Managing Immunocompromised Animals

This unit describes some commonly used methods for maintaining immunocompromised rodents, specifically mice, to prevent infections from adventitious pathogens. The Basic Protocol describes the microisolation housing unit, while alternate procedures cover individually ventilated isolator housing, flexible-film or semi-rigid isolator housing, and barrier facility housing.

MICROISOLATION HOUSING

Materials

- Sterilant (ABQ Sterilant-Disinfectant, Alcide, or CLIDOX-S, Pharmacal Labs; http://www.pharmacal.com/), placed in spray bottle and tray
- Microisolation housing unit comprised of the following: autoclavable plastic cage, plastic filter top, stainless steel wire bar lid, water bottle with rubber stopper and stainless steel sipper tube, autoclavable feed and bedding
- Autoclavable flasks (250- to 1000-ml capacity) filled with water
- Disposable lab coat and gloves
- HEPA-filtered laminar-flow workbench
- Rubber-tipped forceps and forceps tray containing sterilant

Prepare the microisolation housing unit

1. Assemble the microisolation housing unit with the components shown in Figure 1.2.1. Do not include the animal and water.

2. Autoclave the assembled microisolation housing unit in a vacuum autoclave set to the sterilization or pasteurization cycle.

   Monitor the autoclave’s performance with both instrumentation and biological indicators. The autoclave cycle should end with a 15- to 20-min drying period (under vacuum) to ensure that the filter medium is dry before the autoclave door is opened. This will also dry the bedding. Do not place microisolation unit on the floor.

3. Autoclave flask filled with water (on liquid cycle).

Figure 1.2.1  Microisolation housing unit (Lab Products, 1984).
Prepare the operator and workbench

4. Put on a disposable lab coat and gloves. Tape gloves to the coat to form a tight seal.
   *Check gloves first for pinholes by inflating with air.*

5. Transfer autoclaved microisolation housing unit, water flask, and animals to the vicinity of the HEPA-filtered laminar-flow workbench using a service cart.

   *Gloves must be wet with sterilant at all times while working in the workbench.*

7. Wipe down the internal surfaces of the workbench hood, walls, and floor (but not the back filter wall) with sterilant and paper towels. Allow sterilant to sit for 3 min.

Load the microisolation housing unit

8. Place the autoclaved microisolation housing unit in the workbench and remove the filter frame top (Fig. 1.2.2).
   *Remove the top as though removing a Petri plate lid. To place the filter frame (plastic filter top) to the left of the cage bottom, tilt the filter top off the cage bottom to the left and all the way upside down, laying it upside down on the surface of the workbench. Reverse direction to place filter top to right of cage bottom. This procedure prevents dust particles from blowing from the top of the filter frame into the cage bottom.*

9. Spray the water flask with sterilant and place in the workbench. Fill the water bottle, add the stopper with sipper tube, and place in the cage.
   *Avoid dripping sterilant into the water bottle.*

10a. For filter-crated animals (obtained from a specific pathogen-free colony compatible with research needs), inspect the crate carefully for any holes or breaks in its integrity. If completely intact, thoroughly spray the crate (*including the filter*) with sterilant and place in the workbench. Open the crate. If a tool is used, it must be kept in a tray containing sterilant in the workbench.

10b. If animals are currently in a microisolation unit, place the unit in the workbench and open as in step 8. Do not spray the cage or cage filter.

11. Take a pair of totally submerged rubber-tipped forceps from the forceps tray in the workbench and shake off excess sterilant in the hood but away from the back of the workbench and the back filter wall.

![Figure 1.2.2](image.png)

*Figure 1.2.2* Loading the microisolation unit. To place the filter top to the left of the cage bottom, tilt filter top off the plastic cage to the left and completely upside down, laying it upside down on the work surface.
workbench to avoid wetting the filter. Lift the wire bar lid, grab animals by the tail, carefully transfer to the cage, and replace the filter frame on the cage bottom.

_The unit is now ready and can be returned to the animal rack. Soiled microisolation units should be washed in a cagewash. Restock and autoclave before use._

12. Spray and wipe workbench with sterilant after all animals have been transferred to clean microisolation units and placed on the animal rack.

**INDIVIDUALLY VENTILATED ISOLATOR HOUSING**

This caging system for housing immunocompromised animals incorporates a microisolation cage with the addition of several options for ventilation to each cage through manifolds in the rack. The cages are mounted on a rack equipped with a blower that delivers HEPA-filtered air via the manifold system (Fig. 1.2.3). The air is then exhausted directly from the cage or is refiltered prior to exhausting into the room or the building exhaust. An additional option uses convection to allow air flow across the cages. This housing system maintains animals free of pathogens and prevents buildup of ammonia and other gaseous pollutants in the cage microenvironment (Dillehay et al., 1990; Lipman et al., 1993; Huerkamp and Lehner, 1994). Cages, filter tops, and other materials are sterilized before animals are introduced. All handling of animals, cages, and other materials is performed in the laminar-flow workbench using the methods described for microisolation housing (see Basic Protocol).

**ALTERNATE PROTOCOL 1**

**INDIVIDUALLY VENTILATED ISOLATOR HOUSING**

This caging system for housing immunocompromised animals incorporates a microisolation cage with the addition of several options for ventilation to each cage through manifolds in the rack. The cages are mounted on a rack equipped with a blower that delivers HEPA-filtered air via the manifold system (Fig. 1.2.3). The air is then exhausted directly from the cage or is refiltered prior to exhausting into the room or the building exhaust. An additional option uses convection to allow air flow across the cages. This housing system maintains animals free of pathogens and prevents buildup of ammonia and other gaseous pollutants in the cage microenvironment (Dillehay et al., 1990; Lipman et al., 1993; Huerkamp and Lehner, 1994). Cages, filter tops, and other materials are sterilized before animals are introduced. All handling of animals, cages, and other materials is performed in the laminar-flow workbench using the methods described for microisolation housing (see Basic Protocol).

**ALTERNATE PROTOCOL 2**

**FLEXIBLE-FILM ISOLATOR HOUSING**

The flexible-film isolator was developed as a containment chamber to house gnotobiotic animals (Trexler and Reynolds, 1957). Immunocompromised animals may be housed in this instead of the microisolation housing unit. The isolator consists of a flexible polyvinyl plastic chamber equipped with filtered air supply and exhaust ports, glove ports with rubber gloves, and an entry port (Fig. 1.2.4). The entry port mounted on the isolator wall is an air-lock chamber sealed on either end with vinyl caps. Newer semirigid units consist of rigid polypropylene on all sides but one, that being flexible polyurethane.
After sterilizing the inside of the isolator with sterilant, the presterilized packaged cages, feed, bedding, and other materials are introduced into the isolator through the entry port. The inside of the entry port and the outside surfaces of the packaged materials are sterilized by spraying with sterilant and waiting 3 min for maximum efficacy. Animals are introduced upon receipt from the vendor in a special, presterilized transfer unit that attaches with a vinyl sleeve to the entry port. After sterilization of the inside of the entry port and attached sleeve, a Mylar cover on the transfer unit is broken and removed, and the animals are transferred into cages in the isolator. Filter tops are not routinely used for cages inside the isolator, and a laminar-flow workbench is not needed with this protocol.

**BARRIER FACILITY HOUSING**

Of the four protocols described for housing immunocompromised animals, the barrier facility housing system is the most expensive and labor-intensive method. A separate positive-pressure, HEPA-filtered air handling system for the barrier room or rooms is required. All materials enter the barrier through a double-door autoclave or sterilizable entry port similar to the one described for the flexible-film isolator. Personnel enter the barrier through a series of air locks, removing all street clothing, showering, and redressing in sterilized clothing, cap, mask, shoes, and gloves.

**COMMENTARY**

**Background Information**

Animals that are immunocompromised—either because of genetic defects (e.g., athymic nude mice, NOD-scid/scid and some genetically-engineered strains) or because of irradiation or treatment with immunosuppressive agents—cannot survive when housed in conventional animal facilities. Their increased susceptibility to bacterial, viral, and fungal infections leads to high morbidity and frequent mortality. The use of microisolation housing units is the simplest, most labor-efficient, and least expensive of the different systems that have been developed. The alternate methods described all have disadvantages when compared to the microisolation system. The use of laminar-flow and individually ventilated housing racks is limited by the expense of purchasing the units. Flexible-film or semi-rigid isolators are maximally protective, but have a high labor requirement, are awkward to work in, and hold a limited number of cages. Barrier facilities require a major investment in money for construction and maintenance. In addition, management of personnel to follow strict barrier procedures can be burdensome.
Orcutt (1987) provides a review of housing methods for managing immunocompromised animals free of infectious pathogens. Sedlacek et al. (1980, 1981) developed the design and management concept using microisolation housing units to maintain a pathogen-free mouse colony. Individually ventilated housing and its effectiveness in maintaining the cage microenvironment is presented by Huerkamp and Lehner (1994) and in preventing inter-cage viral transmission is presented by Dillehay et al. (1990) and Lipman et al. (1993). Foster et al. (1983) provide a review of flexible-film isolator technology and barrier facility technology.

**Critical Parameters**

The following points cannot be emphasized enough in maintaining the integrity of the microisolation housing unit:

1. Do not open the microisolation unit once it is autoclaved, except in the laminar-flow workbench.
2. Keep gloves wet with sterilant at all times when working in the laminar-flow workbench.
3. Except for the microisolation housing unit, all items entering the laminar-flow workbench should be sprayed or wiped with sterilant.
4. Do not place the microisolation unit on the floor.

**Disclaimer**

This unit was prepared by Patricia Brown in her private capacity. No official support or endorsement by the National Institutes of Health or the United States Department of Health and Human Service is intended or should be inferred.

**Literature Cited**


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