Let's talk about another type of containment device that is used quite commonly, micro-isolators. This involves containment at the cage level. One thing to remember about micro-isolators is that there is an imperfect seal of the lid to the cage which allows uncontrolled escape or input of air. It allows inflow of contaminants from the cage surface or, conversely, things going out during manipulation, especially in a laminar flow hood. Proper use of a micro-isolator system is very training dependen. It is technique dependent and everybody has to follow the same technique.

We can demonstrate imperfect seals in the following manner: take a micro-isolator cage and put a heavy rubber band around it to make sure it is seated well; then release smoke from a smoke stick, and you will find smoke inside the cage and not because its coming in through the filter at the top.

When you open a micro-isolator cage in a hood with all the supplies that you need to change the cage, you're going to be removing whatever is in that cage all over the supplies, all over the operator's arms, etc. You need to do the cage changing in a vertical flow hood, not a horizontal flow hood. The operator must be protected from hand and arm contamination. It is usually a two person process if you're dealing with bio-hazardous materials. Depending on the nature of the biohazard, you may need complete personnel gowning and post task decontamination.

Filters on micro-isolators come in different types. Most cage filters are dust filters. They're usually spun bond polyester made with a continuous thread lying down in a random fashion to form a sheet, but the average pore sizes can range all over the place. Those filters get more efficient the longer they're used because the pores get blocked up with dust and other particles. They are not high efficiency particulate air (HEPA) filters. How often you change filters and the type of filter material make a difference in the degree of bio-containment you really have. Organisms such as bacteria can lodge on filters and grow through them; sometimes you can find the bacteria on the other side of the filter.

Isolators hold small groups of cages in a controlled environment. They're easier to monitor than a barrier room or micro-isolators, falling somewhere in between. Isolators are under negative pressure in the bio-containment mode. They are available as flexible films or semi-rigid. There are more designs than you know what to do with. Some are even available for rather large animals, though they are generally used with small or very young animals. Isolators insure minimal operator exposure to the agent. The risks are primarily through breaks in the glove. Exhaust air filtration can be matched to the type and level of the hazard. Generally you need to fit them with HEPA filters.

Let me end with this concept. You can begin to combine various pieces of equipment into what I call a barrier within a barrier. It combines a barrier room with other forms of containment devices, such as isolators or micro isolators, resulting in additional barriers to keep the organism from escaping. These systems are generally expensive to operate and are usually only suitable for small numbers of small animals. It may involve total personnel enclosure into ventilated suits. Husbandry practices and waste handling can become very cumbersome. We have one at Charles River in which everything is done inside the barrier, including examination of tissue

cultures, use of incubators, and transfer of materials into bio-containment hoods.

Special Containment Devices for Research Animals
Breakout Session

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Our group first covered barrier ooms which were acknowledged to have considerable value. Barrier rooms, by definition, are sealed rooms that contain what is within. They have air and waste handling capability, allow for disinfection and sterilization of equipment and implements, provide for decontamination of personnel and have back up power. Entry and egress are through air locks. Established standard operating procedures appropriate for the degree of risk and for the task, are rigorously followed. Our group believed that barrier rooms were most appropriate for containment of large numbers of animals or of large animals themselves (i.e., livestock). They are also effective for pathogen or chemical containment. The effectiveness of barrier rooms is heightened when one applies additional layers of protection using, for example, barrier cages, such as micro-isolators, or flexible film isolators. This scenario creates a barrier within the barrier. Barrier rooms are especially valuable for containing biohazards associated with large animals. Livestock containment presents challenges related to the sheer size of the animal and the considerable volume of waste products. Large animals discharge feces and urine from relative great height or pressure. When excrement is splashed, biohazards can be disseminated by aerosol droplet formation. From the perspective of the researcher and the animal caretaker, the use of personal protective devices within a barrier room provides the safest and least encumbered access to livestock.

On the other hand, we acknowledged barrier rooms to have problems. They are expensive to build, cumbersome to use, and energy intensive to operate. One also should not take for granted that they can provide containment or exclusion interchangeably. Often, engineering and physical modifications may be necessary to convert the area to the desired use.

Showers are frequently a component of a barrier room or facility. The value of showering as a decontamination procedure was questionable. For example, body orifices, such as the nose, may not be adequately cleansed by showering. This may create a break in a barrier operated to exclude certain microorganisms. Personnel compliance may be a problem. People may avoid taking a shower. Consequently, we agreed that the greatest value of showering was in forcing people to change their attire and thereby prevent fomite transmission of harmful agents on the clothing.

One participant in our session made the point that simply designing or having a barrier room was not sufficient. One needed to oversee the planning and construction of the facility, because contractors may cut corners and one could end with an ineffectual barrier room.

Flexible film isolators received much attention from our group. These containment devices receive filtered air and contain glove ports and an air lock. They are suitable for housing small numbers of small animals, but are versatile. They can be used for containment or exclusion, for studies involving microbial agents or chemicals, and can adapt to BSL2 or BSL3 activities. Isolators can be used to subdivide large spaces into several smaller spaces. The use of multiple flexible film isolators allows animals to be isolated from others of dissimilar microbial or genetic backgrounds, separate sources or different research uses. The down side to isolator use is that they are labor intensive and present problems with dexterity due to the gloves used. However, vendors are developing thin and durable gloves. Isolator technology has been enhanced by the development of prepackaged food, water and bedding that can be easily introduced into the device.

Some members of our group expressed concern about the containment of livestock in flexible film isolators. These devices can only house animals up to a certain size and present challenges in accessing animals, collecting and handling waste, and maintaining an environment that is wholesome for the animal. Isolator durability was a concern, because large animals can exert tremendous forces of wear and tear.

The cost of isolator containment devices was also a concern. The purchase price is clearly high, but considered relative to the cost of purchasing a barrier caging system with appropriate change-out stations (i.e., Class II laminar air flow cabinets) it may be affordable. Costs of consumable supplies, such as gowns, gloves and disinfectant, should also be less when using flexible film isolators.

Several devices or rooms were considered undesirable for pathogen or chemical containment. Mass air displacement rooms and cubicles were inappropriate for the task. Individually-ventilated microisolator caging systems, open-fronted laminar air flow cabinets and positive-pressure laminar air flow racks were not considered containment devices. Vibration, air turbulence and noise from these units could have an effect on the well-being of animals. With respect to laminar air flow racks and cabinets, variability in device performance depending on make, manufacturer and maintenance of the device was a cause of apprehension. A major concern was that laminar air flow systems can have their protective integrity disrupted by eddying of air when doors or cages were opened or when arms, hands or implements were introduced.

BioBubbles are portable, HEPA-filtered, mass air displacement, laminar air flow containment devices that can serve as barriers or clean rooms. They are popular at a number of institutions. We had no group consensus on BioBubbles. One individual regarded them as inappropriate as containment devices. Several other individuals gave endorsements.

In many of our institutions and in many of our containment operations, micro-isolator cages are our primary caging system. Some concerns were expressed whether micro-isolators were truly exclusion or containment devices when eddying could occur when they were opened in a laminar air flow work station. Individually-ventilated micro-isolator cages were not seen as adequate for containment or exclusion. The manufacturers have been innovative in developing cages with solid lids with gaskets to seal the

lid-bottom interface, but air balance was not considered reliable and leaks are a risk.

Proceedings of the 4th National Symposium on Biosafety

Animal Biosafety Levels 1-4: An Overview

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This presentation will be a very rapid run through of the current biosafety recommendations for persons working with vertebrate animals.

The slides used in this presentation will be available for persons who are involved with training. Since training is a real integral part of almost all successful programs today, we have reproduced them in this Proceedings as CDC's gift to you (see Appendix).

Yesterday Dr. Martin Favero said that the most frightening words in the English language are "I'm here from the government and I'm here to help you". I just wanted to make sure that you realize we really are here to help you. I will be joined in a few moments by Dr. Bobby Brown, a CDC veterinarian, who will pick up where I leave off. I will begin with the introduction to Animal Biosafety Levels 1 and 2. Bobby will talk about ABL-3 and then give you a visual perspective of some of the ABL-4 issues.

Vertebrate Animal Biosafety Levels are designated as ABSL-1, 2, 3 and 4. These are based on a combination of recommendations that have grown out of experience when we talk about the practices and procedures which are so crucial daily.

We talk about safety equipment or the primary barriers; the facilities themselves - how they are designed and run - are the secondary barriers. This would be applicable for all vertebrate animals for experiments where we are using infectious agents, specifically those that will cause infections in humans. The ABSLs provide for increasing levels of personnel and environmental protection, and they are to be considered as minimal standards for safe work with infected laboratory animals. You take the information that's in the CDC/NIH guidelines (Biosafety in Microbiological and Biomedical Laboratories) (BMBL) as Step 1, and then you adopt it to your own facilities and make it user-friendly.

The standard practices and procedures that we follow are simple.

1. We wash hands after handling animals, collecting blood, and before leaving the animal facility.
2. We ban such enjoyable pleasures as eating, drinking, smoking, handling contact lenses, applying cosmetics, or storing human food in the animal facility.
3. Eyes are protected with some kind of glasses, goggles, or face shields, especially when contact lenses are worn.

4. We properly dispose of all sharps.

We need to use procedures to minimize both the splatters and the aerosols such as using a cotton pledget to cover the withdrawal of a needle from a vial. Animal Biosafety Level 1 also defines some special practices such as decontaminating all your work surfaces after use or after a spill of viable materials. We must be cognizant of appropriate decontamination of equipment. For example, enrichment equipment that's used with non-human primates may need to be washed in soap and water and decontaminated. It is essential to be very careful about handling animal waste because animal care personnel may develop medical problems following prolonged exposures to proteins in urine.

Cages can be washed manually or in a cage washer. The final rinse should be at a 180° F, in conjunction with the Guide. Doors to animal rooms should open inward and have a self-closing mechanism on them. Doors should be kept closed when experimental animals are present. We don't like to see the little kick stands on the bottom of doors to keep them propped opened. Obviously, if you have to move cages in and out, you need to hold doors open, but truly the doors should be kept closed. You need to have an insect and rodent control program in place. Many of the newer control programs involve use of a lot fewer chemicals for insect and rodent control, preferable to just continuing with a spray program.

We want to prevent persons who are at increased risk of acquiring infection from entering our animal rooms. We are dealing increasingly with populations of employees who may have lifestyles or medical conditions that put them at increased risk of exposure to certain things. People who are immunocompromised or who may be on certain drug therapies that induce immunosuppression are at increased risk. However, this also creates an opportunity for the supervisor of the facility to really understand the employees working there.

We also want to be careful about allowing animal room access for persons whom infection might be unusually hazardous. For example, we don't believe that it is a good idea to have children come in to look at the new ferret babies. It's much better to visit a petting farm for appropriate animal exposure. There is interest in having our children understand our personal work environments, but we must also minimize their risks.

We have no recommendations for primary barriers of Biosafety Level 1 because they are not needed. Animal Biosafety Level 1 is for work with agents that are not particularly hazardous for healthy adult humans; therefore, we don't need special containment.

For Animal Biosafety Level 2, all of the standard practices indicated for ABL1 remain the same. A key point to keep in mind is that what is said for Biosafety Level 1 applies at 2, and then the sum of those apply at 3 and so on. There are some special practices at Biosafety Level 2:

1. Animal room doors are posted with the universal biohazard symbol.
2. The sign should contain the name of the investigator, the agents in use, and any special precautions required.

For example, if immunization, a baseline serum, or wearing special clothing is required in the animal facility, that all needs to be posted. It's not just the animal care people and the veterinarians who have access to animal facilities. It might also be the maintenance personnel or, heaven forbid, an investigator who actually wants to see their animals.

Serological surveillance programs are of interest, and a later session will speak very specifically about this. If you have a baseline serum storage program, you need to have a formal written program in place. With the emerging litigious society that we're in, things like *informed consent* and other legal documents need to be on file. Things that didn't apply 25 years ago do today, so be aware and be prepared.

You may want to set up specific testing protocols for sera at very defined intervals. This may well be for a pre-exposure or post-exposure incident or for somebody who has just been exposed to some agent. You can take a baseline serum at that point and 28 days later look for rising titer or whatever your protocol says you should do. The key to this is maintaining written records. You need to place those written records in the official medical folder of the employee, and you need to be conveying this information to the supervisor. At CDC we have developed an automatic immunization and medical record tracking system which is kind of nice. If you visit the CDC, we'll be able to talk a bit more about the system we call AIMS. Finally, the employee obviously needs to be kept in the loop recording any medical records and the availability of results of testing.

If you are going to conduct a [medical] surveillance program, you'll need to define things such as:

1. How long you are going to store the serum after it's been collected?
2. The parameters under which it will be stored.
3. The personnel who have access to it.
4. The question of who "owns" the serum needs to be answered.

These are interesting issues that you need to work on with your legal people. Obviously, an appropriate immunizations component could include anything from Hepatitis A to B, and/or it may be very specific. For example, if you have a project on rabies, you would want to vaccinate employees. We would also include TB skin testing in the same medical surveillance program.

You need to maintain the written medical evaluation, any surveillance data that you collect on testing, and any treatment data resulting from any associated occupational injury or illness. Immediately report spills and accidents! Nelson Garnett described a problem with perception as to whether an event had been an accident or an intentional injury.

At CDC, we believe that they all are incidents that need to be reported and evaluated. This is really helpful for the person who may wind up at some point needing to file a worker's compensation claim for insurance reasons, etc.

Training has to be provided, at least annually, to personnel who are involved with animal care and use issues. This training should cover:

1. Potential hazards
2. Precautions to prevent exposure
3. Exposure evaluation procedures
4. Any changes in protocol

If you have an SOP and for some reason you're going to change it, it is crucial to gather personnel together to explain the changes that will occur. You should document the fact that you have training and that specific individuals have been trained. I know that for those of you who are in the training business, it is a difficult thing to make the second and third years of training interesting, exciting and titillating.

You also have to prepare or adopt a biosafety manual. The CDC/NIH guide, (the *BMBL)*, is a minimum guide, and we hope that you will take the information that's there and customize it to work for you. Clearly, I don't know what your Building 47 is, what your Room 3 is, or where the incinerator is, etc. For persons who would like to receive a copy of this book, or if you would like to receive a Word Perfect disk version, we will mail it to you. The reason I offer this is that it's always easier to plagiarize, and I'd prefer that you plagiarize correctly rather than misstate what we have taken so much time to write in the book.

We certainly need to be posting signs for persons who are going to enter the laboratory or animal holding space. Other information that might be included on the sign:

1. Use of radioactive materials
2. Possible chemical hazards, or
3. Anything that may present a unique problem for facility personnel.

People know they must wear appropriate gowns, gloves, respirators, and other kinds of personal protective equipment no matter where they go inside the facility, but you must make sure that they don't wear these items **outside** of the animal facility. The clothing is worn to protect the person, and in some cases it's worn to protect their street clothes. However, protective clothing or equipment should not be worn to the cafeteria, the library, etc. Protective clothing is worn in the animal room and is removed before leaving the facility.

A wide variety of personal protective devices are on the market. You're aware of the incredible array of gloves to protect against a wide variety of njuries. I simply caution you to make sure that the gloves that you are using are appropriate for the agents or the chemicals that may be in use. Other kinds of personal protective equipment would include goggles, eye protection, face shield, possibly a respirator if required and so forth. These protective equipment needs must be worked out in your safety meetings as you plan and initiate the research experiments.

At Biosafety Level 2, we want to be particularly careful about needles and syringes. We want to make sure that we are substituting plastic for glass ware. The other day somebody asked me about the use of Pasteur pipettes, and I suggested that while there are some cardboard sleeves in which you could dispose of those pipettes, there are also plastic Pasteur pipettes with self-contained bulbs that you can use to minimize the problems of sticking

yourself or dropping and breaking glass equipment.

We need to have some mechanism for wrapping up the materials that we move from the animal facility back to the laboratory room. There are a number of devices on the market. This carrying case has a rubber gasket, and it is a commercially available device. You can put in a rack to hold your vacutainers or other tubes, load, close it up, wipe it off with a suitable disinfectant, and transfer it then to the laboratory where you would then process material in a biological safety cabinet. Please decontaminate all contaminated equipment before reuse or, in particular, before removal from the animal facility. Autoclaving is perhaps the most common way to decontaminate something, but we recognize that there are a wide variety of other ways, including use of some chemicals.

Cages get autoclaved before cleaning at ABSL-2. Animals not involved in the work being performed must be kept out of the facility. That's probably not a very big issue, but you'd be surprised how many people like to bring pet animals into laboratories, and that becomes an issue.

Safety equipment, the primary barriers that we recommend, are biological safety cabinets (BSC). We have a new publication that was just released on proper selection and use of biological safety cabinets. More and more you see BSCs popping up in animal procedure rooms. They are most appropriate in some cases for transferring animals and in some cases for dissecting necropsies.

The secondary facilities are basically the same as we would see at Level 1, except we begin to pay some attention to the way air is moved in this environment. We want to maintain directional inward air flow. We want to make sure that the animal room itself is negative to the corridor so that air moves from the corridor (the area of least contamination) to the area of potentially higher contamination. This also does wonders for odor control in the corridors. The air should be exhausted to the outside without recirculation to other rooms. In some of our facilities at CDC, we have cut slots in the doors, and have hung a little piece of surveyor's colored tape. As long as that tape flutters inward, the animal care people know that they are maintaining that inward directional air flow. If the air flow direction falls static or if its reverses itself, then they know to call engineering to rectify the problem.

You want to make sure that the facilities are designed and constructed to facilitate cleaning and housekeeping. This becomes a big issue and has to be appropriate for the species involved. Finally, in many of our animal rooms we have drains with traps in the floor. If you ave drains, you have to have a trap in there; you also want to make sure that you fill that trap with at least water. You can also put a good disinfectant down there, If you aren't hosing down your rooms or if you're basically leaving them static once you've put your disinfectant in, you may want to pour a cup of mineral oil on top to minimize evaporation. Mostly what we're concerned about is growth of bacteria in the sewer, but if a trap dries out, then you get gas in the room and that really is obnoxious.

This very rapid run through of Animal Biosafety Levels 1 and 2, paints the picture for what we do when we have to deal with agents that are beyond

"just agents" such as hepatitis. We're moving into areas like working with tuberculosis in animals. What about work with more hazardous microorganism at Animal Biosafety Levels 3 and 4? I now present Dr. Bobby Brown, head of our Animal Resources Program at CDC, who will continue this dialog.

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