrition:**

Mice in research facilities are generally fed a pelleted rodent diet *ad libitum*. Maintenance diets generally contain 4-5% fat and 14% protein. Young animals and those used for breeding have higher nutrient requirements; therefore, diets for these animals contain 7-11% fat and 17-19% protein. An adult mouse will consume about 15 grams of feed per 100 grams of body weight per day. Food for immunodeficient rodents should be autoclaved or irradiated to prevent introduction of infectious agents. Young mice may not be able to reach the food, so it is acceptable to put food on the floor of the cage until they are able to eat from the hopper. If the food is too hard for young mice to chew, it may be moistened. Food should not be used more than 6 months after the milling date.

Water may be provided by bottles and sipper tubes or by the use of automatic watering systems. Cages should be checked every day to ensure that water is not leaking into the cage and soaking the bedding; wet mice become hypothermic very rapidly. An adult mouse will consume about 15 ml of water per 100 grams of body weight per day. Mice dehydrate very rapidly when they do not have access to water. Water may in some instances be hyperchlorinated (10 ppm) or treated with hydrochloric acid (pH 2.5) to control bacterial growth; this is especially important when dealing with immunodeficient rodent strains or mice that have been experimentally irradiated. Young mice may require a longer sipper tube in order to reach the water source.

**Animal Identification:**

Identification schemes vary according to the needs of the researcher. Cage card identification may be adequate for many circumstances; however, more precise methods may be required when individuals within a cage need to be readily identifiable. Ear notching is probably the most commonly used technique and the numbering system is shown in Figure 1.
Fighting mice may on occasion obliterate their numbers by chewing on the ears, but in general this system works well. Appropriately sized, numbered metal ear tags may also be used when properly applied. Freeze branding and tattooing are less practical methods due to the small size of mice but are sometimes employed. Toe clipping may only be used to identify neonates. Temporary identification of a mouse may be accomplished by dyeing the fur, clipping the fur, or marking the tails with an indelible marker. Subcutaneously implanted microchips can be used to identify extremely valuable animals (e.g., transgenic founder animals).

Acclimatization:

Transportation of animals is stressful and leads to physiologic changes, such as increased cortisol levels, which may potentially alter research results. Mice received from another site need to have adequate time to recover from shipping stress, and the length of time required may depend on the distance/time involved in transporting the mice. Generally a minimum of 48 hours is required for blood cortisol levels to return to baseline values. A quarantine/holding period allows the mice to adapt to their new surroundings and permits observation for any signs of infectious disease.

Special Anatomical and Physiological Features:

Vertebral formula: C7, T13, L6, S4, C28 (some variation occurs between strains). 13 pairs of ribs.

Both the front and rear paws have 5 toes; on the front paw the first digit is represented by a flattened nail (Figure 2).

Dental formula: 2 (I1/1, C0/0, P0/0, M3/3). The incisors grow continuously throughout life, and are referred to as open-rooted incisors. The molars are rooted. Mice with malocclusion of the incisors require periodic trimming of the incisors so that the animal can eat. Such animals should not be used as breeding stock as the condition is inherited.
Mice have a simple intestine. Mice have a gall bladder, while rats do not. The inguinal ring remains open throughout life, and the testes can be withdrawn from the scrotum into the abdomen. The locations of major lymph nodes are shown in Figure 3. Mice have no tonsils.

Figure 3. From The Mouse in Biomedical Research edited by Foster, Small, and Fox.
On the left side the lung consists of a single lobe, while there are 4 lobes on the right (Figure 4).

The mouse has a large surface area per gram of body weight when compared to larger species of animals; as a result it has a very high metabolic rate and at rest uses 22 times more oxygen per gram of body weight than the elephant. To provide adequate oxygenation to body tissues, the mouse has a rapid respiratory rate (average 163 breaths/minute), short air passages, a high RBC concentration (hematocrit 40-50%), and a high hemoglobin concentration. Mice are obligate nasal breathers, meaning that they cannot breathe through their mouths. The heart rate is also extremely rapid, at 400-500 beats/minute.

Although mice have a wide range of temperature adaptation in the wild, sudden temperature variations in the laboratory setting can result in death. The mouse has a low tolerance for acutely increased temperatures. Mice do not have sweat glands and cannot pant, and they depend primarily on the vascularization of their ears and tail for loss of heat. Wild mice deal with environmental temperature increases by seeking shade or underground shelter. Ambient temperature is therefore a major consideration when mice are being shipped or transported even for short distances. Temperatures greater than 28°C can result in heat prostration and death.

The mouse produces very concentrated urine (average urine specific gravity, 1.058), and water conservation is very important for survival of wild mice. As mentioned earlier, mice dehydrate very rapidly when access to water is restricted. Mouse urine normally contains large amounts of protein.

The spleen may be up to 50% larger in male than in female mice. Mature male mice have higher granulocyte (neutrophil, eosinophil) counts in their peripheral blood than female mice of the same age. Basophils are only rarely seen in the peripheral blood.
Reproduction:

The age of puberty in mice varies according to the strain of mouse, nutritional status, and environmental influences, but in general occurs between 28 and 49 days of age. Signs of puberty in the female mouse include opening of the vagina and the presence of cornified epithelial cells in the vaginal smear. Fertility of the female mouse is greatest between 75 and 300 days of age.

Mice are polyestrous and breed year round. If the female is maintained on a constant light-dark cycle she will ovulate once every 4-5 days (with some variability) at approximately 3-5 hours after the onset of the dark period. The onset of male receptive heat generally occurs between 10 p.m. and 1 a.m. Ovulation is spontaneous and usually occurs 8-11 hours after the onset of estrus (estrus generally lasts 14 hours. Ovulation does not always occur in every estrous cycle. A postpartum estrus occurs 14-28 hours following parturition, at which time the female may be rebred to maximize production. If a timed pregnancy is required, breeding the female at the postpartum estrus should be avoided because implantation of the embryos is delayed for a variable period of time (4-10 days).

Mating is detected by the presence of a milky white to yellow vaginal plug due to secretions from the male’s coagulation glands. This plug normally persists 16 to 24 hours and can last up to 48 hours. If timed pregnancies are required by the investigator, the mice should be checked for the presence of a vaginal plug at least every morning, and if possible two times daily because some females will mate during the day. Hidden, deep copulation plugs may be found in some mice bred during the postpartum estrus or routinely in some stocks of mice. A magnifying loupe and a small probe may be useful in detecting vaginal plugs.

When the cervix and vagina are stimulated by breeding, prolactin is released from the anterior pituitary which in turn causes the corpus luteum to secrete higher levels of progesterone for about 13 days. If fertilization has occurred, the placenta will take over the production of progesterone. If no fertilization has occurred, the female will still appear to be pregnant for this period of 13 or so days; this phenomenon is referred to as pseudopregnancy. Grouping of females may also induce pseudopregnancy (see pheromone section following).

The average gestation is 19-21 days. Implantation of the embryos usually occurs on the fifth day postbreeding. Litter size varies from 1-13 pups, with the first litter often being smaller than subsequent litters. Although female mice do not commonly mutilate or cannibalize their pups, whelping animals or those which have recently whelped should be undisturbed for at least 2 days postpartum. Mouse pups are altricial at birth; that is, they are born blind, deaf, and naked. Newborns are often referred to as “pinkies”. The “milk spot”, which consists of the stomach filled with milk, can be seen through the pup’s thin skin and can be used to determine whether nursing has occurred. Fine hair covers the pup by 10 days of age and their ears are open at this time. By day 12 the eyes are open as well. Lactation lasts for approximately 3 weeks; pups are generally weaned at 21 days of age, at which time they weigh 10-12 grams. Sexing of the pups is done by observation of the anogenital distance, which is 1.5 to 2 times greater in males than in females (Figure 5, see page over).

Commonly used mating systems include pair mating, trios (one male and 2 females), and harems (one male and multiple females). Males left continuously with the females are more likely to re-breed the females at the postpartum estrus, thereby decreasing the time between litters. Within harem systems, females with litters near the same age tend to pool the pups and share in their care. This makes identification of pups and their dams more difficult. If identification of individual pups is required for research purposes, the pregnant female should be isolated in her own cage near the time of parturition.
Pheromones:

Pheromones are chemical substances produced by one animal which provide olfactory stimuli and communication to another animal. Pheromones play important roles in the behavior and reproduction of the mouse. Mice secrete two types of pheromones, known as signaling and priming pheromones. **Signaling pheromones** include the fear substance, male and female sex attractants, and aggression-inhibitor. The preputial gland pheromones of male mice provide female attractant stimuli. Urine from dominant male mice contains both aversion- and aggression-promoting pheromones. Application of urine from dominant males discourages investigation of the area by subordinate animals and incites aggression in other dominant males.

**Priming pheromones** include the estrus-inducer, the estrus-inhibitor, and adrenocortical activator. These pheromones can affect the estrous cycle of the female mouse; therefore, an understanding of these pheromone effects is crucial for successful management of mouse breeding colonies. Three effects have been well characterized in the mouse: the Bruce effect, the Lee-Boot effect, and the Whitten effect.
The **Bruce effect**, or strange male pregnancy block, occurs when a recently mated female is housed with or near a strange male. Implantation is inhibited in 30% of the females and pregnancy is blocked when the strange male is introduced within 24 hours of mating. The affected females return to estrus in 4-5 days. The maximum effect occurs when the strange male is of a different strain than the breeding male. Direct contact with the male is not required for the block to occur. Males castrated prior to puberty cannot induce this effect. This phenomenon is not seen in rats.

The **Lee-Boot effect** is induced by housing female mice in groups of 4 or more. A higher incidence of pseudopregnancies is observed in these groups of female mice than in singly housed females in the absence of matings, suggesting that female mice produce a pheromone which influences the estrous cycle. Anestrus (cessation of cycling) can occur if female mice are housed in groups of 30 or more. This phenomenon is not observed in rats.

The **Whitten effect** is seen when female mice are paired with male mice for breeding after an extended time of housing with other females only. Most of these female mice will mate on the third night after being introduced to the male. For comparison, mating is normally evenly distributed over the first 4 nights after pairing females with males when the females were previously caged individually. This effect can be taken advantage of by the mouse breeding manager to orchestrate timed pregnancies. This phenomenon also occurs in the rat.

**Restraint and Handling:**

Juvenile and adult mice may be caught and picked up by grasping the base or middle third of the tail with the fingers or smooth forceps. Once caught, the mouse can be restrained by placing it on a wire cage lid, grasping the loose skin behind the neck and ears with the thumb and forefingers, and holding the tail against the palm of the hand using the fourth and fifth fingers (Figure 6). Mice can also be held using a two-handed technique (Figure 7). Use care to make sure that the skin around the neck is not pulled so tightly that the mouse cannot breathe. This technique is commonly used to quickly examine a mouse or to administer an injection. Pregnant or obese mice should be handled gently and supported with a hand under their feet.
Injection Sites:

Intraperitoneal (i.p.) injections: These should be given in the lower right quadrant of the abdomen to prevent puncturing the spleen which is located on the left side (Figure 8). The mouse’s head should be tipped downward while the mouse is held upside down to prevent puncturing the intestines (not demonstrated by Figure 8). A small gauge needle (23-25 gauge) should be used, and a maximum volume of 2-3 ml in adult mice may be injected. This is the most common method of administering drugs and anesthetics to mice.

Subcutaneous (s.c.) injections: These may be administered ventrally or dorsally depending on the substance or cells to be injected. When given dorsally the most common location is between the shoulder blades (Figure 10). Care should be taken to avoid sticking the needle into your finger when holding the mouse as shown in Figure 10.
Intravenous (i.v.) injections: The tail vein is the most common site for i.v. injections. A tail vein is present at 90° on either side of the central tail artery (Figure 11 and 12). It is helpful to warm the mouse or the tail to induce vessel dilation prior to attempting injection; use care to avoid inducing heat prostration in the warmed mice. Use a 26-30 gauge needle and introduce the needle bevel up. This technique requires some practice to become efficient at hitting the vein, and good restraint of the mouse is essential. Commercial restrainers are sold which facilitate tail vein injection.

Figure 11. From The Laboratory Animal Technician Training Manual, edited by Lawson.

Figure 12. Distribution of the artery and veins in the mouse tail
**Intramuscular (i.m.) injections:** This technique is **NOT** commonly used in mice, and is generally **NOT** recommended because of the small size of the animal and the correspondingly small muscle masses. The quadriceps muscle can be used, but the volume should not exceed 0.2 ml per site, and a needle 25 gauge or smaller should be used to avoid muscle damage. Irritating substances such as ketamine should not be administered i.m. as they may lead to self mutilation of the affected limb if muscle or nerve damage occurs.

**Blood collection:** A safe maximum for a single sample is 1.25% of body weight (1.25 ml/100 grams of body weight) taken every 2 weeks. Animals on chronic studies requiring multiple blood samples may require hematocrit monitoring. Several techniques may be employed to collect blood samples from the mouse.

**Retroorbital bleeding:** This technique is one of the most commonly used for routine blood collection. The mouse must be anesthetized for this procedure. Pressure is placed on the top and bottom lids of one eye to keep the eye open and the globe pushed forward slightly. A glass microcapillary tube is placed in the medial canthus of the eye at a 30°-45° angle toward the back of the eye (Figures 13 and 14). Use firm, steady forward pressure and rotate the tube between the thumb and forefinger to cut through the conjunctiva at the back of the eye and enter the retroorbital sinus, at which time blood should flow into the tube. If no blood is obtained gently back off on the position of the tube. After collecting the sample, close the eyelids and apply pressure with a piece of gauze until hemostasis has been achieved. A small amount of ophthalmic ointment containing an antibiotic may be placed on the eye after bleeding has stopped to act as a “bandage” and help prevent infection. If the technique of the sampler is good the eye should not be damaged. If frequent samples must be collected, alternate the eye used for sampling.

**Tail vein:** This is another site commonly used to collect small blood samples from mice. Older mice should probably be anesthetized for the procedure, but the drawback is that blood is harder to obtain from the tail vein when the blood pressure drops. A small nick is made in the tail vein with a needle. Care must be taken to avoid cutting into the artery or amputating the tail with a scalpel blade. Blood can be collected using a microcapillary tube or may be allowed to drip into a small eppendorf tube. After blood has been collected the tail incision should be compressed with a piece of gauze until hemostasis occurs.
If bleeding is difficult to stop a silver nitrate stick may be applied. This technique can result in scarring to the tail.

*Saphenous vein:* A technique for obtaining blood from the saphenous vein of mice and other small animals has been recently described and is rapidly becoming the technique of choice for many investigators. This procedure does not require that the mouse be anesthetized to collect a blood sample, and is much less invasive than the two previously mentioned techniques. A tube can be used to restrain the mouse, and the hind leg is extended by applying gentle downward pressure just above the knee. The hair over the tarsal area is shaved with clippers followed by a number 11 scalpel blade, and the vein is pricked with a needle (25 gauge is usually adequate). Blood can be collected in a microcapillary tube. Smearing a small amount of silicone grease over the area to be punctured helps to prevent the blood from coming into contact with the skin and minimizes blood clotting. When the blood has been collected, gentle pressure applied with a piece of gauze should be used to effect hemostasis. Pictures demonstrating this technique may be found at the web site:
http://www.uib.no/vivariet/mou_blood/Blood_coll_mice_.html

*Facial vein (limited to adult mice):*
This is a relatively new technique and repeated sampling is possible by using alternate sides of the face in the area of the mandible. The sample may be a mixture of venous and arterial blood. The method is said to require less training than tail, retro-orbital sampling to reliably withdraw a reasonable quantity of blood. It may be performed on awake animals that are properly restrained to allow proper site alignment and venous compression for good blood flow (Figure 15). A minimal amount of equipment is required and can be performed relatively rapidly. 20G or smaller size needles should be used to prevent excessive bleeding.

*Note: Eyes are not bulging here. Risk of not obtaining blood.*
Terminal bleeding procedures: Two techniques are commonly used to collect larger volume samples from anesthetized mice at the time of euthanasia. In the first technique, the brachial vessels are exposed by removing the skin in the axilla and the vessels are then cut. Pooling blood is collected using a pipette, and blood collection must be done quickly because clotting is initiated by contact of the blood with the tissue. Terminal bleeding can also be done by collecting blood directly from the heart. Heart puncture may be done “blindly” by directing a needle into the thoracic cavity from the outside after palpating the beating heart at the level of the 5th intercostal space. Alternatively, the chest cavity may be opened so that the heart can be visualized, at which time the needle can be introduced into the right ventricle to collect blood (Figure 16).

Miscellaneous Techniques:

Tail clipping: This technique is frequently used to obtain tissue for DNA analysis of transgenic mice. Less than 10 mm of the tail tip should be removed. The tail ossifies between 2-4 weeks of age; therefore, mice older than 3 weeks of age must be anesthetized for the procedure at NUS. Employ adequate hemostatis to stop bleeding at the amputation site. Frequently, pressure on the cut end of the tail with a piece of gauze until the bleeding stops is inadequate; please use a styptic (e.g., silver nitrate). A product called “Quick Stop” powder also works well to assist with hemostasis, and is used by dipping the cut surface of the tail into the powder after the major bleeding has been arrested by pressure.

Oral gavage: This technique is commonly used in drug and toxicology studies to deliver a precise amount of test material, and can also be used to administer medications. A special gavage needle with a ball at the end is used to deliver materials directly into the stomach. The ball on the needle prevents entry into the trachea. The length of the gavage tube required is determined by measuring the distance from the mouth to the last rib (Figure 17). The mouse should be held with the head and neck extended (Figure 18). If the gavage tube does not easily pass into the esophagus, remove it and try again.
Anesthetic and Surgical Considerations:

The general physical health of the animal should be evaluated prior to any surgical procedure or anesthetic event; sick mice are not good candidates for such procedures and should not be used. It is not necessary to fast mice prior to anesthesia unless the surgical procedure involves the GI tract. Either injectable or inhalant anesthetics may be used, however, post-procedure recovery is much more rapid when inhalant anesthetics are used. For a list of suitable drugs and dosages, please consult the LAC Staff and/or the Animal Care and Use Training Manual.

After the mouse has been anesthetized, a small amount of bland ophthalmic ointment is placed in the eyes to prevent the corneas from drying. If the surgery or procedure will last more than 15 minutes, administer fluids to the animal. 0.01-0.02 ml/gram body weight of either warm lactated Ringer’s solution or normal saline should be given subcutaneously to prevent hypovolemia. Mice have a high ratio of body surface area to mass; therefore, they lose heat rapidly after being anesthetized. Always maintain the animal on a surface that helps conserve body heat and supply an external source of heat as well.

Always determine anesthetic depth before initiating surgery. When a mouse is adequately anesthetized, touching the medial corner of the eye should not result in a response. Likewise, the withdrawal response cannot be elicited when pressure is applied to the back foot of an animal in a suitable plane of anesthesia. Respiration should be monitored to ensure that it is of adequate depth and normal character. Mucous membranes and foot pads should remain a pink color indicating that the animal’s perfusion is adequate. Heart rate is too rapid in the mouse to be a useful parameter to monitor during surgery.
The IACUC requires the use of analgesics to control pain following major surgical procedures for the first 48 hours post-surgery, or longer if the animal still appears painful. A veterinarian can help you select an analgesic that will be appropriate for your particular research needs.

Recognition of Pain and Distress in Mice:

Because animals cannot volunteer to participate in medical research, we are ethically constrained to provide humane care, and to alleviate as much pain and distress as is possible in such animals. We must always work with the assumption that if a procedure causes pain in human beings it will also cause pain in an animal, and indeed, this concept is mandated by the NACLAR. Para 8.4.1 (a) of the “Guiding Principles” in the NACLAR Guidelines state that “Pain and distress cannot always be adequately evaluated in animals and investigators must therefore assume that animals experience pain in a manner similar to humans. Decisions regarding their welfare in experiments must be based on this assumption unless there is evidence to the contrary.” The proper use of anesthetic and analgesic drugs helps to alleviate pain and distress during procedures. It is imperative that researchers learn to recognize the signs of pain and distress in mice. Inconvenient or not, the benefit of the doubt must always go to the animal.

The most common signs of pain and distress in mice listed in order of increasing severity, include: (1) ruffled or “spikey” fur (mouse looks unkempt); (2) weight loss which may be mild to severe, anorexia, dehydration; (3) ocular discharge; (4) lethargy, depression, or reluctance to move; (5) sitting with the back in a hunched position; (6) ataxia (uncoordinated muscle movements), regional or generalized weakness; (7) tremors, which may be intermittent to persistent depending on the condition of the animal; (8) hypothermia; (9) labored respiration; and (10) cyanosis, or a blue tinge to the mucous membranes. Any animals exhibiting combinations of 2 to 3 minor signs, or a single major sign should be euthanized immediately.

Animals in pain and distress may not interact with their cage-mates, or may interact with them in a more aggressive manner. They may also become more aggressive towards human handling. Female mice may cannibalize litters in response to pain and distressing situations. Animals may squeal when picked up or when an affected area is touched. Persistent vocalization and crying indicates substantial pain or distress that should be relieved immediately. Moribund animals should require immediate euthanasia.

Malocclusion:

Malocclusion is a frequently occurring problem in mice, and seems to be more prevalent with the advent of transgenic technologies. The condition results when the incisors are misaligned, and thus do not undergo normal wear. Because the incisors of mice grow continuously, an animal with malocclusion cannot eat and often appears runted after weaning. Euthanasia is suggested for such animals, as the condition is heritable. After identification of such animals, LAC will initially trim the teeth, but if the investigator wishes to keep such animals they will be responsible for monitoring the condition of the teeth and performing the incisor trimming every two weeks for the life of the animal. If the investigator does not wish to trim the teeth the mouse must be euthanized.

Guidelines for Growth of Implanted Tumors and Cell Lines:

The NUS IACUC has set guidelines for the condition and maximum allowable size of implanted tumors and cell lines. These guidelines also apply to tumors that arise spontaneously or that arise due to genetic alterations in the mouse being used for study. The maximum allowable size for a tumor in a mouse is 1.5 cm. If scientific justification is provided to the IACUC, and they approve this justification, then tumors larger than 1.5 cm may be grown. No mouse may be implanted with more than one tumor or injected with
a neoplastic cell line in more than one location. Please keep in mind that not all locations on or within the mouse can bear a 1.5 cm tumor. The presence of large tumors in body cavities such as the cranium or thoracic cavity, or behind the eye places greater limitations on the maximum acceptable size of a growth, and greatly impedes the normal functions of the animal. Large tumors in sites such as the thoracic or abdominal cavity may interfere with vital functions such as respiration. Mice bearing tumors in locations that interfere with the animal’s ability to ambulate must be euthanized.

Most tumor lines are implanted subcutaneously, and the phenotype of some tumor lines is such that the mass will eventually become ulcerated. An ulcerated tumor may have one of two appearances. The ulceration may present as an open, moist lesion or as a scabbed area, which is indicative of a break in the underlying epithelium. Ulcerated tumors will not heal, and are not amenable to surgical repair; thus, any animal bearing an ulcerated tumor must be euthanized immediately. Tumor growth may also result in cachexia (extreme weight loss) due to the intense metabolic needs of neoplastic masses. Any mouse bearing a tumor that has reached 1.5 cm or is ulcerated, that is cachectic, or displays any of the signs listed in the section on recognition of pain and distress must be euthanized immediately.

Please note that it is the responsibility of the investigator to monitor tumor bearing animals on a daily basis, including weekends and holidays.

Euthanasia:

Investigators and their personnel are reminded to use the euthanasia method outlined in their IACUC approved protocol. If a different method of euthanasia is required the investigator must first seek IACUC approval.

Mice may be euthanized by placing them into a CO₂ chamber until they have expired (Figure 19).
Please note that a compressed gas cylinder is the only source of CO\(_2\) that may be used for euthanasia purposes. According to the most recent Report of the American Veterinary Medical Association Panel on Euthanasia, CO\(_2\) for euthanasia may not be generated by the use of dry ice, fire extinguishers, or antacids as these methods are unreliable in producing the required concentration of CO\(_2\) and insuring a fast and painless death. *Mice may not be crowded into cages before euthanasia by this method as it induces stress and may result in inefficient euthanasia.* No more than five mice (i.e. the maximum original number in the cage) may be euthanized together. In addition, you may not leave cages of mice next to the euthanasia areas for the husbandry staff to euthanize. Persons violating these rules will be referred to the IACUC.

Currently, compressed CO\(_2\) gas in cylinders is available at the following locations:

AHU Rear Loading Bay
sAHU Procedure Room 2

MD9 and MD11 are independent vivariums, thus, animals housed in those areas should be euthanized in the appropriate rooms for each facility and not in LAC areas.

If you are planning to euthanize animals in one of the common vivarium areas, please plan your work accordingly so that the last task completed in the vivarium is euthanasia of the animals. Because the euthanasia chambers are common areas, and someone may have placed animals infected with rodent pathogens into the chamber prior to your arrival, you should consider yourself to be contaminated after working in one of these areas, and exit the vivarium immediately after completing euthanasia. You should not re-enter the same or a different vivarium within the same day. Return empty cages to the appropriate cagewash facility. *Under no circumstances should you take animals to a different vivarium or vivarium area to euthanize them, nor should you return dirty cages to a cagewash area within another vivarium. Mice taken from a barrier facility may never be returned to those areas for euthanasia as they are barrier areas. If it is unclear where dirty cages should be taken please contact a LAC Laboratory Officer for more information.*

A chamber prefilled with a volatile inhalation anesthetic such as isoflurane may be used in lieu of CO\(_2\). Because the liquid forms of volatile anesthetics are an irritant to the skin and mucous membranes, care must be taken to ensure the mouse does not come into direct contact with the agent. An overdose of the injectable anesthetic pentobarbital may also be used (150 mg/kg body weight intraperitoneally).

Cervical dislocation and decapitation are considered to be physical methods of euthanasia and may be used; however, the mouse must first be anesthetized unless the IACUC has granted an exception based on scientific justification. If cervical dislocation is used as a means of euthanasia, investigators must be responsible for ensuring personnel have been properly trained and consistently apply the technique humanely and effectively. If decapitation is used as a method of euthanasia, the equipment used must be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. When decapitation is used as the means of euthanasia, the blade must be dropped quickly and forcefully so that a clean cut and not a slow crush is obtained. The use of plastic restraint cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine.
Euthanasia of neonates requires special considerations. Neonatal rodents are extremely resistant to hypoxia induced by CO\textsubscript{2}, therefore, this is not considered to be a valid method of euthanasia during at least the first postnatal week. Pups up to one week of age may be anesthetized using isoflurane or halothane and then decapitated, or may be euthanized with an overdose of the inhalant anesthetics. (Figure 20.) As with adult mice, pups greater than one week of age may be euthanized with a gas inhalant anesthetic, CO\textsubscript{2}, or pentobarbital alone.

**Figure 18.** A precision vaporizer used to deliver isoflurane.

*It is always the responsibility of the investigator to ensure that the animal is dead before disposal of the carcass.* Cessation of the heartbeat by palpation of the thoracic cavity is used to determine that the animal is no longer alive if the thoracic cavity has not been opened to obtain a terminal blood sample or to perform perfusion. Cessation of respiration alone is not a reliable indicator of death, and may only indicate an extremely deep plane of anesthesia.

**Occupational Health Concerns:**

Development of allergies to species of animals used in research, especially rodents and rabbits, is one of the most common problems encountered by both animal care workers and investigators. While the most common manifestations of this sensitivity are nasal symptoms, itchy eyes, and rashes, it is estimated that up to 10% of chronically exposed individuals will develop asthma which can be life-threatening. The majority of allergies induced by mice are due to a protein found in the urine. This protein can become airborne, and individuals that are extremely sensitive can be adversely affected by simply walking into a room where mice are housed. The use of gloves, laboratory coats, and other protective clothing helps to minimize exposure and prevent the development of allergies. The use of filter top cages also helps to minimize the amount of aerosolized protein. Wash well with soap after working with the mice.
Anaphylaxis may occur in extremely allergic individuals if they are bitten by a mouse or receive a puncture wound from a needle that has mouse proteins on it. Development of allergies should be reported to your supervisor and the Occupational Health Program.

Anyone being bitten by a mouse should report the injury immediately to the University Health and Wellness Centre, which is located at Yusof Ishak Hall, Level 4. The phone numbers are 6776-1631 (Nurses Station) and 6516-2880/6516-2390 (Admin Office). For on-campus emergencies and after office hours, please proceed to NUH Accident and Emergency Unit. A bite from a mouse may result in a puncture wound, and any bite wound should be cleaned thoroughly to prevent bacterial infections. A current tetanus immunization is recommended for anyone working with mice, as such injuries may provide entry for the tetanus bacterium.

Transmission of infectious diseases from mice to man is rare today because of the care used in rearing and housing laboratory mice; however, the introduction of wild-caught rodents or laboratory mice from questionable sources into a facility may provide the potential for zoonotic diseases to occur. Diseases which may be readily transmitted from infected mice to humans include lymphocytic choriomeningitis, salmonellosis, and leptospirosis. Of special concern to those handling wild mice in the southwest are Hantavirus infection (pulmonary form in the US, renal form in SE Asia) and bubonic plague. Wild rodents should never be handled without gloves and protective clothing.

References:


Guide for the Care and Use of Laboratory Animals by the National Research Council. National Academy Press. 1996


