THE UNIVERSITY OF HONG KONG

LABORATORY ANIMAL UNIT

VETERINARY CARE FOR LABORATORY ANIMALS

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CONTENTS

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1. INTRODUCTION

1.1 All personnel who care for or use animals in research, teaching or testing must assume responsibility for their well-being in terms of minimizing pain and distress by following the recommendations listed in the "Guide for the Care and Use of Laboratory Animals, 1996, NRC" *(http://nap.edu/readingroom/books/labrats/)* and the "Code of Practice for the Care and Use of Animals in Experimental Animals, 2004, Agriculture, Fisheries and Conservation Department (AFCD)" *(http://www.afcd.gov.hk/quarantine/am/dog/code.htm).*

1.2 Veterinary care is an essential part of an animal care and use program. Adequate veterinary care for laboratory animals consists of effective programs for:

- Preventive medicine.
- Surveillance, diagnosis, treatment, and control of disease, including zoonosis control.
- Management of protocol-associated disease, disability, or other sequelae.
- Anesthesia and analgesia.
- Surgery and post-surgical care.
- Assessment of animal well-being.
- Euthanasia.

2. PREVENTIVE MEDICINE

2.1 Disease prevention is an essential component of comprehensive veterinary care. Effective preventive medicine programs enhance the research value of animals by maintaining healthy animals and minimizing non-protocol sources of variation associated with disease and inapparent infection. These programs include quarantine, stabilization and separation of animals by species, source and health status.

2.2 Quarantine, Stabilization and Separation

2.2.1 Quarantine is the separation of newly received (e.g. imported) animals from those already in the facility until the health and preferably the microbial status of the newly received animals have been determined. An effective quarantine minimizes the chance for introduction of pathogens into an established colony.

2.2.2 It is necessary to obtain a Special Import Permit from the local authority (viz AFCD) in advance for importation of laboratory animals from overseas countries and comply fully with AFCD's "quarantine and serology screening" requirements.

- The imported animals have to be kept in a quarantine area after arrival and tested (by serology) for a specified list of diseases not less than 30 days after arrival.
- The animals can only be released from quarantine upon AFCD's receipt of negative test results.
- The Laboratory Animal Unit (LAU) can handle importation procedures and arrange serological testing of imported animals for investigators (*http://www.hku.hk/launit/content/procedures/import_procedure.pdf*).

2.2.3 Newly received animals should normally be given a period of at least 14 days for physiologic, psychologic, and nutritional stabilization before they are used in experimentation.

2.2.4 Physical separation of animals by species is recommended to prevent inter-species disease transmission (e.g. the bacterium *Bordetella bronchiseptica* characteristically produces only subclinical infections in rabbits but severe respiratory disease might occur in guinea pigs) and to eliminate anxiety and possible physiologic and behavioral changes due to inter-species conflict (e.g. rats are natural predators of mice).

- Such separation is usually accomplished by housing different species in separate rooms; however, cubicles, laminar-flow units, cages that have filtered air or separate ventilation (e.g. individually ventilated cages) and isolators (e.g. flexi-film isolators) might be suitable alternatives.
- In some instances, it might be acceptable to house different species in the same room, for example, if two species have a similar pathogen status and are behaviorally compatible.

2.3 Surveillance, diagnosis, treatment and control of diseases

2.3.1 *All animals should be observed for signs of illness, injury or abnormal behavior by a person trained to recognize such signs. A copy of LAU's Standard Operating Procedures on Health Checking of Animals is attached at Appendix A.*

- *As a rule, this should occur daily, but more frequent observations might be warranted, such as during post-operative recovery or when animals are ill or have a physical deficit.*
- Unexpected deaths and signs of illness, distress, or other deviations from normal in animals should be reported promptly to ensure appropriate and timely delivery of veterinary care.

2.3.2 Diagnostic laboratory services facilitate veterinary care and can include gross and microscopic pathology, clinical pathology, hematology, microbiology, parasitology, clinical chemistry and serology. The LAU provides veterinary advice and disease investigation/diagnosis services for all departments in the University on request (http://www.hku.hk/launit/)

2.3.3 The choice of medication or therapy should be made by a veterinarian in consultation with the investigator. The selected treatment plan should be therapeutically sound and when possible, should cause no undesirable experimental variable.

2.3.4 Sub-clinical microbial, particularly viral, infections occur frequently in conventionally maintained rodents but also can occur in facilities designed and maintained for production and use of specific-pathogen-free (SPF) rodents if a component of the "microbial barrier" is breached (e.g. malfunctioning of autoclaves or non-compliance of disease control rules/regulations). Examples of infectious agents that can be sub-clinical but induce profound immunologic changes or alter physiologic, pharmacologic, or toxicologic responses are Sendai virus, Kilham rat virus, Mouse hepatitis virus, Lymphocytic choriomeningitis virus and *Mycoplasma pulmonis.*

- The principal method for detecting viral infections is serologic testing.
- Other methods of detecting microbial infections, such as bacterial culturing and histopathology and DNA analysis using the polymerase chain reaction (PCR), should be used in combinations that are most suitable for specific requirements of clinical and research programs.
- Transplantable tumors, hybridomas, cell lines, and other biologic materials can be sources of murine viruses that can contaminate rodents. Screening of biologic materials for microbial contamination should be performed before they are introduced into a barrier animal facility.

3. SURGERY

3.1 Pre-surgical planning, pre-operative care, aseptic technique, surgical preparation & post-operative care

The essential requirements for a successful survival surgery include:

- *adequate surgical skills*
- *knowledge of procedures to ensure asepsis*
- *familiarity with the anatomy, physiology, pharmacology, anesthesia and basic care of the animal species undergoing surgery*

3.1.1 Pre-surgical planning & pre-operative care

3.1.1.1 Pre-surgical planning

- Appropriate attention to pre-surgical planning, personnel training, aseptic and surgical techniques, animal well-being and animal physiologic status during all phases of a protocol will enhance the outcome of surgery. The individual impact of those factors will vary according to the complexity of procedures involved and the species of animal used. A team approach to a surgical project often increases the likelihood of a successful outcome by providing input from persons with different expertise.
- Pre-surgical planning should include input from all members of the surgical team, including the investigator, veterinarian, technicians and animal-care staff. The surgical plan should identify:
  - Personnel and their roles and training needs;
  - Equipment and supplies required for the procedures planned;
  - The location and nature of the facilities in which the procedures will be conducted;
  - Pre-operative animal health assessment and post-operative care.
- If a non-sterile part of an animal, such as the gastrointestinal tract, is to be surgically exposed or if a procedure is likely to cause immuno-suppression, pre-operative antibiotics given prophylactically for certain surgical protocols might be appropriate. However, *the use of antibiotics should never be considered as a replacement for aseptic procedures.*

3.1.1.2 Pre-operative care

- Good pre-operative care will reduce the incidence of many of the complications that can occur during anesthesia.
- Ensure all equipment and supplies necessary are available and in good working order (i.e. anesthetic machines [and anesthetic gases], monitoring equipment, heating pads/blankets, fluids, etc.).
- Ensure all personnel involved are familiar with the equipment, techniques and post-operative care required, including after-hours observation and treatment.
- Ensure that only healthy animals are used.
  - Animals should be obtained 14 days (acclimatization) prior to surgery.
  - Pre-operative fasting:
    - Pigs and dogs - They should receive no food during the 8-12 hours before anesthesia to minimize vomiting during anesthesia and recovery.
    - Ruminants - Withholding food for 12-24 hours before anesthesia may help to reduce the incidence of ruminal tympany or bloat (i.e. the accumulation of gas in the stomach).

- Rabbits and small rodents - Pre-anesthetic fasting is unnecessary since vomiting during induction does not occur in these species. For guinea-pigs, withholding food for 6-8 hours before anesthesia may be required because they may retain food in their pharynx after being anesthetized. Fasting may be required for gastro-intestinal surgery but rodents and rabbits are coprophagic, so measure to prevent them from ingesting their own feces may be necessary to provide an empty stomach.
- Birds - Large or medium-sized birds may be starved for 6-12 hours to reduce the risk of regurgitation of crop content. Smaller birds should not be fasted for longer than 2 hours to avoid the risk of hypoglycemia.
- Reptiles and amphibians - Fasting is generally unnecessary.
- Fish - Pre-anesthetic fasting of 1-2 days is required.
- Pregnant animals - Withholding food can produce severe metabolic disturbance which may prove fatal.

- All animals should be provided with drinking water until approximately 60 minutes before induction of anesthesia.
- All animals should be weighed prior to anesthesia to allow accurate calculation of drug dosages and to facilitate monitoring of post-operative weight loss.

3.1.2 Aseptic technique

3.1.2.1 It is important that the investigator and his team members have had appropriate training to ensure that good surgical technique is practised. Proper surgical technique is important in preventing wound infection, promote wound healing and ensuring likelihood of a satisfactory outcome of the surgical procedure. *Good surgical technique includes:*

- *asepsis,*
- *gentle tissue handling,*
- *minimal dissection of tissue,*
- *appropriate use of instrument and monitoring equipments,*
- *effective hemostasis and maintenance of sufficient blood supply to tissues,*
- *accurate tissue apposition and correct use of suture materials and patterns,*
- *expeditious performance of the surgical procedure.*

3.1.2.2 In general, surgical procedures are categorized as “major” or “minor” and in the laboratory setting can be further divided into “survival” and “non-survival”.

- *“Major survival surgery” penetrates and exposes a body cavity, or produces substantial impairment of physical or physiologic functions, or involves procedures associated with orthopedics or extensive tissue dissection or transection (such as laparotomy, thoracotomy, craniotomy, joint replacement and limb amputation). Appropriate aseptic technique is necessary if a body cavity is penetrated.*
- *“Minor survival surgery” does not penetrate or expose a body cavity and causes little or no physical or physiological impairment (such as percutaneous biopsy, wound suturing, peripheral vessel cannulation). Minor procedures are often performed under less stringent conditions than major procedures but still require aseptic technique and instruments and appropriate anesthesia.*
- *“Survival surgery” involves a surgical procedure from which the animal recovers from anesthesia and becomes conscious.*

- *In "non-survival surgery", an animal is euthanatized before recovery from anesthesia. It might not be necessary to follow all the techniques outlined in this section if non-survival surgery is performed; however, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean.*

3.1.2.3 A*septic technique is defined as surgery performed using procedures that limit microbial contamination to the lowest possible practical level so that significant infection or suppuration does not occur.* No procedure, piece of equipment, or germicide alone can achieve that objective. Aseptic technique requires the input and cooperation of everyone who enters the operating suite. *Aseptic technique includes:*

- *preparation of the patient, such as hair removal and disinfection of the operative site;*
- *preparation of the surgeon such as the provision of decontaminated surgical attire, surgical scrub, sterile surgical gloves and masks;*
- *sterilization of instruments, supplies, and implanted materials;*
- *use of operative techniques to reduce the likelihood of infection.*

3.1.2.4 To achieve aseptic technique, pre-surgical planning is required and begins during the development of the animal research protocol in consultation with a veterinarian. This planning should include:

- identification of personnel making up the surgical team,
- the roles and training needs for the surgical team,
- equipment and supplies required for the procedures planned,
- the location and nature of the facilities in which the procedures will be conducted,
- pre-operative and post-operative care of the animals.

3.1.2.5 Specific sterilization methods should be selected on the basis of physical characteristics of materials to be sterilized.

- Autoclaving (using steam) and gas (e.g. ethylene oxide) sterilization are common effective methods.
- Sterilization indicators should be used to identify materials that have undergone proper sterilization.
- Liquid chemical sterilants (e.g. chlorine dioxide) should be used with adequate contact times, and instruments should be rinsed with sterile water or saline before use.

3.1.2.6 Most bacteria are carried on airborne particles or fomites, so *surgical facilities should be maintained and operated in a manner that ensures cleanliness and minimizes unnecessary traffic.*

- In some circumstances, it might be necessary to use an operating room for other purposes. In such cases, it is imperative that the room be returned to an appropriate level of cleanliness before its use for major survival surgery.

3.1.2.7 Careful surgical monitoring and timely attention to problems increase the likelihood of a successful surgical outcome.

- Monitoring includes checking of anesthetic depth and physiologic function, and assessment of clinical signs and conditions.
- Maintenance of normal body temperature minimizes cardiovascular and respiratory disturbances caused by anesthetic agents and is of particular importance.

3.1.2.8 The species of animal influences the components and intensity of the surgical program.

- In rodents, subclinical infections can cause adverse physiologic and behavioral responses that can affect both surgical success and research results. *A dedicated surgical facility is not required for rodents but the facility should be clean and surgery must be performed using aseptic techniques (i.e. using sterile instruments, surgical gloves and aseptic procedures to prevent infection). A good reference entitled "Guidelines for Survival Rodent Surgery" can be downloaded at* http://oacu.od.nih.gov/ARAC/surguide.htm.
- In general, *non-rodent aseptic surgery should be conducted only in facilities intended for that purpose. Surgical procedures on farm animals maintained for biomedical research require the use of appropriate aseptic technique, sedatives, analgesics, anesthetics, and conditions commensurate with the risk to the animal's health and well-being.*

3.1.3 Preparation of surgical instruments and the animal

3.1.3.1 Planning the surgical procedure requires consideration of the instruments necessary for the procedure and what type of instrument pack will be utilized.

- A simple peel-pack can be used and typically contains small numbers of small to medium sized instruments.
- A complex pack consists of overlapping cloth or paper drapes folded together and sealed with autoclave tape. It can contain a large collection of instruments of various sizes.
- Tip protectors are recommended for use with delicate instruments or those with sharp points.

3.1.3.2 When performing *multiple rodent surgeries* it is a good idea to have staging areas for the different steps of the procedure.

- *Whenever possible, animals waiting for surgery should be kept at a visual and olfactory distance from those animals undergoing surgery.*
- *Surgical preparation of the animal should occur in a location different than that used for performing the surgeries. This will help to prevent hair and dander from getting on the sterile packs.*
- If space constraints or requirements for use of a down-draft table necessitates a single location for preparing the animal and performing the surgery, then it is recommended that the bench towel used to prepare the animal be replaced before performing the surgery.
- The surgical pack, if already open, must be covered during the preparation of the surgical site to prevent contamination of the pack with hair or dander.

3.1.3.3 *The area selected for performing surgery should be sanitized with an appropriate disinfectant such as chlorine dioxide or a chlorhexidine product as part of the pre-surgical preparations. A dedicated surgical site is not required for rodent surgery.* Once the location is prepared and all supplies and equipment are organized, the animal is then anesthetized and the surgical preparation follows (Note: The NIH has produced an informative CD on "Training in Survival Rodent Surgery", please write to rodentcd@od.nih.gov for a copy of this CD):

- The first step in surgical preparation of the animals is removal of hair from the surgical site when the animal is under anesthesia. Bathing some animals (e.g. farm animals) before preparation of the surgical site might be necessary to remove excessive dirt and hair, and might be done the day before the scheduled surgery.

- The most common method is to use an electric clipper. A piece of adhesive tape or moistened gauze dabbed over the clipped area will pick up loose hair that could otherwise migrate into the incision.
- Hair removal from the operative site should be done carefully to avoid causing small skin abrasions that could become infected.
- An easy alternative to clipping the fur in rodents is to remove it by plucking. Hair follicles in rodents are usually in telogen or the resting phase, and hair can be removed without injury. It is a fast and easy method that does not leave stubble.

- Following hair removal, the standard surgical preparation of the skin consists of alternating scrubs of a povidone iodine disinfectant and 70% isopropyl alcohol. Alternative products such as chlorhexidine may also be used.
  - Using a gauze sponge or cotton-tipped applicator, cleansing should be done in a circular motion beginning at the center of the shaved area and working toward the periphery.
  - To avoid contamination of the already scrubbed area, do not go back to the center area with the same sponge.
  - The scrubs should be alternated between an iodophor scrub and alcohol and repeated at least three times, ending with the iodophor.
  - To avoid hypothermia (especially in small animals) care should be taken to not excessively wet the animal.
  - For small incision sites in mice, cotton-tipped applicators work best for applying the scrubs to the skin.
- Depending on the anticipated duration of surgery, as well as size and species of animal, warming devices or insulating materials should be placed between the table and the animal to prevent loss of body heat.
  - Circulating warm water heating devices or heat lamps are better than electrically heated pads which are more likely to cause burns because the animals cannot move away from them.
- To avoid unnecessary post-operative discomfort, try to ensure that the animal is placed in as a normal a posture as possible.
  - Avoid tying out the limbs and use positioning pads instead.
  - Try to protect pressure points such elbow, hock and the wings of the pelvic bones.

3.1.3.4 Once the animal is prepared, the "surgical pack" is opened. This should occur prior to donning sterile surgical gloves.

- The sterilization indicator should be observed to ensure it has turned the appropriate color before the pack is used.
- For simple-peel packs, the pack should be opened with care to avoid contact with the inside surface as it can be used as a sterile surgical field on which to keep the instruments.
- Likewise, complex surgical packs are opened to keep the inside surface of the wrapping sterile so that it can be used as a sterile field.

3.1.3.5 If cold sterilant solutions (e.g. chlorine dioxide, glutaraldehyde) are used to sterilize instruments, it is important that the instruments are exposed for the proper length of time and that expiration dates of solutions are observed. When the instruments are removed from the solution, they must be rinsed with sterile water, saline or alcohol as the sterilization solution is very irritating to tissues. Rinsed instruments can then be placed on a sterile field.

3.1.3.6 Hot (glass or metal) bead sterilizers (using dry heat) may also be used for sterilizing instruments. This method sterilizes only the tips of the instruments.

- To ensure proper sterilization, the beads must be pre-heated to the recommended temperature and the instruments exposed for the recommended time according to the manufacturer's recommendation.

- To avoid damage to the instrument and possible contamination of the surgical site, gross debris must be removed from the instrument before sterilizing.
- When removing instruments from the bead sterilizer, allow them to cool before touching tissues to avoid tissue injury.
- The hot bead method is best reserved for sterilizing instruments between surgeries.

3.1.3.7 Once instrument packs are opened, other sterile supplies, such as scalpels and suture material, can be opened and placed on the sterile field.

3.1.4 Preparation of the surgeon

Proper surgical attire consists of cap, mask, and clean laboratory coat. Hands should be washed with an anti-bacterial soap and sterile gloves donned. Examination gloves used for handling animals and working in the laboratory are not the same as sterile surgical gloves and should not be substituted. It is important to don the gloves in such a way that prevents contamination of the outer surface of the glove.

3.1.5 Draping and Instrument Handling

3.1.5.1 After the surgical gloves are donned, the prepared surgical site on the animal must be draped to protect the exposed tissues (caused by the incision) from contamination.

- The most common drape is a paper drape. It may be pre-cut or intact and a hole must be cut in it.
- Plastic (transparent) drapes, usually with an adhesive, offer the advantage of more visibility and better patient monitoring.
- Sterile gauze sponges can also be used for drapes in small animals.

3.1.5.2 Once the previous steps are completed, surgery can be performed.

- It is important to be aware of the space that is not sterile between the surgical pack and the draped animal. Instruments should not be laid in this space as contamination could occur.
- While performing surgery, care should be taken to not get paper or cloth instrument drapes wet. Wet material acts as a wick to pull bacteria through from the non-sterile surface below. This wicking is known as "strike-through" and any instruments in contact with the wet surface should be considered contaminated and re-sterilized before further use.

3.1.6 Suture Selection

The selection of the type and size of suture material should be done in advance based on the type of surgery and species of animal.

- For small animals, a “000” (3 - 0) suture thickness or smaller is recommended.
- Cutting and reverse cutting needles have sharp edges and are best used for skin suturing.
- Non-cutting, taper or round needles are used for suturing easily torn tissues such as peritoneum, muscle or intestine.
- If ligation of vessels or suturing of tissues other than skin is necessary during surgery, an absorbable material such as polyglactic 910, polyglycolic acid, polyglyconate, polydioxanone or chromic gut should be used.
- For skin closure, non-absorbable suture such as polypropylene or nylon, stainless steel wound clips or staples may be used.
- Most rodents will gnaw at any externalized sutures, so a buried suture line or wound clips are recommended.

- Cyanoacrylate surgical adhesives may be used to close incisions or to close the area between sutures.
- Silk is a non-absorbable suture material that can cause tissue reactions and may wick micro-organisms into the wound. Silk is best used for cardiovascular procedures only and not recommended for skin closure.

3.1.7 Post-operative care

3.1.7.1 Pre-surgical planning should specify the requirements of post-surgical monitoring, care, and record-keeping, including the personnel who will perform these duties. Adequate post-operative care enhances the animal's recovery by improving its physiological status and minimizing pain and distress. *The application of prophylactic antibiotics is not a substitute for the practice of proper aseptic surgery.*

- The investigator, veterinarian, technicians and animal-care staff share responsibility for ensuring that post-surgical care is appropriate. Good communication is essential to the animal's welfare.
- *An important component of post-surgical care is observation of the animal and intervention as required during recovery from anesthesia and surgery. The intensity of monitoring necessary will vary with the species and the procedure and might be greater during the immediate anesthetic recovery period than later in post-operative recovery.*

3.1.7.2 During the anesthetic recovery period, the animal should be in a clean, dry, warm and quiet area with subdued lighting where it can be observed often by trained personnel.

- It is important to keep the animal patient warm, particularly small animals whose large surface area in relation to body mass results in rapid heat loss. This can be achieved in small animals by using incubators. For adults, the temperature should be 25-30^{o}C, and for neonates 35-37^{o}C.
- Recovering animals should not be placed directly on sawdust and similar-size bedding as they may aspirate bedding particles or asphyxiate and the bedding may stick to the surgical wound.
- Particular attention should be given to thermoregulation, cardiovascular and respiratory function, and post-operative pain or discomfort during recovery from anesthesia.
- During anesthetic recovery, animals should be positioned in a manner to avoid compromising cardiovascular and/or respiratory function. The swallowing and cough reflexes are usually suppressed during anesthesia and these gradually return as anesthetic lightens.
    - If an endotracheal tube is present, it should be removed when the animal begins to swallow spontaneously or attempts to cough.
    - Improper positioning can lead to aspiration pneumonia, obstruction of airways, tissue necrosis, or edema at pressure points.
    - Non-ruminant species (e.g. pigs) should be placed on their sides, with head and neck extended, to try to minimise the risk of airway obstruction. If the animal is recumbent for more than 4 hours then it should be repositioned to lie on its other side to prevent lung congestion and hypostatic pneumonia, and protect the elbow/hock areas with padded bandages to prevent pressure sores developing.
    - Ruminants (e.g. goats) should be propped up on their sternums to minimise the risk of overdistension of the rumen with gas (i.e. ruminal tympany, this can be relieved by passing a stomach tube) and of inhalation of regurgitated rumen contents.
- Recovery of the animal from anesthesia can be aided by the administration of warmed parenteral isotonic fluids (e.g. 0.9% normal saline, Lactated Ringer's solution) given subcutaneously or by the intra-peritoneal route

for maintenance of water and electrolyte balance. The maintenance requirement for fluid is approximately 40ml/kg/24hr in normal animals.

- Analgesic and other drugs like antibiotic should be administered if indicated.
- The surgical incisions should be checked for wound dehiscence and the need for dressing changes.
- In neonates or animals recovering from prolonged surgical procedures, hypoglycemia can be a problem leading to post-surgical complications. These animals may benefit from the administration of glucose given orally. Glucose solutions should not be given subcutaneously or by the intra-peritoneal route.

3.1.7.3 After anesthetic recovery, animals may be returned to their holding area once they are awake, able to move about in the recovery cage/pen and appear to be making normal behavioral adjustments.

- An animal should not be placed in a group cage/pen unless it is capable of protecting itself from cage/pen mates. The cage/pen card should be labeled with the surgical procedure performed and the operation date.
- Post-operative care should continue beyond the return of the animal to its home environment. *Animals should be monitored closely for several days after the surgical procedure to ensure their healing and recovery*.
- *Animals must be monitored for the continued need for analgesics to alleviate post-operative pain. This assessment should be made at least twice daily in the first few post-operative days.*
- Daily weighing of the animal during the post-operative period is a sensitive method of monitoring the animal. While subtle changes in the animal's activity or appetite may not be clinically observed, changes in weight will be quickly detected allowing appropriate clinical intervention to be instituted.
- Food intake may be difficult to monitor in rodents, especially if group housed. However, if post-operative animals are singly housed and food rations are supplied in measured amounts this can be a useful monitoring tool. Supplying a softer, more palatable, easily accessible diet may encourage the animal to eat.
- The animal's hydration can be monitored by "tenting" the skin along the back of the animal. In a well-hydrated animal, the skin should quickly fall back into place when released. If an animal is dehydrated, the skin will be slow to return to its original position and appropriate fluids have to be given to the animal.
- Animals should be monitored for post-surgical infections (including infected or dehisced surgical incision), self-inflicted trauma and wound dressing/bandaging problem.
- *Wound closures (i.e. skin sutures, clips, or staples) should be removed at 10 to 14 days post-operatively unless requirements from the animal research protocol necessitate otherwise.* Suture scissors or staple removers should be used for ease and minimal discomfort to the animals.

3.1.7.4 Proper records on surgical procedures and post-operative observation/ treatment must be maintained and kept close to where the animal is held. A sample copy of LAU's "Surgical Operation and Post-operative Record" for rodents/rabbits and goats/pigs is attached at Appendix B & C.

3.2 Facilities for aseptic surgery

3.2.1 In planning the location to perform surgery the best possible surgical area should be selected. *The following points should be considered in choosing a good surgical area:*

- *an uncluttered area that is easily organized and disinfected, and free of debris and equipment not related to surgery.*

- *the area should be dedicated for the duration of the procedure, but can be used for other purposes when not being used for surgery.*
- *Locations that are beneath supply ducts should be avoided to minimize contamination from dust.*
- *high traffic areas such as those near doorways should be avoided to prevent unnecessary interruptions and creation of air turbulence.*

3.2.2 The design of a surgical facility should accommodate the species to be operated on and the complexity of the procedures to be performed.

- *For most rodent surgery, a facility may be small and simple, such as a dedicated space in a laboratory appropriately managed to minimize contamination from other activities in the room during surgery.*
- The facility often becomes larger and more complex as the number of animals, the size of animals, or the complexity of procedures increases (for instance, large-volume rodent procedures), the need for special restraint devices, hydraulic operating tables, and floor drains for farm animal surgery, and procedures that require large surgical teams and support equipment and thus large space.
- The relationship of surgical facilities to diagnostic laboratories, radiology facilities, animal housing, staff offices, and so on should be considered in the overall context of the complexity of the surgical program.
- *Surgical facilities should be sufficiently separate from other areas to minimize unnecessary traffic and decrease the potential for contamination.*
- Centralized facilities provide important advantages in cost savings in equipment, conservation of space and personnel resources, reduced transit of animals, and enhanced professional oversight of facilities and procedures.

3.2.3 For most surgical programs, *functional components of aseptic surgery include:*

- *surgical support,*
- *animal preparation,*
- *surgeon's scrub,*
- *operating room,*
- *post-operative recovery.*

3.2.4 The number of personnel and their level of activity have been shown to be directly related to the level of bacterial contamination and the incidence of post-operative wound infection. The areas that support the above-listed functions should be designed to:

- minimize traffic flow in the operating room by the installation of an observation window, a communication system (such as an intercom system) and judicious location of doors.
- separate the related non-surgical activities from the surgical procedure in the operating room by physical barriers, distance between areas or by the timing of appropriate cleaning and disinfection between activities.

3.2.5 Control of contamination and ease of cleaning should be key considerations in the design of a surgical facility.

- The interior surfaces should be constructed of materials that are monolithic and impervious to moisture.
- Ventilation systems supplying filtered air at positive pressure can reduce the risk of post-operative infection.
- Careful location of air supply and exhaust ducts and appropriate room-ventilation rates can minimize contamination.
- *To facilitate cleaning, the operating rooms should have as little fixed equipment as possible.*

- Floor drains are generally discouraged because they can serve as a source of bacterial contamination and noxious gases.
- Other features of the operating room to consider include surgical lights to provide adequate illumination, sufficient electric outlets for support equipment and gas scavenging capability.
- *Equipment should be kept physically clean, and only equipment that is used regularly should be kept in the surgical facility.*
- *The operating room and support areas should be cleaned and disinfected at frequent intervals to keep potential microbiological contamination at a minimum.*

3.2.6 The surgical support area should be designed for washing and sterilizing, and for storing instruments and supplies.

- Autoclaves are commonly placed in this area.
- It is often desirable to have a large sink in the animal preparation area to facilitate cleaning of the animal and the operative site.
- A dressing area should be provided for personnel to change into surgical attire; a multipurpose locker room can serve this function.
- There should be a scrub area for surgeons, equipped with foot, knee, or electric-eye surgical sinks. To minimize the potential for contamination of the surgical site by aerosols generated during scrubbing, the scrub area is usually outside the operating room.

3.2.7 A post-operative recovery area should provide the physical environment to support the needs of the animal during the period of anesthetic and immediate post-surgical recovery and should be so placed as to allow adequate observation of the animal during this period.

- The electric and mechanical requirements of monitoring and support equipment should be considered.
- The type of caging and support equipment will depend on the species and types of procedures but should be designed to be easily cleaned and to support physiologic functions, such as thermo-regulation and respiration.

3.3 Maintenance of the Surgical Facility

In addition to proper design and construction of a surgical facility, the development of standard operating procedures and other general practices for maintenance of the facility on a day-to-day basis is also a key component of a good veterinary care program. The same principles and practices should apply to research laboratories which are used for performing rodent surgery. These include:

- Efficient coordination and schedule on the use of the operating room.
  - Identify the personnel involved (i.e. investigator and technical and other support staff, including emergency contact information.)
  - Identify the animals, equipment, instruments, drugs and other supplies required.
  - Ensure the project has been approved by CULATR (Committee on the Use of Live Animals in Teaching and Research).
- Proper maintenance of inventory on purchase and supply items (e.g. drugs, fluids, sutures), including regular checking on proper storage and expiry dates of all supplies, status of packaged sterile items and proper storage).
- Proper maintenance of the surgical facility/laboratory, equipment and instruments
  - Checking the anesthetic machines (e.g. leakages, soda lime status), operating lights and tables, ventilators, monitoring equipment, etc.
  - Cleaning and package of surgical instruments (including checking on sharpness, use of tip protectors)
  - Requests for repair and maintenance works for the surgical facility/laboratory should be submitted to the Estates Office promptly with follow up monitoring.
- The facility should be kept clean and uncluttered at all times.

- The operating room/laboratory should be regularly disinfected (e.g. monthly and prior to/after each surgery), including wiping down walls, ceilings and other surfaces as well as equipment like anesthetic machines, surgical lights, tables and chairs..

- Proper records should be maintained.
  - Physical examination record (i.e. date of receipt, species, sex, weight, ID number, source, investigator name, CULATR approval number)
  - Surgical record (i.e. procedures performed, post-operative instructions regarding analgesic, antibiotic, fluid and food restriction, and special care)
  - Anesthesia record (i.e. pre-anesthetics, anesthetics, fluids & other drugs including dosage, routine and time of administration, animal vital signs and monitoring parameters).
  - Post-operative record (i.e. daily observations on vital signs, appetite, surgical site and complications, and treatments; the duration of observation depends on the invasive of the procedure [observation may continue for 7-10 days for invasive surgery]).
- Other records
  - Log book/register for dangerous/scheduled drugs.
  - Log book for operation room booking, usage of X-ray facility/other large equipment items.
  - Equipment maintenance/repair record.
  - Record on specimens sent for laboratory examination.

4. PAIN, ANALGESIA AND ANESTHESIA

4.1 Pain and analgesia

4.1.1 An integral component of veterinary care is prevention or alleviation of pain associated with procedural and surgical protocols. Pain is a complex experience that typically results from stimuli that damage tissue or have the potential to damage tissue. A painful stimulus prompts withdrawal and evasive action. Pain is a stressor and, if not relieved, can lead to unacceptable levels of stress and distress in animals. The proper use of anesthetics and analgesics in research animals is an ethical and scientific imperative.

4.1.2 Fundamental to the relief of pain in animals is the ability to recognize its clinical signs in specific species. Critical to the assessment of the presence or absence of pain or distress is having the ability to distinguish between normal and abnormal animal behaviour. This is especially true when dealing with rodents and rabbits that often exhibit pain and distress with only subtle changes in their behaviour.

- Species vary in their response to pain, criteria for assessing pain in various species differ.
  - Mice
    - Increase in sleeping time and weight loss
    - Pilo-erection and hunched appearance
    - Isolated from rest of group
  - Rats
    - Behavioural change (increase in aggressiveness, resist handling)
    - Vocalisation and struggling
    - Lick or guard painful area, sit crouched
    - Increased sleeping time
    - Ocular discharge (*Chromodacryorrhoea*:- 'reddish' haematoporphyrin-stained exudate)
  - Guinea-pigs
    - Unusual sign of acceptance (i.e. less alert and less apprehensive)
    - Loud vocalization
    - May appear sleepy
  - Hamsters
    - Weight loss
    - Extended sleep period
    - Increased aggression or depression

        - Ocular discharge and diarrhoea
    - Gerbils
        - Ocular discharge, partially closed eyelids
        - Changes in activity and burrowing behaviour
        - Arched back/hunched posture
        - Weight loss and hair loss on tail
    - Rabbits
        - Often react to pain with stoic appearance
        - Reduced food and water intake
        - Limited movement
        - Photosensitivity, ocular discharge and protruded third eyelid
        - Faecal staining of hair coat
        - Digestive disturbance
        - Dehydration
    - Ruminants (goats/sheep)
        - Dull and depressed
        - Inappetance and weight loss
        - Rapid shallow respiration
        - Grunting and grinding of teeth
        - Reduced rumination and eructation
    - Pigs
        - Changes in behaviour (e.g. tolerate handling more readily)
        - Changes in gait or posture
        - Decrease in appetite
        - Adults may become aggressive
        - Depression, unwilling to stand or move
    - Birds
        - Changes in escape behaviour/vocalization when approached
        - Partially closed eyelids
        - Vocalisation, wing flapping/droop
        - Anorexia, ruffled feathers, "mouth breathe" (gasping)
- It is essential that personnel caring for and using animals be very familiar with species-specific (and individual) behavioral, physiologic, and biochemical indicators of well-being.
- *In general, unless the contrary is known or established it should be assumed that procedures that cause pain in humans also cause pain in animals.*

4.2 The selection of the most appropriate analgesic should reflect professional judgment as to which best meets clinical and humane requirements without compromising the scientific aspects of the research protocol. Reduction of research-associated pain/distress in animals can have an effect on the speed with which animals return to normal behaviour following surgical procedures.

4.2.1 Pre-operative or intra-operative administration of analgesics might enhance post-surgical analgesia. The selection depends on many factors, such as

- the species and age of the animals,
- the type and degree of pain,
- the likely effects of particular agents and on specific organ systems,
- the length of the operative procedure,
- the safety of an agent for an animal, particularly if a physiologic deficit is induced by a surgical or other experimental procedure.

4.2.2 Analgesics which are commonly used in laboratory animals include:

- *Opioids like morphine and buprenorphine. Buprenorphine is the opioid analgesic of choice in most laboratory animal species. It is usually administered subcutaneously in rodents and rabbits, and intra-muscularly in goats and pigs. It has a short duration of action of 4 – 12 hours depending on the animal species*

- *Non-steroidal anti-inflammatory drugs like ketoprofen, carprofen and flunixin. These analgesics have longer duration of action of 12 - 24 hours depending on the animal species. They can be administered subcutaneously or via drinking water.*

4.3 Anesthesia

The two primary aims of anesthesia are to prevent pain and provide humane restraint.

4.3.1 Choice of anesthetics

No anesthetic regime is suitable for all surgeries. Depending on the type of surgical procedure, the anesthetic should be selected based on the length of the surgical procedure, the biological characteristics of the animal species to be operated on, the equipment available and the expertise of those who will be responsible for administering the anesthetic. The compatibility of the anesthetic with the experimental design should be considered; however, the overriding concern should be well-being of the animal.

- Each anesthetic used must be approved in the animal research protocol.
- Injectable anesthetics like ketamine, xylazine, fentanyl/fluanisone, propofol and tiletamine and pentobarbitone are widely used in laboratory animals. To ensure proper dosing when using injectable anesthetics, each animal should be weighed and dosed according to its body weight.
    - Some anesthetics, such as ketamine, abolish the blink reflex. Application of an ophthalmic lubricant is recommended to protect the corneas of anesthetized animals from desiccation.
    - Pentobarbitone produces severe cardiovascular and respiratory depression. It also has poor analgesic activity and considerable between-strain variation in anesthetic dose.
- Inhalant anesthetics like halothane and isoflurane are best administered using precision calibrated vaporizers and waste anesthetic gases must be scavenged to limit exposure by:
    - Down-draft tables which are under negative exhaust pressure and draw excess gases away from the operator.
    - Chemical fume hoods.
    - Type II Bio-safety cabinets that are vented to the outside.
- *Ether is flammable/explosive and irritating to the respiratory tract. It is therefore not recommended for use an anesthetic agent.*
- *Chloroform has numerous side-effects and is not suitable for laboratory use.*

4.3.2 Anesthetic Support and Monitoring

4.3.2.1 Anesthetized animals must be monitored during the procedure to assure that they stay in the proper anesthetic plane.

- The animals should not be too lightly anesthetized that they experience pain or regain consciousness, or too deep that vital functions are compromised.
- Once the anesthetic has been given time to take effect, the anesthetic plane can be assessed by pinching the toes (i.e. pedal withdrawal reflex/limb withdrawal response), tail or ear which is suitable for rodents and rabbits, or by touching the edge of the eyelids (palpebral reflex) which is commonly used in goats and pigs. Any reaction from the animal indicates that the animal is too lightly anesthetized and that additional anesthesia should be given.
    - Palpebral reflex is lost during the onset of light surgical anesthesia with barbiturates. Use of ketamine will also cause the loss of this reflex at higher levels of anesthesia.
- It is important to visually inspect the animals and not rely solely on monitoring instruments.

        - The color of the mucous membranes and exposed tissues is easy to monitor. This will give an indication of tissue perfusion and oxygenation.
        - The color should be a bright pink to red and not dusky gray or blue. Pink mucous membranes indicative of good tissue perfusion and oxygenation.
    - Respiratory pattern and frequency are also easily monitored and will give an indication of anesthetic depth and other potential complications.
    - Core body temperature can be monitored in rodents, including mice using properly sized rectal thermometer probes.
    - Pulse oximetry can be used in larger rodents, rabbits and farm animals to monitor pulse and oxygenation. When instruments such as pulse oximeters are used regular calibration is recommended to ensure accurate information is reported on the animal's true condition.
    - Electrocardiograms and capnography can also be used.

4.3.2.2 The most frequent complication of anesthesia is hypothermia which may result in prolonged recovery or possibly death in small animals like rodents.

- Animals should be provided with a heat source during the pre-operative, intra-operative, and postoperative periods.
- In farm animals, electronic heat pads/blankets are available with thermostats linked to the animal’s body temperature.
- If fluid administration is required, the fluids should be warmed first (e.g. a bag of intravenous fluid can be warmed to body temperature by immersing it in warm water or by using an instant heat pouch).
- Because of the high air flow, the risk of hypothermia is heightened when chemical fume hoods or biosafety cabinets are used as the location to conduct rodent surgery.
- For rodents, proper use of warming devices should be practiced to prevent injury to the anesthetized animal.
    - Heat lamps can be very dangerous and are not recommended.
    - Temperature at the level of the animal should be held between 30 - 35°C.
    - Use of a dry-bulb thermometer is recommended to measure the temperature adjacent to the animal to monitor that the temperature is kept in the desired range.
    - Electric heating pads are not recommended for use with rodents due to the variation in temperatures across the pad surface.
    - The safest devices for providing heat are circulating warm water blankets or instant heat devices or hand warmers. These devices should be covered with a paper towel or other insulation so that the animal does not come in direct contact with the heating device. Animals should be monitored to prevent gnawing or ingestion of the warmers.
    - Electric slide warmers can be used as a heat source during recovery.
- Animals must be watched very closely and be placed in the recovery cage as soon as they begin to awaken.

4.3.2.3 Some classes of drugs such as sedatives and neuromuscular blocking agents are not analgesic or anesthetic and thus do not relieve pain; however, they might be used in combination with appropriate analgesics and anesthetics.

- Neuromuscular blocking agents (e.g. pancuronium) are sometimes used to paralyse skeletal muscles during surgery in which general anesthetics have been administered. When these agents are used during surgery or in any other painful procedure, many signs of anesthetic depth are eliminated because of the paralysis. However, autonomic nervous system changes (e.g. sudden changes in heart rate and blood pressure) can be indicators of pain related to an inadequate depth of anesthesia.

- If paralyzing agents are to be used, it is recommended that the appropriate amount of anesthetic be first defined on the basis of results of a similar procedure that used the anesthetic without a blocking agent.

4.4 For experiments involving the production of genetically modified mice, please refer to the following document on the choice of analgesics and anesthetics, and recommendations on refinement of procedures to improve animal welfare:

Refinement and reduction in the production of genetically modified mice, Sixth Report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement, Laboratory Animals (2003), Vol. 37, Supplement 1 http://www.catchword.co.uk/rsm/00236772/v37n3x1/contp1-1.htm. (This document is also posted on the CULATR website http://www.hku.hk/facmed/research/culatr.html)

5. ANTIBIOTICS

Bacterial infections in animals used for experimental studies are undesirable because they will lead to morbidity/mortality, discomfort and experimental variation. However, the use of antibiotics to treat infected animals may itself interfere with the experiment.

5.1 Aseptic technique should be used as standard practice but there is a role for the rational use of peri-operative antibiotics where aseptic technique cannot be absolutely assured or inadvertent breaks in aseptic techniques occurs. Animals in the post-operative period are often at increased risk of infection.

- Antibiotic use is recommended for clean-contaminated surgery and when chronic implantation of foreign material takes place.
- When wound contamination occurs during surgery, it takes several hours for the bacteria to grow. Therefore, antibiotics should be administered pre-operatively to allow peak blood levels to be achieved at the time of wound contamination before significant bacterial proliferation takes place.

5.2 *Post-operative infections will be favoured by the following:*

- *Contamination from dirty equipment, a dirty room, dirty hands and poorly cleaned wounds.*
- *Injured tissues - Poor surgical technique results in excess bruising. Damaged tissues cannot fight infection well.*
- *"Dead space" - Pockets will be left between tissues after surgery (if wound closure is adequate) where blood and serum can accumulate predisposing the animal to infection*
- *Immunosuppression - May be caused by drugs (e.g. corticosteroids), immunodeficient animal strains (e.g. nude mice and SCID mice) and stress.*

5.3 *Antibiotic coverage is not a substitute for poor surgical technique and inadequate hygiene.* The choice of antibiotics depends on the animal species, surgical procedure and knowledge on antibiotic resistance. Antibiotic toxicity in rodents and rabbits is well documented:

- Ampicillin is toxic to guinea-pig, hamster and rabbit;
- Penicillin, lincomycin and cephalosporins are toxic to guinea-pig, hamster and rabbit;
- Streptomycin is toxic to mouse, guinea-pig and hamster;
- Trimethoprim-sulphamethoxazole is toxic to hamster;
- Vancomycin is toxic to rabbit.

6. PROPER USAGE OF DRUGS

6.1 Dangerous Drugs Ordinance (Cap. 134) and related legislation

- A person in charge of a laboratory used for the purposes of research and attached to a university is authorized under the Dangerous Drugs Ordinance of the Laws of Hong Kong

(http://www.justice.gov.hk/Home.htm) to be in possession and to supply dangerous drugs (e.g. ketamine, buprenorphine, diazepam, midazolam, fentanyl/fluanisone). Dangerous drugs can be obtained from the LAU, subject to the approval of the Head of Unit, for use in CULATR-approved experiments only.

- Investigators using dangerous drugs are required to comply with the storage and record keeping provisions of the Dangerous Drug Ordinance as follows:
  - Dangerous drugs should be kept in a locked receptacle which can only be opened by the person authorized under the Dangerous Drugs Ordinance to possess them.
  - The authorized person in possession of dangerous drugs must keep a "Dangerous Drugs Register" in which all transactions of dangerous drugs must be recorded. The format of this Register is fixed by the Ordinance as follows (a copy of this Register can also be downloaded from http://info.gov.hk/dh/useful/1tod/ketamine.htm).
  - Other drug-related legislation in Hong Kong includes the Antibiotics Ordinance, Cap. 137 and the Pharmacy & Poisonous Ordinance, Cap. 138.
- Drugs should be stored according to manufacturers' recommendation to ensure that their full activity will be maintained (e.g. some drugs have to be kept away from light, others have to be stored at 2-5^{o}C and some drugs have to be kept in plastic bottles).
- *It is a good practice to keep a record of all drugs held and used. Expired drugs should be properly labeled and should not be used on animals.*
- A copy of LAU's "Standard Operating Procedures on Storage and Record Keeping of Drugs" is attached at Appendix D.

6.2 Dosage and Route of Administration

Drugs should be used according to manufacturers' instructions. The recommended dosages and routes of administration of commonly used anesthetics, analgesics and anti-bacterial drugs in rats, mice, hamsters, guinea-pigs, rabbits, goats and pigs are listed in Appendix E-J.

6.3 Disposal

Expired and un-wanted drugs should be disposed of properly according to Safety Office's instruction.

7. HUMANE END-POINTS

7.1 These are observable changes which indicate a continuing and possibly irreversible deterioration in an animal's condition. At this point the animal should be removed from the procedure and receive the appropriate treatment.

7.2 Humane endpoints can also be implemented when the animals reflect suffering that cannot be justified or when particular clinical signs affirm a specific outcome such as pending death (e.g. decreased body temperature in mice with bacterial infections, slow circling movements in mice with neurotropic viruses like rabies).

7.3 As soon as an animal ceases to be scientifically useful in an experiment, or the objective of the experiment has been achieved, then it should normally be euthanased.

7.4 Experimental studies may involve procedures that cause clinical symptoms or morbidity in animals. The selection of the most appropriate endpoint(s) requires careful consideration of:

- the scientific requirements of the study,
- the expected and possible adverse effects the research animals may experience (pain, distress, illness, etc.),
- the most likely time course and progression of those adverse effects,
- the earliest most predictive indicators of present or impending adverse effects.

7.5 The effective use of endpoints requires that properly qualified individuals perform both general and study-specific observations of the research animals at appropriate time points.

- Optimally, studies are terminated when animals begin to exhibit clinical signs of disease if this endpoint is compatible with meeting the research objectives. Such endpoints are preferable to death or moribundity since they minimize pain and distress.
- Efforts must be made to minimize pain and distress experienced by animals used in research.

7.6 For experimental protocols that involve *morbidity as an endpoint*, the clinical signs (depending on severity and duration) that may constitute an endpoint include, but are not limited to:

- Rapid weight loss.
- Diarrhoea, if debilitating.
- Progressive dermatitis.
- Rough hair coat, hunched posture, lethargy or persistent recumbency.
- Coughing, labored breathing, nasal discharge.
- Jaundice and/or anemia.
- Neurological signs.
- Bleeding from any orifice.
- Self-induced trauma.
- Any condition interfering with eating or drinking (e.g. difficulty with ambulation).
- Excessive or prolonged hyperthermia or hypothermia.

7.6.1 Selected clinical observations that are used in *toxicological studies* for determination of an endpoint:

- General appearance
  - Dehydration, weight loss, abnormal posture, hypothermia, swelling.
- Skin and fur
  - Ruffled hair, discoloration, urine stain, cyanosis, icterus, ulcer, alopecia.
- Eyes
  - Exophthalmos, microphthalmia, lacrimation, discharge, opacity.
- Nose, month and head
  - Tilted head, nasal discharge, malocclusion, salivation.
- Respiration
  - Sneezing, dyspnoea, rales.
- Urine and faeces
  - Discoloration, blood in urine/faeces, polyuria, anuria.
- Locomotion
  - Hyperactivity, hypoactivity, circling, tremor, convulsion, paralysis, coma.

7.6.2 Signs in *neoplasia studies* that may constitute an endpoint (as recommended in the UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia, second edition, 1997, UK Co-ordinating Committee on Cancer Research, http://www.ncrn.org.uk/csg/publications.htm#Animal) include:

- A tumor burden greater than 10% body weight.
- In an adult 25gm mouse, a mean subcutaneous flank tumor diameter exceeding 17 mm or in an adult 250gm rat, a mean tumor diameter exceeding 35 mm.
- Tumors that ulcerate, become necrotic or infected.
- For ascitic tumours, the ascitic burden is greater than 10% normal body weight in mice and rats.
- For metastatic tumours, when animals develop symptoms of impending morbidity, e.g. dyspnoea due to lung deposits.
- In tumour therapy experiments using adult rodents, when weight loss is greater than 20% body weight at the start of the experiment for 72 hours.
- Persistent anorexia or dehydration.
- Unable to maintain upright position or to move.
- Moribund, lethargy or failure to respond to stimuli.
- Hypothermia, unconscious or comatous.
- Blood stained or mucopurulent discharge from orifice.
- Labored breathing with nasal discharge and/or cyanosis.
- Enlarged lymph nodes and spleen.

- Anaemia, ulcerated tumours or large tumours that interfere with normal movement.
- Ascitis burden greater than 10% baseline body weight or significant abdominal distension.
- Incontinence or prolonged diarrhea.

7.6.3 *For experimental protocols that involve morbidity as an endpoint, one criterion for determination of an endpoint is when an animal is found unexpectedly to be moribund, cachectic, or unable to obtain food or water.*

7.6.4 *Animals should be monitored regularly for signs of suffering, including monitoring on weekends and holidays. Proper records should be maintained.*

7.7 While it is preferable to use the earliest endpoints compatible with the scientific requirements of each study, there are studies that require *moribundity or mortality as an endpoint.*

- The moribund condition is defined as a clinically irreversible condition leading inevitably to death. In these studies, animals are permitted to die or become moribund, as a result of experimental procedures.
- In some cases, pain relieving measures are not used because such measures may compromise the experimental integrity of the study.
- Examples of research proposals that may have death or moribundity as an endpoint include: infectious disease studies, drug and toxicity studies, and cancer research.

7.7.1 Animals involved in experiments that may lead to moribundity or death should be monitored daily by personnel experienced in recognizing signs of morbidity (illness, injury, or abnormal behavior). Selected criteria for euthanasia of moribund animals are listed as follows:

- Rapid weight loss (15-20% within a few days),
- Extended period of weight loss (progressing to emaciated state),
- Spreading area of alopecia caused by disease,
- Rough hair coat, haunched posture, distended abdomen, lethargy, especially if prolonged (3 days),
- Diarrhoea, especially if debilitating or prolonged (3 days),
- Coughing, rales, nasal discharge,
- Distinct icterus and/or anemia,
- Rapid growth of mass or masses, or clinical signs of neoplasia,
- Central nervous system signs such as head tilt, tremors, seizures, circling, paralysis or paresis,
- Frank bleeding from any orifice,
- Markedly discolored urine, poluria or anuria
- Persistent self-induced trauma
- Lesions interfering with eating or drinking
- Clinical signs of suspected infectious disease requiring necropsy for diagnosis

7.7.2 Consideration should be given to moving animals to individual cages when their condition deteriorates to the point that injury from other animals is likely. Dead animals must be promptly removed.

7.7.3 The frequency of observation should be increased when animals exhibit the above or other signs of moribundity. Monitoring of the animals on weekends and holidays is required. Proper records should be maintained.

7.8 For guidelines on humane end-points, please refer to the following documents which are posted on the CULATR website (http://www.hku.hk/facmed/research/culatr.html):

- Institute for laboratory animal research report: Guidelines for the care and use of mammals in neuroscience and behavioral research (2003).
- CCAC guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing (1998).

- Institute for Laboratory Animal Research Report: Humane endpoints for animals used in biomedical research and testing (2000).
- The UKCCCR guidelines for the welfare of animals in experimental Neoplasia, United Kingdom Co-ordinating Committee on Cancer Research (1997).

8. EUTHANASIA

8.1 Euthanasia is the act of killing animals by methods that induce rapid unconsciousness and death without pain or distress. Unless a deviation is justified for scientific or medical reasons, methods should be consistent with the following guidelines which are posted on the CULATR website:

- 2000 Report of the AVMA Panel on Euthanasia
- NIH Guidelines for the euthanasia of rodent feti and neonates (2004)

8.2 In evaluating the appropriateness of methods, some of the criteria that should be considered are:

- ability to induce loss of consciousness and death with no or only momentary pain, distress, or anxiety;
- reliability;
- non-reversibility;
- time required to induce unconsciousness;
- species and age limitations;
- compatibility with research objectives;
- safety of and emotional effect on personnel.

8.3 Euthanasia might be necessary at the end of a protocol or as a means to relieve pain or distress that cannot be alleviated by analgesics, sedatives or other treatments. Protocols should include criteria for initiating euthanasia, such as degree of a physical or behavioral deficit or tumor size that will enable a prompt decision to be made by the veterinarian and the investigator to ensure that the end point is humane and the objective of the protocol is achieved. For guidelines on humane end-points, please refer to the documents which are posted on the CULATR website as mentioned in Section 7.8 above.

8.4 Euthanasia should be carried out in a manner that avoids animal distress. In some cases, vocalization and release of pheromones occur during induction of unconsciousness. For that reason, other animals should not be present when euthanasia is performed.

8.5 The selection of specific agents and methods for euthanasia will depend on the species involved and the objectives of the protocol.

- Generally, inhalant or non-inhalant chemical agents (such as barbiturates, non-explosive inhalant anesthetics, and CO_2) are preferable to physical methods (such as cervical dislocation, decapitation, and use of a penetrating captive bolt). Acceptable methods of euthanasia include:
  - Pentobarbitone: For most species (at 150-200mg/kg, i/v or i/p).
  - Halothane: For small laboratory animals and neonates.
  - Carbon dioxide: For most small animals (using compressed cylinder gas at a rising concentration to above 70%) but not suitable for fish, cold-blooded vertebrates and neonates <2 wk-old.
  - MS222/TMS (Tricaine methane sulfonate): For fish and amphibians.
  - Cervical dislocation: For poultry, birds, mice and fish.
- Ether is flammable/explosive and irritating to the respiratory tract. It is therefore not recommended for use a euthanasia agent.
- Isoflurane has pungent odour and animals often hold their breath, resulting in delay of unconsciousness.
- However, scientific considerations might preclude the use of chemical agents for some protocols.
- All methods of euthanasia should be reviewed and approved by the CULATR.

8.6 It is essential that euthanasia should be performed by personnel who are skilled in methods for the species in question and that it will be performed in a professional and compassionate manner. Death should be confirmed by personnel who can recognize cessation of vital signs in the species being euthanatized.

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Laboratory Animal Unit, April 2, 2005

Appendix A

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Routine Health Checking of Animals

1. All cage tops should be partially opened to see if there are animals hiding underneath the hoppers / bottles.

2. All animals (except lactating females and their sucklings) should be dispersed to see if there are animals hiding underneath the hoppers / bottles.

3. Each animal should be checked for signs of illness and abnormalities.

4. The body parts of each animal to be examined include: eyes, pinnae, mouth, nose, head, face, neck, back, sides of chest, sides of abdomen, hip, tail and 4 limbs.

5. Abnormalities to be looked for include:

   5.1 Abnormal behaviour (e.g. circling, Shivering; listlessness);
   5.2 Body weight (emaciation, inappetance) / size;
   5.3 Hair coat;
   5.4 Dehydration;
   5.5 Anaemia;
   5.6 Gasping;
   5.7 Difficulty in parturition;
   5.8 Diarrhoea / vomiting;
   5.9 Swelling / budding / tumour;
   5.10 Wound / ulceration / abscess / pus;
   5.11 Whisker-loss / hair-loss;
   5.12 Overgrown incisors;
   5.13 Discharge (from eyes, ears, nose, mouth, vagina, wounds);
   5.14 Arched back;
   5.15 Collapsed abdomen;
   5.16 Other strange appearance / lesions

6. Animals suspected to be abnormal should be removed from the cages and subject to further detailed examinations.

7. Submit an “Animal Health Report” to the Area Head/Head of Unit.

Appendix B

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Surgical Operation and Post-Operative Record for Rodents & Rabbits

Department : _____ CULATR No.: _____

Principal Investigator: _____ Position: _____

LAU User: _____ Position: _____

Species/Strain: _____ Date of First Operation: _____

A. ***Description of surgical procedures*:**

Note: (1) To be completed by Principal Investigator/LAU User immediately after the operation, please use separated sheet if required.

(2) Separate record form is required for operations which involve different surgical procedures even though they are under the same CULATR No.

_____

_____

_____

_____

_____

_____

_____

_____ _____ _____

Name of Principal Investigator(PI)/LAU User Signature of PI/LAU User Date

B. ***Anaesthesia***

Drug (Please √)	Dosage (please specify)	Route (Please √)
□ Ketamine		□ Intraperitoneal injection
□ Xylazine		□ Intramuscular injection
□ Hypnorm® (Fentanyl/fluanisone)		□ Subcutaneous injection
□ Dormicum® (Midazolam)		□ Intravenous injection
□ Isoflurane		□ Inhalation
□*Hypnorm / Midazolam mixture		□ Others (please specify):-
□ #Ketamine / Xylazine mixture		
□ Others (please specify):-		

***** 1 part Hypnorm + 1 part Midazolam + 6 parts water for injection

2 parts Ketamine + 1 part Xylazine

C. <u>Post-operative Observation and Treatment</u>

(Please use additional sheets if required)

Date		Rack no.	Cage no.	Animal no. *((Note 1)*	Health Condition *(Note 2)*	Treatment			Principal Investigator/ LAU User Signature	LAU staff Signature
Operation	Treatment					Drug	Dosage	Route		

<u>Note</u>:-

(1) Identification methods include ear marking (ear punch / notch /tattoo), fur / tail marking

(2) Abbreviations for Health Condition:

A – Abscess	HL – Hair loss	R – Recovering	WE – Surgical wound exudates
C – Cough	IA – Inappetance	SM – Suture missing	WR – Surgical wound edges reddish
D – Diarrhoea	L - Lameness	WD – Surgical Wound dehiscence	WS – Surgical wound swelling

Appendix C

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Surgical Operation and Post-Operative Record for Large Animals (Goats and Pigs)

Species/Breed	:	Department	:
Colour	:	User	:
Code	:	CULATR No.	:
Sex	:	Commencement Date	:
Age / DOB	:	Completion Date	:

A. *Description of surgical procedures*: (To be completed by researcher/user performing the operation, please use overleaf if required)

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____

_____	_____	_____
Name of Researcher/User	Signature of Researcher/User	Date

B. *Pre-operative Record / Procedures*

1. Body weight (kg): _____ Body condition: _____
2. Appetite: _____ Fasting (hours): _____
3. Stomach Tube (size) : _____ Endotracheal tube (size): _____

C. *Anaesthesia*:

1. Pre-anaesthetic Medication

Drug	Dose	Route	Time

2. Induction

Drug	Dose	Route	Time

3. Maintenance

(a) Inhalational Anaesthetic

□ O_2 _____ L/m □ Halothane _____ %
□ N_2O _____ L/m □ Isoflurane _____ %

Anaesthesia Machine Setting

Pmax 60hpa	Vt 10ml-15ml/kg	fiPPV Vent. Freq.	Ti:Te 1:2	Tip:Ti 5	PEEP 3hPa

Monitoring reading

Time after induction	Heart rate	SpO_2	Paw

Abbreviations:
Pmax – Maximum pressure
fiPPV – Breathing frequency
Tip : Ti – Inspiratory pause
SpO_2 – Saturated pulse oxygenation
hpa – Pressure unit = 0.01 Bar
Vt – Tidal volume
Ti : Te – Insp/Exp time ratio
PEEP – Positive end expiratory pressure
Paw – Airway pressure

(b) Injectable Anaesthetic

Time after induction	Drug	Conc.	Route	Dosage	Vol. (ml)

D. *X Ray Examination*

No. of radiographs taken	Location	kV setting	mAs setting	Remarks

E. *Recovery*

State	Time
Off Inhalational Anaesthetic	
Extubation	
Animal wake up	

F. *Post-operative Observation and Treatment*

Date	Health Condition	Treatment			Remarks	LAU staff signature
		Drug	Dose	Route		

Abbreviations for Health Condition:

A – Abscess	EI – Exudates from incision	L – Lameness	WD – Wound reddish edges
C – Cough	HL – Hair loss	R – Recovering	WD – Wound dehiscence
D – Diarrhoea	IA – Inappetance	SM – Suture missing	WS – Wound swelling

G. *Euthanasia*

Date	Drug	Dose	Route	Remarks

H. *Others*

_____

_____

_____

_____

_____ _____ _____

Name of LAU staff Signature of LAU staff Date

Appendix D

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Storage and Record Keeping of Drugs

1. All controlled and uncontrolled drugs should be kept inside drawers/cabinets in the Technician Office by senior technical staff before they are dispatched to concerned Areas. The expiry date and required storage condition of the drugs should be checked on arrival. The Technician Office should always be locked when it is not under attention.

2. Drugs should be given to the Area Head or the First Technician of the concerned Area directly for use in CULATR-approved projects only.

3. Area Heads and First Technicians are responsible for proper storage and usage of controlled and uncontrolled drugs.

4. All drugs should be stored in the cabinets or refrigerators (according to manufacturer' recommendations) in the Preparation Rooms or Area Offices which should be locked outside office hours.

5. Drugs that require low temperature storage should be kept in refrigerators according to manufacturer's instruction.

6. Drugs which are sensitive to light should be kept in an opaque container.

7. All the controlled drugs (e.g. anesthetics and analgesics) must be kept under lock and key. Keys should be kept by Area Heads, Technicians and the Head of Unit.

8. Proper record of usage should be maintained for all drugs.

9. Details such as issue date, quantity, user name, department, order number, CULATR Number should be recorded in the "Drug In/Out Record" form when drugs are issued (refer Annex 1).

10. A bi-monthly drug inventory check should be done to replace expiring/expired drugs.

11. Expired drugs should be properly labeled while awaiting disposal and should not be used in animals.

12. Expired drugs should be disposed of as chemical waste and according to Safety Office's instruction.

Annex 1

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

DRUG USAGE RECORD

Date	**Drug Name**	**In** Bottle(s)/ Volume	**Out** Bottle(s)/ Volume	**Balance** Bottle(s)/ Volume	**User / Department**	**CULATR No.**	**Signature**	**Remark**

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Anesthesia and Post-operative Care of Mouse

1) Pre-emptive analgesia by Temgesic® (buprenorphine 0.3mg/ml)

Inject 0.01ml Temgesic, s/c [for mouse with body weight = 20-30gm (~0.1mg/kg)] **one hour before** administration of anaesthetic.

2) Anaesthesia by Hypnorm® / Dormicum® mixture

a) Preparation of Hypnorm® (fentanyl citrate 0.315mg/ml / fluanisone 10mg/ml) & Dormicum® (midazolam 5mg/ml) anaesthetic mixture for mouse

Drug	**Volume** (ml)
Hypnorm, 10mg/ml	1
Dormicum, 5mg/ml	1
Water for injection	6

b) Dosage of by I/P

Mouse	**Volume (ml)**	
Body Weight (gm)	***Hypnorm®/Dormicum® mixture*** (Hypnorm - 12.5 mg/kg Dormicum - 6.25mg/kg)	**Remark**
15	0.15	0.1ml/10gm body wt.
20	0.2	
25	0.25	
30	0.3	

3) Post-operative Care

3.1) Fill cage with soft "towel" bedding and nesting material, and keep warm.

3.2) Analgesic:

a) Buprenorphine (Temgesic®): 0.1mg/kg s/c (0.01ml/mouse with b.wt. 20-30 gm), 8-12 hourly for 3-5 days, if necessary; or

b) Carprofen: 5 mg/kg, q12-24hr, in drinking water (by adding 0.1-0.15 ml of 50 mg/ml carpofen to 100 ml water); or

c) Flunixin: 2 mg/kg, q12hr, in drinking water (by adding 0.1 ml of 50 mg/ml flunixin to 200 ml water).

3.3) Fluid : Dextrose-saline (4% dextrose, 0.18% saline) or saline (0.9%) at 1-2ml s/c or i/p (for a 30g mouse), if necessary.

3.4) Observe abnormalities daily and fill in the Post-Operative Care Record Form.

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Anesthesia and Post-operative Care of Rat

1) Pre-emptive analgesia by Temgesic® (buprenorphine 0.3mg/ml) or **Ketoprofen®** (100mg/ml)

Inject analgesic **one hour before** administration of anaesthetic.

Rat	**Volume (ml)**	
Body Weight (gm)	***Temgesic®*** (0.05mg/kg s/c, 8-12 hourly)	***Ketoprofen®*** (5mg/kg s/c, daily)
150	0.025	0.0075
200	0.033	0.01
250	0.042	0.0125
300	0.050	0.015

2) Anaesthesia by Ketamine/Xylazine mixture

a) Preparation of Ketamine/Xylazine anaesthetic mixture

Drug	**Volume** (ml)	
	Method A	**Method B**
Ketamine HCl, 100mg/ml	1.2	2
Xylazine, 20mg/ml	1	1
Water for injection	7.8	-

The mixture can be stored at 2-8 ℃ for three months

b) Dosage of Ketamine/Xylazine anaesthetic mixture by I/P

Rat	**Volume (ml) - Ketamine/Xylazine mixture**	
Body Weight (gm)	***For Method A*** (Ketamine-60mg/kg Xylazine-10mg/kg, i/p)	***For Method B*** (Ketamine-67mg/kg Xylazine-6mg/kg, i/p)
150	0.75	0.15
200	1.00	0.2
250	1.25	0.25
300	1.5	0.3

3) Anaesthesia (for rat with B. Wt. ~200g) by Hypnorm® (I/M) / Halothane or Isoflurane (inhalational)

3.1) Inject 0.2 ml Hypnorm for induction.
3.2) Set oxygen flow rate at 200 ml/min.
3.3) Set Halothane / Isoflurane at 0.5-2% to maintain the animals under anaesthesia.

4) Ventilator

4.1) Tidal volume: 10-15 ml/kg
4.2) Ventilation rate per minute: 60-100

5) Post-operative Care

5.1) Fill cage with soft "towel" bedding and nesting material, and keep warm.
5.2) Temgesic® : 0.05mg/kg s/c, 8-12 hourly for 3-5 days, if necessary.
5.3) Fluid : Dextrose-Saline (4% dextrose, 0.18% saline) or saline (0.9%) at 10ml s/c or i/p (5 ml per site for a 200g rat), if necessary.
5.4) Terramycin LA® (Oxytetracycline 200mg/ml, 60mg/kg, q72 hrs), s/c, if necessary.
5.5) Observe daily for abnormalities and fill in the Post-Operative Care Record Form.

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Anesthesia and Post-operative Care of Hamster

1) Pre-emptive analgesia by Temgesic® (buprenorphine 0.3mg/ml)

Inject analgesic **one hour before** administration of anaesthetic.

Hamster Body Weight (gm)	Volume (ml) *Temgesic*® (0.05mg/kg s/c, 8-12 hourly)
80	0.013
90	0.015
100	0.016
110	0.018
120	0.020

2) Anaesthesia by Ketamine/Xylazine mixture

a) Preparation of Ketamine/Xylazine anaesthetic mixture

Drug	Volume (ml)
Ketamine HCl, 100mg/ml	2
Xylazine, 20mg/ml	1

The mixture can be stored at 2-8 ℃ for three months

b) Dosage of Ketamine/Xylazine anaesthetic mixture by I/P

Hamster Body Weight (gm)	Volume (ml) - Ketamine/Xylazine mixture (Ketamine-100mg/kg, Xylazine-6mg/kg, i/p)
100	0.15

3) Post-operative Care

5.1) Fill cage with soft "towel" bedding and nesting material, and keep warm.
5.2) Temgesic® : 0.05mg/kg of b.wt, s/c, 8-12 hourly for 3-5 days, if necessary.
5.3) Fluid supplementation: Dextrose-saline (4% dextrose, 0.18% saline) or saline (0.9%) at 3 ml, s/c or i/p (for a 100g hamster), if necessary.
5.4) Terramycin LA® (Oxytetracycline 200mg/ml, 60mg/kg, q72 hrs), s/c, or Oxytetracycline 250mg.liter drinking water, if necessary.
5.5) Observe daily for abnormalities and fill in the Post-Operative Care Record Form.

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Anesthesia and Post-operative Care of Guinea-pig

1) Pre-anaesthetic fasting: for 6-8 hours before anaesthesia.

2) Pre-anaesthetic medication:

a) **Temgesic®** (buprenorphine 0.3mg/ml): pre-emptive analgesia
0.05mg/kg b.wt, s/c one hour before anaesthesia

b) **Atropine**: for minimizing bronchial and salivary secretions
0.02 – 0.05mg/kg b.wt., s/c or i/m, 30 minutes before anaesthesia

3) Inhalational Anaesthesia:

A. Anaesthetic Chamber		
1. Induction:		
Initial Gas flow rate (L/min)	O_2	2
	N_2O	1
Halothane / Isoflurane		1% → 5% (in 2 minutes)
2. After loss of consciousness		
Gas flow rate (L/min)	O_2	1
	N_2O	0.5
Halothane / Isoflurane		2 % (for 2 minutes)
B. Maintenance: Using close-circuit during surgery		
Gas flow rate (L/min)	O_2	0.5
	N_2O	1.0
Halothane / Isoflurane		2-3% Observe respiration, if drop, adjust Halothane (%) accordingly
After Surgery		Turn off Halothane/Isoflurane, then N_20 Supply O_2 at 0.5L/min until righting reflex resumes

4) Post-operative Care

4.1) Provide soft "towel" bedding and nesting material, and keep warm.
4.2) Check animals at least twice daily:
  i. look for abnormal discharge from eyes, nose and mouth,
  ii. check appetite and excreta &
  iii. monitor body weight and check surgical wound.
4.3) Inject **Temgesic®** (buprenorphine 0.3mg/ml, 0.05mg/kg, s/c), 8-12 hourly for 3-5 days if necessary.
4.4) Fluid Supplementation: Dextrose-saline (4% dextrose, 0.18% saline) or saline (0.9%) at 10-20ml, s/c or i/p (for a 1kg guinea-pig) and Multi-Vitamins (0.3ml/kg, s/c, daily), if inappetance and dehydration observed (check skin tone for sign of dehydration).
4.5) Record observation & treatment daily on the Post-Operative Care Record Form.

5) Surgical Wound Care

5.1) Spray with wound dressing
5.2) Clean with disinfectant (eg. diluted Savlon®, H_2O_2)
5.3) Provide "Elizabethan" collar (if wound biting occurs)

THE UNIVERSITY OF HONG KONG
LABORATORY ANIMAL UNIT

Anesthesia and Post-operative Care of Rabbit

1) Pre-emptive analgesia by Temgesic® (buprenorphine 0.3mg/ml) or **Ketoprofen®** (100mg/ml)

Refer to Dosage Table in item (8) below.

2) Anaesthesia by Ketamine/Xylazine/ACP mixture

a) Preparation of Ketamine/Xylazine anaesthetic mixture

Drug	Volume (ml)
Ketamine HCl (100mg/ml)	7.0
Xylazine (20mg/ml)	5.0

The mixture can be stored at 2-8 ℃ for three months

b) Dosage of Ketamine/Xylazine anaesthetic mixture and ACP (acepromazine,10 mg/ml) **by I/M**

Rabbit Body Weight (kg)	Volume (ml) Ketamine/Xylazine Mixture (Ketamine-35mg/kg Xylazine -5mg/kg)	ACP (1mg/kg)
1	0.6	0.1
1.5	0.9	0.15
2	1.2	0.2
2.5	1. 5	0.25

3) Anaesthesia by *Hypnorm*® (fentanyl citrate 0.315mg/ml / fluanisone 10mg/ml) by **I/M** (0.3ml/kg) and ***Dormicum*®** (midazolam,5 mg/ml) by **I/V** (0.2mg/kg)

Refer to Dosage Table in Para.(8) below.

4) Sedation

Sedation by using Hypnorm® (0.2 - 0.5ml/kg, i/m), Xylazine (2-5mg/kg, i/m), & Dormicum® (midazolam, 0.5-2 mg/kg, i/m) can be considered prior to anaesthesia - refer Dosage Table in Para.(8) below.

5) Ventilator

5.1) Tidal volume: 10-15 ml/kg
5.2) Ventilation rate per minute (for a 1-5 kg rabbit): 25-50

6) Post-operative Care

6.1) Provide soft "towel" bedding for animal and keep warm.
6.2) Check animals at least twice daily :
  i. look for abnormal discharge from eyes, nose and mouth,
  ii. monitor the respiration rate (normal = 30 - 60/min.),
  iii. check appetite and excreta &
  iv. monitor body weight and check surgical wound
6.3) Inject **Temgesic®** (buprenorphine 0.3mg/ml, 0.05mg/kg, s/c), 8-12 hourly for 3-5 days or **Ketoprofen®** (100mg/ml, 3mg/kg, s/c) daily, for 3-5 days if necessary.
6.4) Inject **Terramycin LA®** (Oxytetracycline 200mg/ml, 30-60mg/kg), s/c, i/m, q72 hrs if necessary.
6.3) Provide corn husk or Timothy hay to stimulate appetite, if required.
6.4) Fluid Supplementation: Dextrose-saline (4% dextrose, 0.18% saline) or saline (0.9%) at 30-50ml, s/c or i/p (for a 3kg rabbit) and Multi-Vitamins (0.3ml/kg., s/c daily) if inappetance and dehydration observed (check skin tone for sign of dehydration).
6.5) Record observation & treatment daily on the Post-Operative Care Record Form.

7) Surgical Wound Care

7.1) Spray with wound dressing
7.2) Clean with disinfectant (eg. diluted Savlon®, H_2O_2)
7.3) Provide "Elizabethan" collar (if wound biting occurs)

8) Drug Dosage Table

Rabbit Body Weight (kg)	***Volume (ml)***							
	Anesthesia		***Sedation***			***Analgesia***		***Treatment***
	Hypnorm® (0.3ml/kg, i/m)	**Dormicum®** (2mg/kg, i/v)	**Hypnorm®** (0.3ml/kg, i/m)	**Xylazine** (3mg/kg, i/m)	**Dormicum®** (1mg/kg, i/m)	**Temgesic®** (0.05mg/kg, s/c)	**Ketoprofen®** (3mg/kg, s/c)	**Terramycin LA®**(30mg/kg, s/c)
1	0.3	0.4	0.3	0.15	0.2	0.17	0.03	0.15
1.5	0.45	0.6	0.45	0.225	0.3	0.25	0.045	0.2
2	0.6	0.8	0.6	0.3	0.4	0.33	0.06	0.3
2.5	0.75	1	0.75	0.375	0.5	0.42	0.075	0.4

The University of Hong Kong
Laboratory Animal Unit

大動物手術之有關程序
Anesthesia and Post-operative Care of Large Animals (Pig and Goat)

1. **根據附件一，作手術前之準備工作**
   Follow Annex 1 for pre-operative care and preparations.

2. **替做手術之動物磅重及計算注射麻醉藥之劑量**

   Weighing animals and calculate anaesthetic doses

A. 用於豬之麻醉藥及止痛藥劑量
Anaesthetics and Analgesics for Pigs

a1. *Zoletil 50®* (A/C: Tiletamine + Zolazepam)： 50 mg/ml, 9 mg/kg (i/m)
Xylazine® (A/C: Xylazine)： 20 mg/ml, 1.5 mg/kg (i/m)
混合劑 (mixture)： 5 ml Zoletil® + 2 ml Xylazine (i/m)

a2. *Zoletil 100®*： 100 mg/ml, 9mg/kg (i/m)
Xylazine®： 20 mg/ml, 1.5 mg/kg (i/m)
混合劑 (mixture)： 5 ml Zoletil® + 4 ml Xylazine (i/m)

a3. *Zoletil 100®*： 100mg/ml, 12mg/kg (i/m)
Xylazine®： 20 mg/ml, 1.5 mg/kg (i/m)
混合劑 (mixture)： 5 ml Zoletil® + 3 ml Xylazine (i/m)

a1、a2 用於復原性手術，其後以 Propofol 作延長麻醉，而 a3 則用於終止性手術，手術後動物不會復原】

[a1, a2 for recovery surgeries using Propofol as top-ups, a3 for non-recovery surgeries]

b. *Atropine®* (A/C: Atropine) 如需要，用於抑制分泌）0.6 mg/ml, 0.05 mg/kg（s/c）

c. *Dorminal®* (A/C: Pentobarbital) 如需要用於剔殺動物）200 mg/ml, 4 ml/10 kg （i/v）

d. *Temgesic®* (A/C: Buprenorphine) 如需要，手術後鎮痛用）0.3 mg/ml, 0.01 mg/kg （s/c），每天二到四次

e. *Ketoprofen®* (A/C: Ketoprofen) 如需要，手術後鎮痛用）100mg/ml, 3mg/kg（s/c），每天一次

BWt.體重	混合劑量(（a1）	混合劑量（a2）	混合劑量（a3）	Atropine (b)	Dorminal (c)	Temgesic (d)	Ketoprofen (e)
10 kg	2.5 ml	1.6 ml	2 ml	0.8 ml	4 ml	0.3 ml	0.3 ml
20 kg	5 ml	3.2 ml	4 ml	1.6 ml	8 ml	0.7 ml	0.6 ml
30 kg	7.5 ml	4.8 ml	6 ml	2.4 ml	12 ml	1.0 ml	0.9 ml
40 kg	10 ml	6.4 ml	8 ml	3.2 ml	16 ml	1.3 ml	1.2 ml
50 kg	12.5 ml	8.0 ml	10 ml	4.0 ml	20.0 ml	1.6 ml	1.5 ml

B. 用於山羊之麻醉藥及止痛藥劑量
Anaesthetics and Analgesics for Goats

a. *Ketamine®* (A/C: Ketamine) 100mg/ml, 10mg/kg (i/m)

b. *Dormicum®* (A/C: Midazolam) 5mg/ml, 2mg/kg (i/m)

c. *Propofol®* (A/C: Propofol) 10mg/ml, 4mg/kg (i/v)

d. *Xylazine®* (A/C: Xylazine) 20mg/ml, 0.1mg/kg (i/m) [adult]

e. *Xylazine®* (A/C: Xylazine) 20mg/ml, 0.025mg/kg (i/m) [lamb]

f. Temgesic® (A/C: Buprenorphine) 0.3 mg/ml, 0.005-0.01 mg/kg (i/m) bid

g. Ketoprofen® (A/C: Ketoprofen) 100mg/ml, 2mg/kg (i/m) oid

BWt 體重	Ketamine(a)	Dormicum （b）	Propofol（c）	Xylazine (d)	Xylazine (e)	Temgesic (f)	Ketoprofen（g）
5 kg	0.5	2 ml	2 ml	0.025 ml	0.00625 ml	0.17 ml	0.1 ml
10 kg	1 ml	4 ml	4 ml	0.05 ml	0.0125 ml	0.33 ml	0.2 ml
20 kg	2 ml	8 ml	8 ml	0.1 ml	---	0.67 ml	0.4 ml
30 kg	3 ml	12 ml	12 ml	0.15 ml	---	1 ml	0.6 ml
40 kg	4 ml	16 ml	16 ml	0.2 ml	---	1.33 ml	0.8 ml
50 kg	5 ml	20 ml	20 ml	0.25 ml	---	1.67 ml	1 ml
60 kg	6 ml	24 ml	24 ml	0.3 ml	---	2 ml	1.2 ml

3. **根據附件二，作手術後之護理及記錄程序。**

Follow Annex 2 for post-operative care and record keeping.

Annex 1

（附件一）

大動物手術前之程序

Pre-operative care of and Preparations for Large Animals

1. 進行手術之動物需於隔晚及手術當日餓肚
   Fast the animals the night before surgery

2. 確保剪毛器有足夠電源
   Ensure clippers fully charged

3. 進行手術之動物需於前一天把身體潔淨，動物欄、洗手盆、紙巾箱等需保持清潔
   Clean the animal's body, pen, sink and paper towel holder one day before surgery

4. 檢查紙帽、口罩及鞋套有足夠存量
   Ensure sufficient stock of paper caps, masks and shoe-covers

5. 確保 X 光機操作正常，沖片藥水及韮林備用
   Ensure the X-ray machine and the film processor (including developers and films) are in normal operation

6. 檢查氣體供應是否足夠
   Ensure sufficient supply of oxygen gas (and other anaesthetics)

7. 預備及消毒（如需要）應用之手術物品
   Prepare and autoclave all necessary surgery items

8. 替做手術之動物磅重及計算注射麻醉藥之劑量
   Weigh the animal and calculate the required anaesthetic doses

Annex 2

（附件二）

大動物手術後之護理程序

Post-operative care of Large Animals

1. 注射 T*erramycin LA®* (A/C: Oxytetracycline) (200mg/ml)，可參閱大動物房之檢疫程序內，藥物之注射劑量
   Inject *Terramycin LA*® (a/c oxytetracycline, 200mg/ml) according to the dosage given on the Quarantine Procedure Form

2. 注射 *Temgesic®* (A/C: Buprenorphine)（0.3mg/ml）或 *Ketoprofen®* (A/C: Ketoprofen)（100mg/ml），可參閱大動物手術之有關程序內，藥物之注射劑量
   Inject *Temgesic*® (a/c buprenorphine, 0.3mg/ml) or *Ketoprofen*® (a/c Ketoprofen, 100mg/ml) according to dosages listed in the "Large Animal Surgery Procedures" document

3. 在動物耳上打下號碼作記錄，需要時可替動物修剪長甲
   Apply ear tag and trim the hooves if required

4. 清楚把資料填上＂手術後記錄表＂上
   Enter all relevant and necessary information on the "Post-operative Care Record Form"

5. 預備布料或暖風機於手術後替動物保溫，需要時房內之温度可預早調高1-2度
   Get ready "clothes" or heater to keep the animals warm after surgery, raise the room temperature by one to two degrees if necessary

6. 將欄內之食斗、磚頭移離動物（避免動物醒時撞傷），改放密盒盛載糧食
   Remove the feeder and concrete stepping blocks from the pen (to prevent physical injuries to animals recovering from anaesthesia), use solid-bottom cages to hold the feed

7. 留意動物手術後之健康復原狀況，有問題時需報告區域主管或技術員
   Pay attention to the health condition of the animals, report to Area Head or the Technicians if there are any problems