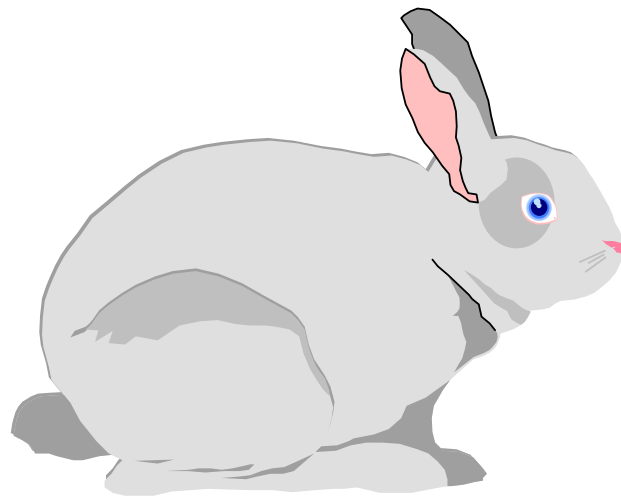


Guidelines for use of Laboratory Animals in Medical Colleges



**Indian Council of Medical Research
New Delhi
2001**

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PREFACE

*L*aboratory animals used in medical colleges play a vital role in teaching / research as well as developing skills for diagnosis. Here the animal is almost exclusively used as a substitute or model for man as most laboratory animals have the same set of organs-heart, lungs, liver and so on which work in the same way as they do in humans. Knowledge gained from animal experiments enhances the understanding of the subjects like Physiology, Microbiology, Pharmacology, Biochemistry etc. Animal experiments give an insight to the students about the etiology, diagnosis, progression and methods of prevention of various diseases. Commonly used animals in medical colleges are frogs, rats, mice, rabbits, guinea pigs, cats, dogs, monkeys and to lesser extent sheep.

Use of defined animals in appropriate conditions will reduce the stress on the animals and will result in generating reproducible and reliable results. A thorough knowledge of the biological characteristics and husbandry requirements of the species to be used is essential to ensure animal welfare. It is obligatory on the part of Investigators/students to handle the animals gently, following the guidelines of ethical consideration for animal use.

These guidelines provide the basic minimum provisions for animal care in medical colleges using animals for teaching / research purposes and those where breeding of such animals is also undertaken.

It is hoped that the medical fraternity will find these guidelines useful to ensure the welfare of the animals.

Animal facilities for medical colleges for teaching undergraduates and postgraduates

According to the Medical Council of India requirement, it is mandatory to have animal facilities attached to every teaching medical college.

Commonly used animals in the medical colleges

The various departments which use animals for teaching purpose in undergraduate and post graduates courses are :-

Anatomy	Physiology	Biochemistry
Microbiology	Pathology	Biophysics
Pharmacology	Surgery	Cardiology
Neurology	Cardio-thoracic surgery	Lab Medicine
Neuro Surgery	Orthopedics	Psychiatry
Ophthalmology	Pediatric	Paed.Surgery
Endocrinology	Biotechnology	Medicine
Gastroentrolgy	Gastrointestinal	Surgery

The most commonly used animals are :-

Frog		
Mouse	Rat	Hamster
G.Pig	Rabbit	Cat
Dog	Monkey	Sheep

Procurement of animals

It will be economical to procure animals from reliable sources rather than breeding them if the requirement of animals is minimal.

The various species of animals required for medical colleges should be procured from recognised sources. Local procurement sources should be identified by the medical colleges for the supply of non-laboratory bred animals. Medical Colleges with breeding facilities, should procure the breeding stock from a reliable source for initiating a colony ensuring that genetic makeup and health status of animals is known. Additionally the following aspects has to be taken care of :-

- Healthy animals should be obtained from a recognised source.
- Acceptable methods and norms of transportation should be followed, considering the distance, seasonal and climatic conditions and the species of animals.

- The animals should be given a reasonable period for physiological, psychological and nutritional stabilisation before their use.

Food and Water

- Animals should be fed palatable, non-contaminated, and nutritionally adequate food.
- Feed should be procured from reliable source.
- Good quality Feed and water should be provided ad libitum.
- Areas in which feed are processed or stored should be kept clean and enclosed to prevent entry of insects and wild rodents.
- Watering devices, such as drinking tubes should be examined routinely to ensure their proper operation.
- Feeders should allow easy access to food and watery while minimising contaminating by urine and faeces.

Sanitation and cleanliness

- Animal rooms, corridors, storage spaces, and other areas should be cleaned with appropriate detergents and disinfectants.
- Animals should be kept dry except for those species whose natural habitation need water. Where larger animals and non-human primates are housed soiled litter material should be removed routinely.
- Cages should be cleaned each time before animals are placed in them. Animal cages, racks and accessory equipment, such as feeders and watering devices, should be washed and cleaned frequently to keep them free from contamination.
- Cages, water bottles, sipper tubes, stoppers and other watering equipment should be washed and disinfected regularly.
 - Deodorisers or chemical agents other than germicides should not be used to mask animal odours.

Veterinary care :

- Wherever required, adequate veterinary care must be provided under the supervision and guidance of a registered veterinarian or a person trained and experienced in laboratory animal sciences.
- Animals should be observed regularly and problems of animal health and behaviour, recorded and addressed.
- For animals kept for experiments of longer duration, the following steps should be adopted :
 - All animals should be observed for signs of illness, injury or abnormal behaviour by the animal house staff and reported to the attending veterinarian.
 - Diseased animals should be isolated from healthy ones .

Personnel hygiene and Training of staff

- Initial in-house training should be imparted to the staff associated with animals facility
- Appropriate and protective gears (gloves, masks, head cover. Coat,shoes, etc.) be used by the personnel in the animal facility as per requirement.
- Personnel should have periodic medical check ups to ensure there health status.

Surgical procedures and duration of experiment

- Multiple surgical procedures on an animal for any experiment are not to be practiced unless specified in a protocol.

Restraint

- Devices, wherever required, suitable in size and design for holding animals for examination and collection of samples should be made available to minimize stress and avoid injury to the animals and handlers.

Records keeping

Animal facilities should have the following records :

- Animal House plan
- Name & addresses of the staff including the facility incharge and contact telephone nos.
- Health records of staff
- Training record of staff involved in animal care & procedures
- Records pertaining to the items in stock
- Animal stock procurement and supply register
- Records of experiments or procedures conducted with the number of animals used in each experiment.
- Clinical record of sick animals and any treatment administered
- Mortality and ailing record.

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 administered to sustain 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 euthanised by accepted methods.

In the event of a decision to euthanise an animal on termination of an experiment or otherwise, an approved method of euthanasia should be adopted and the investigator must ensure that the animal is clinically dead before it is sent for disposal. Carcasses should be disposed off either by incineration or deep burial method.

Anaesthesia

Sedatives, analgesics and anaesthetics (Annexure I) should be used to control pain or distress of the animal under experimentation. Species characteristics and biological 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 the care of an appropriately experienced person till it completely recovers from anaesthesia and post operative stress. Animals during post recovery period should be housed individually till they recover fully from the surgical stress.

Euthanasia

The procedure should be carried out quickly and painlessly in an atmosphere free from fear or anxiety. The choice of a method will depend on the nature of study, the species of animal and number of animals to be sacrificed (Annexure II). The method should in all cases meet the following requirements :

- Death, without causing anxiety, pain or distress with minimum time lag phase.
- Minimum physiological and psychological disturbances
- Compatibility with the purpose of study and minimum emotional effect on the operator,
- Location should be separate from animal rooms, method should be reliable, safe to the personnel and simple and economical.

Animal Houses

- Animal houses should be made of durable and preferably moisture – proof material and should have adequate space to facilitate free movement of personnel as well as equipment.
- Doors should be rust & vermin proof with provision for door closure. Rodent barriers should be provided at all entry points of animal houses.
- Walls and ceilings should be free of cracks.
- Floors should be smooth, non – absorbent and skid proof.
- Temperature and humidity in animal facilities should be controlled for the comfort of the laboratory animals. As far as possible the usage of smaller animal during the extreme weather conditions should be avoided.

- Proper lighting system with adequate illumination at cage level should be maintained in the animal room.

- The animal cages should provide adequate space to permit freedom of movement and normal postural adjustments, and have a resting place appropriate to the species; provide a comfortable environment; have an escape-proof enclosure that confines animal safely; have easy access to food and water; provide adequate ventilation; meet the biological needs of the animals; keep the animals dry and clean, be consistent with species requirements. (Annexure III A, B, C & D) However, aquatic animals like frogs and toads need to be kept in clean water free from chlorine and copper, preferably in containers attached to running tap water to prevent the accumulation of waste products.
- Houses, pens, boxes, shelves, perches, and other furnishings should be constructed in a manner and made of materials that allow cleaning or replacement in accordance with generally accepted husbandry practices.
- Physical separation of animals by species, wherever possible, is recommended to prevent inter-species disease transmission and to eliminate anxiety and possible physiological and behavioural changes due to inter-species conflict.
- Population density and group composition should be maintained as stable as possible, particularly for canines, non-human primates, and other social mammals.
- Animal facilities should be maintained free from pests and vermins

Bedding

- Bedding wherever prescribed, should be absorbent, free of toxic chemicals or other substances that could injure animals or personnel.
- Bedding should be removed and replaced with fresh materials as often as necessary to keep the animals clean and dry.

Quality of Experiment

There are several factors, which can influence the outcome of experimental results. Some of these factors are listed in Annexure IV.

BUDGET REQUIRED

Animal Housing Facility and Staff (for which animals to be procured and no breeding facility is available)

It is essential to have appropriate housing facility for holding different species of animals. The minimum basic requirements are the following :

Rooms for accommodating animals	:	3
Room for quarantine	:	1
Room for storage of feed / cage and other items	:	1
Room for cleaning cages etc.	:	1

In addition to the physical facilities appropriate cages, racks, feed hoppers, water bottles etc. also have to be provided. To attend to the care and husbandry of the animals and the maintenance of hygienic conditions the following staff have to be provided.

Staff :

Animal Attendants	-	3
Technical Staff	-	1
Veterinarian (Part time for breeding facilities)	-	1

For meeting the above requirement the following minimum funds have to be provided :

Non Recurring

A. For creating 7 rooms	-	15.00 lakhs
Air condition (5 nos)	-	2.00 lakhs
Cages, racks etc.	-	2.00 lakhs

Recurring

B. Diet	-	1.00 lakhs
Animals	-	1.00 lakhs
Staff salary	-	3.00 lakhs
Contingency	-	1.00 lakhs

		25.00 lakhs
		=====

Animal House for Medical Colleges where both breeding as well as teaching and research are being under taken

1. Building area required for accommodation of breeding animals	- 6 rooms
2. Area for experimental Animals	- 4 rooms
3. Area for laboratory , wash and change for staff	- 3 rooms
4. Area for Stores and cleaning	- 3 rooms
Total rooms	-16 rooms

(Room size 10' x 20')
Total area : 4000 sq. ft.

• **Staff**

Post	No. of Post	Salary per annum
1. Veterinarian	- 1	2,00,000/-
2. Technical staff	- 4	4,00,000/-
3. Animal Attendant	- 8	6,00,000/-

BUDGET

A. NON RECURRING

Building	-	30,00000/-
A/c	-	20,00000/-
Cages /Rack etc.	-	10,00000/-
Total	-	60,00000/-

B. RECURRING

Staff Salary	-	12,00000/-
Diet	-	4,00000/-
Animal Cost	-	2,00000/-
Contingencies	-	1,00000/-
Total	-	19,00000/-

Grand Total (A + B)- 79.00 lakhs

Annexure-I

Commonly used Anaesthetics in Laboratory Animals

Drug	Mouse	Rat	Hamster	G.Pig	Rabbit	Cat	Dog	Monkey
Ketamine (mg/kg)	75 (I/p)	90 (I/p)	40 (I/p)	100(I/p)	22-24(I/m)	30(I/m)	30(I/m)	10/25(I/m)
Ketamine (mg/kg) + Acepromazine (mg/kg)	100 (I/m) 2.5	75 2.5	125 to 150 5	12 5	30 01	- -	- -	- -
Ketamine (mg/kg)I/m + Xylazine (mg/kg)I/m	80-100 10	90 10	200 10	40 5	35 5	- 1 to 2	5 2 to2	5 to 10 0.5
Ketamine (mg/kg)I/m + Diazepam (mg/kg)I/m	200 (I/m) 5	- -	70 2	125 5	- -	- -	- -	- -
Alphaxalone / Alphadolone (I/P)	10 to15	10 to 12	-	40	6 to 9	-	-	12 to 18 I/m
Pentobarbitone mg/kg	35 I/v 50 I/p	25 I/v 50 I/p	- 35 I/p	30 I/v 40 I/p	30 I/v -	25 I/v -	20-30 I/v -	35 I/v -
Thiopentone mg/kg	30 to 40 I/v	20 to 30 I/v	20 I/v	20 I/v	20 I/v	10 to 15 I/v	25 I/v	25 I/v
Urethane (g/kg)I/m	-	1 to 2	1 to 2	0.5 1.5 I/p	1 I/p I/m	1.25 I/v	1.0 I/v	1.0 I/v
Atropine 0.02 to 0.05 mg./kg For all species by s/c or I/m or I/v used to reduce salivary / bronchial secretions and protect heart from vagal inhibition, given prior to anaesthesia.								

N2O + O2	-	-	-	-	-	-	-	-
Induction & Maintenance	1.1	1.1	-	-	1:1 & 3:2	-	-	1.1
Halothane (%)	-	-	-	-	-	-	-	-
Induction & Maintenance	4 1.25	4 to 5 1 to 2	-	4 1 to 2	1 to 2.5 1 to 2.5	4 1.25	1 to 2%	-
Isoflurane (%)	-	-	-	-	-	-	-	-
Induction & Maintenance	3 to 4 1.5	4 1.5 to 3	-	3 to 4 1.5	-	-	-	2 to 3 2 to 3
Methoxyflurane (%)	-	-	-	-	-	-	-	-
Induction & Maintenance	-	4 1.5 to 3	-	-	-	-	-	1 to 1.5 1 to 1.5

Source :

- 1) *The UAFW hand Book on the Care and Management of laboratory Animal, Trevar Poole (Ed.) 7th Edn. Vol. Blackwell Science, 1999*
Fundamental of Experimental Pharmacology, M. N. Ghosh (Ed.) 2 edn. Scientific book Agency, Calcutta, 1984.
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ANNEXURE – II
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
PHYSICAL METHODS								
Decapitation (for analysis of stress)	A	A	A	NR	NR	NR	NR	NR
INHALATION OF GASES								
Carbon Dioxide	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

Intraperitoneal

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 embolus

IP =

IV = Intravenous

IM= Intramuscular

Annexure-III A

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	77.4	
	>25	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-III (B)

Recommended Space for Cats and Dogs

Animals	Weight,kg	Floor Area/Animal, Ft ²	Height in
Cats	<4	3.0	24
	>4^e	>4.0	24
Dogs	<15	8.0	-
	Upto 30	12.0	-
	>30^e	>24.0	

Annexure-III (C)

Minimum floor area and height recommended for Monkeys (Rhesus and Bonnet) based on their weight (size) and behavioral activity

(For Langurs, the recommended space is in the foot note below)

Weight (in Kg)	Floor area		Height (Cm)
	Ft ²	Cm ²	
Upto 1	1.6	1440	50
Upto 3	3.0	2700	72
Upto 10-12	4.3	3870	72
Upto 12-15	6.0	5400	72
Upto 15-25	8.0	7200	90

- Note: a) The height of the cage should be sufficient for the animals to stand erect with their feet on the floor, whereas the minimum height of the cage for langurs has to be 90 cm as mentioned in INSA guidelines.
- b) The floor area for langur upto 6 kg weight, 5000 Cm² and above 6 kg, 6000-9000 Cm² is recommended. The height of the cage in either case remains the same, i.e. 90 Cm., as mentioned in INSA guidelines.
- c) If the experimental protocol demands caging more than 6 months, animals should be provided with double the floor space mentioned above.
- d) All primate facilities should have one or more runs as big as possible with a minimum floor space of 150 sq.ft. and height not less than 2 metres for free ranging activities.

Annexure III D

Recommended Space for Commonly Used Farm Animals

Animals /Enclosure	Weight. Kg	Floor Area/Animal Ft²	Height(Ft.)
Sheep			8
	<25	10.0	
	Up to 50	15.0	
	>50	20.0	
2-5	<25	8.5	
	Up to 50	12.5	
	>50	17.0	
>5	<25	7.5	
	Up to 50	11.3	
	>50	15.0	

Annexure IV

Factors which could influence the results of animal experiments

Factor	watch for
Genetic quality	<ul style="list-style-type: none">- strain / stock- breeding system- quality breeder / supplier
Biological status	<ul style="list-style-type: none">- sex- age- body weight
Health status	<ul style="list-style-type: none">- quality breeder / supplier- constant level of quality- hygiene barrier in maintenance
Nutrition	<ul style="list-style-type: none">- quality supplier- constant composition (lot-nr.)- quality drinking water
Maintenance	
- Cage	<ul style="list-style-type: none">- type (dimensions)- bedding- number of animals per cage
- animal room	<ul style="list-style-type: none">- ventilation- temperature- relative humidity- lighting- noise- other animals
Transportation	<ul style="list-style-type: none">- Means of transportation- Transport cage- Food supply
Animals care	<ul style="list-style-type: none">- qualification of Animal Care Taker
Experimental techniques	<ul style="list-style-type: none">- qualification of animal technician- standardisation of techniques- time of intervention

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