Up-gradation of Animal House from a Conventional one to a State-of-the-art Facility

Experience at BARC's Radiation Medicine Centre in Mumbai

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Introduction

The 'Animal House facility' (AHF) in a research healthcare setting is a broad terminology that encompasses the housing of the small or large laboratory animals. By the definition it connotes a place where animals are housed either for breeding or for experimental work or both for the purpose of pre-clinical use. In the guidelines, the laboratory animals (also referred to here as animals) are defined as any vertebrate animal produced for or used in research, testing, or teaching (Guide, 2011). Generally, laboratory rodents are extensively used for biomedical research and pharmaceutical industries for testing and evaluation of safety margin for human and veterinary medicine (Barre-Sinoussi & Montagutelli, 2015).

At the Radiation Medicine Centre, BARC, Parel, the Animal house facility is running since 1963, keeping the view of its importance towards the development and evaluation of new radiopharmaceuticals and examining its in vivo quality control before administration in the patients. Principally, the RMC Animal House facility is a conventional experimental animal facility, where conventional work culture was adopted as compared to barrier facility or animal bio-containment facility protected by providing additional barriers, advanced instruments with multiple controlling systems like environmental control, engineering control and enrichment facility for the animals. However, in each animal facility, the risk factors are different and it depends on the use. To mitigate the risk factor, it must be identified and a strategy plan must be made to define an acceptable level (NIH guidelines, 2019).

Over the period of time, new advancements and updated guidelines have been formulated for animal usage used with the intent of its application with humane care and they are followed worldwide. To ensure ethical humane care and scientific requirements in laboratory animals, a defined program is started to address the esteems of animal value. This forms the core requirements of the guidelines and an important system and support for self-regulation (Klein and Bayne 2007). To have better scientific outcome from the animal experiments, animal husbandry practices, animal house designs, layout planning and minimum requirement needs to be defined as per the national and international guidelines (AALAAC, CCSEA guidelines 2018).

The animal house facility at RMC is involved in various animal experiments primarily for development of new PET, SPECT and therapeutic radiopharmaceuticals, their biodistribution studies and development of polyclonal antibodies for radioimmunoassay (Fig.1). Different laboratory animals like rats, mice, rabbits and immunocompromised animals are employed to this purpose. The use of small animal models constitutes an important & integral part of testing of new radiopharmaceutical agents and its mandatory requirement from the regulatory clearance authority for human trials (Pawar et al., 2020). As mentioned before, the animal bio-containment and radioactivity are the two hazards of working within the facility. Therefore, the modification of conventional animal house layout to demarcate specialized work areas was planned. All the necessary approvals obtained from different safety committees of BARC like RMC local safety committee, ULSC-RO and OPSRC, and additionally, from the animal ethics committee from BARC. For minor structure changes approval from DCSEM, Civil Engineering Department, Vikram Sarabhai Bhavan, Anushaktinagar and the Civil Engineering Department, Tata Memorial Hospital, Mumbai was obtained.

The main Objective and Spectrum behind the AHF Modification was Characterized as follows

Animal research facilities involved with animal experiments & radioactivity work are considered to harbour bio-hazardous factors. Therefore, the facility associated concerns and risk factors are accordingly considered and addressed. To meet all the requirements at an acceptable level it is wise to construct the facility with versatility built-in to allow for a wide range of research projects and ensure safe environment.

The existing layout of the facility is as follows

Total number of Rooms- 9; Animal Colony: 3, Radioactive work: 1, Waste storage: 1, Quarantine Room: 1, Autoclave and Washing:1, Staff seating and Feed storage: 1, Animal House Admin: 1.

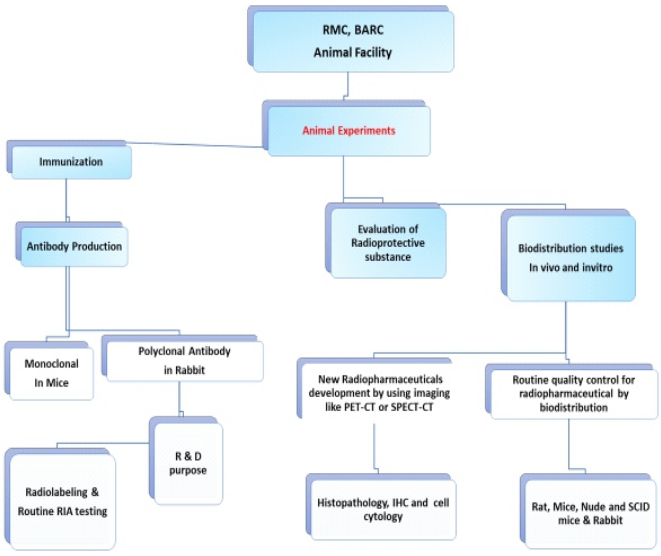

Fig.1: Work function of RMC, Animal House Facility.

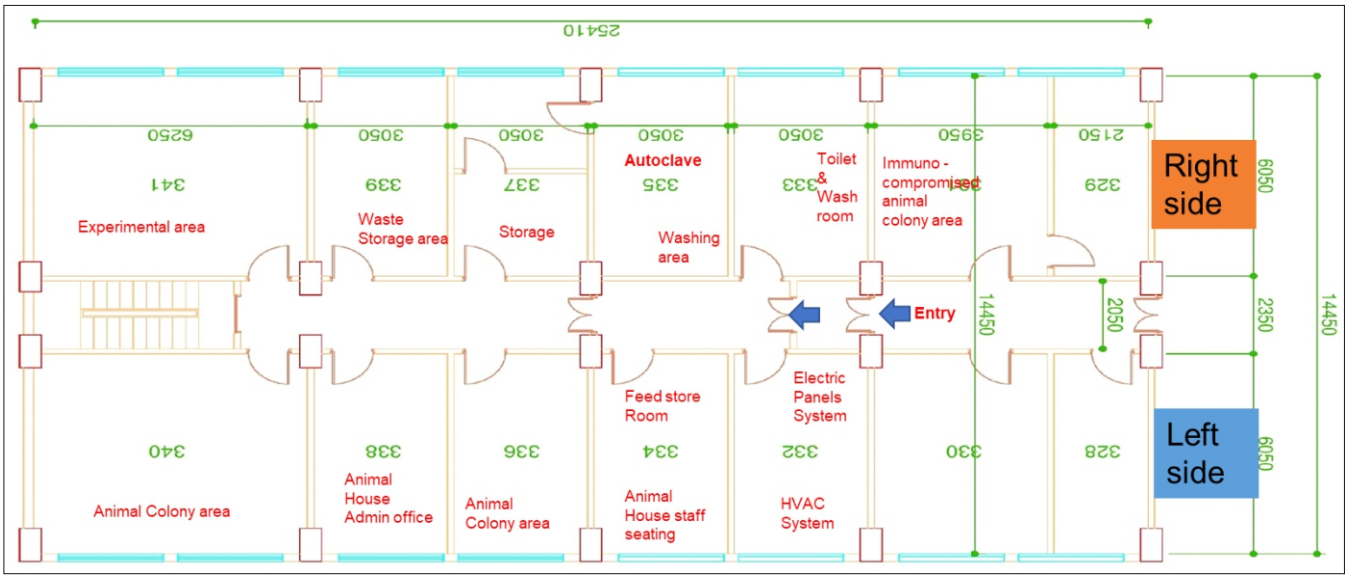

Fig.2: Current Layout of the RMC Animal House Facility.

Considering the current layout and mandatory requirement of safety guidelines the above modification was planned and approved by the different BARC safety councils.

To manage the work-safety and control or reduce the hazards elimination, substitution, engineering controls, good work practices and use of personal protective equipment kit must be considered (Wald and Stave, 2003). However, in view of the space constraints and necessity of the facility, the elimination and substitution of hazards components were unable to be implemented. Therefore, a modification of plan was made with the AHF design rearrangement, engineering control, good work practice, advanced instrument control, barrier construction like air barrier, physical barrier, use of PPE kits and training for the staff. These were considered to mitigate the associated risk factors and generate a safe working environment.

Modified layout of the Animal House Facility at RMC (BARC), Parel

Total Rooms - 9; Animal Colony: 3, Radioactive work: 1, Waste storage: 1, Quarantine Room: 1, Autoclave and Washing :1, Analyzer and Feed storage: 1, Animal House Admin and Staff seating: 1, Access control with read circle.

***Limited access control to the facility*:** The animal house staff and the administrative authority have access control cards for the facility. The electronic access control cards are provided to the animal house staff and facility related authority to have limited authorised access during working hours. Radioactive work areas with limited access control with manually operated systems are provided. There is no access to the other staff/common public. The facility common passage is under CCTV surveillance monitored by RMC, security staff and security have main door key withdrawal authority post working hours for the general surveillance (Fig.4).

***Engineering control in facility*:** There are controls on air flow, the flow rate being done by the central heating, ventilation, and air conditioning (HVAC) system and effective centralized exhaust system. The air flow within the animal colony will be maintained. A standard 15 to 18-air changes per hour is maintained in the animal colony. The additional air curtain lock was planned to fit outside each animal housing room.

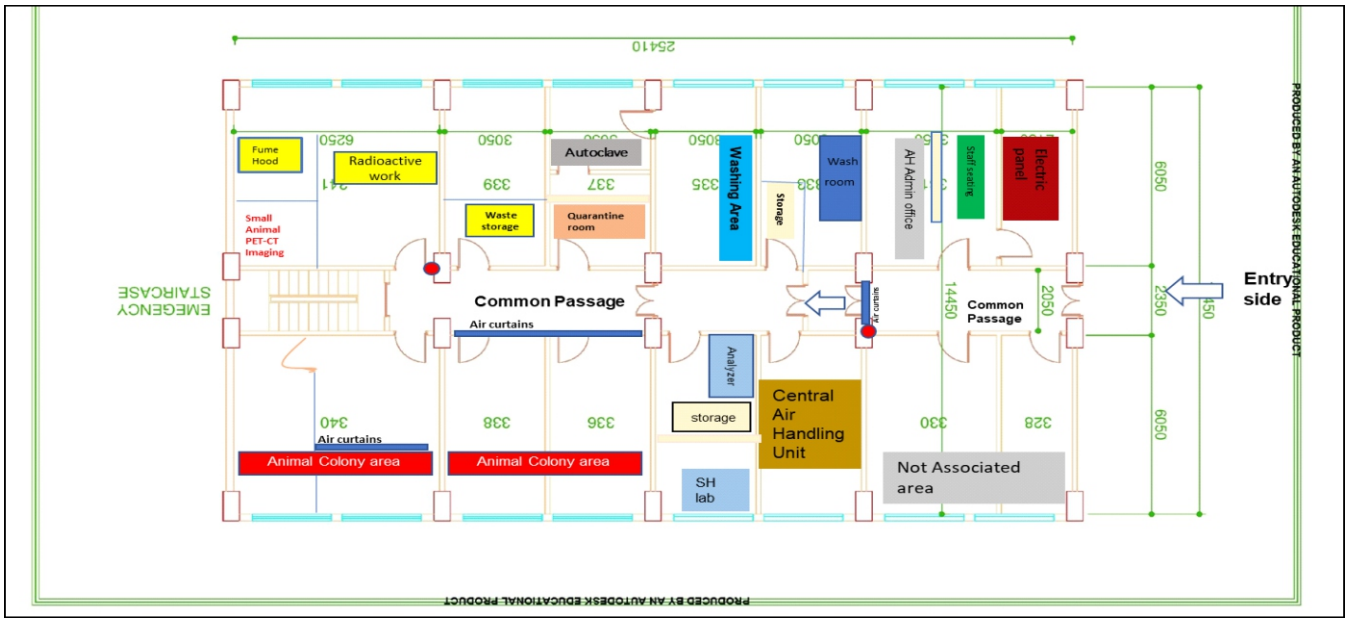

Fig.3: Modified layout of the Animal House Facility at RMC (BARC), Parel.

Fig.4: Electronic Door Lock: Access control door lock facility for animal house main entrance door.

Conventional cage system *IVC cage system*

Fig.5: Conventional cage system upgraded to IVC cage system for rats.

Fig.6: Animal cage change station: Daily routine use for cage changes in the rat and mice.

***Instrument control*:** It is employed for animal housing, associated daily husbandry practices and radiation safety. It is introduced as safe work practices when working with animals and radioactivity.

■ Individually ventilated cage (IVC) system: A new individual ventilated cage system is installed for the wistar rats. The system is very much safe for the handlers and animals or vice-versa as filtered air goes inside and filtered air comes outside (Fig.5).

Fig.7: Fume-hood facility: Employed for handling radioactivity for animal experiments.

Fig.8: Single Door High-Pressure High-Vacuum (HPHV) semi-automatic rectangular autoclave: Sterilization of cages, water bottles, bedding material and disposable aprons.

■ Animal Cage Change station: It is used routinely to change the cages of the animals. Due to its pre-filter and HEPA filter system, it protects the staff working with animal allergens. Hence it will provide health safety to the workers and animals (Fig.6).

■ Fume-hood: New fume-hood is fabricated as per the space availability and requirements. The work involving radioactivity in animal experiments is handled, injected and disposed off inside the fume-hood only (Fig.7).

■ Steam sterilizer: Introduction of single door High-Pressure High-Vacuum (HPHV) semi-automatic rectangular autoclave is used on daily basis for sterilization of animal cages, bedding material and water bottles. It helps to keep the bacterial contamination in check (Fig.8).

■ Animal tattooing machine: Animal marking is important in specifying experimental groups. Tattooing is done with AIIMS ink and tattooing machine with recommended ink and pattern. This is useful in identification of animals and prevents the missing of animals or reading wrong data related to human errors (Fig.9).

■ Veterinary Haematology Analyzer: Periodical animal health monitoring is a much-required aspect of AHF facilities. The parameters are standardized for laboratory animals like mice, rats and rabbits. This will be useful for rapid screening of any health issues in the animals.

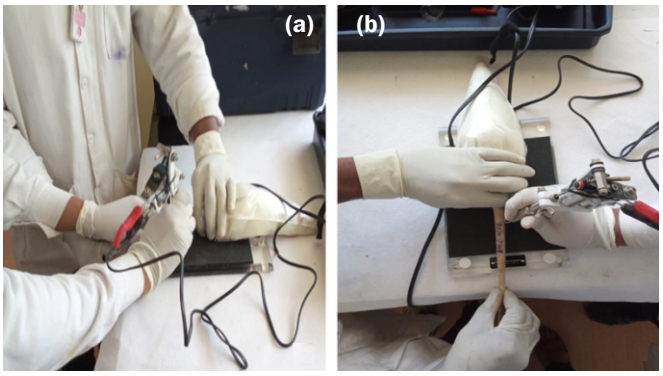

Fig.9: Animal marking with tattooing machine: (a) One person holding the animal cone and another person holding the tattooing gun for marking. (b) Process of 'Tail tattooing' by using tattoo machine.

■ Serum Biochemistry analyzer: Serum biochemistry machine helps in the identification of different organ functions based on levels of enzymes, hormones, proteins and waste products generated during the process of metabolism. They are useful for subclinical case identification and subserve as markers for animal health status (Fig.10).

■ Air curtains: Air curtains are provided outside each animal colony door, radioactive work area and at the main entrance of the facility. It will improve the air quality inside and outside of the working area (Fig.11).

■ Enrichment in the animal cages: Laboratory animals are considered social animals and hence measures taken to enhance natural physical activity and normal behaviours within the captive animals. Minimum enrichment should include nesting material, refuge area, tunnels and cons for small rodents like rats and mice (Fig.12). In rabbits chew sticks, hay blocks and nest boxes can be provided. The enrichment aids in maintaining and improving the quality of captive animals, decreasing the frequency of abnormal behaviour, and having a positive impact on the research outcome (Baumans, 2005).

■ Biosafety cabinet: The biosafety cabinet will be utilized for animal work that is not radioactive, including injections, administration, blood collection, and dissection for organ collection and necropsies. By doing this, contamination in the working area is reduced and thereby staff safety will be enhanced.

Fig.10: Veterinary Haematology and serum biochemistry analyzer: (a) Haematology Analyzer. (b) Serum Biochemistry analyzer.

Fig.11: Air Lock System: Animal colony with air curtain and limit switch at entry door.

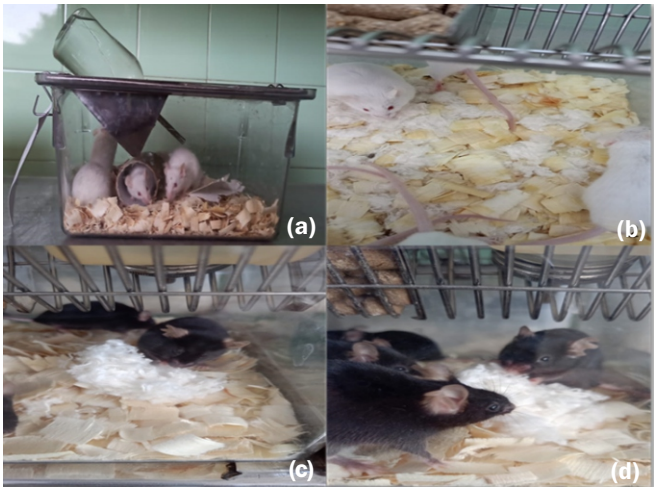

Fig.12: Enrichment of animal cages with tunnels & nesting material: (a) Mice using the tunnels (b) Nesting material collection by individual mice (c) Mice using nesting materials and (d) Tunnels in mice colony.

■ Radiation monitoring unit: Area Gamma Monitor is installed in radiation experimental area for monitoring the area.

Personal Safety

To achieve personal protection disposable apron, disposable face mask, plastic/cotton shoe covers, head cap, double gloves are kept inside the facility. Staff/Person /Students who wish to enter inside the animal house needs to wear before entering inside the animal facility. Staff/Person is wearing personal protective equipment (PPE), which will protect the user against health or safety risks at work and reduce the contamination, generation of aerosols and small dust particles transmission from human to animal and animals to humans. Personal radiation monitoring batches like Thermoluminescent Dosimeter (TLD), Pocket dosimeters used by the staff while handling the radioactivity in animals. Animal house entry and exit must be done in the animal house log book with date and time.

Conclusion

The inclusion of laboratory animals is essential for pre-clinical studies along with maintenance of proper husbandry and in-vivo imaging techniques to assess the data. In pre-clinical veterinary nuclear medicine facilities, similar to other veterinary practices, risk evaluation is a crucial rational process that should safeguard the animals, working staff and the environment. The major experimental work involved is

either imaging or biodistribution. The radioactivity associated risk factor begins with the injections, handling, restraining during procedures like transporting, imaging, biological sample collections and euthanasia during the experiments. To mitigate the experimental risk factors, standard operating procedures like use of sedative drugs during injections, animal restraining instruments, use of a partial containment system with air flow control etc for each study need to be defined. This will enhance the safety of the personnel working with the animal. During the procedure, use of anaesthetics drugs, animal carrying containment box with cotton & absorbent sheets, animal holding baskets increases the distance and shielding from radioactivity injected animals. It additionally safeguards the working environment. The personnel protective clothing, monitoring batch, gloves, mask, safety shoes or shoe cover ensure the workers' safety. Application of safety equipment, good husbandry practices with general radiation safety principle (time, distance and shielding) generate a safe environment for both animals and humans. However, it is a complex approach that necessitates regular evaluation and discussion of hazards that may come from the infectious agents within the animals, radiation safety and different research protocols.

In conclusion, knowledge, expertise, training, and the precise safety of the equipment and features of facility design are crucial factors to perform safe research and disposal of biocontainment within the facility. In this direction, the use of well-defined research and routine protocols in containment are especially important when animals and radioactivity are used and are evolved further based upon unexpected situations that may occur from time to time.

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