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Basic Care Procedures

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Overview

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Mice and rats account for over 90% of the animals used for biomedical research. The proper care of these research animals is critical to the outcome of experiments. There are general procedures that apply to the majority of these mice and rats, but some of the animals, such as the immunocompromised ones, require additional steps to be taken to sustain them for experimentation.

Commonly used immunocompromised mice include those that have naturally occurred in inbred mice and those that have been created through genetic engineering. The first immunocompromised mice used in research were "nude" mice. The BALB/c Nude (nu) mouse was discovered in 1966, within a BALB/c colony that was producing mice lacking both hair and a thymus. These athymic mice have an inhibited immune system that is devoid of T cells. The value of this animal was soon discovered for the use in studies of microbial infections, immune deficiencies, and autoimmunity. Although not as commonly used as the nude mouse, there is also a nude rat. The nude rat is T cell deficient and shows depleted cell populations in thymus-dependent areas of peripheral lymphoid organs. Another naturally occurring immune deficient mouse is the severe combined immunodeficiency mouse (SCID). These mice have a defect that impairs the production of functional B and T cells. With the inability to mount an adequate immune response, these mice can serve as host recipients for transplants of human cells. Several genetically-modified mouse strains with immune deficiencies are commercially available.[1] The severity of the deficiency varies with the genetic modification; however, all of the immunocompromised mice and rats are handled similarly.

This manuscript will describe the care of both immunocompetent and immunocompromised animals. The housing, diet, environmental requirements, and handling will be discussed for both groups of animals.

Principles

Housing/Caging

Mice and rats are primarily housed in shoebox-type caging with a solid bottom that contains bedding material. The typical shoebox mouse cage provides 75 square inches of floor space, which is adequate for a maximum of five adult mice. However, the experimental design may call for groups of four mice. In this case, the mice should be housed upon receiving in groups of four, to avoid the disruption of social groups when the experiment begins. In general, rat caging allows for a maximum of two fully-grown adult animals. If the rats are to be housed long-term, they should be established in pair housing.

The cages can be static or individually ventilated (IVC). Static cages are either covered with a wire bar lid or are settled onto a rack under a shelf that is then the top of the cage. Filter material is added over the cage top either as a bonnet over the wire bar lid, or as a sheet of filter paper over the rack shelves. IVCs are set up as a cage, wire bar lid, and bonnet that are placed on a rack that provides air flow into the cage. The use of IVCs eliminates the need to change the cage as often as static cages are changed.

Standard cage systems provide a source of water and an area for food distribution. Water can be provided via a water bottle placed on the top of the cage or via a sipper tube that is a built in component of the animal cage. When the cage is engaged onto a rack, water is transported into the sipper tube for an automatic watering system that is designed to meet the needs of the animal throughout the animal facility. Caging must be properly prepared to provide proper care for the animals. There are either feeders placed within the animal cage, or a section of the wire bar lid is used for food storage. Food must be placed such that it is not soiled by the animals.

The *Guide for the Care and Use of Laboratory Animals*[2] (the "Guide") states that all caging implements must be sanitized at least every 2 weeks. Some cages and accessories may need to be changed more often as dictated by the level of odor and ammonia in the enclosure. In general, the IVCs are scheduled for changing every two weeks unless there are four or more males or a female with a litter; those enclosures may require weekly changing. As cage changing is an opportunity for the mice to be exposed to pathogens, changing of the caging for immune deficient mice should be done as infrequently as possible.

Diet

There are many compositions of diets available with variations in protein, carbohydrate, and fat levels. Typically, feeds should be comprised of 15-19% protein, 44% carbohydrates, and 4-9% fat. Food with an increased fat content is often used for breeding colonies to enhance reproduction. As the food can be a source of contamination for immunocompromised mice, it must be either steam sterilized or irradiated. Food that is subjected to the autoclaving process must be specially formulated to withstand the process. However, the irradiation process does not alter the food contents.

Water is another potential source of contamination for the immune deficient animals. Water should be processed either through an autoclave or filtration that includes the water passing through a UV filter. To steam sterilize water in an autoclave, individual bottles of water are covered with aluminum foil. Bottle lids with sipper tubes are sterilized separately in an autoclave bag. Acidification of the water to a pH of 2.5-3 helps control the growth bacterial organisms in the water bottles. Water is acidified by adding two drops of 12 mole (M) hydrochloric acid (HCl) to eight

liters of reversed osmolality (RO) water. Automatic watering systems can be used with a series of filtration throughout the system that includes particulate filters and an ultraviolet light filter.

Environment

Environmental conditions within an animal care facility must be tightly controlled. Federal regulations and guidelines have established the standards for the care and handling of commonly used animals. Lighting, ambient temperature, humidity, and air flow are provided to meet the physiological needs of the animals. With the exception of the need for a slightly increased room temperature, the parameters for the immunocompromised animals are the same as those for conventional animals. The athymic nude animals also require additional bedding and shredded paper-type nesting materials in their cages to assist with thermoregulation.

Animal rooms should be maintained at a temperature between 68-72°F with a humidity level of 40-60%.[2] Humidity levels below 40% can result in ringtail of mice and rats, a condition that causes the skin of the tail to dry to the point of it constricting around the tail, which can lead to the tail sloughing. Lighting cycles should be set to avoid stressors to the animals caused by inappropriate photoperiods or light intensity. Normally, photoperiods are 12 hours on and 12 hours off. The lighting intensity should vary in accordance with the activity in the room. When technicians are working in the animal room, higher lighting levels can be used. However, the lighting should be lowered to approximately 325 lux once all activity is concluded.[2] The lights should be on timers and the cycles verified through monitoring systems. As directed by the Guide, animal rooms must have a minimum of 10-15 air changes of 100% fresh air per hour.[2] Air changes decrease microbes and odors in the animal room. In densely populated animal rooms, the number of air changes per hour may need to be increased.

Environmental factors at the cage level include the caging type, the substrate used within the cage, the water delivery system, and the diet. Social housing is mandatory unless scientifically justified, or an animal is incompatible with all others. The provisions of environmental enrichment, such as paper huts and nesting materials, allow the animals to exhibit species-specific behaviors, which in turn decreases the stress level and increases the overall wellbeing of the animals. Nesting materials also provide a sleeping area that is saturated in calming pheromones that assists with the housing of multiple male mice in the same cage. The nesting area is moved to the clean cage upon cage changing to help prevent aggression in the males as they are introduced into a new environment.[3]

Procedure

1. Handling immunocompetent (conventional) mice

It has been demonstrated that immune disorders of mice are very similar to those in humans.[2] As these animals have limited immunological capabilities, procedures in the handling and care of these animals have been designed to minimize the risk of infectious disease through the use of engineering controls, personal protective equipment for the animal care staff, and standard operating procedures; these mitigate contamination of caging and other equipment with which the animals are in contact.

1. Unpacking from shipping containers: Receiving animals from other institutions or animal vendors poses a risk to the animals already housed within the animal facility. During transit, the animal transport boxes can come in contact with pathogenic agents. Therefore, proper procedure should be performed when unpacking the shipping containers.
    1. Minimal Personal Protective Equipment (PPE) requirements are gloves, clean lab coat or scrubs, a particulate mask, and safety glasses when handling the animals.
    2. Select the appropriate cage, including the wire bar lid and the barrier top. The cage should be furnished with appropriate bedding and nesting materials. All caging should be prepared and placed in the receiving area prior to the arrival of the animals.
    3. Upon arrival, the transport boxes are placed in a secure and temperature controlled room. Then, examine the container for damage.
    4. Disinfect the outer surfaces of the shipping container with a choline dioxide product to destroy any harmful pathogens. Make sure that the disinfectant is in contact with the box in accordance with the manufacturers recommendations, usually 15-30 minutes.
    5. Place the container in an Animal Transfer Station (ATS). The ATS provides HEPA-filtered airflow within the hood that provides allergen protection by creating a vacuum air barrier between the user and work zone. This ensures minimal exposure to animal hair/dander and cage bedding material. The unidirectional air also protects the animals from the surrounding room air, minimizing the chance of contamination.
    6. Once the shipping container is opened, assess the overall health of the animals. Make a note of any injured or ill animals.
    7. Place the animals into the assembled cages in the proper group sizes. The grouping should be in accordance to the space regulations, and the experimental protocol to which they will be assigned.
    8. Cover the cage with the wire bar lid. Secure the divider on the wire bar lid to create two areas on the lid.
    9. Select the appropriate food and place the food in one side of the wire bar lid. Leave the second side open to allow for better circulation of air.
    10. Water will be provided via water bottles or an automatic watering system. Water bottles are used for newly weaned mice to ensure that they are able to maintain their hydration levels. For IVC caging with automatic watering, charge the watering tube prior to placing it on the rack.
    11. Place the cage on a rack. If using IVCs, check that the cage is fully engaged onto the rack, and that the sipper tube is secured to the watering system.

2. Cage changing conventional mice
    1. Animal cages are changed in an ATS. The ATS is disinfected at the end of each session.
    2. Only one cage can be opened at a time.
    3. Caging prepared with the appropriate bedding and nesting materials is brought into the animal room. A small number of cages are placed in the ATS.
    4. IVC cages with internal sipper tubes must be charged to force water into the sipper to remove the dead space of air in the sipper tube. If not properly charged, the mice may not be able to provide enough pressure on the sipper tube to remove the air from it.

5. Minimal Personal Protective Equipment (PPE) requirements are gloves, clean lab coat or scrubs, a particulate mask, and safety glasses when handling the animals.
6. A cage of animals to be changed is placed into a new cage, and then in the ATS.
7. Animals are either transferred by hand or with the use of a padded forceps. Gloves are either changed between cages or between groups of mice. Gloves can also be disinfected with a nontoxic chemical between cages.
8. It has been demonstrated that transferring the nesting area of the mice into a clean cage assists with the transition into the clean cage, especially for group-housed males. Other enrichment items are also transferred to the new cage.
9. Once the cage top or feeder is placed in the new cage, food is added.
10. The barrier top is placed on the new cage and the cage is returned to the rack.
11. IVC cages must be properly docked into the rack to ensure that the air and water valves are engaged.

2. Handling immunocompromised mice

1. Unpacking from shipping containers
    1. Immunocompromised animals must be unpacked under sterile conditions. All individuals handling immune deficient mice must wear a steam sterilized gown, mask, hair bonnet, and sterile gloves. Aseptic technique should be used at all times when handing these animals.
    2. All equipment and caging should have been sterilized by steam, UV radiation, or chemically sterilized with a soak in 70% ethanol for at least 30 minutes There are two options for sterile caging: standard caging that has been wrapped and steam sterilized, or caging that is sterilized-usually through irradiation-by the vendor. These cages are one use, disposable units.
    3. Prior to manipulating mice under the laminar flow hood, the hood is sprayed with 70% ethanol with the blower operating. The hood blower should remain on at all times to maintain the sterile environment. After each use, the hood is sprayed with a disinfectant and wiped of all debris and again sprayed with 70% ethanol.
    4. Optimally, two people are required for the proper receiving of the immunocompromised mice: one to handle the animals using aseptic techniques (the sterile technician), and one that will handle contaminated items (the nonsterile technician). The nonsterile person should still wear a clean, long-sleeved gown; lab coat or smock; mask; bonnet; and sterile gloves to prevent contamination of the sterile interior should they need to reach into the hood.
    5. Prepare a laminar flow hood as previously described. It is preferable to have a hood dedicated for immunocompromised animals. It is recommended that hood be equipped with ultraviolet (UV) sterilization lights that can be used to sterilize the interior of the hood between uses. A timer is utilized to ensure that the surface of the hood has adequate exposure to the UV rays.
    6. The exterior of all supplies that are placed into the hood are sprayed with 70% alcohol, including the outer packaging of food, water, and caging.
    7. Shipping containers are sprayed with a chlorine dioxide solution before they are placed in the hood. Once placed in the hood, they are to remain unopened for 15-30 minutes.
    8. The designated animal handler opens the sterile food and water under the hood.
    9. The nonsterile technician opens the shipping box under the hood. Care must be taken by the nonsterile technician to avoid contact with the inner surfaces of the hood and the inside of the shipping box.
    10. The animal handler opens the sterile cage, and prepares the cage by placing food and water onto the cage top. The mice are then removed from the shipping box and placed into the sterile cage.
    11. Animal condition is noted by the sterile animal handler. Any animals that appear ill or have obvious injuries will be segregated. All cages marked with animals requiring veterinary attention will be placed for handling, cage changing, or experimental manipulation last, to reduce potential contamination of other animals.
    12. The nonsterile person will remove the populated cage from the hood. Cages can be placed on a standard rack as static cages, or on a ventilated rack as IVCs. In the IVCs, the mice should be provided with additional nesting materials to assist with thermal regulation.
    13. The animal handler should change gloves after each shipping container to avoid possible cross contamination of groups of animals. The shipping container number is recorded on the cage card to track the origin of the animals in the event of illness.
    14. Unused supplies can be saved for future use provided the original container can be resealed. Unused food is removed from the room.
    15. Mice should undergo a minimum one week acclimation period prior to experimental use.

2. Cage changing for immunocompromised mice
    1. The mouse cages are changed at least once weekly, or more often if deemed necessary.
    2. Steps for hood preparation, cage preparation, and personnel PPE are the same as for the unpacking of the animals.
    3. Animals are either transferred by hand or with the use of padded forceps. The designated animal handler should reglove after every 5-10 cages, if the gloves are torn or contaminated, or if an animal appears ill.
    4. Any cages with animals appearing sick will be placed at the end of the cage changing order, to avoid possible exposure of healthy animals to pathogens or contaminants.
    5. If food and/or water must be added to the cage before the next scheduled cage change, it must be done aseptically under the prepared hood.

Summary

The proper care and handling of animals used in biomedical research is crucial to the outcome of the experiments. Regulations and established guidelines dictate many of the practices used. However, additional measures are required for mice and rats that have immune deficiencies to mitigate accidental exposure to opportunistic pathogens. When practices adhere to established procedures, the health and wellbeing of the experimental animals is enhanced, and better experimental resulted are attained.

References

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