

STANDARD OPERATING PROCEDURES FOR LABORATORY ANIMAL FACILITY

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GOALS

The goal of these SOP is to promote the humane care of animals used in education and biomedical research with the basic objective of providing a detailed descriptions that will enhance animal well-being, quality in the quest of advancement of biological knowledge that is relevant to humans and animals.

GUIDELINES

Animal Care

1. Cages should be checked First thing every day, to note the condition of the animals. A staff member will check the animal cages daily for visible signs of change or distress, such as leaky bottles, birth of new pups, decrease in food or water consumption, blood in cage, wounds, secretions around the eyes, nose and genital area, respiratory distress, constipation, diarrhoea, swelling, sluggishness, gait, dull coat or loss of hair. All concerns will be reported to the Supervisor and depending on the severity of the concern, the PI/ attending veterinarian will be notified.
2. Cages should be changed at least once per week or more often as needed. During cage changing, animals are inspected for any abnormal conditions as listed above. Water bottles should be checked every day and fresh water should be added as needed.
3. Sanitize the water bottles once a week.
4. Shelves, cage holders, lids and bonnets should be cleaned once a month
5. Room should be sanitized every three to six months.
6. Sweep the floor should be and mop weekly or as needed.
7. Feeding plates should be wiped weekly.
8. Each cage must have an identification card with the following information: protocol number, investigator's name, date received, strain, sex, date of birth, and number of animals per cage.
9. Only items that are essential to the animal care of that room should be stored in the animal housing room.
10. The floor drains should be checked every day and flush out if necessary.
11. Doors should be wiped weekly.

VETERINARY CARE

Adequate veterinary care must be provided and is the responsibility of a veterinarian or a person who has training or experience in laboratory animal sciences and medicine. Daily observation of animals can be accomplished by someone other than a veterinarian; however, a mechanism of direct and frequent communication should be adopted so that timely and accurate information on problems in animal health, behaviour, and wellbeing is conveyed to the attending veterinarian.

The veterinarian can also contribute to the establishment of appropriate policies and procedures for ancillary aspects of veterinary care, such as reviewing protocols and proposals, animal husbandry and animal welfare; monitoring occupational health hazards containment, and zoonosis control programs; and supervising animal nutrition and sanitation. Institutional requirements will determine the need for full-time or part-time or consultative veterinary services.

ANIMAL PROCUREMENT

All animals must be acquired lawfully as per the CPCSEA guidelines. A health surveillance program for screening incoming animals should be carried out to assess animal quality. Methods of transportation should also be taken into account (**Annexure - 4**).

Each consignment of animals should be inspected for compliance with procurement specifications, and the animals should be quarantined and stabilized according to procedures appropriate for the species and circumstances.

QUARANTINE

Quarantine is the separation of newly received animals from those already in the facility until the health and possibly 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. The duration at quarantine in small lab animals from one week to one month.

STABILIZATION

Regardless of the duration of quarantine, newly received animals should be given a period for physiologic, psychologic and nutritional stabilization before their use. The length of time stabilization will depend on the type and duration of animal transportation, the species involved and the intended use of the animals.

SEPARATION

Physical separation of animals by species is recommended to prevent interspecies disease transmission and to eliminate anxiety and possible physiological and behavioural changes due to interspecies conflict. Such separation is usually accomplished by housing different species in separate rooms, cubicles or cages. If two species have a similar pathogen status and are behaviourally compatible, it shall be acceptable to house different species in the same room.

People should be restricted from entering in to the facilities unless otherwise required and after handling these animals they should not be handling any other animals in the facilities.

SURVEILLANCE, DIAGNOSIS, TREATMENT AND CONTROL OF DISEASE

All animals should be observed for signs of illness, injury, or abnormal behaviour by animal house staff daily, but more-frequent observations might be warranted, during postoperative recovery or when animals are ill or have a physical deficit. It is imperative that appropriate methods be in place for disease surveillance and diagnosis (**Annexure 1 & 2**).

Post mortem examination and signs of illness, distress, or other deviations from normal health condition in animals should be reported promptly to ensure appropriate and timely delivery of veterinary medical care. Animals that show signs of a contagious disease should be isolated from healthy animals in the colony. If an entire room of animals is known or believed to be exposed to an infectious, the group should be kept intact and isolated during the process of diagnosis, treatment, and control. Diagnostic clinical laboratory may be made available.

ANIMAL CARE AND TECHNICAL PERSONNEL

Institutions should employ people trained in laboratory animal science or provide for both formal and on-the-job training to ensure effective implementation of the program (**Annexure - 7**).

PERSONAL HYGIENE

It is essential that the animal care staff maintain a high standard of personal cleanliness by using appropriate Personnel Protective Equipment (PPE) e.g. change of uniforms, footwear etc.

Clothing suitable for use in the animal facility should be supplied and laundered by the institution. A commercial laundering service is acceptable in many situations. It is acceptable to use disposable gloves, masks, head covers, coats, coveralls and shoe covers. Personnel should change clothing as often as is necessary to maintain personal hygiene. Outer garments worn in the animal rooms should not be worn outside the animal facility.

Washing facilities appropriate to the program should be available. Personnel should not be permitted to eat, drink, smoke or apply cosmetics and perfumes in animal rooms. They should finish the work with animals as early as possible and sit somewhere else outside and not in the animal rooms / areas.

MULTIPLE SURGICAL PROCEDURES ON SINGLE ANIMAL

Multiple surgical procedures on a single animal for any testing or experiment are not to be practiced unless specified in a protocol only approved by the IAEC.

DURATIONS OF EXPERIMENTS

No animal should be used for experimentation for more than 3 years unless adequate justification is provided.

PHYSICAL RESTRAINT

Brief physical restraint of animals for examination, collection of samples, and a variety of other clinical and experimental manipulations can be accomplished manually or with devices be suitable in size and design for the animal being held and operated properly to minimize stress and avoid injury to the animal.

Important guidelines for the use of restraint equipment:

1. Restraint devices cannot be used simply as a convenience in handling or managing animals.
2. The period of restraint should be the minimum required to accomplish the research objectives.
3. Animals to be placed in restraint devices should be given training to adapt to the equipment.
4. Provision should be made for observation of the animal at appropriate intervals. Veterinary care should be provided if lesions or illness associated with restraint are observed. The presence of lesions, illness, or severe behavioural change should be dealt with by the temporary or permanent removal of the animal from restraint.

PHYSICAL FACILITIES

The physical condition, design and size of an animal facility depend on the scope of institutional research activities, animals to be housed, physical relationship to the rest of the institution, and geographic location. A well planned, properly maintained facility is an important element in good animal care.

LOCATION OF ANIMAL FACILITIES TO LABORATORIES

Good animal husbandry and human comfort and health protection require physical separation of animal facilities from personnel areas such as offices, break room, training and education room.

1. Laboratory animals are very sensitive to their living conditions. It is important that they shall be housed in an isolated building located as far away from human habitations as possible and not exposed to dust, smoke, noise, wild rodents, insects and birds.
2. This separation can be accomplished by having the animal quarters in a separate building, wing, floor, or room. Careful planning should make it possible to place animal housing areas adjacent to or near laboratories, but separated from them by barriers such as entry locks, corridors, or floors.
3. While planning an animal facility the space should be well divided for various activities. The animal rooms should occupy about 50-60% of the total constructed area and the remaining area should be utilized for services such as stores, washing, office and staff, machine rooms, quarantine and corridors. The environment of animal room (Macro-Environment) and animal cage (Microenvironment) are factors on which the production and experimental efficiency of the animal depends. Since animals are very sensitive to environmental changes, sharp fluctuations in temperature, humidity, light, sound and ventilation should be avoided.

FUNCTIONAL AREAS

Sufficient animal area required to:

- Ensure separation of species or isolation of individual projects when necessary;
- Receive, quarantine, and isolate animals; and
- Provide for animal housing.
- Specialized laboratories or
- Receiving and storage areas for food, bedding
- Pharmaceuticals and biologics, and supplies
- Space for administration, supervision, and direction of the facility
- An area for washing and sterilization equipment and supplies,
- An autoclave for equipment
- Food, and bedding; and separate areas
- For holding soiled and cleaned equipment
- An area to store wastes prior to incineration or removal

PHYSICAL FACILITIES

Building materials should be selected to facilitate efficient and hygienic operation of animal facilities. Durable, moisture-proof, fire-resistant, seamless materials are most desirable for interior surfaces including vermin and pest resistance.

Corridor(s) should be wide enough to facilitate the movement of personnel as well as equipment and should be kept clean.

Utilities such as water lines, drain pipes, and electrical connections should preferably be accessible through service panels or shafts in corridors outside the animal rooms.

ANIMAL ROOM DOORS

Doors should not be rust, vermin and dust proof. They should fit properly within their frames and provided with an observation window. Door closures may also be provided. Rodent barriers can be provided in the doors of the small animal facilities.

EXTERIOR WINDOWS

Windows are not recommended for small animal facilities. However, where power failures are frequent and backup power is not available, they may be necessary to provide alternate source of light and ventilation.

FLOORS

Floors should be either monolithic or epoxy smooth, moisture proof, non-absorbent, skid-proof, resistant to wear, acid, solvents, adverse effects of detergents and disinfectants.

They should be capable of supporting racks, equipment, and stored items without becoming gouged, cracked, or pitted, with minimum number of joints.

A continuous moisture-proof membrane might be needed. If sills are installed at the entrance to a room, they should be designed to allow for convenient passage of equipment.

DRAINS

Floor drains are not essential in all rooms used exclusively for housing rodents. Floor in such rooms can be maintained satisfactorily by wet vacuuming or mopping with appropriate disinfectants or cleaning compounds. Where floor drains are used, the floors should be sloped and drain taps kept filled with water or corrosion free mesh. To prevent high humidity, drainage must be adequate to allow rapid removal of water and drying of surfaces. At the inlet and outlets of the drains should be fitted with wire mesh guard to prevent wild rodent entry

WALLS & CEILINGS

Walls should be free of cracks, unsealed utility penetrations, or imperfect junctions with doors, ceilings, floors and corners.

Surface materials should be capable of withstanding scrubbing with detergents, disinfectants and the impact of water under high pressure.

STORAGE AREAS

Separate storage areas should be designed for feed, bedding, cages and materials not in use.

Refrigerated storage, separated from other cold storage, is essential for storage of dead animals and animal tissue waste.

FACILITIES FOR SANITIZING EQUIPMENT AND SUPPLIES

An area for sanitizing cages and ancillary equipment is essential with adequate water supply

EXPERIMENTAL AREA

All experimental procedures in small animals should be carried out in a separate area away from the place where animals are housed.

ENVIRONMENT

Temperature and Humidity Control: Air conditioning is an effective means of regulating these environmental parameters for laboratory animals. Temperature and humidity control prevents variations due to changing climatic conditions keeping in view of the variations in the number of room occupants the range should be within or approximately between 18 to 29°C (64.4 to 84.2oF) all times.

The relative humidity should be under control within the range of 30% to 70% throughout the year. During extreme summer appropriate methods e.g. sprinklers should be adopted for cooling.

Ventilation: In renovating existing or in building new animal facilities, consideration should be given to the ventilation of the animals' primary enclosures.

Heating, ventilation, and air-conditioning systems should be designed with 12-15 air cycles per hour so that operation can be continued with a standby system. The animal facility and human occupancy areas should be ventilated separately.

Power and Lighting: The electrical system should be safe and provide appropriate lighting and with sufficient number of power points lighting system be installed provide adequate illumination for people to work in the animal rooms and a lowered intensity of light for the animals. A time-controlled lighting system should be used to ensure a regular diurnal lighting cycle wherever required. Emergency power should be available in the event of power failure.

Noise Control: The facility should be provided with noise free environment. Noise control is an important consideration in designing the animal facility. Concrete walls are more effective than metal or plaster walls because their density reduces sound transmission. Preferably less than 85 dB is desirable for rodents.

ANIMAL HUSBANDRY

Caging or Housing System

The caging or housing system is one of the most important elements in the physical and social environment of research animals. It should be designed carefully to facilitate animal wellbeing, meet research requirements, and minimize experimental variables.

The housing system should:

- Provide space that is adequate, permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species;(Annexure – 3)
- Provide a comfortable environment
- Provide an escape proof enclosure that confines animal safety
- Provide easy access to food and water;
- Provide adequate ventilation
- Meet the biological needs of the animals, e.g., maintenance of body temperature, urination, defecation, and reproduction;
- Keep the animals dry and clean, consistent with species requirements ;
- Facilitate research while maintaining good health of the animals.

They should be constructed of sturdy, durable materials and designed to minimize cross-infection between adjoining units. Polypropylene, polycarbonate and stainless steel cages should be used to house small lab animals.

To simplify servicing and sanitation, cages should have smooth, impervious surfaces that neither attract nor retain dirt and a minimum number of ledges, angles, and corners in which dirt or water can accumulate.

The design should allow inspection of cage occupants without disturbing them. Feeding and watering devices should be easily accessible for filling, changing, cleaning and servicing. Cages, runs and pens must be kept in good condition to prevent injuries to animals, promote physical comfort, and facilitate sanitation and servicing. Particular attention must be given to eliminate sharp edges and broken wires, keeping cage floors in good condition.

SOCIAL ENVIRONMENT

The social environment includes all interactions among individuals of a group or among those able to communicate. The effects of social environment in caged animals vary with the species. In selecting a suitable social environment, attention should be given whether the animals are naturally territorial or communal and accordingly they should be housed single or in groups. When appropriate, group housing should be considered for communal animals. In grouping animals, it is important to take into account population density and ability to disperse; initial familiarity among animals; and age, sex, and social rank.

Population density can affect reproduction, metabolism, immune responses, and behaviour. Group composition should be held as stable as possible, because mixing of groups or introducing new members can alter behavioural and physiological functions.

ACTIVITY

Provision should be made for animals with specialized locomotor pattern to express their natural habitat, especially when the animals are held for long periods. Cages are often used for short-term (up to 3 months) housing for postsurgical care, isolation of sick animal and metabolic studies.

FOOD

Animals should be fed with palatable, non-contaminated, and nutritionally adequate food daily unless the experimental protocol requires otherwise.

Feeders should allow easy access, while avoiding contamination by urine and feces.

Food should be provided in sufficient amounts to ensure normal growth in immature animals and to maintain normal body weight, reproduction, and lactation in adults.

Food should contain adequate nutrition, with proper formulation and preparation; and ensure free from chemical and microbial contaminants; bioavailability of nutrients should be at par with the nutritional requirements of the animal. The animal feed should contain moisture, crude fibre, crude protein, essential vitamins, minerals, crude fat and carbohydrate for providing appropriate nutrition. Diet should be free from heavy metals (e.g., Lead, Arsenic, Cadmium, Nickel, Mercury), naturally occurring toxins and other contaminants.

Areas in which diets are processed or stored should be kept clean and enclosed to prevent entry of insects or other animals. Exposure to extremes of relative humidity, unsanitary conditions, light, oxygen, and insects hasten the deterioration of food.

Food hoppers should not be transferred from room to room unless cleaned and properly sanitized.

BEDDING

Bedding should be absorbent, free from toxic chemicals or other substances that cause irritation, injure animals or personnel, and of a type not readily eaten by animals. Bedding should be used in amounts sufficient to keep animals dry between cage changes without coming into contact with watering tubes.

Bedding should be removed and replaced periodically with fresh materials as often as necessary to keep the animals clean and dry. The frequency is a matter of professional judgement of animal care personnel in consultation with the investigation depending on the number of animals and size of cages. In general it is ideal to change the bedding twice a week or whenever requires.

The desirable criteria for rodent contact bedding is ammonia binding, sterilizable, deleterious products not formed as a result of sterilization, easily stored, non - desiccating to the animal, uncontaminated, unlikely to be chewed or mouthed, non - toxic, non - malodorous, nestable, disposable by incineration, readily available, remains stable during use, optimizes normal animal behaviour, non - deleterious to cage - washers, non - injurious and non - hazardous to personnel, non - nutritious and non - palatable.

Nesting materials for newly delivered pups should be provided wherever needed (e.g. Paper cuttings, tissue paper, cotton etc.).

WATER

Animals should have continuous access to fresh, potable, uncontaminated drinking water, according to their requirements. Periodic monitoring of microbial contamination in water is necessary. Watering devices, such as drinking nozzles and automatic waterers should be examined routinely to ensure their proper operation. Sometimes it is necessary to train animals to drink water from automatic watering devices.

It is better to replace fresh water bottles every day than to refill them, however, if bottles are to be refilled, care should be taken that each bottle is replaced on the cage properly from where it was removed.

SANITATION AND CLEANLINESS

Sanitation is an essential activity in an animal facility. Animal rooms, corridors, storage spaces, and other areas should be properly cleaned with appropriate detergents and disinfectants as often as necessary to keep them free of dirt, debris, and harmful agents of contamination. Cleaning utensils, such as mops, pails, and brooms, should not be transported between animal rooms.

Where animal waste is removed by hosing or flushing, this should be done at least twice a day. Animals should be kept dry during such procedures.

Cages should be sanitized before animals are placed in them. Animal cages, racks, and accessory equipment, such as feeders and watering devices, should be washed and sanitized frequently to keep them clean and contamination free. Generally this can be achieved by washing solid bottom rodent cages and accessories once or twice a week and cages, racks at least monthly.

Wire - bottom cages other than rodent cages should be washed at least every 2 weeks. It is good practice to have extra cages available at all times so that a systematic cage-washing schedule can be maintained. Cages can be disinfected by rinsing at a temperature of 82.2C (180oF) or higher for a period long enough to ensure the destruction of vegetative pathogenic organisms. Disinfection can also be accomplished with appropriate chemicals. Equipment should be rinsed free of chemicals prior to use. Periodic microbiologic monitoring is useful to determine the efficacy of disinfection or sterilization procedures.

Rabbits and some rodents, such as guinea pigs, mice and hamsters, produce urine with high concentration of proteins ammonia and minerals. Minerals and organic compounds in the urine from these animals often adhere to cage surfaces and necessitate treatment with acid solutions before washing.

Water bottles, sipper nozzles stoppers, and other watering equipment should be washed and then sanitized by rinsing with water of at least 82.2oC (180oF) or appropriated chemicals agents (e.g. Sodium Hyperchlorite) to destroy pathogenic organisms, if bottles are washed by hand, mechanized brushes at the washing sink are useful, and provision should be made for dipping or soaking the water bottles in detergents and disinfectant solutions. A two – compartment sink or tub is adequate for this purpose.

Some means for sterilizing equipment and supplies, such as an autoclave or gas sterilizer, is essential when pathogenic organisms are present. Routine sterilization of cages, feed and bedding is also essential besides care is taken to use clean materials from reliable sources. Deodorants or chemical agents other than germicidal agents should not be used to mask animal odours. Such products are not a substitute for good sanitation.

ASSESSING THE EFFECTIVENESS OF SANITATION

Sanitation practices should be monitored appropriately to ensure effectiveness of the process and materials being cleaned; it can include visual inspection of the materials, monitoring of water temperatures, or microbiologic monitoring.

The intensity of animal odours particularly that of ammonia should not be used as the sole means of assessing the effectiveness of the sanitation program. A decision to change the frequency of such bedding changes or cage washing should be based on factors such as the concentration of ammonia, appearance of the cage, condition of the bedding and number and size of the animals housed in the cage. Autoclaving : Chemical Indicator - batch wise assessment; Biological indicator -Periodical assessment.

WASTE DISPOSAL

Wastes should be removed regularly and frequently. All waste should be collected and disposed of in a safe and sanitary manner. The most preferred method of waste disposal is incineration. Incinerators should be in compliance with all central, state, and local Public Health and Pollution Control Board regulations.

Waste containers containing animal tissues, carcasses, and hazardous wastes should be lined with leak - proof, disposable liners. If wastes must be stored before removal, the waste storage area should be separated from other storage facilities and free of flies, cockroaches, rodents, and other vermin. Cold storage might be necessary to prevent decomposition of biological wastes.

PEST CONTROL

Adaptation of Programs designed to prevent, control, or eliminate the presence of or infestations by pests are essential in an animal home environment.

EMERGENCY, WEEKEND AND HOLIDAY CARE

Animals should be cared for by qualified personnel every day, including weekends and holidays, to safeguards their well - being including emergency veterinary care. In the event of an emergency, institutional security personnel and fire or police officials should be able to reach people responsible for the animals. That can be enhanced by prominently posting emergency procedures, names, or telephone numbers in animal facilities or by placing them in the security department or telephone centre. A disaster plan that takes into account both personnel and animals should be prepared as part of the overall safety plan for the animal facility.

RECORD KEEPING

The Animal House should maintain following records:

- Animal House plans, which includes typical floor plan, all fixtures etc.
- Animal House staff record - both technical and non - technical
- Health record of staff animals.
- All SOPs relevant to the animals
- Breeding, stock, purchase and sales records
- Minutes of institute Animals Ethics Committee Meetings
- Records of experiments conducted with the number of animals used (copy of Form D)
- Death Record
- Clinical record of sick animals
- Training record of staff involved in animal activities
- Water analysis report

STANDARD OPERATING PROCEDURES (SOPs) / Guidelines

The Institute shall maintain SOPs describing procedures / methods adapted with regard to Animal Husbandry, maintenance, breeding, animal house microbial analysis and experimentation records.

A SOP should contain the following items:

- Ø Name of the Author
- Ø Title of the SOP
- Ø Date of preparation
- Ø Reference of previous SOP on the same subject and date (Issue no and Date)
- Ø Location and distribution of Sops with sign of each recipient
- Ø Objectives
- Ø Detailed information of the instruments used. in relation with animals with methodology (Model no., Serial no., Date of commissioning, etc)
- Ø The name of the manufacturer of the reagents and the methodology of the analysis pertaining to animals
- Ø Normal value of all parameters
- Ø Hazard identification and risk assessment

PERSONNEL AND TRAINING

The selection of animal facility staff, particularly the staff working in animal rooms or involved in transportation, is a critical component in the management of an animal facility.

The staff must be provided with all required protective clothing (masks, aprons, gloves, gumboots, other footwears etc.) while working in animal rooms. Facilities should be provided for change over with lockers, wash basin, toilets and bathrooms to maintain personal hygiene. It is also important a regular medical check-up is arranged for the workers to ensure that they have not picked up any zoonotic infection and also that they are not acting as a source of transmission of infection to the animals. The animal house in-charge should ensure that persons working in animal house don't eat, drink, smoke in animal room and have all required vaccination, particularly against tetanus and other zoonotic diseases.

Initial in-house training of staff at all levels is essential. A few weeks must be spent on the training of the newly recruited staff, teaching them the animal handling techniques, cleaning

of cages and importance of hygiene, disinfection and sterilization. They should also be made familiar with the activities of normal healthy and sick animals so that they are able to spot the sick animal during their daily routine check up for cages (Annexure -7).

TRANSPORT OF LABORATORY ANIMALS

The transport of animals from one place to another is very important and must be undertaken with care. The main considerations for transport of animals are, the mode of transport, the containers, animal density in cages, food and water during transit, protection from transit infections, injuries and stress.

The mode of transport of animals depends on the distance, seasonal and climatic conditions and the species of animals. Animals can be transported by road, rail or air taking into consideration of above factors. In any case the transport stress should be avoided and the containers should be of an appropriate size so as to enable these animals to have a comfortable, free movement and protection from possible injuries. The food and water should be provided in suitable containers or in suitable form so as to ensure that they get adequate food and more particularly water during transit. The transport containers (cages or crates) should be of appropriate size and only a permissible number of animals should only be accommodated in each container to avoid overcrowding and infighting (**Annexure - 4**)

ANAESTHESIA AND EUTHANASIA

The scientists should ensure that the procedures, which are considered painful, are conducted under appropriate anaesthesia as recommended for each species of animals. It must also be ensured that the anaesthesia is given for the full duration of experiment and at no stage the animal is conscious to perceive pain during the experiment. If at any stage during the experiment the investigator feels that he has to abandon the experiment or he has inflicted irreparable injury, the animal should be sacrificed. Neuromuscular blocking agents must not be used without adequate general anaesthesia (**Annexure -5**).

In the event of a decision to sacrifice an animal on termination of an experiment or otherwise an approved method of euthanasia should be adopted (**Annexure - 6**) and the investigator must ensure that the animal is clinically dead before it is sent for disposal.

Anaesthesia

Unless contrary to the achievement of the results of study, sedatives, analgesics and anaesthetics should be used to control pain or distress under experiment. Anaesthetic agents generally affect cardiovascular, respiratory and thermoregulatory mechanism in addition to central nervous system.

Before using actual anaesthetics the animals is prepared for anaesthesia by overnight fasting and using pre-anaesthetics, which block parasympathetic stimulation of cardiopulmonary system and reduce salivary secretion. Atropine is most commonly used anti-cholinergic agent. Local or general anaesthesia may be used, depending on the type of surgical procedure. Local anaesthetics are used to block the nerve supply to a limited area and are used only for minor and rapid procedures. This should be carried out under expert supervision for regional infiltration of surgical site, nerve blocks and for epidural and spinal anaesthesia.

A number of general anaesthetic agents are used in the form of inhalants. General anaesthetics are also used in the form of intravenous or intra-muscular injections such as barbiturates. Species characteristics and variation must be kept in mind while using an anaesthetic. Side-effects such as excessive salivation, convulsions, excitement and disorientation should be

suitably prevented and controlled. The animal should remain under veterinary care till it completely recovers from anaesthesia and postoperative stress.

Euthanasia

Euthanasia is resorted to events where an animal is required to be sacrificed on termination of an experiment or otherwise for ethical reasons. The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety. For accepting an euthanasia method as humane it should have an initial depressive action on the central nervous system for immediate insensitivity to pain. The choice of a method will depend on the nature of study, the species of animal to be killed (**Annexure- 6**). The method should in all cases meet the following requirements:

- a. Death, without causing anxiety, pain or distress with. minimum time lagphase. . . .
- b. Minimum physiological and psychological disturbances.
- c. Compatibility with the purpose of study and minimum emotional effect on the operator.
- d. Location should be separate from animal rooms and free from environmental contaminants.

LABORATORY ANIMAL ETHICS

All scientists working with laboratory animals must have a deep ethical consideration for the animals they are dealing with. From the ethical point of view it is important that such considerations are taken care at the individual level, at institutional level and finally at the national level.

MAINTENANCE

Housing, feeding, ventilation, lighting, sanitation and routine management practices for such animals are similar to these for the other animals of the species as given in guidelines. However, special care has to be taken with transgenic/gene knockout animals where, the animals can become susceptible to diseases where special conditions of maintenance are required due to the altered metabolic activities. The transgenic and knockout animals carry additional genes or lack genes compared to the wild population. To avoid the spread of the genes in wild population care should be taken to ensure that these are not inadvertently released in the wild to prevent cross breeding with other animals. The transgenic and knockout animals should be maintained in clean room environment or in animal isolators.

DISPOSAL

A record of animal disposal and the manner of disposal should be kept as a matter of routine.

Annexure-I

HAEMATOLOGICAL DATA OF COMMONLY USED LABORATORY ANIMALS

	Mouse	Rat	Hamster	G.Pig	Rabbit	Cat	Dog (Beagle)	Monkey (Rhesus)
RBC ($\times 10^6 / \text{mm}^3$)	7-12.5	7-10	6-10	4.5-7	4-7	5-10	5.5-8.5	3.56-6.96
CV(%)	39-49	36-18	36-55	37-48	36-48	30-15	37-55	26-48
lb(g/dl)	10.2-16.6	11-18	10-16	11-15	10-15.5	8-15	12-18	8.8-16.5
BBC ($\times 10^3 \text{ mm}^3$)	6-15	6-17	3-11	7-18	9-11	5.5-19.5	6-17	2.5-26.7
Neutrophils (%)	10-40	9-34	10-42	28-44	20-75*	35-75	60-70	5-88
Lymphocytes (%)	55-95	65-85	50-95	39-72	30-85	20-55	12-30	8-92
Eosinophils(%)	0-4	0-6	0-4.5	1-5	0-4	2-12	2-10	0-14
Monocytes(%)	0.1-3.5	0-5	0-3	3-12	1-4	1-4	3-10	0-11
Basophils (%)	0-0.3	0-1.5	0-1	0-3	2-7	Rare	Rare	0-6
Platelets ($\times 10^3 \text{ mm}^3$)	160-410	500-1300	200-500	250-850	250-656	300-700	200-900	109-597

*Neutrophils often resemble eosinophils due to granules

(NOTE-The range of normal values may vary in a laboratory using specific species, strain or substrain of these animals. Any major deviation on higher or lower side may be considered as a conditions and not a disease per se)

Annexure-2

BIOCHEMICAL DATA OF COMMONLY USED LABORATORY ANIMALS

	Mouse	Rat	Hamster	G.Pig	Rabbit	Cat	Dog	Monkey
Protein (g/dl)	3.5-7.2	5.6-7.6	4.5-7.5	4.6-6.2	5.4-7.5	6-7.5	6-7.5	4.9-9.3
N (g/dl)	2.5-4.8	2.8-4.8	2.6-4.1	2.1-3.9	2.7-4.6	2.5-4.0	3-4	2.8-5.2
N (g/dl)	0.6	1.8-3	2.7-4.2	1.7-2.6	1.5-2.8	2.5-3.8	2.4-3.7	1.2-5.8
C (mg/dl)	62-175	50-135	60-150	60-125	75-150	80-108	54-99	46-178
Urea nitrogen	12-28	15-21	12-25	9-31.5	17-23.5	3.5-8.0	3.5-7.5	8-40
Nine (mg/dl)	0.3-1	0.2-0.8	0.91-0.99	0.6-2.2	0.8-1.8	<180 (n mol/l)	<120 (n mol/l)	0.1-2.8
Bilirubin (mg/dl)	0.1-0.9	0.2-0.55	0.25-0.6	0.3-0.9	0.25-0.74	<4.0 (m mol/l)	<5.0 (n mol/l)	0.1-2
Sterol (mg/dl)	26-82	40.130	25-135	20-43	35-53	2-4 (m mol/l)	4-7 (m mol/l)	108-263

The range of normal values may vary in a laboratory using species, strain or sub strain of these animals. Any major deviation on higher or side may be considered as a condition and not a disease per se)

Annexure-3A

Minimum floor area recommended for laboratory animals (based on their weight/size and behavioural activity)

Animal	Weight In Grams	Floor area/Animal (CM ²)		Cage height (CM ²)
Mice	<10	38.7		12
	Upto 15	51.6		
	Upto 25 >25	77.4 96.7		
Rats	<100	109.6		14
	Upto 200	148.3		
	Upto 300	187.0		
	Upto 400	258.0		
	Upto 500	387.0		
	>500	≥451.5		
Hamsters/Gerbils/ Mastomys/Cotton Rats	<60	64.5		12
	Upto 80	83.8		
	Upto 100	103.2		
	>100	122.5		
Guinea pigs	<350	387.0		18
	>350	≥651.4		
		Floor area (Sq.ft)	(Sq.meter)	Height (inches)
Rabbits	<2000	1.5	0.135	14
	Upto 4000	3.0	0.27	14
	Upto 5400	4.0	0.36	14
	>5400	5.0	0.45	14
	Mother with kids	4.5	0.40	14

Annexure 3B

Example for calculating the number of Mice to be kept per cage, based on floor area recommended for animal according to their weight (Size) and size of the Cage

Recommended floor Area per animal (Cm ²)	38.7	51.6	77.4	96.7
Weight of animals (Grams)	<10	upto 15	upto 25	>25
Example I Cage size 24 x14 cm i.e. floor area of 336 cm ² maximum number of animals	8	7	4	3 [±]
Example II Cage Size 32.5 x 21 cm i.e. floor area of 682 cm ² maximum number of animals	17	14	9	7

Note : Cage size, specially length and breadth may vary. However, the minimum Floor area and cage height recommended for group housing has to be taken into consideration. Thus, the number of animals which can be housed in a particular cage (of different sizes) can be calculated on the basis of a) floor area of the cage, b) recommended floor area per animal and c) weight of animal.

Annexure 3C

Example for calculating the number of rats to be kept per cage, based on floor area recommended per animal according to their weight (Size) and size of the cage

Recommended floor Area per animal (Cm ²)	109.6	148.3	187.0	258.0	387.0	>451.5
Weight of animal (Grams)	<100	upto 200	upto 300	upto 400	upto 500	>500.
Example Cage size 32.5 x 21 Cm i.e. floor area of 682 (Cm ²) maximum number of animals	6	5	4	3	2	1
Note: Cage size, specially length and breadth may vary. However the minimum floor area and cage height recommended for group housing has to be taken into consideration. Thus, the number of animal which can be housed in a particular cage (of different sizes) can be calculated on the basis of a) floor area of the cage b) recommended floor area per animal and c) weight of animal.						

Annexure 4

REQUIREMENT FOR TRANSPORT OF LABORATORY ANIMALS BY ROAD, RAIL AND AIR

	Mouse	Rat	Hamster	G. Pig	Rabbit	Cat	Dog	Monkey
Maximum No. of Animals per cage	25	25	25	12	2	1 or 2	1 or 2	1
Material used in transport Box	Metal Cardboard, Synthetic Material	Metal Cardboard, Synthetic Material	Metal Cardboard, Synthetic Material	Metal Cardboard, Synthetic Material	Metal Cardboard, Synthetic Material	Metal	Metal	Bamboo/ Wood/metal
Space per Animal (Cm. Sq.)	20-25	80-100	80-100	160-180	1000-1200	1400-1500	3000	2000-4000
Minimum height of box (cm)	12	14	12	15	30	40	50	48

**Annexure 5
COMMONLY USED ANAESTHETIC DRUGS FOR LABORATORY ANIMALS**

Drugs (mg/kg)	Mouse	Rat	Hamster	G. Pig	Rabbit	Cat	Dog	Monkey
KTEAMINE Hel	22-24 i/m	22-24 i/m	-	22-24	22-24	30 i/m	30 i/m	15-40
PENTOBARBITONE SODIUM	35 i/v 50 i/p	25 i/v 50 i/p	35 i/v -	30 i/v 40 i/p	30 i/v 40 i/p	25 i/v -	20-30 i/v -	35 i/v -
THIOPENTONE SODIUM	25 i/v 50 i/p	20 i/v 40 i/p	20 i/v 40 i/p	20 i/v 55 i/p	20 i/v	25 i/v	25 i/v	25 i/v 60 i/p
URETHANE	-	0.75 i/p	-	1.5 i/p	1.0 i/p, i/v	1.25 i/v 1.50 i/p	1.00 i/v	1.0 i/v

ANNEXURE - 6

EUTHANSIA OF LABORATORY ANIMALS

(A : Methods Acceptable for species of animals indicated NR : Not Recommended)

Species	Mouse	Rat	Hamster	Guinea Pig	Rabbit	Cat	Dog	Monkey
a) PHYSICAL METHODS								
Electrocution	NR	NR	NR	NR	NR	NR	NR	NR
Exsanguination	NR	A	A	A	A	A	NR	NR
Decapitation (for analysis of stress)	A	A	A	NR	NR	NR	NR	NR
Cervical dislocation	A	A	A	NR	NR	NR	NR	NR
b) INHALATION OF GASES								
Carbon Monoxide	A	A	A	A	A	A	A	A
Carbon Dioxide	A	A	A	A	A	A	NR	NR
Carbon Dioxide plus Chloroform	A	A	A	A	A	A	NR	NR
Halothane	A	A	A	A	A	A	A	A
DRUG ADMINISTRATION								
Barbiturate Overdose (route)	A(IP)	A(IP)	A(IP)	A(IP)	A(IV, IP)	A(IV,IP)	A(IV,IP)	A(IV,IP)
Chloral hydrate Overdose (route)	NR	NR	NR	NR	A(IV)	A(IV)	A(IV)	A(IV)
Ketamine (Overdose route)	A(IM/IP)	A(IM/IP)	A(IM/IP)	A(IM/IP)	A(IM/IV)	A(IM/IV)	A(IM/IV)	A(IM/IV)
Sodium Pentohol [(Overdose (route))]]	IP	IP	IP	IP	IV	IV	IV	IV

Methods Not Acceptable for any species of animals

a) PHYSICAL METHODS :

i) Decompression ii) Stunning

b) INHALATION OF GASES :

i) Nitrogen Flushing ii) Argon Flushing

c) DRUG ADMINISTRATION :

(i) Curariform drugs (ii) Nicotine Sulphate (iii) Magnesium Sulphate (iv) Potassium Chloride (v) Strychnine (vi) Paraquat (vii) Dichlorvos (viii) Air embolism

IP = Intraperitoneal

IV = Intravenous

IM= Intramuscular