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Microbiological Studies on the Performance of a Laminar Airflow Biological Cabinet

JOSEPH J. McDADE, FRED L. SABEL, RONALD L. AKERS, AND ROBERT J. WALKER

Biohazards Department, Pitman-Moore Division, The Dow Chemical Company, Zionsville, Indiana 46077, and Engineering Department, Behrent Engineering Company, Denver, Colorado 80211

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Engineering and microbiological tests indicated that a typical, commercial laminar airflow cabinet was not effective in providing either product protection or agent containment. The cabinet was modified and tested through a series of alternate configurations to establish a set of design criteria. A mock-up cabinet was developed from these design criteria. The mock-up unit was evaluated for efficiency in providing both product protection and agent containment. In these evaluations, challenge methods were developed to simulate normal, in-use laboratory operations. Controlled bacterial or viral aerosol challenges were used at higher than normal levels to provide stringent test conditions. Test results indicated that the mock-up unit was considerably better in preventing agent penetration (0.1 to 0.2 particles per 100 ft^3 of air) than the commercial cabinet (5 to 6 particles per 100 ft^3 of air) during product protection tests. Similarly, agent containment was considerably better in the new cabinet (particle escape of 2 to 3 per 100 ft^3 of air at only one of the five test sites) than in the commercial cabinet (particle escape of 2 to 14 per 100 ft^3 of air at three of the five test sites).

The laminar airflow principle developed by Whitfield (15) has been widely and successfully applied in industry for the control of particulate contamination during clean assembly work. More recently, laminar airflow clean rooms and clean work benches have been used with success for control of microbial contamination in the aerospace (4, 8-10) and medical fields (11, 14). Use of laminar airflow cabinets is also increasing in the pharmaceutical field and in microbiological laboratories (5).

A variety of horizontal (crossflow) and vertical (downflow) laminar airflow cabinets are commercially available. The general areas of application of such units comprise (i) product protection, i.e., use of laminar airflow cabinets for operations involving manipulations of materials that must be kept sterile or free from unwanted ecological agents, and (ii) agent containment, i.e., use of laminar airflow cabinets for manipulations involving etiological agents, materials, or procedures requiring personnel protection. The item(s) in use must be confined within the working area and must not be allowed to escape from the cabinet.

The horizontal laminar airflow cabinet is best suited for maximal product protection. However, when agent containment is required, the vertical laminar airflow cabinet is more applicable. Air-handling-system modifications have led to the development of downflow units that have been reported to provide product protection and agent containment. In such units, a larger volume of moving air is exhausted from the cabinet than the volume of air supplied through the filter. This creates a negative pressure at the face of the cabinet, thereby drawing room air into the face or front opening of the cabinet. Theoretically, a protective air curtain is established at the cabinet face to provide both product protection and agent containment.

In this study, a commercial vertical laminar airflow cabinet was tested for its ability to establish and maintain laminar flow of air. The cabinet was also tested to establish a quantitative measure of the degree of product protection and agent containment. In general, the unit was deficient in establishing laminar airflow within the cabinet and was not as good as desired for product protection or agent containment.

Since vertical laminar airflow clean rooms were effective in controlling particulate and microbial contamination, it was decided to develop a downflow cabinet that was more nearly laminar in airflow. Laminar airflow is defined in Federal Standard 209a (6) as "Air flow in which

the entire body of air within a confined area moves with uniform velocity along parallel flow lines, with a minimum of eddies." Thus, in our study, the design goal for the cabinet was development of uniform, parallel, and nonturbulent airflow throughout any given cross section of the cabinet. This report describes the engineering approach, tests and analysis, and the microbiological studies conducted to develop and to evaluate the performance of the new cabinet.

MATERIALS AND METHODS

Test cultures. Stock cultures of *Serratia marcescens* (ATCC 14756) were maintained at 4 to 6 C on Tryptose Phosphate Agar (TPA) slants. Routine cultures were propagated at 30 C on TPA slants and were transferred at 24-hr intervals during tests. Test cultures were prepared as suspensions for aerosolization by heavily streaking a plate (100 mm in diameter) of TPA with a 3-mm loopful of inoculum from a routine culture. The freshly streaked plate was incubated at 30 C for 16 hr. Then, the resultant growth was harvested from the plate and resuspended in 200 ml of Tryptose Phosphate Broth (TPB). This suspension contained from 1.0 to 6.0 $\times$ 10^9 cells per ml.

Test bacteriophage was prepared by inoculating 1,000 ml of a 5-hr TPB *Escherichia coli* culture (ATCC 11303-B) in a Fernbach flask with 200 ml of a suspension of T1 coliphage (ATCC 11303-B1) containing 7.0 to 9.0 $\times$ 10^9 plaque-forming units (PFU) per ml. The mixture was placed on a magnetic stirrer and slowly agitated at room temperature until the medium cleared. Upon clearing, 10 ml of chloroform was added, and the lysate was centrifuged for 30 min at 680 $\times$ *g*. The number of PFU in the lysate was determined according to the method described by Adams (1). The phage suspension was stored at 4 to 6 C for periods up to 2 months.

Product protection tests. An aerosol generator (model 200 A; Schoeffel Instruments, Westwood, N.J.) was used to create the challenge aerosols for the product protection tests. This self-contained generator dispenses 0.2 ml of fluid per minute. Particle distribution in the resultant aerosol ranges from 1 to 4 μ in diameter (3, 7). In these tests (Fig. 1), a Schoeffel generator was placed 12 inches (30.5 cm) from the face of the cabinet and 6 inches (15.2 cm) above the level of the perforated work surface. Positioned in this manner, the aerosol produced would closely approximate the location of an aerosol generated by a laboratory technician working at the cabinet. To measure the extent of aerosol penetration into the cabinet, 12 rows of five agar settling plates (100 by 15 mm) each were placed across the perforated work surface. These plates covered approximately one-third of the total area of the work surface. An aerosol was generated for 2 or 3 min per test. A Reyniers slit sampler was placed under the perforated work surface of the hood to sample air from the exhaust portion of the cabinet in order to indicate the presence of viable particles. Control samples were obtained in the challenge aerosol prior

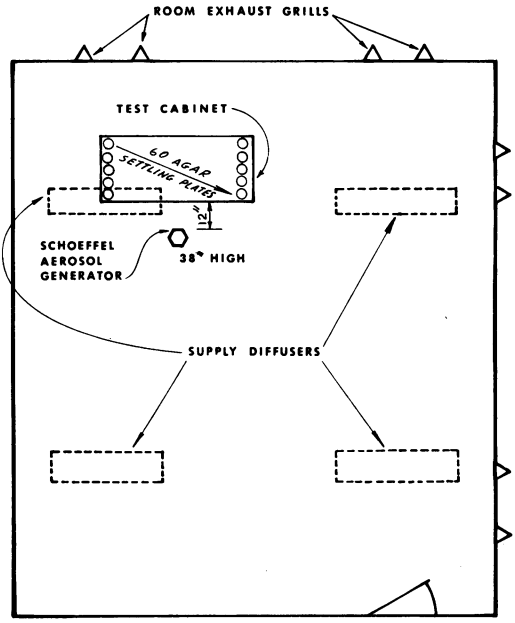

FIG. 1. *Setup for product protection tests.*

to each trial. Aerosols of *S. marcescens* were collected on TPA plates and were incubated at 30 C for 24 hr prior to counting. The T1 coliphage aerosols were collected on TPA plates which had been overlayered previously with a thin layer of 1.0% TPA containing 2.5 to 9.5 $\times$ 10^7 viable *E. coli* cells/ml. The settling plates (100 by 15 mm) were overlayered with 3 ml of the inoculated agar per plate and the Reyniers plates (150 by 25 mm) with 8 ml per plate. After sample collection, test plates were incubated at 37 C, and the number of PFU was counted after 6 and 24 hr of incubation.

Agent containment tests. A model 1001 Waring Blendor was used to create the challenge aerosol during agent containment tests. The blendor was placed in the center of the cabinet on the perforated work surface and 24 inches (61 cm) from the right inside edge. Test aerosols were produced by blending 200 ml of the bacterial or viral suspension within the cabinet for a 1-min period with the top on the blendor. Then, the blendor was turned off, and the top was removed for a 1-min period, after which the top was replaced. The particle size distribution of the resultant aerosol ranged from 1 to 3.5 μ (3). Challenge aerosols were collected with volumetric air samplers (Reyniers) on appropriate medium described above. The air samplers were equipped with a 60-min timing mechanism and were calibrated to sample air at a rate of 1 ft^3 of air per minute. Six air samplers were used per test, and they were located in the following positions (Fig. 2): sampler 1 (R-1) was placed under the perforated work surface of the cabinet, beneath and adjacent to the blendor; sampler 2 (R-2) was placed outside of the cabinet, 12 inches (30.5 cm) from the left corner and 6 inches (15.2 cm) from the face opening, level with the work surface; sampler

3 (R-3) was located outside of the cabinet at the respiratory level of an operator seated at the cabinet, i.e., center front of the unit, 6 inches from the face opening and 18 inches (45.7 cm) above the work surface; sampler 4 (R-4) was located outside of the hood, 12 inches from the right corner and 6 inches from the face opening and level with the work surface; sampler 5 (R-5) was located 12 inches from the left corner, level with the work surface and 6 ft (1.97 m) from the face opening; sampler 6 (R-6) was located 12 inches from the right corner, level with the work surface and 6 ft from the face opening. In general, trials were conducted as follows. The samplers were operated for a period of 10 min to obtain a background count. At the completion of the aerosol cycle, the sampler continued to run for 8 min, making a total for each trial of 10 min or 10 ft^3 of air sampled. Five trials were conducted during each series of plates.

Cabinet airflow studies. A complete description of the engineering phase of this study has been presented elsewhere (*in press*). For continuity, a brief description of the engineering is included. A theoretical analysis indicated that airflow in the commercial cabinet was nonuniform along the bottom edge of the front window panel, at the face of the cabinet, and across the perforated work surface. This analysis also identified areas of extreme eddy or turbulence at the upper edge of the face opening. Actual airflow velocities measured in this cabinet proved the theoretical calculations to be correct. All velocity profiles were measured with an Alnor Thermo-Anemometer employing a special 24-inch (61 cm) probe. Measurements were taken (Fig. 3) at 2-inch (5.1 cm) intervals 2.5 (6.4 cm) inches below the high efficiency particulate air (HEPA) filter bank (filter traverse), at 1-inch (2.54 cm) intervals at the bottom edge of the front window panel (mid-level traverse), at 1-inch intervals 1 inch above the perforated work surface (work surface traverse), and at 1-inch intervals up the face opening (face opening traverse). All traverse planes were 19 inches (49.3 cm) from the left end of the cabinet. The air-handling system of the commercially available cabinet was modified to allow recirculation, and an auxiliary exhaust fan, pitot tube, and manometer were attached to measure the portion of the exhaust air discarded. Velocities at each traverse line were measured. The air quantity for each representative plane was calculated and totaled against the fan-flow totals to confirm velocity readings. A profile of the cabinet face opening was calculated to determine airflow distortion, areas of probable turbulence, and effect of the inner air mass. Airflow patterns within the cabinet were converted through a series of configurations to evaluate the flow conditions typical of a number of commercially available cabinets. After these evaluations, the following set of design criteria were established: (i) airflow paths should be as nearly a straight line as possible; (ii) highly different velocity air masses should not be mixed; (iii) the airflow in the face opening should be turned to more nearly parallel the inner air stream; and (iv) the quantity of air exhausted should be minimal to reduce face turbulence, exhaust filter size, and building conditioned air losses.

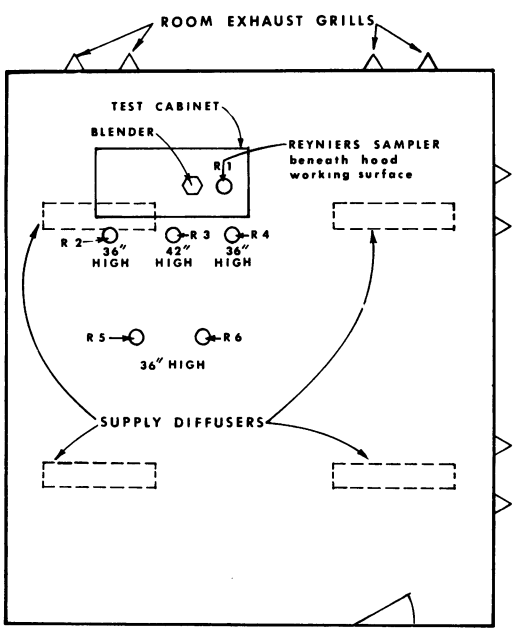

FIG. 2. *Setup for agent containment tests.*

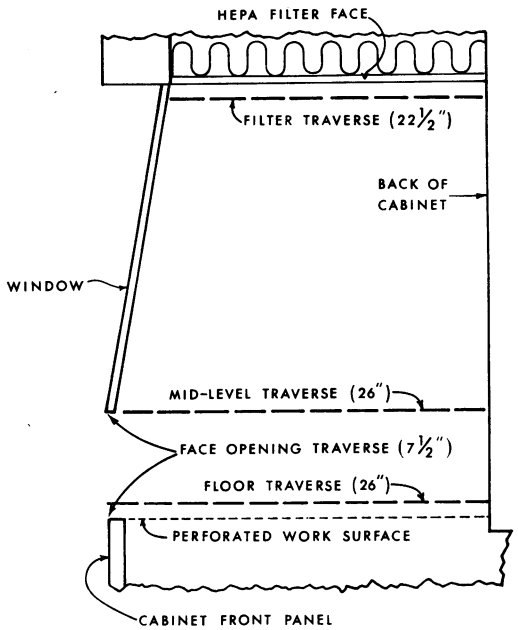

FIG. 3. *Measurement locations for velocity profiles.*

RESULTS

Engineering studies. It was found that the entire airflow of the commercial cabinet was shifted toward the back of the unit. This direction

of flow was due to two factors: the 40% free area perforated work surface, and the fact that the cabinet air was exhausted below the perforated work surface at the back of the cabinet. The 40% free area perforated work surface did not create sufficient back pressure to equalize effects caused by the location of the return air exhaust. The velocity profile of the mid-level traverse was shown to drop near the front of the cabinet due to the expanding air stream formed by the outward sloped window. None of the other configurations evaluated appeared to be better in laminar airflow characteristics nor to have particular advantage over the original test cabinet.

Positioning the window parallel to the inner airstream and the back of the cabinet corrected the variation in velocity caused by the expanding airstream. The free area in the perforated work surface was reduced to correct the backward sweep of the inner mass of cabinet air. Reducing this free area to 10% caused the air that was drawn into the cabinet face to be "pulled" into the work area of the cabinet. However, when the portion of the work surface extending beyond the window was increased to 20% free area (keeping the portion of the perforated work surface behind the window at 10% free area), airflow conditions within the cabinet improved (Fig. 4). Finally, the attachment of an angled front lip further improved flow conditions at the face opening by turning the face flow to more nearly parallel the inner air stream (Fig. 4). At this point, it appeared that all of the established design criteria had been fulfilled, and the design mock-up (Fig. 4) was developed for further microbiological testing.

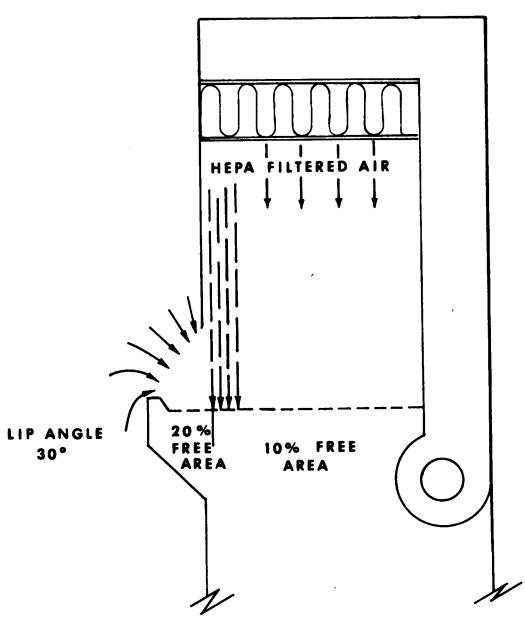

FIG. 4. *Schematic cross section of the design mock-up cabinet.*

TABLE 1. *Product protection tests in the commercial cabinet*[a]

Test no.	Room air-handling system	Total virus penetration (PFU) of cabinet	Viral penetration per 100 ft³
1	On	659	27.8
2	On	1,428	60.2
3	Off	24	1.0
4	Off	230	9.7

[a] The cabinet air supplied during challenge was 2,372 ft³. The titer of T1 coliphage was 5×10^7 PFU/ml. The 2-min aerosol challenge was 2×10^7; the aerosol challenge per 100 ft³ was 8.4×10^5.

Product protection tests. A typical set of results obtained in the commercial cabinet are presented in Table 1. More than 10 tests were conducted with bacteriophage and 8 tests with *S. marcescens*. The data shown for tests 1 and 2 represent the greatest viral penetrations recorded. Results similar to those shown in tests 3 and 4 were observed repeatedly with both the bacterial and the viral challenge aerosols. At present, data are not available to resolve the differences in agent penetration noted when the room air-handling system was on or off. A possible explanation lies in the "induction type" diffuser used for the room air supply. The air velocity produced by such a diffuser was turbulent and at a much greater value than any in the cabinet. Thus, the room turbulence tended to distribute the challenge aerosol more uniformly across the face opening of the cabinet and at velocities greatly in excess of the low-velocity areas at the top of the face opening. When the room air was off, the aerosol was more readily controlled by the higher velocity air flowing into the exhaust grille at the cabinet face.

Table 2 contains a typical set of results obtained during product protection tests in the design mock-up of the new cabinet. Results similar to those given in Table 2 have been obtained repeatedly in some 15 more tests with either *S. marcescens* or T-1 coliphage. In all of these tests with the new cabinet, it should be noted that the room air-handling system was on, the aerosol challenge was three to four orders of magnitude larger, and the aerosol challenge period was an additional 60 sec. All of the above items provided a more stringent test, yet product protection was vastly improved in the new cabinet. The commercial cabinet tests showed an

TABLE 2. *Product protection tests in a design mock-up of the new cabinet*[a]

Test no.	Agent titer	Aerosol challenge (3-min)	Aerosol challenge per 100 ft³	Total microbial penetration of cabinet	Microbial penetration per 100 ft³
1	3×10^{8}[b]	1.8×10^{8}	6.9×10^{6}	0	0
2	1.5×10^{9}[b]	9×10^{8}	3.5×10^{7}	2	0.08
3	4×10^{9}[b]	2.4×10^{9}	9.2×10^{7}	5	0.19
1	5×10^{8}[c]	3×10^{8}	1.2×10^{7}	5	0.19
2	5×10^{8}[c]	3×10^{8}	1.2×10^{7}	3	0.12

[a] The room air-handling system was on; the cabinet air supplied during challenge was 2,598 ft³.
[b] *S. marcescens* expressed as bacteria per ml.
[c] T1 coliphage expressed as PFU per ml.

TABLE 3. *Comparison of viral containment test results in both cabinets*[a]

Reyniers sampler no.	Total no. of particles recovered[b]		Particle recovery per ft³ of room air sampled	
	Commercial cabinet	Design mock-up	Commercial cabinet	Design mock-up
1	TNTC[c]	TNTC	TNTC	TNTC
2	8	3	0.14	0.03
3	1	0	0.02	0
4	0	0	0	0
5	7	0	0.12	0
6	0	0	0	0

[a] Based on 10 trials.
[b] Aerosol: 5.9×10^{9} PFU of T1 coliphage/ml.
[c] Too numerous to count.

TABLE 4. *Comparison of bacterial containment test results in both cabinets*[a]

Reyniers sampler no.	Total no. of particles recovered[b]		Particle recovery per ft³ of room air sampled	
	Commercial cabinet	Design mock-up	Commercial cabinet	Design mock-up
1	TNTC[c]	TNTC	TNTC	TNTC
2	0	0	0	0
3	6	0	0.12	0
4	2	1	0.04	0.02
5	1	0	0.02	0
6	0	0	0	0

[a] Based on five trials.
[b] Aerosol: commercial cabinet, 5.6×10^{7}/ml; design mock-up, 4.9×10^{9}/ml.
[c] Too numerous to count.

average penetration of five to six microbial particles per 100 ft³, whereas the new cabinet indicated an average penetration of 0.1 to 0.2 microbial particles per 100 ft³.

Agent containment tests. The results of a typical set of agent containment tests in both cabinets are compared in Table 3. The test agent was T1 bacteriophage. As presented in Table 3, containment was considerably better in the design mock-up (particle escape 0.03 per ft³ at one site, 0 at the other four) than in the commercial cabinet (particle escape 0.02 to 0.14 per ft³ at three sites, 0 at the other two). Results similar to those given in Table 3 have been obtained in more than 30 trials in the commercial cabinet and in more than 60 trials in the design mock-up cabinet.

Table 4 contains a second set of typical results comparing bacterial containment tests in both cabinets. The bacterial containment efficiency of the design mock-up cabinet was far better than that of the commercial cabinet (Table 4). In this series of tests, the challenge aerosol used with the design mock-up cabinet was two orders of magnitude larger than that used in the commercial cabinet, yet agent containment was superior in the design mock-up cabinet.

DISCUSSION

In the present study, a number of different configurations and airflow patterns were evaluated in an attempt to compare the product protection or agent containment features or both of several commercially available laminar flow cabinets. It was possible to apply a theoretical engineering analysis to all phases of the development of a newly designed laminar downflow cabinet. Assumptions and conclusions made in this theoretical analysis were supported by actual tests and airflow measurements. It was essential to use a long probe (24 inches, 61 cm) to measure airflow. Smaller, hand-held thermo-anemometers would create a cone of turbulence due to the presence of the operator's hand in the airstream. Thus, it was felt that the measurements taken in our study represented realistic, repeatable values. Early in the study, design criteria were estab-

lished and, through a series of modifications, it was possible to meet the design criteria. When this was accomplished, the design mock-up cabinet functioned very satisfactorily during both product protection and agent containment tests.

The degree of agent containment or product protection is dependent on the airflow pattern and the degree of turbulence within the hood. Smoke has been used successfully by Turner (13) and Schulte et al. (12) to evaluate fume hoods with respect to operating conditions and hood design. Unfortunately, there is a paucity of published information concerning the biological evaluation of safety cabinets and particularly of laminar flow hoods. Williams and Lidwell (16) used *Bacillus subtilis* spores and *Chromobacterium prodigiosum* cells to evaluate the containment efficiency of an open-face cabinet. Barbeito (2) presented a detailed evaluation of a laboratory handling infectious microorganisms. His tests included evaluation of safety cabinets, filters, and exhaust systems. In each case, the spread of the challenge aerosol was limited by the pressure differential of the units being evaluated and not by a laminar airflow movement. The containment efficiency and the protection afforded a biological product by a laminar flow unit has not, until this time, been described in detail. It is felt that the product protection or the agent containment tests or both represent a very high challenge in typical in-use situations. In the product protection tests, the aerosol generator was positioned to simulate the location of an operator. In these tests, the units were exposed to a microbial aerosol considerably in excess of that normally encountered, yet the newly designed cabinet provided excellent product protection. Similarly, in the agent containment tests, the use of a blendor was considered to be a realistic aerosol generator. The repeated sequence of hand insertion and removal during the creation of each challenge aerosol was felt to simulate the normal operations of a technician using the cabinet.

The engineering phase of this study centered around development of a more nearly laminar airflow cabinet. The microbiological phase centered around the development of tests and procedures to evaluate the performance of the new cabinet. Taken collectively, the results obtained in this study represent a joint effort between engineering and microbiology to produce a laminar airflow cabinet that functioned very efficiently in providing agent containment and product protection.

Design drawings of the new cabinet may be obtained from William E. Barkley, National Cancer Institute, Wiscon Building, Room 600. 7550 Wisconsin Ave., Bethesda, Md. 20014.

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