Modified Laminar Flow Biological Safety Cabinet

GERARD J. MCGARRITY AND LEWIS L. CORELL
Department of Microbiology, Institute for Medical Research, Camden, New Jersey 08103

Received for publication 5 August 1974

Tests are reported on a modified laminar flow biological safety cabinet in which the return air plenum that conducts air from the work area to the high efficiency particulate air filters is under negative pressure. Freon gas released inside the cabinet could not be detected outside by a freon gas detection method capable of detecting $10^{-4}$ cc/s. When T3 bacteriophage was aerosolized 5 cm outside the front opening in 11 tests, no phage could be detected inside the cabinet with the motor-filter unit in operation. An average of $2.8 \times 10^4$ plaque-forming units (FFU)/ft$^2$ (ca. 0.028 m$^2$) were detected with the motor-filter unit not in operation, a penetration of 0.0%. Aerosolization 5 cm inside the cabinet yielded an average of 10 PFU/ft$^2$ outside the cabinet with the motor-filter unit in operation and an average of $4.1 \times 10^4$ PFU/ft$^2$ with the motor-filter unit not in operation, a penetration of 0.002%. These values are the same order of effectiveness as the positive-pressure laminar flow biological safety cabinets previously tested. The advantages of the negative-pressure return plenum design include: (i) assurance that if cracks or leaks develop in the plenum it will not lead to discharge of contaminated air into the laboratory; and (ii) the price is lower due to reduced manufacturing costs.

Various laminar flow biological safety cabinets (LFBSC) are available commercially and in laboratory use. Although there are slight variations in design, the essential features of such cabinets are high efficiency particulate air (HEPA) filtration, an average vertical velocity of 50 cm/s inside the cabinet, open access to the work at the front, and an air curtain with a calculated face velocity of 37 cm/s to restrict passage of aerosols into and out of the cabinet. Such cabinets have been evaluated and shown to be effective under static conditions (3, 4, 5, 7), and there are specifications for design, construction, and performance of such units (2). LFBSC are in wide use for microbiology, cell culture, and tumor virus work. The basic performance criteria of LFBSC have also been incorporated into a safety cabinet to house rodents (6).

There are some difficulties associated with use of these cabinets. Accessibility to the work is somewhat reduced compared to open bench work, and introduction of large items into the cabinet is limited by the size of the front opening or side door. An area of concern is the possibility of leaks from the positive-pressure return air plenum which recirculates potentially infectious air from the work area of the cabinet back to the HEPA filter. A LFBSC developed to correct these deficiencies utilized a return air plenum under negative air pressure with regard to room air. The front of the cabinet is a movable safety plate-glass window that facilitates entrance of bulky items. The purpose of this study was to evaluate this negative-pressure LFBSC for comparison with positive-pressure LFBSC to contain bacteriophage aerosols and freon.

MATERIALS AND METHODS

Laminar flow biological safety cabinet. The LFBSC under evaluation (Fig. 1) (Sterilgard model VBM 600, The Baker Co., Inc., Sanford, Me.) measured 132 by 76 by 234 cm high; the work area measured 117 by 61 by 97 cm high. The airflow through the cabinet is diagrammed in Fig. 2 and 3. Before entering the work area, air passes through a HEPA supply filter that is 99.97% efficient in removal of particles with an average diameter of 0.3 μm. The filtered air travels vertically through the cabinet at an average velocity of 50 cm/s (100 ft/min). Air exits from the work area via two exhaust grills across the entire width of the cabinet in the rear and front of the work surface measuring 5 by 117 cm and 10 by 117 cm, respectively. This air travels back to the motor-filter units through three return air plena located behind the two side walls and the rear wall. Air from one side wall and half of the rear wall plena moves to each of two motor-filter units. All return plena and the two motor-filter units housings are under negative pressure. Upon leaving the motor-filter unit housing, the air enters a positive-pressure zone that is surrounded
The negative-pressure return plenum. Approximately 90% of the air in the positive-pressure area passes through the HEPA supply filter to the work area. The remainder passes through a HEPA exhaust filter. A volume of room air similar to that exhausted is taken into the cabinet at the front opening. This air does not penetrate the work area, but passes into the exhaust grill at the front of the work surface and into the return air plenum.

The cabinet is equipped with a movable front-view window of 0.64-cm safety plate glass that can be raised to a maximal height of 60.9 cm to permit entrance of large items. During working conditions, the window was raised 20 cm. Under these conditions, the calculated face velocity of the air curtain across the 20-cm opening, determined by dividing the exhaust airflow quantity by the front work access opening area, was 37 cm/s (75 ft/min; 2).

**Generation and enumeration of bacteriophage aerosols.** Aerosols containing approximately 10⁸ plaque-forming units (PFU) of T3 bacteriophage of *Escherichia coli* were generated in a DeVilbiss no. 40 nebulizer. Samples were collected in all glass impingers (AGI-4) and assayed by methods previously reported (4). Results were expressed as the number of PFU per cubic feet (ft³) of air.

**Freon testing.** To determine if air in the return air plenum system leaked into the laboratory, freon gas (Freon-22, DuPont DeNemours Co., Inc., Wilmington, Del.) was continuously bled inside the cabinet at the rear center of the work area while a freon detector (H-2 Halogen Leak Detector, General Electric Co., Schenectady, N.Y.) simultaneously scanned all joints outside the cabinet. For this testing, the air leaving the exhaust HEPA filter was ducted to the exterior. The freon detector was employed on a nonquantitative mode; range was 10⁻⁶ cc/s. All freon tests were performed after working hours to minimize background traces of gas. Controls consisted of a freon leak standard, release of freon with the motor-filter unit not in operation, and insertion of the freon detector inside either the cabinet or exhaust ducts during freon release.

**RESULTS AND DISCUSSION**

In 11 tests when phage was aerosolized 5 cm outside the front opening, no phage particles could be detected by AGI-4 samplers positioned at two locations 5 cm inside the opening (average penetration = 0.0%). The sensitivity of this assay was 50 PFU/ft³ (ca. 0.028 m²), based on a sampling rate of 0.44 ft³/min and a collecting volume of 22 ml in the impinger. Control values with the motor-filter unit not in operation yielded an average of 2.8 × 10⁵ PFU/ft³ in 11 tests (range: 2.2 × 10⁴ to 8.5 × 10⁵ PFU/ft³).

When phage was aerosolized 5 cm inside the cabinet and simultaneous AGI-4 samplers were obtained at two locations 5 cm outside the cabinet, an average of 10 PFU/ft³ was detected in 10 tests. Nine of the tests failed to detect phage and one test yielded 10² PFU/ft³. In control studies with the motor-filter unit off, an average of 4.1 × 10⁵ PFU/ft³ were detected in 10 tests (range: 1.2 × 10⁴ to 7.0 × 10⁵ PFU/ft³). Average penetration of the phage in these studies was 0.002% (10/4.1 × 10⁴). There was no significant difference between the phage penetrations of the present studies and those reported on a positive-pressure airtight return plenum LFBSC from the same manufacturer and of the same overall dimensions (4).

No freon could be detected outside the cabinet while the gas was being released inside the cabinet in six separate tests. A full scale response was obtained when the motor-filter unit was turned off and when the detector was positioned either inside the cabinet, in the return air plenum, or in the exhaust duct. A full scale response was also obtained when a leak standard was employed. When the sampling probe was placed inside the cabinet upstream of freon release, a response was obtained in approximately 7 s. Calculations show that under conditions of this test, the freon gas recirculated through the cabinet and reached the site of the detector in 5.5 s, indicating the accuracy and
sensitivity of the freon detection method. This test was performed four times, always with similar results. Freon tests were also performed in a LFBSC equipped with a positive-pressure airtight return plenum. In these tests, the positive-pressure LFBSC was completely sealed by bolting a metal plate over the access and pressurizing to 1-inch water pressure with freon. No freon could be detected outside the cabinet.

In separate studies, freon could not be detected at any point outside the front access opening of the cabinet during release of the gas inside. Moving the sampling probe 2.54 cm inside the opening yielded a full scale response, clearly delineating the zone of freon-laden cabinet air from room air.

The results of the studies with phage aerosols and freon show that the cabinet provides protection to materials and personnel equal to a LFBSC cabinet with a positive-pressure airtight return plenum tested under similar conditions (4). Since the return air plenum is not airtight, manufacturing costs can be reduced.

Although the freon detection technique for locating leaks and zones of contamination is widely employed in engineering, it is less familiar to microbiologists who are used to having results expressed in biological units. The sensitivity of the AGI-4 impingers as used in these studies was 50 PFU/ft³, based on a sampling rate of 0.44 ft³/min. The overall sensitivity of the phage assay was \(3.7 \times 10^{-1}\) PFU/s (50 PFU/ft³ × 0.44 ft³/min × \(\frac{1}{60}\) s). This is approximately 4.5 logs less sensitive than the freon detection method used in this study (10⁻⁶ cc/s). Detection of freon can be performed easily with immediate readout to locate air imbalances and leaks, and there is no biological decay. The imbalance or leak can be quantitated if necessary.

The only area inside the cabinet that contains contaminated air under positive pressure is the gasketed supply plenum immediately upstream of the HEPA supply filter. However, this area is surrounded by a plenum of negative pressure, so small air leaks in the positive-pressure zone recirculate to the negative-pressure plenum and then back to the positive-pressure zone and the HEPA filter.

The movable front-viewing window facilitates entrance of large and bulky items. The window

---

**Fig. 2. Diagrammatic airflow in LFBSC with negative-pressure return plenum (front view).** (1) HEPA supply filter; (2) view window; (3) open access, 20 cm high; (4) return air plenum under negative pressure; (5) motor; (6) positive-pressure plenum; (7) HEPA exhaust filter; (8) work area. Solid arrows indicate positive-pressure air flow; open arrows indicate negative-pressure airflow.
was placed at a height of 20 cm during these studies, based on National Institutes of Health specifications (2). Greater window heights and different air velocities may result in improper air balances at the front opening. To guard against this, the cabinet is equipped with an audio alarm system that is triggered when the window is not in its proper position when the motor-filter unit is in operation. There is also a suction pressure gauge to indicate pressure drop across the HEPA filters. Increase in pressure drop can be compensated for by increasing the motor speed by solid state controls or by changing HEPA filters.

Proper maintenance of LFBSC is important. It has been shown that a significant number of LFBSC in laboratory use are faulty, having either leaks in the HEPA filter or gaskets, improper velocity profiles, or air imbalances (8). Recommendations to minimize and detect these problems are available, along with proper operating procedures (1).

Personnel should familiarize themselves with the maintenance of these cabinets and periodically have them certified by specified procedures to insure proper operation (1). Failure to do so can negate all advantages of the equipment and represent waste of time and research funds.

ACKNOWLEDGMENTS

We thank V. Ammen, J. Sarama, and V. Vanaman for technical assistance.

This study was supported by a grant-in-aid contract from the state of New Jersey and Public Health Service grant 5 S01 RR0582-06 from the Division of Research Resources.

LITERATURE CITED

1. Anonymous. 1973. Effective use of laminar flow biological safety cabinets. National Audiovisual Center (Government Services Administration), Washington, D.C.
2. Anonymous. 1974. Laminar flow biological safety cabinet. NIH Specification NIH-03-112a, amended, Jan., 1974. National Institutes of Health, Bethesda, Md.
3. Barbeito, M. S., and L. A. Taylor. 1968. Containment of microbial aerosols in a microbiological safety cabinet. Appl. Microbiol. 16:1225–1229.
4. Coriell, L. L., and G. J. McGarrity. 1968. Biohazard hood to prevent infection during microbiological procedures. Appl. Microbiol. 16:1885–1900.
5. McDade, J. J., F. L. Sabel, R. L. Akers, and R. J. Walker. 1968. Microbiological studies on the performance of a laminar airflow biological cabinet. Appl. Microbiol. 16:1086-1092.
6. McGarrity, G. J., and L. L. Coriell. 1973. Mass airflow cabinet for control of airborne infection of laboratory rodents. Appl. Microbiol. 26:167–172.
7. Staats, F. H., and J. W. Beakley. 1968. Evaluation of laminar airflow microbiological safety cabinets. Appl. Microbiol. 16:1478–1482.
8. Wedum, A. G., W. E. Barkley, and A. Hellman. 1972. Handling of infectious agents. J. Amer. Vet. Med. Ass. 16:1557–1567.