Mass Airflow Cabinet for Control of Airborne Infection of Laboratory Rodents

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A mass airflow cabinet for handling and housing of laboratory rodents has been developed and tested. The unit consists of a high-efficiency particulate air filter and uniform distribution of air at a velocity of 19 cm per s. Animals are maintained without bedding in mesh-bottomed cages that rest on rollers for rotation inside the cabinet. There is an air barrier of 90 cm per s separating the cabinet air from room air. Sampling for airborne bacteria yielded an average of 0.03 colony-forming units (CFU) per ft² of air inside the cabinet, whereas 28.8 CFU per ft² was simultaneously detected outside the cabinet during housekeeping, a reduction of almost three logs. The efficiency of the air barrier was tested by aerosolization of T3 phage. When phage was aerosolized 5 cm outside the cabinet, no phage could be detected 5 cm inside when the fans were operating; with the fans off an average of $1.6 \times 10^4$ plaque-forming units (PFU) per ft² was detected in six tests. Aerosolization of phage inside the cabinet yielded an average of $9 \times 10^9$ PFU per ft² outside; an average of $4.1 \times 10^9$ PFU per ft² were detected with the fans not in operation, a reduction of more than four logs. In-use studies on effectiveness showed that the cabinet significantly reduced the incidence of mice originally titer-free to Reo-3 virus. Hemagglutination inhibition antibodies to Reo-3 were detected in 9/22 (42%) mice housed in a conventionally ventilated animal laboratory while no seroconversion was detected in any of 22 mice housed in the mass air flow cabinet in the same laboratory.

Laminar airflow (LAF) and modifications of it have been applied to a variety of medical environments, including operating rooms (5, 9), tissue culture laboratories (7, 10) and safety cabinets for handling hazardous biologicals (6, 17).

In animal laboratory applications, we have previously demonstrated that a mass airflow (MAF) system significantly reduced or eliminated airborne spread of bacterial infections depending on conditions of the experiment (11, 12). van der Waaij adapted horizontal and vertical laminar airflow cabinets for housing axenic mice without the use of germ-free isolators, and maintained them germ free when the cages were 10 cm from cages of conventional mice (16). Beal et al. reported studies on a laminar-flow animal rack to maintain animals in an environment free of exogenous airborne microorganisms (4). These laminar-type airflow systems for animals protect the animals against exogenous airborne infection. However, they do not cover two situations that arise.

First, if the animals inside the LAF units are already infected, there is risk of spreading infection if the air from these units passes into the open laboratory where other animals are housed. The other problem is that the cages must be removed from the LAF unit for inspection, cage changing, injection, bleeding, or other manipulation of the animals. The ideal caging system would permit the maintenance of both isolation and containment barriers during quiet periods and during housekeeping and manipulation of the animals. Ideally, it would permit investigations with different viruses in the same room, containment of infectious agents, and work with potentially onogenic or mutagenic agents, or both.

The objective of the present study was to develop and evaluate a recirculating MAF safety cabinet equipped with high-efficiency particulate air (HEPA) filtration, uniform distribution and an air barrier separating the animals from the laboratory air. The intended use of this equipment was to protect animals...
from airborne infection, especially during periods of greatest aerosol risk, i.e., during routine housekeeping, animal handling during inoculation, blood sampling, and other procedures.

MATERIALS AND METHODS

Mass airflow cabinet. The MAF cabinet (Anigard Cabinet, Baker Co., Sanford, Me.) measures 122 by 66 by 61 cm. The cabinet shell is stainless steel; side walls and the removable front viewing screen is plexiglass 6.4 mm thick. The octagonal cage rests on plastic rollers for easy rotation inside the cabinet, and is separated into eight compartments by solid stainless steel; each compartment can accommodate up to four mice. The cage top and bottom is steel mesh; bedding is not used. Urine and feces drop into the mesh floor to a pan 10 cm below. An automatic flushing device removes collected wastes at regular intervals. Automatic watering valves are located in each compartment.

Airflow patterns in the hood are shown in Fig. 1. Air is passed through a HEPA filter (30 by 60 cm) that is at least 99.97% efficient in removal of particles with an average diameter of 30 μm. The filtered air passes to a plenum and then through a perforated steel grid that comprises the entire ceiling of the cabinet. The air velocity, determined with an Anlor thermoanemometer (Anlor Inc., Chicago, Ill.), can be varied from 13 to 19 cm per s (26 to 38 ft/min). A velocity of 19 cm per s was used in these studies. Air leaves the cage area through the entire bottom of the cabinet and is recirculated through the HEPA filter. Approximately 10% of the filtered air is exhausted. A similar volume of room air is drawn into the cabinet through the 15.5-cm opening at the front of the cabinet; the velocity of this air barrier averaged 90 cm per s. This room air does not enter the cage area of the cabinet, but enters the 3.2-cm wide exhaust grill at the front of the cabinet and passes through the return air plenum to the HEPA filter. The air barrier was designed to restrict the passage of aerosols into and out of the hood. There is a working area inside the cabinet 20 cm deep between the cages and the air intake at the front that is used to inspect and handle the animals for experimentation and during cage changing.

Sound levels, determined with a sound level meter type 1565-A (General Radio Co., Concord, Mass.), were 47 db (A) throughout the room with the cabinet motor turned off and 51 db (A) with it in operation. A level of 67 dB (A) was detected inside the cabinet during operation.

Bacterial air sampling. Airborne bacteria were measured with the Andersen air sampler by procedures outlined previously (1, 6). Results were recorded as the number of colony-forming units (CFU) per ft² of air using the positive hole correction factor for dense aerosols collected with the Andersen sampler.

Phage aerosolization and sampling. Aerosols of T3 bacteriophage of Escherichia coli were generated with a DeVilbiss no. 40 atomizer. Aerosols were sampled with all glass impingers (AGI-4) (Ace Glass Co., Vineland, N.J.) that contained tryptose phosphate dextrose broth and Dow Antifoam liquid. The collection fluid was assayed for content of phage by previously reported methods (6). Temperature in the room was 22 C (±2 C); relative humidity averaged 65% (±5%).

Animal room. All studies on the Anigard cabinet were performed in an animal laboratory (457 by 335 cm) that housed four racks of cages of breeder C57/B16 mice with approximately 48 cages per rack. Ventilation consists of 12 fresh air changes per h through medium-efficiency filters. Cages were changed weekly and water bottles (pH, 3.5) were changed twice weekly.

Animals. Specific pathogen-free (SPF) mice were obtained from National Animal Laboratories (NAL), Creve Coeur, Mo. Axenic mice were obtained from Charles River Breeding Laboratories, Wilmington, Mass.

Serological studies. Hemagglutination inhibition (HI) tests were used to detect circulating antibodies to Reo-3 and polyoma viruses (15). Animals were bled orbitally and HI tests were performed in micro-titer (Cooke Engineering, Alexandria, Va.). Human group O erythrocytes (1%) screened for sensitivity to hemagglutination (HA) with Reo-3 were used for the HI test for this virus. Guinea pig red blood cells (1%) were used to hemagglutinate polyoma antigen. Reo-3 and polyoma HA antigens, control antigens, and hyperimmune antisera were obtained from Microbiological Associates, Inc., Bethesda, Md. HI tests included antigen controls, positive and negative antisera. Negative antisera were obtained from axenic mice. Eight HA units were used in all Reo HI tests; 16 units were used in polyoma HI tests. Sera tested for anti-polyoma antibodies were treated with 4 volumes of receptor-destroying enzyme (RDE) (100 U/ml) per volume of serum. An HI titer of 1:10 was considered positive for Reo-3 virus; 1:20 was considered positive for polyoma virus.

Studies on airborne infection in mice. Experiments were designed to simultaneously test whether Reo-3 virus could be transmitted through the air in the animal room under study and, if so, whether the ventilated cabinet could significantly reduce transmission. NAL mice free of detectable HI antibodies in tests in our laboratories were used as monitors of airborne infection. The C57/B16 mice housed in this animal room possess antibodies to Reo-3 virus.

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**FIG. 1. Diagram of airflow through MAF cabinet.** Approximately 10% of the filtered air is exhausted and replaced by a similar volume of room air at the front opening of the cabinet.
ally occurring aerosols of Reo-3 virus in the room were the challenge inocula. These aerosols were generated by personnel during routine procedures of animal handling, cage changing, and housekeeping activities. The NAL mice used to monitor for airborne Reo-3 infection were housed in two environments: experimental mice were housed inside the MAF cabinet and control animals were placed on an open rack nearby. The control animals were housed in sterilized cages with sterile bedding, food, and water. The cages were placed on the top shelf of an animal rack, 152 cm above the floor. One animal was housed per cage. To insure that these animals were exposed only to airborne, but not to contact infection, they were handled by one person who had no further duties in the animal laboratory. These animals were handled once a week when cages, food, water, and bedding were changed and animals were orally bled. Gloves, gowns, masks, and aseptic techniques were observed during these procedures, and gloves were changed between cages. Animals of the experimental group inside the cabinet received sterile food and were watered via an automatic watering device. These animals were handled weekly for orbital bleeding as above, without removing them from the MAF cabinet. Each experiment lasted 4 weeks.

RESULTS

Twenty hourly samplings for airborne bacteria with Andersen samplers yielded an average of 0.03 CFU per ft² (range: 0.0-0.8) inside the cabinet during cage changing in the animal room, whereas simultaneous sampling outside the cabinet averaged 28.8 CFU per ft² (range: 13.0-73.0). Similar tests with the cabinet fans turned off yielded an average of 3.3 CFU per ft² (range 1.1-6.3) inside the cabinet. The cabinet was approximately 150 cm from the site of activity.

Particle size analysis of the bacterial aerosols generated during cage changing indicated that 60% of the airborne bacteria were carried on relatively large particles with an average median diameter larger than 9.2 μm.

To test the efficiency of the air barrier at the front of the cabinet, T3 phage was aerosolized on one side of the air barrier and samples collected on the other side. The aerosolized spray was directed toward the right of the hood in all tests. Results of phage tests are presented in Table 1. When phage was aerosolized 5 cm outside the hood, no phage could be detected 5 cm inside the hood in any of six tests although approximately 10⁷ PFU per ft² were detected in control studies under the same conditions. The hood was evaluated for containment potential by aerosolization inside the hood and phage sampling outside. Phage was detected outside the hood on three of the seven tests but the air curve produced a 4 to 5 log reduction over controls. In other containment studies, freon released inside the cabinet could not be detected when all outside joints were probed with a freon detector.

In animal studies, HI tests showed that 37% (24/65) of the mice in the laboratory had circulating antibodies to Reo-3 virus, and these animals served as the source of airborne infection. The effect of this cabinet on development of anti Reo-3 antibodies is represented in Table 2. Approximately 41% (9/22) of the animals exposed to the air in the animal laboratory developed anti Reo-3 antibodies during the 4-week study periods. HI antibody to Reo-3 was not detected in any of the 22 animals housed in the cabinet (P < 0.001). Titters in the positive animals averaged 1:40 and generally developed toward the end of the 4-week study.

### Table 1. Effect of air barrier of MAF animal cabinet on aerosolization of T3 phage

| Expt. | PFU/ft² | Penetration |
|-------|---------|-------------|
|       | Airflow off | Airflow on |            |
| 1⁰    | 2.7 x 10⁶ | 1.5 x 10⁶  | 6 x 10⁻⁴ |
| 2     | 2.0 x 10⁷ | 6.3 x 10⁶  | 3 x 10⁻⁴ |
| 3     | 4.8 x 10⁶ | 0           | 0.0       |
| 4     | 5.5 x 10⁶ | 0           | 0.0       |
| 5     | 3.3 x 10⁷ | 1.0 x 10⁸  | 4 x 10⁻⁵ |
| 6     | 8.0 x 10⁶ | 0           | 0.0       |
| 7     | 1.0 x 10⁸ | 0           | 0.0       |
| Avg   | 4.1 x 10⁷ | 9.4 x 10⁶  | 10⁻³     |

| Expt. | PFU/ft² | Penetration |
|-------|---------|-------------|
| 1⁰    | 1.5 x 10⁴ | 0           | 0.0       |
| 2     | 1.1 x 10⁴ | 0           | 0.0       |
| 3     | 3.2 x 10⁴ | 0           | 0.0       |
| 4     | 3.2 x 10⁴ | 0           | 0.0       |
| 5     | 1.5 x 10⁴ | 0           | 0.0       |
| 6     | 3.2 x 10⁴ | 0           | 0.0       |
| Avg   | 1.5 x 10⁴ | 0           | 0.0       |

* In this group of experiments, T3 phage was aerosolized 5 cm inside cabinet, and AGI-4 sampling was performed 5 cm outside cabinet.

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### Table 2. Effect of mass airflow cabinet on development of anti REO-3 in nal mice

| Expt no. | No. positive per no. tested |
|----------|----------------------------|
|          | In cabinet | In laboratory |
| 1        | 0/6       | 2/6           |
| 2        | 0/6       | 2/6           |
| 3        | 0/6       | 2/6           |
| 4        | 0/4       | 3/4           |

* In 4-week study period.
Similar results were obtained in separate experiments on airborne transmission of polyoma virus. In three experiments, 71.8% of the mice (23/32) in the normally ventilated animal laboratory developed anti-polyoma titers, whereas 15.8% (3/19) of the mice housed in the cabinet developed titers ($P < 0.001$). Two of the seroconversions in mice housed in the cabinet occurred during a single experiment in which the motors were shut off for 2 h to correct a mechanical difficulty. These two seroconversions were detected in the second week of the 4-week study, and these animals did not infect animals in adjacent cages inside the cabinet. The details of the polyoma study will be published separately.

Control studies showed that mice housed in the cabinet with the motor not in operation developed antibody in approximately the same percentages as those animals housed in the outside laboratory.

**DISCUSSION**

The cabinet in this study has been shown to restrict passage of aerosols into and out of the animal containment area. This is based on environmental sampling, challenge with microbiological aerosols, and by controlled studies on natural airborne infection with Reo-3 and polyoma viruses in mice.

Dense microbiological aerosols are generated in the animal laboratory by housekeeping activities (11), and this was confirmed in the present studies. The majority of these airborne bacteria were carried on particles larger than 9.2 μm and were not detected inside the cabinet when the motor was not in operation. Most of these larger particles sedimented before diffusing to the area of the air sampler inside the cabinet and are not important in airborne infection. Enough of the smaller particles evidently remained airborne to infect animals with Reo-3 virus, even though animals were housed in cages on the top shelf of a rack. Reo-3 is a prevalent virus in mouse colonies, and these studies demonstrate that the virus can be readily transmitted through the air.

It is believed that aerosolizing phage inside the cabinet yielded higher control values than those outside because the cloud was generated into a smaller volume. Penetration of the air barrier occurred when larger clouds ($3.3 \times 10^4$, $2.7 \times 10^4$, and $2.0 \times 10^4$ PFU/ft$^3$ air) were generated.

Although microbiological sampling and aerosol challenge can yield useful information, the most meaningful data come from in-use studies under controlled conditions. Results from the present studies show that Reo-3 and polyoma viruses can be readily transmitted via the airborne route and that the cabinet prevents or eliminates transmission of airborne infection with these agents. The finding that these viruses can survive in the air and infect susceptible animals indicates the need for strict control measures.

An advantage of this type cabinet is that animals can be handled, fed, inoculated and manipulated inside the cabinet so they never have to leave the protected atmosphere. There is an area 20 cm deep between the cages and the front exhaust grill where examination, inoculation, and necropsy can be performed. In the systems reported by van der Waaij and Andreas, all animal handling was done through glove ports (16). In the LAF rack reported by Beal et al., animals would presumably have to be removed from the unit for housekeeping and other procedures. In the study of Beal et al., bedding was not changed during the 26-day evaluation for efficacy against cross-infection by an unidentified mycoplasma (4).

No animal studies were performed to quantify the containment capability of the cabinet. The fact that bedding was not used in the cabinet should minimize aerosol generation inside and the probability of spreading contamination to the outside. In the three instances when seroconversion to polyoma occurred in the cabinet, there was no evidence of cross infection occurring in animals in adjacent cages inside the cabinet. This is in spite of the fact that in the two experiments where infections occurred, it took place in the second week of the 4-week study, and polyoma virus has been shown to be excreted in the urine, saliva, and feces of infected animals (14, 15). This restriction of infectious foci inside the cabinet may be due to the unidirectional air flow and the absence of bedding. We have previously demonstrated how these two factors can influence spread of airborne infection (11).

No physiological studies were performed on the animals maintained in this cabinet although Beal et al. have shown normal physiological findings in rats maintained in mesh-bottomed cages in a LAF rack (4).

No major problems have been encountered in the routine use of the prototype MAF cabinet over a 12-month period. All animals housed in the cabinet to date were used to evaluate its effectiveness. Minor problems in the prototype were either corrected in the laboratory or recommendations made to the manufacturer to make
necessary changes on later units. Some blockage of the automatic flushing system was experienced and this system has been redesigned. Alternatively, urine and feces could be collected on absorbent material and removed periodically.

Temperature inside the cabinet is approximately 3°C above ambient; no temperature increase was noted in the room which housed the unit.

The HEPA filter in the cabinet was tested at the beginning and at the end of the study and found to be free of detectable leaks. A recent survey showed that a substantial number of HEPA filters in laminar-flow biohazard hoods had serious leaks (17). The Office of Biohazard and Environmental Control of the National Cancer Institute recommends that HEPA filters, airflow patterns, and air curtains in laminar-flow biohazard hoods be checked after installation in the laboratory, after moving the unit, following installation of a new HEPA filter, and routinely on a semi-annual basis (2, 3). A similar program for ventilated cabinets for laboratory animals is hereby recommended.

To obtain maximum benefit from the aerodynamics of this cabinet, stringent aseptic techniques must be practiced when handling animals in the cabinet. The fact that many mouse pathogens can be isolated from bedding, urine, feces, air, and other environmental sources indicates the potential of infecting mice in the cabinet by contact with hands and fomites.

The vertical air velocity used in this hood (19 cm/s) is substantially lower than that conventionally employed in laminar flow systems (50 cm/s), but we have shown that lowered air velocities are effective in elimination of airborne contamination in tissue culture laboratories and operating rooms and elimination of airborne infection in animal laboratories in controlled studies (5, 7, 11, 12).

Potential applications of this cabinet include quarantine; isolation; maintaining animals hyper-susceptible to infection because of therapy, surgery, genetics, or other reasons; and for protecting animals from exposure to human pathogens. The cabinet can also serve as a containment facility for housing infected animals and to protect animals and humans in the outside laboratory. Even though tests with nebulized phage and two mouse viruses show it to be effective, additional tests with other pathogens are desirable. The cabinet is not a substitute for class III hoods which utilize complete gastight containment and are recommended where the agents studied carry a high risk to personnel.

Each of the eight cages in the cabinet will hold three to four mice, depending on weight. The number of individual cages inside the cabinet could be reduced to allow more space per cage so that larger animals could be housed. A larger unit is now available to house more rodents or to accommodate other animals including small primates.

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