A Guide to Research Rodent Housing

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BACKGROUND

Laboratory rodents have been used in the United States as research animals since the late 1800s. In the past, housing of rodents used for research was designed primarily to prevent escape, to provide easy access to the animals by researchers, to allow the animals ready access to food and water, and to allow efficient dirty bedding removal and cage cleaning. At first, little thought was given to disease control; in fact, little was known about naturally occurring diseases of rodents that could affect research results. Through the 1950s, significant mortality of laboratory rodents prior to and during the course of experimentation from naturally occurring diseases was commonplace and accepted as part of the course of experimentation.

As the sophistication of biomedical research increased during the 1950s and 1960s, a few of the more common rodent diseases with obvious clinical signs were described. Rudimentary control measures were instituted to control these diseases. These procedures included: disinfecting of housing materials, subdividing populations of animals into many small rooms, and developing of commercial sources of rodents free of some pathogens.

Prior to 1970, only limited diagnostic testing methods were available for rodents. Serologic screening methods were limited to a few rodent viral agents, and this screening did not became common until the late 1970s. Animal facility design reflected the philosophy that recognition of clinical signs of disease was to be followed by removal of affected individuals from the group for treatment or culling them from the population in an effort to control disease spread. Animal facility design and housing methods focused more and more on cost of manipulation of caging and ease of its movement to and from central cage washing facilities which were the focal point of cleaning and disinfection. Hence, a clean/dirty (supply/return) corridor system connecting a series of rectangular animal rooms to a cage wash and other support facilities became a common design feature. Doors were thought to represent significant microbiological barriers, and wearing protective clothing such as lab coats or shoe covers was deemed important as a means for controlling disease spread. These concepts were philosophically in keeping with disease control measures used in veterinary practice at the time. Suspended caging (a wire grid floor in a cage suspended over a catch pan containing bedding that could then be frequently changed) and the advent of automatic watering to eliminate the labor associated with filling water bottles were two important developments initiated in the 1960s and 1970s. They helped provide a better environment for laboratory animals while reducing cost of operation.

As early as the 1960s and 1970s, many animal facilities contained portions of the facility which were physically separated and operated autonomously from the other portions of the animal facility. These barrier facilities employed many of the barrier production features originally developed by Dr. Henry Foster and others in the 1950s as part of commercial

breeding operations. A research barrier facility depends upon the existence of a microbiologically impervious perimeter enclosing a room or group of rooms for housing animals, often with the incorporation of support facilities such as cage washing and even laboratories within the barrier. Air and water entering the barrier were treated to remove some or all known infectious agents. Commonly, the air entering the facility was highly filtered, and depending upon the purpose of the barrier (either to exclude agents or contain agents within the barrier), the exhaust air was also filtered. As with the air, water was also treated by filtration or other means to remove infectious agents, and in addition water was also sometimes treated by addition of chlorine or other chemicals to further disinfect it. A through-the-wall autoclave for heat-based disinfection of material was a common feature. Spray locks, spray ports or pass-through dip tanks for the application of chemical disinfectants, such as peracetic acid, formaldehyde, or chlorine based agents for disinfection of non-autoclavable supplies and equipment, were commonly incorporated. Entrance to the barrier facility (which also served as its only exit) was limited to a single entry lock system requiring a clothing change and often a shower. Dedicated, disinfected clothing was worn in the barrier facility and could have varying degrees of completeness of personnel coverage ranging from dedicated footwear and a scrub suit to total gowning covering all surfaces of the individual. Such barrier facilities usually were limited in size and restricted in access to just a few individuals. The costs for operating such barrier facilities were high, and their use was limited.

By the mid-1980s, the number of organisms causing clinical or sub-clinical disease in rodents or organisms known to have the ability to alter research results had risen substantially due, in large part, to research efforts conducted at a few diagnostic laboratories, as well as at a number of pharmaceutical companies using rodents. Diagnostic methodologies such as serology had become much more reliable through the development of better diagnostic reagents, and regular screening of rodent colonies had become more commonplace by both suppliers and by research institutions. The use of rigorous barrier room methods and the use of isolators for maintaining foundation stocks known to be free of certain microorganisms was employed more extensively during the mid-1970s through the mid-1980s by commercial suppliers of laboratory rodents, resulting in an increasingly reliable source of supply of animals free of a growing list of undesirable microorganisms. By using reliable supplies of disease-free rodents and regular microbiological testing of in-housing colonies, it was possible to locate, eliminate and replace animals harboring such microorganisms.

In order to further protect the health status of research animals, a new type of housing—a cage level barrier—was developed in the 1980s. This housing method, termed *microisolation caging*, was first applied to the production of immunologically deficient mice and rats and was subsequently expanded for use in immunocompetent animals. Although the basics of microisolation cage design had really been present since the early 1970s, it was not until the rigorous imposition of complete disinfection by autoclaving or chemical disinfection of all cage components and materials to which the animal had potential access, coupled with manipulation of the animals and the cage components aseptically in a disinfected workspace (laminar airflow hood) in a constant stream of disinfectant air, that a truly effective system became available.

Initially, such caging depended upon air passing into the cage passively (static microisolation caging), principally through convection of hot air generated by the animals' body mass, out of the filter or other openings. The exiting air would be replaced with fresh air flowing through the same passages. Since the cage contained a filtered top made of coarse filter material, other than the movement of this air, nothing, including infectious particulates, penetrated the sealed cage environment.

In the early 1990s, the addition of forced ventilation and subsequent exhaust extraction from each cage using a variety of systems improved the static microisolation caging system. This provided high air exchange rates within individual cages, refreshing the animals' environment and substantially drying it out. Intervals between bedding changes in such an environment could be increased allowing less manipulation and greater operating economies than the static microisolation systems, which often require much greater cage/bedding change frequencies as compared to conventional open caging that has better air exchange.

In the early 1990s, the technology that had been around almost as long as laboratory animals—isolator housing—reappeared as another means of providing greater microbiological exclusion than conventional open caging. Isolators, which place a barrier around a group of cages while allowing manipulation of the cages and the animals therein through the use of built-in gloves, proved to be particularly useful in managing immunologically compromised animals and certain breeding colony operations. Isolators proved particularly useful in housing animals intended to be progenitors of larger colonies and have gained recent acceptance for housing founder lines of transgenic animals or other valuable animals in a highly secured environment. Isolators have also been used for quarantining animals of uncertain microbiological status (biocontainment) and provide the advantage of allowing experimental manipulations to be conducted with ease while the animals are still in quarantine.

The explosion in transgenic animal production coupled with the free exchange of animals between a variety of institutions, as well as the movement of untested biological materials between institutions, has caused an upsurge in the prevalence of rodent diseases/infections with agents that can have significant impact on research results. The use of conventional open cage housing, while efficient and acceptable for some types of research, is becoming less and less the norm due to increasing prevalence in the research environment of highly infectious rodent disease causing organisms. In an effort to control unwanted microorganisms in rodents, many institutions are relying more heavily on microisolation cage housing and isolators and to a lesser extent on true barrier facilities for the maintenance of rodents.

ROOM LEVEL BIOEXCLUSION—BARRIER ROOM / FACILITY

Barrier facilities can either be used to exclude (bioexclusion) or contain (biocontainment) unwanted or hazardous microorganisms. At most research institutions that employ barrier rooms, seldom does the barrier encompass a single room, but usually a series of rooms maintained within a barrier to exclude the entrance of rodent disease causing microorganisms. This differs substantially from barrier facilities at commercial suppliers of laboratory animals where, commonly, individual animal production rooms are autonomous barriers that do not

share any services or personnel between them. The barrier room is a sealed room that can be disinfected / sterilized. Generally, personnel access is limited to only one entrance, and all personnel undergo the same level of decontamination and gowning. All supplies are disinfected/sterilized as they enter (bioexclusion) or exit (biocontainment). All animals crossing the barrier are free of agents to be excluded or contained. People entering (exiting) the room are decontaminated and covered with protective clothing using an entry lock system.

To enter the barrier, personnel should go through a series of completely autonomous locks that are separated from each other by tightly sealing doors and which have independent air supply and exhaust. The first lock, which may be split into two depending upon whether the barrier is entered from the exterior of the building or from an interior area, is where street clothing is removed and placed within storage containers/lockers. In the case of entry from the exterior or an uncontrolled and potentially contaminated space, an initial air lock is often provided to trap insects and to provide a location for removing coats and shoes.

Following disrobing, a second lock compartment—a shower—is entered. While a water shower can temporarily reduce microorganisms on the external surfaces, significant decontamination of the human body by this method is not achievable. Its principal purpose is to ensure that complete clothing removal has occurred since people do not like getting wet with their clothes on.

Following a three to five minute shower, the third component of the lock system—the clean dressing area—is entered. In this area, the individual dries off and puts on a suitably disinfected or sterilized set of garments. Often, this area is used to conduct a final hand washing in order to ensure adequate disinfection of hands and forearms before gloving. Upon leaving this area, personnel either enter the barrier room directly or go through a final air lock or air shower before entering the room.

In order to prevent people from acting as fomites for carrying in microorganisms that may contaminate animals housed within open caging in a barrier room, people must be gowned in such a way that all body surfaces are covered with protective clothing to minimize the risk of introducing microorganisms through direct animal contact or through the release of infectious aerosols. In general, this means that all surfaces of the employee from the tips of the toes to the top of the head (with the possible exception of the eyes and perhaps a small portion of the face or neck) should be covered completely. Special attention needs to be paid to areas such as the junction of the uniform to the gloves and to footwear, to prevent exposure of skin surfaces to the animal's environment.

The shower-based entry system (wet entry) limits the speed by which personnel can enter the barrier room, and interposes a wet environment that may not be regularly or completed disinfected of organisms of concern. The wet entry process also requires additional laundry and supplies, and the shower step can easily be circumvented unless electrical interlocks or other preventive measures are incorporated.

As an alternative, the shower component can be eliminated or be used as an optional component for people leaving the barrier who might use it for personal hygiene. Instead, if

personnel can be trusted to use the undress component of the lock system correctly, then they can proceed directly to the clean gowning lock without the imposition of a wet shower. This assumption becomes less certain as the number of personnel that must pass through the lock system increases. Final gowning is often followed by a separate air lock, or more commonly an air shower, before entering the barrier room.

An air shower is a double-door chamber that contains a number of jets that deliver high velocity, highly filtered air in a manner that washes particles from the surfaces of the protective clothing worn by the employee. The assumption is that particulates, which could consist of microorganisms or inanimate particulates carrying microorganisms, are washed off the clothing and exhausted out of the chamber. While the process is not 100 percent efficient, there is a substantial reduction of particulates from the exterior surfaces of the clothing. In addition to the cleansing function upon entry, the air shower also removes particulates upon exit from the barrier which is a safeguard against spreading infectious particulates through the rest of the lock system and the animal facility should the barrier become contaminated. In addition, the air shower serves as a positive safeguard to the entry and exit of rodents or pests into or from the barrier facility. It also serves as a 30 to 45 second psychological reminder of the clothing requirement for the entry process for personnel while the air shower is in operation.

While some research barrier facilities have restrooms, laboratories and break areas or other support facilities oriented towards personnel rather than animal care located inside the barrier area, this is not particularly logical with respect to disease and microorganism control. Such areas may reduce operational costs and are convenient to people, but they are usually areas in which people remove some of their protective clothing inviting external contamination of the room's surfaces. These are also areas in which large amounts of material and equipment are stored or used that can easily become contaminated and spread contamination to all personnel and animals subsequently coming in contact with them. The use of restrooms and lunch or break rooms is particularly hard to rationalize, especially if open caging is used in the barrier.

All equipment coming into the barrier room generally enters through an autoclave (double-door) or a pass-through chemical decontamination chamber. The autoclave used in such operations must be calibrated and, if appropriate, validated initially and then at least annually for each specific load configuration and cycle to be used in order to ensure that disinfection/sterilization conditions have been achieved. One cannot assume just because the autoclave completes a cycle that the necessary disinfection or sterilization conditions have been met, especially if the process has not been regularly calibrated and load configured. While perhaps there is a reduced risk for newly acquired items that have not been in the animal facility, any materials that have passed into the non-barriered areas of an adjacent animal facility pose a significant contamination risk.

Some materials used in barrier facilities/rooms cannot be autoclaved. In such instances, chemical agents are used to decontaminate their external surfaces. However, simply placing items, which may have been used in other locations and have the risk of potentially being contaminated with unwanted microorganisms, in a bag and then spraying the outside of the bag with disinfectants does nothing to disinfect materials contained within that bag. The same

holds true for equipment with complex interior surfaces. Electronic equipment with cooling fans that suck air into interior components are disinfection challenges. Moreover, the chemical agents used for disinfection must be active against the organisms that are to be excluded. Just like the autoclave process, chemical disinfection should also be calibrated in order that contact times, application procedures and adequate concentrations of the disinfectant are used. Manufacturers' claims can sometimes be based on extrapolations from other organisms or conditions, or based on assumptions that have not been verified.

Static air pressure differentials are considered by many to be important in the operation of barrier rooms, but by themselves will not control organism movement across the barrier. It must be remembered that many of the microorganisms of concern are less than 1 micron in size and are shed in great numbers by infected animals. While air movement may help to move particulates from one location to the next, other means of movement of infectious particulates, such as direct contact or the use of contaminated equipment, are equally important in transmission. Commercially available air handling systems are incapable of controlling airflow once doors are opened. In general, negative pressures are recommended in order to keep things in, while positive pressures are recommended to keep things out of a barrier.

Ideally, all sanitation procedures are done within the barrier. The more caging and other equipment that must move across the barrier for cleaning in a central facility and then moved back again, the greater the risk that something will be incompletely disinfected and will serve as a source of infection to the barrier facility. This is particularly true when support areas also serve non-barrier animal holding areas.

Similarly, quarantining animals of unknown health status within the barrier is not recommended due to the increased risk associated with logistics of moving potentially contaminated animals into the barrier and then into containment equipment. Moreover, the handling of waste materials from these animals, as well as routine animal manipulation that may be required, all increase the risk of escape of disease causing microorganisms within the barrier and potential contamination of other animals. Finally, removal of animals found to be infected and subsequent disinfection of their housing area poses further risk of disease spread in the barrier, even if cage or group level bioexclusion is used.

It is important to remember that in the case of a barrier facility with open caging, as might be found in more conventional housing environments, once an agent gains entrance, there is no means to control the spread. The population within the barrier is naïve to the agent, and unless there is some other form of bioexclusion housing being used in conjunction with the barrier, spread throughout the facility is often quite rapid. This can be advantageous from the standpoint of detection of the organism by health monitoring programs which rely on random sampling of a population in which there are no restrictions to spread, but at the same time, the rapid spread of contamination can be devastating to the research program.

Barrier facilities are seldom effective in excluding non-rodent specific organisms. If an organism is a human or an environmental commensal, there are too many opportunities for exposure to occur, even if personnel are appropriately attired. If such opportunistic organisms

are to be excluded, a more rigorous group or cage level bioexclusion system such as microisolation cages or isolators must be used.

Overall, barrier rooms are not particularly effective in a research setting and are prone to periodic failure. They can be effective for housing large numbers of animals that do not require frequent experimental manipulation or access by a large number of personnel and to which there is not regular introduction of new animals or biological materials that could serve as a source of unwanted infection. Compromises that are often necessary in a research setting to make barrier operation feasible, such as: allowing a large number of people to enter the barrier, inconsistent personnel entry and disinfection procedures, location of research and personnel areas within the barrier, conducting of cage cleaning outside of the barrier, and the assumption that supplies such as food and bedding are adequately decontaminated by the manufacturing processing, or that cage washing provides sufficient decontamination for entry into the barrier without further treatment; all increase the risk to the barrier and the likelihood of failure. Some or all of these risks may be worth taking as long as occasional failure can be accepted and a plan of action for eliminating the unwanted microorganisms has been developed before the contamination occurs.

MICROISOLATION CAGING

The principle upon which microisolation cage housing of rodents is based is very similar to that of petri dishes used in bacteriology. Physical or chemical methods are used to assure that the internal surfaces of the cage are free of undesirable microorganisms, and the cage is subsequently handled in an aseptic fashion to ensure that the interior surfaces do not become contaminated with these organisms during use. For this reason, every process associated with such caging must be carefully examined and calibrated to assure that contamination does not occur during handling. Any assumptions made about such processes that cannot be confirmed, or any short cuts taken for convenience or in an effort to save operating costs, can risk the entire system. Similarly, the operating assumption must be that the health status of the animals placed within the microisolation caging is known and is appropriate. If this assumption is incorrect, infected animals can reside within one or more cages interspersed with cages containing other animals that are not infected.

In theory, such an occurrence or contamination should reside only within the infected cages and should not be passed between cages if perfect operating procedures are followed. Unfortunately, perfection is not always achievable; consequently, some risk of spread is possible when contaminated animals are maintained within microisolation caging. This risk is further magnified in proportion to the length of time that the infected animals remain within the caging system. Extended housing of animals in microisolation caging such as breeding colonies or when it is used on long-term experimentation such as product registration studies increase the risk. Conversely, short-term studies lasting a few weeks or a few months with subsequent replacement of animals by new ones from a known contamination-free source will dilute/wash out the contamination from the colony providing that other techniques are strictly adhered to.

Removing animals from the protected environment of a microisolation cage for experimental manipulation using equipment of uncertain disinfection status that may or may not have been used in association with other animals also increases risk. Regrouping animals, as may occur in the course of breeding experiments, can pass infection on to other cages increasing the risk and sustaining infection. Similarly, the more times a cage is opened and the greater the number of people manipulating a cage, the greater the chance of introduction of unwanted microorganisms.

While there are many opportunities to introduce contaminants into a microisolation caging system, they are surprisingly effective in a research environment in controlling and often eliminating the spread of infection within a facility even when operating technique is less than perfect. Their relatively simple design and operation makes their use easy for personnel to master, and the equipment required for maintaining them is not unusual in a biomedical research environment. Unfortunately, their cost of operation is significantly higher than open caging and can be even further increased if steps are not taken to house such caging in large rooms with a logical flow pattern to and from services areas, or if sufficient space for cage and rack manipulation is not provided.

A key concept in the use of microisolation caging is that each cage represents its own microbiological unit that is independent of all other caging in the room. This means that the barrier to microbiological containment is at the cage level, not at the room level, which is desirable since control of contaminants by air pressure differentials, doors, and protective clothing for personnel is much more prone to failure than strict adherence to aseptic microisolator technique. Hence, every effort should be made to modify facilities to allow larger numbers of cages to be maintained in rooms and to provide sufficient space and aseptic change stations (e.g., laminar flow hoods) to allow both husbandry and investigative work to go on within the animal holding room.

In theory, there is no specific need for animal procedure rooms, providing that techniques are compatible with the controlled environment within a laminar flow workstation. Special procedural rooms may be required when volatile anesthetics are used or when other chemicals that are not appropriately handled in a laminar flow hood are required (although hoods with 100 percent venting to dedicated room exhaust will address this problem), or in those instances where specialized equipment is needed that is not well suited for use in such hoods. When such conditions exist, it is important that every effort be made to assure that the animals are exposed only to adequately disinfected surfaces and are, to the greatest extent possible, handled aseptically and protected exposure to other animals housed in other caging units during such manipulation. If this cannot be adequately guaranteed, the animals should be considered as potentially infected, and may require additional restrictions or limitations to their movement as well as increased health surveillance. Since the barrier is at the cage level, however, any such contamination should be limited to one group of animals and one researcher's experiments rather than potentially dispersed throughout the entire population—all dependent, of course, on adherence to handling with proper aseptic technique.

The subdivision of animals into cage level microbiological groups, while useful in greatly reducing the chance of infectious agents spreading through a colony, also tremendously limits

the ability to detect the presence of such agents by conventional health monitoring methods. Microorganisms are traditionally detected by using sampling methodologies that make the assumption that all of the animals from a population to be sampled have equal probability of being exposed to the agent in question and, further, that there are no other limitations to acquiring infection. Animals should be all of the same immunologic status, and there should be no age, sex or other biological factors influencing the spread of the agent. Finally, such sampling methods rely upon the population being greater than 100 individuals, which is not the case with microisolation caging wherein the population in the cage seldom exceeds 5 animals. Under such circumstances, small populations require disproportionately large samples to get the same probability of detecting an agent. In order to address the limitations of a small sampling population, a sentinel health monitoring program can be used to hopefully fulfill the exposure assumptions imparted by a larger population (i.e., in effect linking the population of many cages).

In such a program, a small number of immunocompetent animals are exposed to soiled bedding or other cage components from multiple, well-identified cages over a period of weeks or even months. The sentinel animals should be obtained from a source known to be free of the organisms to be screened, or they should be selected from colony animals in the unit to be monitored. To be truly reflective of animals housed with a microisolation caging system and not to be misleadingly contaminated by organisms that exist outside the system, sentinel animals are maintained within their own microisolators using aseptic technique. The addition of exposure practices, such as the use of soiled bedding from other cages, the use of soiled cages, the rotation of animals that are surplus to a breeding program through the sentinel cages or the actual use of these animals as sentinel animals, increases the ability of the system to detect the presence of unwanted microorganisms.

In the end, however, the sentinel program will only alert the researchers to a failure in technique in one or more of the caging units, and not specifically identify which unit or units are affected. Moreover, there is often a significant time lag from the introduction of the unwanted organisms until their detection, especially with organisms that do not reach a high prevalence in a population. This period of vulnerability is further widened if inadequate sample numbers are used or exposure practices are limited or infrequent. In the case of certain organisms, the use of fomites such as soiled bedding, or even soiled cage components, may not be sufficient for transmission. In such instances (e.g., *Helicobacter* sp., *Mycoplasma pulmonis*, CAR bacillus sp.), only results from direct contact sentinels will be reflective of the presence of those organisms in the population. Since there can be a time lag of months between exposure of sentinels to an agent and their sampling in a health monitoring program, not only could an agent be spread more widely between microisolation cages due to poor technique or movement of animals between cages, but it is also possible that the contamination may have been limited to a few cages which were eliminated during the normal course of experimentation, making verification of a single positive result impossible.

Static Versus Ventilated Microisolation Caging

Static Microisolation Caging

Microisolation caging can be of two types: static or ventilated. In the case of static microisolators, no provisions are made to move air under pressure into or out of the cage. By contrast, ventilated microisolators provide some degree of forced air movement either actively or passively through the cage. Static microisolators are essentially a conventional plastic cage with a filtered lid covering the top of the cage. The filter in the lid is often made of spun-bond polyester with an average pore diameter ranging between 3 and 15 microns. The filter is replaceable and should be replaced periodically, as it becomes readily plugged with dust particles and other materials that are subsequently bonded into the filter during autoclaving or other disinfection processes thereby increasing the efficiency of the filter by decreasing the airflow through it. This gradual reduction in air movement through the filter causes moisture accumulation and poor cage air quality.

The plastic filter tops on static microisolator cages often do not fit perfectly or securely. Air striking the cage can easily move up under the lid and drop into the cage itself; a process which is increased when the cage is put in a laminar airflow stream as might occur in a workstation but does occur even under standard room ventilation conditions. When this occurs, particulates clinging to the outside of the cage can be caught up in the air stream and moved into the cage itself even with the lid on the cage. To minimize this, it is a common practice to lightly spray the outside of the cage with a suitable, quick-acting disinfectant prior to placing the cage in a change station.

The laminar airflow hood that serves as the change station provides an easily disinfected surface that is supplied with clean air that has been HEPA filtered. No assumption should be made regarding the disinfection status of the hood surfaces that might have direct or indirect contact with the microisolation cage, and hence, they should be adequately disinfected prior to each use. The disinfection status of the hood surfaces can be tested either by bacteriological plates (RODAC) or by the use of an ATPase meter that looks for cellular material containing ATP. Neither of these methods can determine whether viral contamination exists. An ATPase meter gives an immediate yes/no answer of disinfection status in terms of relative counts. RODAC plates take 48 hours or more to develop and read—well past the point to take corrective action.

Caging and other equipment placed in the hood should be kept to a minimum to prevent cross contamination through the process of eddying, which occurs when laminar flowing air strikes an object and becomes turbulent. This turbulence can move contaminants from one location to the next. All utensils used in the hood should be regularly disinfected between cages if they will come into contact with the interior surfaces of the cage or the animals. Commonly, animals are handled with forceps that are disinfected between usage in different cages, and the

disinfectant is rinsed off in sterile saline or sterile water prior to handling animals. If gloved hands or other instruments are to be used, they should also be disinfected between cages.

Opening microisolation cages outside of the disinfected and protected environment of a laminar flow workstation, such as on a table in an animal room, exposes their contents to an unreasonable risk from infectious particulate contamination. Repeated openings under such conditions invite eventual contamination when combined with other unavoidable risks.

The use of freshly prepared disinfectant that is allowed adequate contact time is the only way to assure that appropriate disinfection is being achieved. While various claims are made by manufacturers of how long a disinfectant will remain active, there are many variables that can affect this. Moreover, while disinfection can be achieved by older disinfectant preparations, the contact time may be unacceptably extended. Since this is a critical component of the process, it would seem illogical not to refresh such solutions frequently, perhaps daily.

The selection of the type of disinfectant is important since some microorganisms are highly resistant to certain types of disinfectants while very susceptible to others. For example, alcohol only kills vegetative forms of bacteria and is only marginally effective against certain viruses. When aerosolized, it poses a fire hazard. By contrast, chlorine based agents, such as dilute solutions of bleach or chlorine dioxide containing solutions, are rapidly active and effective against a broad spectrum of agents. Moreover, some disinfectants are active in the vapor phase, while others only disinfect what they contact directly.

In general, the poor air exchange found within static microisolation caging requires frequent bedding change and cage cleaning. At usual housing densities for mice, cage changing as a general rule needs to occur at least once a week, and in many cases two or three times a week, in order to maintain minimum standards for animal care (although there are some circumstance in which cage change frequencies can be extended). The type of bedding material can influence, to some degree, the interval between bedding changes and is particularly useful in addressing problems with high urine output associated with conditions such as diabetes found in some animal models.

Most bedding materials are waste products of wood or paper processing (Ref. 9). Commonly, they undergo some degree of processing with heat and, in some instances, chemical treatment that is effective in decreasing the numbers of microorganisms, as well as drastically decreasing the concentration of a variety of volatile organic compounds. This treatment, while useful, does not completely disinfect the bedding, and as a result, it must be further disinfected if it is to be used with complete confidence in microisolation cage housing. This is most commonly accomplished by autoclaving or through the purchase of gamma irradiated bedding. The bedding can either be autoclaved as part of the microisolation cage unit following the cleaning process, or it can be added at the time of cage changing. In the latter case, bedding is dispensed from a larger container that has been disinfected and placed in the laminar flow hood. Care must be taken not to contaminate the contents of the container during the process of removing bedding from it, as it could serve as a means of transmitting infection between subsequent cages.

Like bedding, food must also be disinfected. This is most commonly done by autoclaving, or it to can be purchased as a vacuumed-packed, gamma irradiated product. Food is usually of a natural ingredient type that is processed under clean, but not sterile conditions (Ref. 9). While food is unlikely to be contaminated with rodent specific pathogens, the possibility for such contamination does exist. More likely is the presence in the feed of various environmental and commensal bacteria that could prove troublesome for immunologically deficient animals, as well as the possibility of contamination with zoonotic organisms, such as *Salmonella* sp., that contaminate a wide range of food ingredients.

Commercially available rodent feed is usually provided in a pelleted form which is very dense and difficult to penetrate with steam during the process of autoclaving. While a significant degree of disinfection is possible, total sterility is difficult, if not impossible, to achieve and even more difficult to verify. Autoclaving causes significant degradation of labile nutrients within the food that are required for the health of the animal. This effect is less with gamma irradiation but is dose dependent. It is advisable to buy autoclavable rodent feed that has been fortified with additional amounts of nutrients that are destroyed by autoclaving in order to assure that nutritional deficiencies do not develop. If the feed is to be autoclaved with other cage components in the form of an assembled cage, it will likely be necessary to adjust the autoclave cycle times, temperatures and vacuum pulses to ensure that the degree of disinfection required for the feed is achieved. How the cages are placed in the autoclave, as well as whether they are assembled or unassembled, can significantly affect whether the food in all the cages will be adequately disinfected.

It is important to remember that in using microisolation caging, the degree of disinfection, whether it be by physical means such as autoclaving, irradiation or by chemical means, need only be to the extent required to assure that the materials are free of those organisms considered to be health or research threats to the animals. Absolute sterility may not be required in all cases. For example, in some instances it may not be necessary to autoclave certain cage components if they can be suitably treated with a disinfectant that effectively and completely kills all of the organisms on the institution's bioexclusion list. Similarly, if the process of mechanical cage washing and post-cage wash handling is such that organisms are killed by the process, either through hot water or the application of disinfectants, and if the organisms of concern are not human or environmental commensals, then such a process may provide sufficient decontamination. Further decontamination measures may be necessary if the potential for re-contamination after washing is unacceptable. Clearly, such actions must be predicated on a thorough and honest evaluation of the risk associated with such modifications of standard microisolation technique that relies on post-washing disinfection.

All systems for bioexclusion or biocontainment housing of animals have the potential to fail. It is the frequency and extent of such failures that must be weighed against convenience and operational costs. The decision to decrease the level of biosecurity associated with microisolation cage housing by making alterations in technique needs to be done in concert with those using the animals for research and with a clear action plan in place should an organism on the institution's bioexclusion list be found.

Ventilated Microisolation Caging

While microisolation caging that is unventilated (static) provides a usable bioexclusion housing method, it is more expensive to acquire and operate than conventional open caging. This stems in part from the need to change the cages more frequently due to the severely restricted air exchange that occurs within the cage. Some studies have indicated that as few as two air exchanges per hour may occur in a microisolation cage compared to 10 or more that can occur in open caging within a conventional animal holding room (Ref. 10). This results in wet bedding, elevated intracage environmental temperatures and increased relative humidity over ambient conditions in the room. In turn, there is an increased rate of bacterial decomposition of urine and feces and the development of aesthetically unpleasing environmental conditions, which may adversely impact some research protocols and animal well-being. The rate at which these conditions develop is dependent upon a number of factors including the number and size of animals within the cage as well as the frequency of bedding change, cage sanitation frequency, and ambient environmental conditions.

Much of the cost associated with using microisolation caging is centered around the cage/bedding changing process, and for that reason, the more frequently this must be done, the more costly the system is to operate. The addition of forced ventilation into microisolation caging effectively dries out the bedding material within individual cages allowing a greater interval between cage changing—perhaps 14 days or more. Moreover, this increased air exchange rate, which can be as high as 30 to 70 changes per hour, will effectively dilute out noxious gases generated from the bedding, as well as remove heat and humidity from the cage itself. Some ventilated microisolation caging also incorporates captured exhaust systems whereby air exiting the microisolation caging is drawn up and filtered for subsequent discharge into the room or into the exhaust system exiting the room. Such systems can reduce the amount of infectious material that could be released into the room during the course of normal operation of the unit or filter it out before discharge. If the discharge from the individually ventilated racks is channeled into the exhaust system for the room, heat and humidity, as well as odors, will be removed from the animal holding room thereby allowing lower ventilation rates and reduced utility costs. Such discharge will also minimize the chance of build up of odor causing gases as well as heat and humidity in the room, which is particularly important if the ventilated racking system does not have an independent air supply but rather takes its air supply from the room itself.

There are many designs of ventilated microisolation caging currently available. They differ principally in how air is propelled through them and the tightness of coupling of the cage to the ventilation system. A common design of ventilated microisolation caging utilizes a portable blower and HEPA filter to provide highly filtered air directly to the cage through a coupling that attaches to the cage, penetrating the cage barrier. This can be done at the level of the filter top or through the base of the cage itself, where it may be combined with automatic watering. The use of automatic watering can be labor saving, but does pose difficult challenges for disinfection, especially if immunologically deficient rodents that are susceptible to opportunistic organisms are housed in the caging system. Methods for assuring that the automatic watering system is adequately disinfected on a continuous basis are far

from risk free, and hence, this type of caging is more commonly used with immunocompetent rodents.

Air may also be supplied to the cages in an indirect fashion by discharging an air stream from an individually mounted rack blower system at high velocity above the filter on the cage, thereby pushing air through the filter into the cage itself. This type of system eliminates the chance of contamination occurring through inadequate disinfection of ventilation couplings to the cage, but the ventilation efficiency is directly dependent upon the resistance of the cage filter, which in turn is affected by its cleanliness and length of use as discussed previously. Such systems should have their cage top filters changed on a regular basis.

In addition to having forced air supply, some ventilated microisolation systems also have captured exhaust. Under such circumstances, there is either a direct cage connection pulling air out through a filtered connection into the cage, or the system may utilize a small vacuum port located above the filter top in an area of the cage not associated with the air supply to the cage. In the latter circumstance, there is no direct connection between the exhaust port over the cage and the cage itself. Rather, air must pass out of the cage through the filter, and then be pulled into the exhaust port above the filter. Additional filters are often located on or in the rack itself. This latter type of exhaust will not capture all air passing out of the cage though the filter, but will provide a significant reduction in heat load, moisture, and gaseous contaminants that pass out of the cage through the filter and escape into the room.

Recently, a hybrid between static microisolation and ventilated microisolation caging has been developed. This type of cage contains a filter on the front of the cage low in the cage base and a second filter in the back near the top of the cage base. The filter in the back of the cage connects directly into a chamber in the back of the rack into which a slight vacuum is drawn through attachments to the room exhaust system. Air is drawn into the front of the cage by the convective loss of hot air generated from the warm animal bodies contained within. It then exits the cage through a second filter high up in the back of the cage and is drawn into a chamber in the cage rack that is connected to the room exhaust. Hence, airflows in the front of the cage and out through the back, with the exhaust air containing heat, moisture, and gaseous materials being captured and directed into the room exhaust. This system eliminates the need for a separate HEPA filtered air and exhaust blower system.

With the exception of this latter system, most ventilated microisolation systems require that the internal components of the cage be under positive pressure with respect to the exterior of the cage. Hence, air delivered directly into the cage or blown in through the filter will cause the internal volume of the cage to be under positive pressure and force air from inside the cage to come out through the filter or any other areas from which it can escape. Thus, improperly fitted lids or poor seals in other areas will allow particulates from within the cage to escape and contaminate exterior surfaces. Rips or other defects in the cage filter will also allow the forced release of particulates from within the cage. This is particularly important if such systems are considered for use in biohazard containment, since absolute containment cannot be guaranteed. This can be addressed to some extent if the cage is directly coupled to the exhaust system by running the exhaust at a greater negative pressure than the positive

pressure of the supply air. This causes air to be drawn in through the filter either from a lower pressure positive supply or from the room itself.

A significant part of the purchase cost of ventilated microisolation systems resides in the HEPA filtered supply and exhaust systems mounted on each rack. If a sufficient number of ventilated microisolation racks are to be purchased, the installation of a central HEPA filtered air supply and exhaust may be more cost effective. This also eliminates the risk of blower failure at unpredictable times allowing the possibility of heat build up within the caging and adverse effects to the animals. If a central system is installed, provisions for pressure and mechanical failure monitoring of the system, as well as redundant blowers and auxiliary filtration equipment, are essential in additon to the necessary alarm systems and automatic transfer equipment. Such an air handling system can also be used to power other bioexclusion and biocontainment systems such as isolators. It is critical that emergency power provisions be available for such a system in the same way that it is critical that emergency power also be made available for individual blower systems on ventilated racks.

Ventilated caging, in addition to having the advantage of providing a more refreshed and drier environment, will allow greater animal density per square foot within holding rooms as compared to static microisolation or conventional open caging since little or no space between the cage and shelves on the rack above the cage is required or desirable. Moreover, if captured exhaust is provided rather than allowing exhaust air from the caging or the exhaust blower to return to the room in which the rack is held, the ventilation requirements for the room will be less and allow both operational cost savings as well as construction cost savings.

While there are many advantages to ventilated microisolation caging systems, there are a number of significant disadvantages that must be addressed. One of these is the initial purchase cost of such equipment. Individual rack costs, including portable HEPA filtered blower systems and all associated cage components, can be as high as $30,000 a unit. In addition, all of the support requirements for static microisolation caging must also be met for ventilated microisolation caging. These include: HEPA filtered laminar flow workstations, sufficient autoclave/disinfection capabilities, and the requirement for aseptic technique in manipulating caging. As with microisolation caging, cage changing is often slowed significantly by the need to only open one cage at a time for manipulation and the need to maintain the cages, following disinfection, in a manner that ensures that they will not be re-contaminated. The same difficulties associated with health monitoring programs in static microisolators apply to ventilated microisolators.

However, even considering these issues, when operated correctly, this type of animal housing will provide more animal housing capacity in the same space as compared to both open cage and static microisolation housing, which may override the initial purchase price and high operating cost issues in those institutions were additional animal housing space is not available or prohibitively expensive to construct. Moreover, in the research environment, it provides effective protection from the introduction of unwanted microorganisms from surrounding cages and the ambient environment, providing that the appropriate microisolation procedures are adhered to.

ISOLATORS

Isolator technology has been available since the early 1900s and has been used for maintenance of laboratory rodents since the 1950s. Its use was initially confined to the maintenance of germ-free animals, but has expanded to use with animals of other microbiological profiles. Initial equipment designs and processes were very cumbersome, and failure to maintain the appropriate microbiological environment was not uncommon due to equipment and technique limitations. Over the last 20 years, the use of isolators has increased dramatically due in large part to improvements in their construction and the availability of pre-disinfected, vacuumed-packed supplies to make it possible to operate an isolator without the use of an autoclave.

An isolator is an enclosure constructed of either flexible or rigid material (often plastic) that is used to surround a group of cages that contain animals. In order to manipulate the animals inside the isolator, built-in sleeves with attached gloves are placed on one or more sides of the isolator. Decontaminated/disinfected supplies and equipment are passed into the isolator and waste materials removed from the isolator through a transfer port in which appropriate disinfectants can be applied to the materials to be transferred. This disinfection process chemically disinfects only the surfaces of the material; therefore, it is important that the materials themselves be known to be free of the organisms to be excluded. Air that enters or exits the isolator is filtered most commonly with a HEPA filter, although older isolators may use multiple layers of paper or polyester filter material to achieve varying degrees of filtration and pressure control.

While flexible film isolators are the most common, semirigid isolators, which are rigid boxes with the exception of one flexible side, are being used in increasing numbers due to their rugged construction and the more efficient use of space allowing greater numbers of animals to be contained in the same area as compared to flexible isolators. Semirigid isolators are also easier to stack on top of each other, allowing for more efficient use of floor space.

Isolators come in all sizes and can hold from two to several hundred cages. Each isolator functions as a small animal holding room in which animals are maintained in open cages. Since the cages share the same microbiological space, problems associated with health monitoring of microisolation cages are avoided. Since there is no direct contact between the animals and the exterior environment surrounding the isolator, the chance of fomite transmission from exterior sources is essentially eliminated. As long as the disinfection status of all materials passing into the isolator is assured, there is little chance that contamination will unknowingly be brought in on such materials. As with any other animal housing system, animals remain the most likely source of animal related diseases, as a result, animals used to populate isolators must be free of the microorganisms to be excluded.

Isolators are commonly kept under positive air pressure in order to minimize the chance of any contaminants being pulled into the isolator should a small hole develop in a glove or other surface of the isolator. Isolators may, however, be run under negative pressure for biocontainment purposes. Negative pressure prevents hazardous materials from escaping

should a hole occur in the isolator or the gloves. Flexible film isolators must be specially constructed to support their operation run under negative pressure.

After set up, the isolator must be disinfected in such a manner that all internal surfaces (including filters) are free of organisms that are to be excluded. The integrity of the isolator should be regularly assessed not only visually, but also through leak detection methods. For example, by introducing helium gas in small quantities into the isolator, the external surfaces can be scanned with a helium gas detector to discover leaks. Regular replacement of the gloves while the isolator is in service also will help to minimize the likelihood of breaks in the system since gloves are the most common point of failure. A varying percentage of new gloves from manufactures already have holes or manufacturing defects that will develop into holes. For this reason, all gloves to be used on isolators must be visually screened and/or pressure/leak tested.

For bioexclusion, isolators have a number of advantages. Compared to barrier rooms, the risk of contamination introduced by personnel into an isolator is greatly reduced since direct human contact is eliminated. In addition, fewer animals are placed at risk due to the size limitations of the isolator. Specialized decontamination procedures and clothing are not required for personnel, and personnel access does not need to be restricted. In comparison to microisolation caging systems, isolators are not very technique dependent, since during the course of normal operations, cages are not regularly opened to the external environment. As a result, there is essentially no possibility of direct human or environmental contact.

Unlike microisolation caging systems, husbandry manipulations, including cage and bedding changing, are done within the barrier (i.e., isolator) itself. If animals with limited microflora are being maintained, it is common to actually clean the cages within the isolator. This is because the more often material is brought across the barrier, the greater the chance that incomplete disinfection will occur and allow for the introduction of unwanted microorganisms.

Some isolators are operated with the use of external cage cleaning, whereby cages are removed from the isolator, cleaned, wrapped, autoclaved, and then sprayed back into the isolator either through the transfer port or by using a transfer isolator to hold larger amounts of clean cages or supplies. This latter method increases the time and labor costs associated with normal husbandry practices—and imparts more microbiological risk.

Wherever possible, all experimental manipulations are done within the isolator itself. With a bit of practice, most manipulations that would be done outside of an isolator can be done in the isolator. This includes such delicate procedures as tail vein injection in mice, ear punching, blood sampling, and even some surgical procedures. Some financial assessments of isolator housing indicate that operating costs can be significantly less than ventilated microisolation housing; however, true cost comparisons are difficult to come by.

Isolators do pose certain disadvantages. Isolators do not provide as great a housing density per square foot as ventilated microisolation caging. Housing density can be even more restrictive if the rooms in which the isolators are placed do not have ceiling heights that allow

them to be stacked two high. However, unlike other animal housing systems, isolators can be placed in relatively inexpensive building space since they do not require as sophisticated mechanical systems as those needed for typical animal housing. The ventilation system for isolators, like ventilated microisolators, must be provided with emergency power; and if a centralized ventilation system is used, some redundancy must be planned for in the event of mechanical failure.

Animals removed from isolators and taken to investigator laboratories then returned to isolators can be contaminated in the laboratory and can contaminate the isolator if returned, even if removed and transported in microbiologically secure containers. Under such circumstances, transfer of animal between isolators should not occur. Isolators do not allow instant removal of animals for manipulation outside the isolators. The process of transferring animals out and back into isolators is somewhat more cumbersome than the use of microisolator caging systems, but is not prohibitive. If one cage in an isolator is contaminated, all cages in the isolator will likely become contaminated since there is no barrier to stop transfer.

Overall, isolators can be an effective alternative to microisolation cage housing. They are well-suited for both biocontainment and bioexclusion roles. The lack of direct contact with animals greatly reduces risk of contamination, as well as risk of exposure of employees to allergens and other materials. Given their high degree of contamination control protection, they are well-suited for the role of quarantining newly arrived animals since this function can be done in undedicated or less sophisticated space and still allow manipulation of animals during quarantine, even for research purposes. They also serve well in maintaining valuable stocks of animals in a secured environment. Founder animals from transgenic programs can be securely maintained in isolators as a safeguard to disastrous loss from infectious disease of animals maintained in other housing systems. Their role in biocontainment has been well-established and in the case of very hazardous agents, may be further enhanced by placing them within a limited access area or barrier.

Finally, they may provide an effective alternative to microisolation cage housing where ultimate separation between groups of animals while maintaining ease of manipulation is important. Even when complex manipulations cannot be carried out within an isolator, it is relatively easy to remove the animals from the isolator in disinfected containers to a secured work area such as a laminar flow hood for more complex manipulations, and then return the animals to the isolator using aseptic techniques and a suitably disinfection container. In such cases, lapses in any technique would be limited to that isolator and can be easily traced.

OTHER ANIMAL HOUSING SYSTEMS

Ventilated Cabinets

A variety of cabinets suppling HEPA filtered air to a small enclosure accessed through doors or through a zippered curtain have been used for many years. Most recently, they have been used in conjunction with microisolation caging, but are still used by some with open caging. These systems provide little other than a source of HEPA filtered air to the environment

directly around the caging. They provide no reasonable contamination control between cages (other than if microisolation cages are used; in which case, those cages themselves are the point of contamination control) or contamination control when the doors to the cabinet or the access panel to the cage housing area is opened. When the system is opened, all caging is exposed to the room in which contaminants can reside. In the case of open caging, there is no control of airborne cross-contamination or particulate contamination between cages, and exposure is relatively easy once the cabinet is opened. Microisolation caging housed in such cabinets must be handled using the same techniques and procedures as previously described.

Ventilated cabinets may or may not filter exhaust released from them, and they will generate heat by virtue of blower motors used to provide HEPA filtered air. This heat must be compensated for by the room ventilation system. If directly vented out of the facility through HEPA filtered exhaust, they may provide some degree of personnel exposure protection for static microisolation caging in biocontainment facilities. The purchase cost for such units is quite high, and durability, as well as maintenance issues, are an important consideration. Overall, there seems to be little rationale for their use and little protection provided by them from a microbiological standpoint.

Mass Air Displacement Rooms

Small mass air displacement enclosures the size of small rooms that can be erected within an existing space have been used for many years. Commonly, a blower with HEPA filter extracts air from the existing space outside of the room and discharges it into the room in large volumes. In some systems, the air within the room is recycled through the unit much in the same fashion as would be done by a portable room air cleaner used in some homes. Depending upon the ventilation system supplying the larger room in which the enclosure is construced, heat generation by the HEPA filtered blower may be an issue in maintaining an appropriate temperature within the space. Exhaust air is released under a gap several inches high around the perimeter of the room.

Entrance to the space is usually by a flexible or zippered door, and the construction of the room itself is that of a large plastic bag with the bottom open, hung on a frame often constructed of plastic piping. Other than providing HEPA filtered air in large volumes to the space that theoretically washes out particulates, there is little that such enclosures do to control airborne cross contamination or fomite contamination. Once an unwanted organism enters, there is little to prevent it from spreading rapidly between cages. Their use with static microisolator caging or open caging is hard to rationalize.

Cubicles

A cubicle is a small room within a room that is accessed through a set of doors that either slide up vertically or open outward similar to a pair of French doors. Cubicles may have their own independent air supply and exhaust, or may obtain their air supply from the room in which they are located by pulling it under the doors and up through exhaust registers mounted in the ceiling of the cubicle. In this context, they act much like a chemical fume hood in that

the space under the door develops a face velocity in excess of 100 feet per minute; theoretically preventing the outflow of particulates into the larger room.

In theory, cubicles will prevent airborne cross-contamination between adjacent cubicles. In practice, such separation is lost during normal husbandry procedures. Once the cubicle door is open, all control of airflow is lost. There are no provisions for preventing fomite or direct contact transmission of agents. There is probably some limitation of airborne cross-contamination while the doors are closed, and hence, there may be some limited benefit as compared to open cages within a barrier or conventional holding room. The use of cubicles is best combined with other bioexclusion systems. Cubicles may have some limited value where separation of species is important. From a construction standpoint, they are costly and provide limited animal holding space. Other design variations which provide independent air supply and exhaust to each cubicle suffer from the same limitations as previously described.

COMBINING BIOEXCLUSION HOUSING METHODS

Under certain circumstances, the combining of two bioexclusion housing methods can be used to further reduce the risk of contamination by unwanted microorganisms. By combing two methods, the weakness of one system can be negated by the strengths of the second system. Most commonly, this is configured by placing either microisolation caging or isolators within a barrier. By doing this, the barrier room/facility serves as a means to limit personnel access and to assure that personnel and equipment entering the barrier have a reduced risk of acting as fomites for transmission of unwanted microorganisms into the barrier. This in turn decreases the concentration of contaminants (reduces contaminant pressure) around the primary bioexclusion system—the microisolation cage or isolator. Moreover, the required use of clothing of a known disinfection status by personnel entering the barrier minimizes the likelihood that their clothing will serve as a mechanism of transmitting microorganisms into the immediate environment surrounding the microisolation cages or isolators. Similarly, by applying strict disinfection procedures to equipment or other materials entering the barrier in a disinfection lock or port, contamination from these sources can also be greatly reduced. Entry into the barrier is by its very nature inconvenient; discouraging unnecessary use of the facility, and hence, the number of entries into the barrier.

The value of combining a barrier with other bioexclusion housing systems is lost if entry and exit of personnel and materials from the barrier is compromised for the sake of convenience or operational efficiency. This is an important consideration since the cost of erecting a barrier around microisolation caging or isolators is significant, as is the unproductive space occupied in doing so. This is translated into increased operational costs and per diem rates. For this reason, such dual systems may only be appropriate when the consequences of failure are so significant that it would devastate critical research programs or resources.

Some examples of when such compounded bioexclusion practices may be appropriate are maintenance of critical founder transgenic animals, operation of breeding colonies used for distribution to multiple programs and institutions, and production of biological materials for potential widespread human or animal use. Similarly, such dual rodent housing systems may be appropriate for certain types of biocontainment housing of animals, especially if the agents

being used are rodent specific or have important human zoonotic implications. Obviously, the systems used in these circumstances would have to serve a dual role: acting as bioexclusion as well as biocontainment. Both barrier entrance and exit would need to take this dual role into consideration, as would operations such as cage changing and waste handling, regardless of which systems were used.

SELECTING THE APPROPRIATE HOUSING SYSTEM

One must take into account a variety of factors in deciding upon the most appropriate rodent housing system to use. The weight placed on any of these factors is specific to each institution and its resources as well as research programs. While some generalizations can be made, none would assure or exclude the use of any system for housing rodents destined to be used in research.

Institutional Exclusion List

No decision regarding rodent housing systems can be made without first deciding what organisms will be excluded to prevent any possible adverse impact to research programs at the institution. Making such choices is not simple since there is only a limited amount of peer-reviewed literature demonstrating research interference in the absence of clinical disease. Moreover, some organisms would only be of concern to a very limited number of research projects, in which case it may be more cost effective to provide animals used in such projects with a higher level of bioexclusion housing than the rest of the population at the institution. This approach would minimize costs to the majority of the research programs by not imposing an unrealistic exclusion standard on the entire institution.

A detailed review of the organisms of concern and factors to be considered in ranking them, as well as the risk associated with them, is beyond the scope of this discussion; however, useful information is available from a number of sources to assist in this deliberation. (Ref. 1-9). The construction of such lists should be based on sufficient evidence documented in the peer-reviewed literature in order to establish a range and magnitude of effects caused by any organism considered for exclusion. Extrapolations from other organisms in related species, genera or families, as well as the presumption that if something can be detected it should be excluded even if there is no hard evidence to suggest widespread effects, should be avoided. This only leads to an ever lengthening list of organisms that upon detection would have to be properly acted upon. The consequences of taking the necessary steps to eliminate the organism could dramatically affect the research program, perhaps much more than whatever effects the organism may have had.

Institutional Response to Positive Findings

In selecting a rodent housing method, an institution must consider how it will respond to finding any organism on its exclusion list in the population. Since laboratory testing is not perfect (i.e., false positive results can occur), no action should ever be taken to destroy a colony unless the results are confirmed by testing additional samples and testing by alternative means. If an open caging system is used either in a barrier room or in a non-

barriered area, the detection of a positive finding could easily mean that the organism is spreading through the naïve population; and depending upon the nature of the organism, it may already be too late to take any steps to prevent further or complete exposure. Under such circumstances, the whole population will likely be affected; and if the organism was placed on the list, the response would likely have to be to eliminate most, if not all, of the animals. If this course of action has not been previously considered and decided upon for each organism, then one must question whether it was appropriate to put the organism on the exclusion list at all. If some lesser measure with significant recurring risk is decided upon after finding the organism or if the institution decides not to take any action, then all of the costs associated with detecting the organism, and to some degree the efforts put in place to exclude it, were wasted.

Research Requirements

Not all types of research have the same requirements when it comes to animals and risk factors. Clinical disease that results in transient or permanent disability or death of the animals will unquestionably cut across most types of research. Fortunately, relatively few rodent diseases result in clinical manifestations; although, the number of organisms capable of such actions can be increased if certain predisposing conditions are applied to the animals, such as immunodeficiency. Some organisms, particularly certain viruses, can have subclinical effects on a cellular or sub-cellular level, especially on the immune system. In the case of many viruses, infection and research effects are transitory and may disappear following the development of immunity, while others cause persistent infection that may result in persistent effects. In any event, the variable spread of infection through a population during the course of experimentation can introduce unwanted variation into certain studies, altering the magnitude of results and increasing the number of animals needed to demonstrate significance.

Investigator access to animals must also be considered, as well as the risk imposed by common-use equipment and extensive manipulations. In some cases, the types of equipment needed in a particular research project will inevitably introduce greater risk of contamination to the animals used in that research project than would be acceptable for the rest of the research population of that species at an institution. If the number of studies that would be potentially affected by the organism are limited, then it may be appropriate to use a more restrictive bioexclusion housing system (e.g., isolators) for those studies, while applying another type of housing (such as microisolation caging or even open caging within a barrier) to the rest of the studies in the institution.

At some institutions, very short term studies are the only type of research conducted. The duration of stay at the institution for any group of animals may be only a few weeks to a few months with no long-term presence of any group of animals. Such rapid turnover will often result in the elimination of animals well before an unwanted microorganism can spread beyond a single infected group. Housing of animals in such circumstances might not require extensive bioexclusion housing equipment, but rather a frequent and comprehensive disinfection program to assure that any unwanted microorganism does not reside in the holding facilities, the caging or the experimental equipment.

Available Physical Facilities

While new research facilities are always being erected somewhere, renovation of older facilities to meet new research needs is more common. Although in many cases, funds are not available to renovate facilities, and instead may only be available for caging and some associated support equipment. Development of barrier facilities under the latter instance can be difficult and fraught with compromises that detract from the integrity of the barrier.

Facilities with many small rooms are extremely inefficient for microisolation cage or isolator housing. Commonly, in such small rooms, a choice must be made between providing the necessary laminar flow change station or the addition of another rack of microisolation cage housing. Such limitations become particularly onerous if double sided racking or ventilated microisolation caging is used. The ability to manipulate racks of caging within small rooms makes cage changing, as well as research manipulation, difficult and encourages incorrect use of the system. Without dedicated central air supply or exhaust to the ventilated microisolator racks, the addition of a laminar flow workstation to small room and the heat released from ventilated microisolation caging using portable blowers can result in unacceptable heat build up and odor retention in the room. While on paper, housing densities in such small rooms may seem to be acceptable and perhaps appear to increase overall research animal housing within the facility, the inefficiencies in animal husbandry and research manipulation add substantial cost to the operation, which is eventually reflected in per diem rates and lost research time/productivity.

It is important to realize that with microisolation caging, bioexclusion is accomplished at the microisolation cage level. For this reason, constructing larger rooms that accommodate the larger numbers of units, as well as laminar flow hoods and the necessary open floor space, can greatly increase the efficiency of operation. Similarly, flow of people, equipment, and materials through each room can be facilitated in larger rooms and needs to be considered as well.

Likewise, isolators, while perhaps not requiring a change station, often require access to at least three for their sides for husbandry and research manipulations. Narrow rooms may preclude placement of isolators across from each other while still allowing sufficient aisle space for movement of equipment and supplies or turning of the isolators for access to their sides. To be truly efficient, double stacking of isolators should be considered. Under such circumstances, a ceiling height of 10 to 14 feet is required, but in many cases is not available in existing structures.

Some types of cages, such as microisolation caging, require significant disinfecting or autoclave capacity close to their area of use. Logistically, this should also be within a convenient distance of any cage washing equipment used in processing soiled caging. Limitations imposed by the structure of the building on the location and size of any such equipment can affect its usefulness, as well as its cost of operation. Moreover, if clean or disinfected caging must travel through areas of the animal facility where it can become

potentially contaminated, significant steps may have to be taken to ensure its continued disinfection status which will increase the potential risk and cost.

Operational Limitations

Operational limitations are often the result of funding limitations. Many rodent housing systems represent very large capital equipment expenditures. Some institutions may only be able to afford a limited number of such systems and will decide to use a system that is less costly to acquire but may have greater operating costs.

Similarly, funds may not be available to acquire sufficient autoclave capacity for optimal operation of a microisolation caging system, resulting in the need to find alternative means and levels of disinfection. Such operational shortcuts may introduce more risk into animal housing operations, which may or may not be acceptable. In some cases, labor intensive systems may not be sustainable at certain research institutions due to the difficulty in finding adequate personnel or funding for the necessary positions. Under these circumstances, housing systems that impart a greater level of risk may become necessary in order to sustain the research program.

Available funding for health monitoring may also affect the choice of housing system. Systems such as microisolation housing make health monitoring difficult and can greatly widen the period of vulnerability in detecting the presence of an unwanted microorganism. In come cases, cages containing infected animals could be eliminated from the colony by the time the health monitoring system detects their presence. If detection is further compromised by limiting the number of animals that can be sampled, the frequency of sampling or the number of cages included in the sentinel monitoring system during any given sampling time, undesirable research interactions caused by unwanted microorganisms can occur and persist, thereby defeating the purpose of more costly but more biosecure housing systems which decrease the likelihood of cross-contamination or detect it more rapidly. Unfortunately, it is all too easy to be lulled into the assumption that contamination is not present as long as animals are housed in some form of bioexclusion system in the absence of regular or thorough evaluation of animal health to test this assumption. In choosing a rodent housing system, one also has to make the determination of how hard the institution will look for evidence of failure of the system.

Level of Risk that is Acceptable

All rodent housing systems are subject to failure. In turn, there is always some risk to the research program from such a failure. Some components of the research program can tolerate less risk than others. Unfortunately, the willingness to accept some level of risk and to define those factors in the housing system chosen that introduce such risk is seldom seriously undertaken. If given a choice, most researchers would prefer not to have any risk at all from the presence of microorganisms. That choice, however, would require the screening for and elimination of an almost unending list of organisms and the imposition of extremely stringent and multilevel rodent bioexclusion housing.

To make the choice as to what type of bioexclusion housing and practices are to be used, the institution must decided what balance is to be struck between capital equipment and operating costs as compared to the costs associated with research interruption due to contamination with certain organisms. The willingness of researchers to accept the inconvenience imposed by complex bioexclusion housing systems must also be considered. If the exclusion list is confined to rodent specific organisms and if certain opportunistic infections with limited research impact can be dealt with on a statistical basis in most research projects, the type of bioexclusion housing selected may be less complex or the system may be less complete than if more loftier goals are selected.

Conclusion

The decision as to what type of housing system is to be used for rodents at a research institution is filled with complexity. In general, the large numbers of personnel requiring access to the animals, as well as the wide range of research programs present in most research institutions, make some form of bioexclusion housing that limits cross contamination necessary. A system that prevents or at least greatly reduces the chance of cage-to-cage transmission or group to group transmission of microorganisms is increasingly appropriate in an era of complex transfers of animals and biological materials between researchers both within and between institutions. The recent dramatic increase in epizootics of common rodent infections suggest that previous control strategies that rely on a well-screened supply of disease free rodents housed in open cages in many small rooms may no longer be appropriate. The choice of what housing system to use under what circumstances is institution specific and depends heavily upon a variety of factors and resources. An institution should not rush into adopting a system or systems without developing a sound institutional philosophy as to what the systems are expected to exclude and are capable of accomplishing, as well as the operational assumptions and costs associated with these systems.

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