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An overview of the key concepts required for managing a laboratory animal (rodent) facility

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Abstract

The use of laboratory animals primarily rodents (mice) has increased over the past 20 years especially with the availability of transgenic and mutant strains. This increased use of mice along with novel research models with unique needs, has contributed to the evolution of the modern vivariums, decontamination equipment, imaging cores, ultra clean barriers, decontamination of feed and water etc. In addition, the industry has also made significant improvement in operating animal facilities. Automation of cage processing is just one example that signifies the sophistication which is an ongoing process. This sophistication and availability of better options not only improved animal welfare and ergonomics, but also efficiency in operating the modern animal facilities. As the animal facility operation involves several components, it could be challenging for an entry level manager to be knowledgeable about the best options that suits the needs of the facility. This article provides an overview of the most popular practices for the crucial aspects of animal care involving housing, animal health monitoring, feed and watering systems in addition to quality aspects. To provide a complete review of the laboratory animal facility operations and management would exceed the scope of this article. However, the topics discussed here are worthy of note when assessing the essential care and welfare of laboratory animals

Key words : laboratory animals, rodents, facility, management, feed and water quality, health monitoring, decontamination.

Introduction

Laboratory mice constitute more than 90 percent of all the animals used in research. Over the years the professional field has made significant progress in understanding the physiology, pathology and quality of care for the valuable strains of research mice. Here, we discuss broadly the key factors that could impact the micro and macro environments in a rodent facility. The daily operation in the facility involves humane care for lab animals, protecting the animals from pathogens, to keep them well fed and watered, and to maintain their genetic integrity, in addition facilitating the ongoing research activities in the facility. One should never forget that the pathogen protection can never be achieved without mandatory adherence to pathogen protection standards of all activities related to animal care at the institution.

Animal health monitoring and pathogen protection plan

There is no universal agreement on the desired health status of mice used in research. Scientists and laboratory animal professionals are in agreement and feel the need to avoid

Animal Housing

Summary of common types of mouse caging

System and description	Advantages	Disadvantages
Conventional cages and shelving		
• Boxes sit on, or fit into, open metal shelves • Boxes have wire lids that contain bins for feed and water bottles; lids may or may not have covers • Air circulation in cages is determined by room circulation and by airflow caused by the thermal heat load of the mice (*Reeb et al.* 1997).	• Is least expensive • Allows easy access to mice • Allows cage changing and any work with mice on open tables	• Even with filter covers, provides lowest level of pathogen protection. • With filter covers, poor circulation can affect intra-cage temperatures, ammonia levels, humidity • Requires more frequent cage changing than other systems.
Microisolator cages and conventional shelving:		
• Similar to conventional systems, except individual boxes have covers containing high efficiency particulate air (HEPA) filters	• Is a cost-effective, flexible way to provide higher level of pathogen protection than elsewhere in a mouse room.	• To maintain higher level of pathogen protection, cage changing and any work with mice must be done in HEPA-filtered hood. • Air circulation may be poor, usually worse than conventional cages and shelving. • May require more frequent cage changing.
Ventilated, HEPA-filtered caging systems, with heating, ventilation and cooling (HVAC) functionality:		
• Boxes fit snugly into closed, forced ventilated shelving system.	• Provides highest possible pathogen protection for the mice and allergen protection for the technicians. • Provides good air exchange. • Cages remain dry, which may reduce cage change frequency and labor expense.	• Requires capital expense. • cage changing and any work with mice must be done in HEPA – filtered hoods. • Ventilation can result in drafts within the cages, which might stress some mice
Portable ventilated caging systems:		
• Similar to stationary ventilated units, but • boxes fit into stand alone units on wheels, and • Incoming air is filtered room air; exhaust is filtered back into the room.	• Provides flexible solution for pathogen protection. • Works well for light mouse loads. • Cages remain dry, which may reduce cage change frequency and labor expense. • Cages can be pushed together for floor space economy with out inhibiting air circulation for animals.	• Numerous portable racks can increase the heat load in the room. An option is to modify rack ventilation so it is exhausted outside the room.

microbial contaminants that could impair the performance of mice in research either directly by causing clinical disease or indirectly by causing physical or physiological changes that could alter or confound data. Although there is widespread, if not universal, interest in excluding the pathogenic murine viruses from research facilities, there is no consensus on the importance of excluding organisms such as pinworms, *Helicobacter, Pneumocystis murina, Staphylococcus and Pseudomonas.* To complicate matters, some of the scientific evidence and animal models requiring certain agents for expression of desired pathology/phenotype etc. is undeniable and emphasizes the reality for lack of universal exclusion list of agents. There are many examples where a desired phenotype of a mouse model was lost following rederivation in to an ultra-clean barrier facility. For example, in most mouse models of inflammatory bowl disease (IBD), diabetes, immunology and enterocolitis etc. requires a complex enteric flora and sterile conditions may negatively affect the desired phenotype. In addition, the absorption and toxicity of the investigational new drug compounds can be affected by altered gut flora and or pathogens. Recent publications support the role of normal flora and their impact on immune

Animal Housing

Advantages and disadvantages of different types of bedding for mice

Type of bedding	Advantages	Disadvantages
Hardwood shavings	• Are cost effective • Are physiologically inert; do not elevate cytochrome p450. (Weichbrod *et al.* 1988)	• May be dusty and irritating. • Is not as absorbent as other materials • May be from an environment in which chemicals were used.
Softwood shavings (cedar, pine)	• Are cost effective.	Same as hard wood shavings, plus • Cedar and pine can be dusty and irritating. • Cedar, in particular, elevates hepatic p450 enzyme systems (Wade *et al.* 1968)
Fractions or pellets of corn cob	• Are non irritating. • Are physiologically inert; do not elevate p450 system	• Is moderately expensive • Is not good for nesting; additional nesting material may be required. • May be prone to mold. • Is less absorbent than other bedding.
Recycled paper, cellulose chips	• Provides superior absorbency and ammonia control	• Is moderately expensive • Can be dusty • Fibers may be irritating to certain strains (e.g., nude or SKH-1) that have no eye lashes (White, 2007)
Cotton –based material	• Is highly absorbent. • Is good for ammonia control when shredded	• Is expensive • Is effective against odors only if shredded. • Fibers may entangle infant limbs and cut off circulation.
Paddy husk	• Inexpensive, readily available. • Is a good absorbent	• Very light and could be dusty • Potential for pesticide residues depending on source • Not well studied compared to other materials

function. These findings will only complicate the validity of research data published from different facilities across the globe as one can anticipate variability in the gut flora among research facilities.

Given these complications, unique scenarios and research types, it will be up to the facility to establish suitable standards for their research needs and should develop an animal health program that meets their standards. The four essential components for any program should be (1) The exclusion agent list (2) Preventive measures (3) Monitoring procedures (4) Containment and eradication procedures (in the event of an outbreak). Stringent standard operating procedures (SOPs) for each of these components are vital and all vivarium staff including management staff and research personnel should be trained on the SOPs.

In the event of an out break, appropriate notification and communication to researchers at academic institutions, to customers by animal breeders or suppliers and to clients by contract research organizations is critical to save valuable time and resources.

Once the exclusion list is defined for the program appropriate preventive measures should be considered for protecting the animals. In reality, these measures should be envisioned at the facility design stage, and use of multiple strategies should be considered. These aspects can be logically delineated by concentrating on the macro and micro environments and also port of entry and port of exit (with respect to animals, staff, supplies and reagents). Macro environment components with respect to pathogen protection includes attention to separation of clean and dirty supplies and traffic, and air quality from HVAC system. Micro environment components include, quality of feed, water, bedding, cage processing and cage changing techniques. The port of entry components should address the quality of animals and appropriate quarantine and or rederivation measures, disinfection of supplies at receiving dock and or in to barrier facilities, proper garbing of staff entering the facility and proper screening of reagents especially biologicals prior to injecting the mice in facility.

Rodent health monitoring

As most of the rodent viral agents do not produce clinical signs, serological monitoring for the presence of antibodies for the detection of exposure to an agent is the most commonly

used method. As one cannot monitor each and every animal, using targeted sentinel animals typically per rack is most commonly used method. Sentinels are of two types: Dirty bedding sentinels are exposed to dirty bedding from research animals at every cage change (a small amount of dirty bedding collected from the colony animals cumulatively transferred to sentinel cage). Contact sentinels are other type where the sentinel animal lives with in the same cage/pen of the research animal and is in direct contact with the colony animals. The contact sentinel system is used in limited settings for example quarantine facilities, etc.

Although use of sentinel animals has been the most pragmatic and so far the widely acceptable approach for disease surveillance, this system has several major caveats and concerns. 1) The choice of sentinel animals is very crucial, they should be not only clean but also immuno-competent for the agents that are surveyed. 2) The dirty bedding exposure system is ineffective in transmitting the agents to sentinels. This is because either the agent is not shed into bedding (e.g. Sendai virus) or the agent is shed intermittently (e.g. Mouse Parvo Virus-MPV) and or the agent may be unstable in the environment and loose efficacy to mount immune response in sentinels (e.g. Helicobacter, Mouse Hepatitis Virus-MHV). 3) In case of the contact sentinels, unwanted mating and fighting with the colony animals are obvious issues (castrated animals are available to eliminate mating issues but still fighting are a problem). Contact sentinels, if rotated among several cages they may actually spread the agent in case of an outbreak. 4) Regardless of the type of sentinels, it takes at least 4 weeks exposure to mount effective immune response for antibody detection. Hence, the sentinel method is not indicative of the current health status but the status 4 to 12 weeks prior (based on frequency of sentinel screening). One pragmatic approach that is being used to overcome the inefficiencies in sentinel system is to use the littermate or adult female (mother) after weaning for diagnostics. Typically in quarantine settings when the concern of pathogen entry is high, we can set up the breeding with in quarantine and only the weaned litter is allowed in facility with negative results from full necropsy of the mother. Of course, this scenario may not be possible in all settings but is effective wherever possible. Hence, the facilities using sentinel health monitoring system should critically evaluate serology data of any positives with respect to the pathogenesis of the agent to logically arrive at the current status. This comprehensive understanding is critical for development of best effective containment and remediation plan. In addition, the sensitivity and specificity of the assays used should be factored in for an in-depth analysis of the prevalence and incidence of any agent detected.

Cage changing, feeding and watering of animals

Cage changing is the most common and routine operation that could pose a threat to disease prevention. Ideally the cage changing should be performed using microisolation technique in a biosafety cabinet or in a cage change station with hepa filtered laminar flow. The best practice would be to use forceps to transfer mice and precautions should be taken not to touch the mice or bedding (even with gloved hands). Using hands for collecting sentinel bedding can facilitate transfer of agents from cage to cage as the disinfectants used to spray the gloves are ineffective due to lack of sufficient contact time in between cages. Hence, using forceps that is dipped in a disinfectant is the most ideal method. In addition, use of forceps also prevents the spread of pheromones (minimizing Bruce and Whitten effects) between cages there by improving breeding performance. Use of autoclaved cages and bedding for cage change outs is gaining popularity and could become the norm in the near future.

Types of diets and quality control aspects

Mouse diets are broadly classified in to types based on ingredients (natural, purified, or chemically defined) and the formula (open or closed).

Natural:

The most common type of diet from natural ingredients contain agricultural products and by-products such as whole grains, mill by-products, high-protein meals (animal and vegetable), yeast and mined or processed mineral sources (NRC,1995).

Purified and Chemically defined diets:

Purified diets also known as semi-synthetic diets contain ingredients that represent single nutrients. For example casein, starch, dextrins, sucrose, glucose, specific oils, cellulose vitamins and minerals. Purified diets are expensive than natural ingredient diets, but are useful and an absolute requirement when the exact composition of a diet must be controlled. In addition, nutritional and toxicological studies may require purified diets to eliminate the variability in results from the low concentration of contaminants and immunogens. Recently, the concern of phyto-estrogens from natural ingredient diets and its effects on reproductive and physiological research is being debated. In addition, the concern of Bisphenol A contaminant in diet is also being investigated. Purified diets could help to reduce such concerns and variability in studies where ever required.

A chemically defined diet is a type of purified diet in which individual amino acids are used in place of a protein source and specific fatty acids are used in place of oils. Chemically defined diets are very expensive are used mainly in amino acid and fatty acid metabolism studies.

Formula types – Open and fixed:

Open formula diets can be made of either natural ingredients or purified ingredients, however the formula is fixed i.e., the macronutrient source is consistent and it is not variable. Manufacturers openly publish the exact proportions of ingredients.

Formula types – Closed and variable:

Closed formula diets are always made of natural ingredients and the proportions/formula is proprietary. Manufacturers publish the ingredients but not specific concentrations or

formulations. The source of ingredients and proximate analysis of protein, fat and ash content may be found on these types of diets. It is important to keep in mind that the macronutrient source can be variable according to considerations such as a market price. The protein source is especially liable to vary in the closed formula diet. For example milk protein may be substituted for fish meal. Knapka (1997) has argued that the varying ingredient inclusion rates in variable formula diets poses a considerable risk of experimental variability. Researchers that are concerned about the possible effects of varying dietary nutrients should use purified fixed formula diets or have the relevant nutrients assayed independently by commercial laboratories. The biology of mouse can be affected by varying the source of protein. For example varying the protein source, not the amount, could double the inducible tumor incidence (Guo *et al.* 2004). In addition, the gut micro flora composition and its effect on immunology, obesity etc. is gaining importance. Variability in diets could alter the gut micro flora that eventually could lead to altered research results.

Type of feed based on Physical Form

The most common physical form of feed is pellets and extrusions (collets). The manufacturing process for both these forms start the same way: raw food material is ground, sifted and mixed with vitamin and mineral supplements into a meal.

Pellets:

For pelleting, the meal is mixed with steam raising the temperature to 65-80 °C and gelatinizes the starches, thus binding the diet ingredients together. Heat also reduces the microbiological load by about 2 folds in magnitude. The meal is forced through a die and cut to specific length. The resulting pellets are dried so that moisture content typically is about 12%, a level at which free water content is too low to support growth of microbes. Hence, pelleted feed when protected from moisture can remain on a shelf for about 6 months before nutritive loss or growth of mold becomes a concern.

Purified diets because of the ingredients, for example simple carbohydrates and casein, are very sensitive to the temperatures during pelleting process. Hence, to minimize loss of purified ingredients, the meal is mixed with water, pelletized, and then dried at a low temperature (less than 60 °C) in a vacuum. Purified pelleted diets generally have a shelf life of about 4 months when refrigerated or frozen.

Extrusions:

For extrusions, the meal is ground finer than for pelleting. Meal is mixed with steam and hot water to a temperature of 80-95 °C and extruded under pressure (about 35 atmospheres) to temperatures of 150°C. As steam is trapped within food during extrusion, the extruded pellets (collets) become honey combed. The collets are dried by hot air to a moisture content of 8-12%. Due to the high temperature for this process, the loss of vitamins is greater, at the same time the mold and bacterial spores are also destroyed there by facilitating longer shelf lives than pelleted diets.

Quality control

Decontamination of feed:

The risk of contamination by insects and microbiological organism is unavoidable in feed manufacture, packaging, storing and shipping. Anecdotal evidence and personal communications from facility directors, it has been shown that mouse parvo out breaks are controlled by switching the feed to irradiated diet. The scientific reasoning and data to explain the mechanism is not available although some studies are under way. From practical point of view, the ingredients (for example corn) get stored in silos that are infested with wild rodents. The parvo virus that could get into the ingredients, if survived (not known) the manufacturing process could cause random positivity in the facilities. Irradiating the diets could eliminate the active infection that cannot be spread to sentinels. This reasoning seems unlikely but at least points to the steps and complications in the manufacturing and storage conditions that could lead to contamination.

The two most common methods used for decontamination of feed are autoclaving and irradiation. Pasteurization has been used historically, as this process only eliminates vegetative bacteria and some spores, this is currently not a popular method.

Autoclaving:

Autoclaving is the most common method of sterilizing mouse feed. The process involves sterilizing with steam at a specific temperature and pressure for a specific length of time. Typically, this process is carried out on site using double-door autoclaves facilitating the pass through of sterilized feed into barrier facility. Manufactures produce feed specifically to be autoclaved and mark the feed bags accordingly.

Nutrients that are susceptible to damage by heat, moisture, and oxygen are especially affected by autoclaving. “Browning” reactions among amino acids, especially methionine, cysteine and lysine and between amino acids and carbohydrates, alters the structure of the amino acids and can substantially diminish protein bioavailability.

Manufacturers supplement the feeds to be autoclaved with increased protein content and supplemental amino acids.

Heat labile vitamins (B_1, B_2, B_{12}, B_6 and Pantothenate) are particularly sensitive to autoclaving; modest losses of vitamins A, D_3 and folate also occur. Manufacturers generally add additional vitamins to diets that will be autoclaved. Because of this reason potential toxicity can result if mice are fed autoclavable feed that is not autoclaved. Autoclaving also affects the hardness of the pelleted diets which should be monitored. Clumping of diets specifically the ones that had coatings is also another problem evidenced from autoclaving.

Irradiation:

Irradiation is the process of exposing feed to radiation for the purpose of destroying microorganisms. The radioactivity damages the genetic material (DNA) that prevents replication.

There is no transfer of radioactivity to the irradiated diet during the irradiation process. This process requires specialized facilities.

Gamma irradiation is the most commonly used form of irradiation for diet decontamination. Irradiation at doses less than 10kGy (radicidation or radurization) is equivalent to pasteurization. A minimum dose of 21kGy is sufficient to kill most bacteria, molds and fungi, and is considered a sterilizing dose (however *clostridium* and *bacillus* spores may require above 30 kGy). Doses at 30kGy may be necessary to inactivate some viruses (Baldelli, 1967). The sensitivity of many pathogens to irradiation is provided by the world health organization (WHO, 1999).

Most mouse diets are irradiated at doses of 20-25kGy which does not have an effect of protein bioavailability (Eggum, 1979; Ford, 1979) and loss of vitamins are less than 20 percent (Ford 1979; Isler and Brubacher, 1999). The most concerning factor in irradiated diets is the free radical induced oxidation of fats and production of peroxides. The peroxide values increased 6- 8 fold in a high fat irradiated diet at 25kGy (Ford, 1979). The increase was reduced to 3-4 folds by irradiating under vacuum (peroxide level continued to increase during storage).

Provision of Water:

Quality of water:

Water provided for mice should be free of microbial contamination. Although there are no set guidelines for safe water, the common treatment methods used are – autoclaving, acidification, hyper-chlorination, reverse osmosis and ultra violet light exposure.

There is no set standard for the type of water with respect to laboratory mice. Tap water has wide spread variations, such as mineral content, general hardness, presence of fluoride. The generally accepted notion is that if water is good enough for the community, it is good enough for the laboratory mice.

Water delivery systems

Type of system	Advantages	Disadvantages
Automatic	• Individual cages will never run low • No sterilization of water bottles and lids is required	• It requires a capital expense. • System must be maintained to prevent buildup of mold and bacteria • Some mice may need training, to get used to the system • It is difficult to determine water usage for specific cages • Leakage in some systems may cause mice to drown if cages fill with water. • Cages must be checked daily for evidence of leaks
Glass or plastic bottles in different configurations: • Rubber stopper with sipper tube. • Solid stopper or solid lid with gasket; hole in bottle to provide water • Metal lid with gasket; “sipper” hole in the middle of the cap.	• Water usage per cage can be easily monitored • Sterilization of bottles and Lids is easy. High pathogen protection. • Delivery of medications and or reagents is easy.	• Individual bottles and lids must go through sterilization process. • Technicians must deal with individual bottles and check for air bubbles in sipper tubes (labor intensive). • Bottles and cages must be checked daily for any leaks.
Plastic bags/pouches with water: • Plastic bag punctured with a sipper stem and encased in a mouse proof holder	• Water can be stored long term, treated or untreated. • Disposable bags eliminate labor and expense to wash and process bottles. • Bags can be sterilized separately. • Good for emergency planning	• Capital expense • Maintenance costs for the machine and materials in case of disposable bags.

This notion does not recognize the variability in specific research studies. The best strategy is to use consistent source of water and consistent treatment method. The GLP studies and toxicity studies should pay special attention to the quality of water as the contaminants could potentially affect the results. In addition, micro flora of the gut could be altered with quality of water, an issue that is gaining popularity. Regardless of the source and treatment method, the delivery mechanism, for example, bottle, auto water, pouches etc. should be cleaned and sterilized for maintaining overall quality of the program.

Conclusion

Although we talked about several aspects, this certainly is not comprehensive information and each topic can be a book chapter by itself and the reader is recommended to explore additional resources for a thorough understanding as needed. In addition to these aspects there are several other areas for example facility design, traffic patterns, cage wash setup, decontamination procedures, animal procurement, quarantine and rederivation facilities, handling-identification and basic procedures, security and disaster planning etc. which also are an integral component of any animal care program. From this, it should be understood that animal facility management is complex and requires knowledge and expertise in several aspects, eventually facilitating valuable research studies in physiologically normal animals handled in a humane way.

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