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Influence of Crossdrafts on the Performance of a Biological Safety Cabinet

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A biological safety cabinet was tested to determine the effect of crossdrafts (such as those created by normal laboratory activity or ventilation) upon the ability of the cabinet to protect both experiments and investigators. A simple crossdraft, controllable from 50 to 200 feet per min (fpm; 15.24 to 60.96 m/min), was created across the face of the unit. Modifications of standardized procedures involving controlled bacterial aerosol challenges provided stringent test conditions. Results indicated that, as the crossflow velocities exceeded 100 fpm, the ability of the cabinet to protect either experiments or investigators decreased logarithmically with increasing crossdraft speed. Because 100 fpm is an airspeed easily achieved by some air conditioning and heating vents (open windows and doorways may create velocities far in excess of 200 fpm), the proper placement of a biological safety cabinet within the laboratory—away from such disruptive air currents—is essential to satisfactory cabinet performance.

The expanding field of biological research with its simultaneously expanding hazards has made the biological safety cabinet a standard piece of equipment in many laboratories. The purpose of this cabinet is to protect experiments against airborne contamination (product protection), to protect researchers and the surrounding laboratory from the possibility of aerosol exposure due to manipulations of experiments (personnel protection), or to protect both concurrently through the use of controlled airflows. The class II biological safety cabinet, most commonly used today, provides both personnel and product protection and is recommended for use in handling infectious agents (21), with tissue culture procedures (11, 14), and in some work involving recombinant DNA technology (17, 18).

Some currently marketed cabinets have been tested with various biological challenge aerosols under static conditions (1, 5, 10, 12, 13, 15, 20), but few have been tested quantitatively under dynamic or adverse conditions. Because the function of the cabinet depends upon the critical control of airflows, it would be expected that anything that blocked or disrupted these airflows would have a direct effect upon performance. Kukla (12) found that moving arms in and out or back and forth in front of the cabinet, as well as movement of the laboratory door, had adverse effects upon the personnel protection of a unit. Coriell and McGarrity (5) noted that rapid removal of arms or contaminated articles caused organisms to escape from the unit and should be avoided. Testing a class I safety cabinet (which uses an inward flow of air to protect personnel but exposes the experiment to room contamination), Barbeito and Taylor (3) found that working in the cabinet, walking past it, laboratory door movement, and a combination of all three had an adverse effect upon the personnel-protecting capabilities of a cabinet.

Other factors affecting safety cabinet performance are the airflow pattern and the degree of air turbulence within the room. McDade et al. (13) reported poorer results from the product protection testing of their commercial cabinet when the room airflow system was turned on. They attributed this to the "induction-type" diffuser for the room air supply, which created a turbulent airflow of velocities greater than any used by the cabinet. All laboratories contain potentially disruptive room air currents arising from open doors and windows, fans, air conditioning and heating vents, and personnel traffic; these air currents may have velocities far in excess of those used by the safety cabinet. This has been a recognized problem in the installation and use of biological safety cabinets for some time (7), but no one has defined quantitatively where problems begin to occur.

In this study, a simple crossdraft, controllable between 50 and 200 feet per min (fpm; 15.24 and 60.96 m/min), was created across the face of a biological safety cabinet. The objectives were to define the velocity at which such disruptive room airflow would render the personnel and product protection capabilities of the cabinet unsatisfactory and, above this point, to describe

the relationship between performance and crossflow velocity.

MATERIALS AND METHODS

Biological safety cabinet. The test unit was a 4-foot (ca. 1.22-m), class II, type A (formerly called type 1) biological safety cabinet (model 112c-4; The Baker Co., Inc., Sanford, Maine) designed to the 112c specification of the National Institutes of Health (16). Airflow patterns in the hood are shown in Fig. 1. Both high-efficiency particulate air filters meet government standards by removing 99.97% of dioctyl phthalate particles having an average diameter of 0.34 μm (6). These filters have been shown to be of equivalent efficiency in removing both bacterial and viral aerosols (8, 9). Filtered air descends uniformly through the work zone at an average rate of 78 fpm and at the approximate center of the work surface splits, exiting through either the front air intake or the rear exhaust grille. Air is swept through the blower and up the back positive-pressure plenum to the top of the unit, where approximately 70% is recirculated through the supply filter to the work zone and 30% is exhausted through a second filter at the top of the cabinet. This exhaust air is replaced by an equal volume of room air drawn through the 10-inch (25.4-cm) front opening at an average velocity of 80 fpm. This air curtain does not enter the work area of the hood but passes down through the grille in front of the work area and is primarily responsible for the containment properties of the unit. The balance of airflow at this point is critical because if too great a positive pressure or any negative pressure is created within the work zone, the result will be an outflow of organisms or an inflow of room contamination, respectively. The unit was checked out completely by all the physical and biological procedures listed in the 112c specification (16) to ensure that it was a gas-tight, properly functioning cabinet at the start of the study. Airflow readings were periodically checked to make certain that they remained unchanged.

Crossflows. A horizontal flow module (model CG-56; The Baker Co., Inc.) with the diffusion panels removed (Fig. 2) was used to create a simple crossdraft across the face of the safety cabinet. This module operated by pulling air from the floor level up through a blower and pushing it out through a large high-efficiency particulate air filter. The crossflow speed was controlled between 50 and 200 fpm by a simple adjustment of a solid-state speed controller. Crossflow speed was set and checked daily with an Alnor thermoanemometer (Alnor Instrument Co., Niles, Ill). No other airflow was occurring in the room at the time of the test other than that created by the horizontal flow module and the class II cabinet. Background and/or residual airborne contamination was not a problem in the test laboratory because all air entering or leaving the laboratory was filtered with a high-efficiency particulate air filter. Room airflow plus a horizontal flow clean bench (model EG-6220; The Baker Co., Inc.) was on at all other times and represented a cleaning rate of 28 room air volumes per h. Room construction permitted the testing to be carried out in complete absence of laboratory personnel because all electrical outlets, and thus all testing apparatus, were controlled by an electrical panel in an outer laboratory. The additional factors of personnel and their movements were thus not introduced into the experimental design.

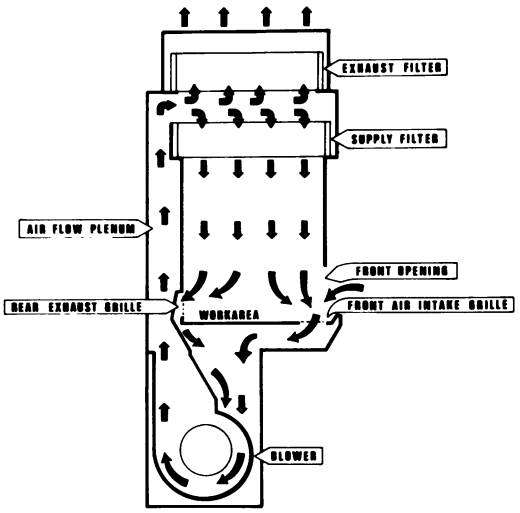

FIG. 1. *Schematic cross section of the class II, type A biological safety cabinet.*

Personnel protection testing. To test the ability of the safety cabinet to contain aerosols created within the work zone, a modification of the biological tracer or containment test of the National Cancer Institute was utilized (16). The test organisms were spores of *Bacillus subtilis* var. *niger* obtained from Frederick Cancer Research Center, National Cancer Institute, Detrick, Md., which yielded characteristic orange colonies on Trypticase soy agar (TSA; Baltimore Biological Laboratory, Cockeysville, Md.) after 24 h at 37°C. A suspension of approximately 3×10^8 spores per ml of distilled water was aerosolized with a DeVilbis no. 40 nebulizer (DeVilbis Corp., Somerset, Pa.) at an air pressure of 10 lb/in^2 (703.1 g/cm^2) and a flow rate of 0.34 ml/min. During the 6.5-min aerosolization period the total challenge to the system was 6.6×10^8 spores. Figure 2 illustrates the test setup. The nebulizer was centered in the cabinet at 14 inches (35.56 cm) above the work surface and 4 inches (10.16 cm) back from the view screen, with the spray directed outward. Directly below this nebulizer was a 3-foot (0.91-m) stainless steel pipe with closed ends elevated slightly above the work surface, representing an arm or similar airflow blockage. Beneath this "arm" (shown better in Fig. 3) was a standard TSA plate elevated on flat-headed stainless steel screws over the front perforated air intake grille to act as a control. Bracketing this arm at 2.5 inches (6.35 cm) outside the cabinet were four all-glass impingers (AGI-4; Ace Glass, Vineland, N.J.), each containing 22 ml of sterile distilled water and operating at a calibrated sampling rate of 12.5 liters per min. Immediately after the sampling period the fluid from these four samplers was pooled and passed through a 0.45-μm membrane filter (type HA; Millipore Corp., Bedford, Mass.), which was then rolled on a TSA plate; the culture was incubated for 24 h at

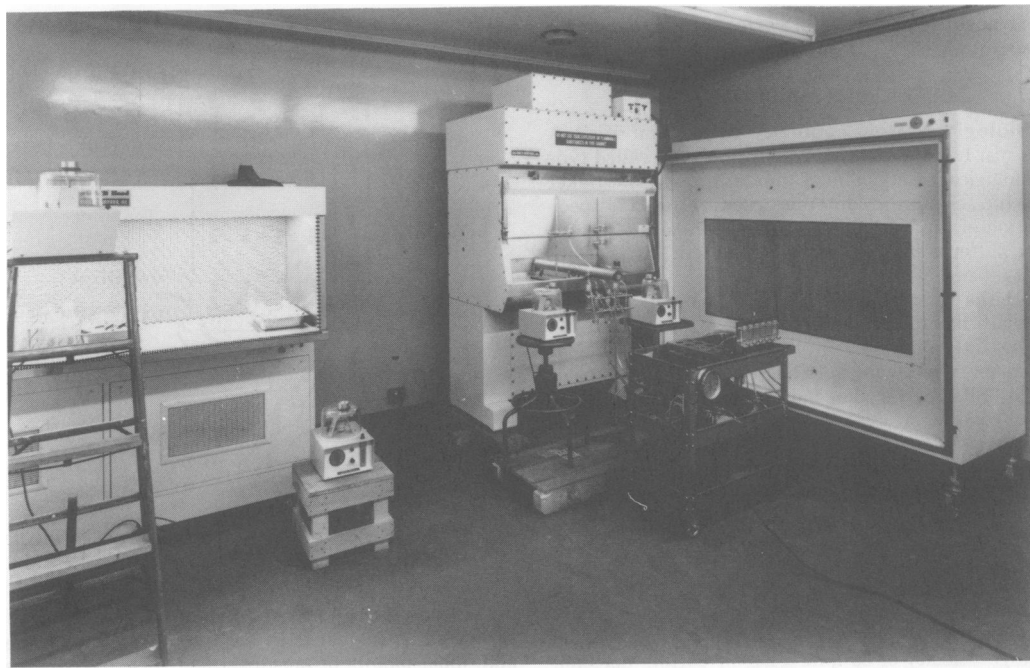

FIG. 2. *Modified personnel protection test setup.*

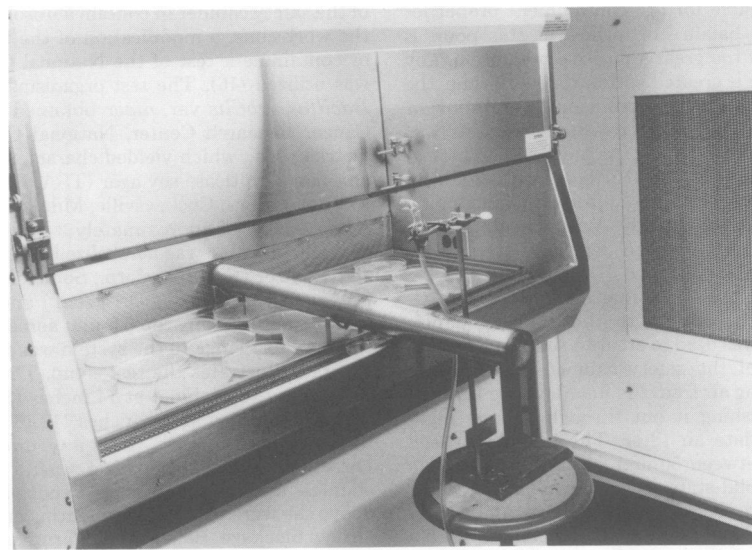

FIG. 3. *Modified product protection test setup.*

37°C, and the colonies were enumerated.

Two slit samplers (model 200 air sampler; Mattson-Garvin Co., Maitland Fla.) were placed such that their inlets were 6 inches (15.24 cm) out from the cabinet face and 8 inches (20.32 cm) from the inner side wall at the work surface level. These operated at a sampling rate of 28.3 liters per min, revolving one TSA plate (150 by 15 mm) once under the slit in 30 min. Two additional slit samplers were located as shown in Fig. 2 because smoke used as an airflow tracer showed these positions to be most advantageous to spore recovery. In fact, due to the crossflow, these two "downwind" locations recovered the most bacteria per test. Also due to this downwind phenomenon, the control plate had to be moved farther to the left of center with increasing crossdraft speed to pick up the 300+ colony limit arbitrarily selected as a positive control. In practice, a confluent plate usually resulted

from such placement. Immediately after the 30-min test, all plates were covered and incubated for 24 h at 37°C, and the colonies were enumerated.

The slit samplers ran for the entire 30-min test period. At 14 min into this sample time, the nebulizer was turned on, followed 1 min later by the impingers. These ran for 5 min and were shut off, and the nebulizer was shut off 30 s later. The 14-min slit sampler time before the spore challenge represented a control for background room contamination, and results from this portion of the slit sampler plate were not included in the total count. If *B. subtilis* var. *niger* colonies appeared in this section of the plate, the data were discarded, and the test was repeated. The horizontal flow module handled between 650 and 2,100 cubic feet per min, depending upon crossflow velocity, and, when added to the room airflow between tests, effectively removed all airborne organisms before the beginning of the next trial run. The total colony-forming units (CFU) recovered per test represent the sum of all CFU retrieved by the four impingers, which sampled a total air volume of 8.8 cubic feet (250 liters) during 5 min, plus all CFU retrieved by the four slit samplers, which sampled a total of 64 cubic feet (1,812 liters) during the 16 min from the start of the nebulizer until the end of the test. The primary purpose of the impingers was to recover organisms which might "creep" out along the top of the stainless steel arm, and they accounted for a very small amount of the total bacteria recovered, especially at the higher crossflow velocities. From 10 to 30 replicate tests were performed at the following crossflow velocities: 0, 50, 80, 90, 100, 110, 120, 130, 140, 150, 175, and 200 fpm.

Product protection testing. To test the ability of the safety cabinet to prevent outside contaminants from entering the work zone, a modified version of the product protection test developed by the National Cancer Institute and detailed in a National Sanitation Foundation standard (19) was performed. Figure 3 illustrates the test setup. A suspension of 3×10^6 spores per ml of distilled water was aerosolized by the same DeVilbis no. 40 nebulizer described above operating at 10 lb/in^2; this represented a total challenge to the hood of 5.1×10^6 spores in 5 min. The nebulizer was located directly above the stainless steel arm (positioned as before) and 4 inches directly outside the cabinet at the bottom edge of the view screen, with the spray directed toward the cabinet. The control plate was located as before. The work surface of the cabinet was covered with 21 open TSA settling plates (150 by 15 mm). The timing sequence consisted of a 5-min nebulization period, followed by 10 min with the system left running undisturbed. The plates were then immediately covered and incubated for 24 h at 37°C, and the colonies were enumerated. From 10 to 20 replicate tests were run at the crossflow velocities listed earlier.

RESULTS

The effect of crossflow velocity on the personnel protection of a biological safety cabinet is shown in Fig. 4. Although the linear-regression line crosses the x-axis at a 70-fpm crossflow, in actual testing no organisms were recovered outside the cabinet until the 80-fpm cabinet airflows were exceeded. Beyond 90 fpm the number of bacteria escaping the unit and recoverable by the sampling apparatus rose logarithmically with increasing crossdraft velocity. At approximately 160 fpm the number of organisms leveled off because the upper efficiency limit of the sampling apparatus, i.e., slit samplers, was reached. For this reason the last two points (175 and 200 fpm) were not calculated into the linear-regression line.

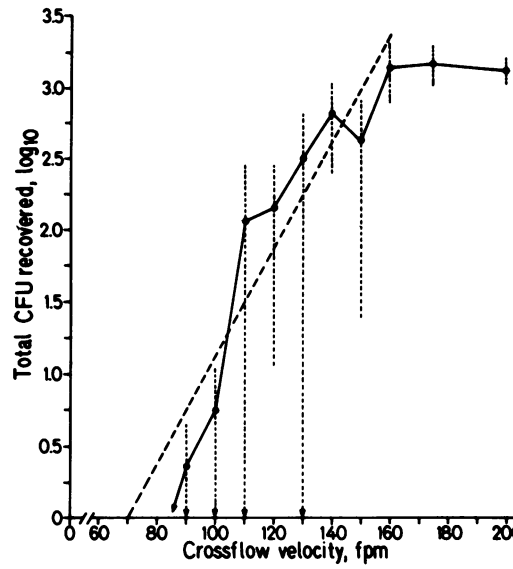

FIG. 4. *Effects of crossflow velocity on the personnel protection of a class II, type A biological safety cabinet. Vertical dotted lines represent the $\log_{10}$ range of total CFU recovered at a particular crossflow velocity (mean ± one standard deviation). The solid line connects mean values. The oblique dashed line represents the calculated linear regression (correlation coefficient, 0.9265; significant at 99.9% level).*

Figure 5 illustrates the effect of crossflow velocity on the product protection of the safety cabinet. Challenge spores were not recovered on the cabinet work surface until a 120-fpm crossflow velocity was reached. Beyond this point the product-protecting capability of the cabinet decreased logarithmically with increasing crossflow velocity.

DISCUSSION

From these results it appears clear that room crossflows may seriously compromise the performance of a biological safety cabinet. As the velocity of these disruptive airflows rises above 90 or 120 fpm, the cabinet loses its ability to protect either operator or experiment, respectively, on a logarithmic scale. This point was 30 fpm higher in the product protection tests due

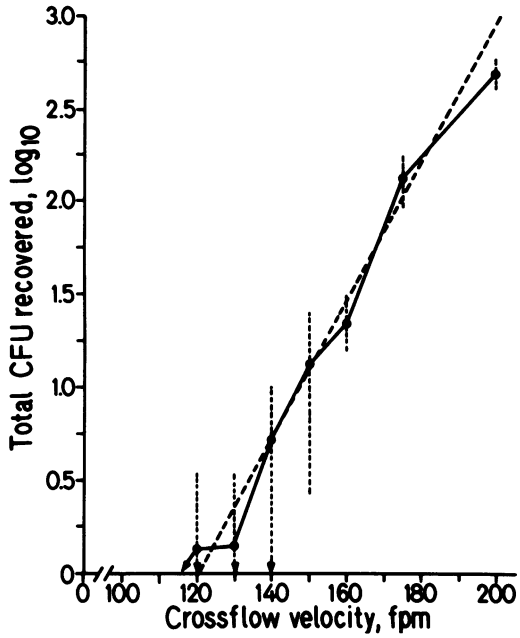

FIG. 5. *Effects of crossflow velocity on the product protection of a class II, type A biological safety cabinet. Vertical dotted lines represent the $\log_{10}$ range of total CFU recovered at a particular crossflow velocity (mean ± one standard deviation). The solid line connects mean values. The oblique dashed line represents the calculated linear regression (correlation coefficient, 0.9919; significant at 99.9% level).*

to one or both of two possible mechanisms: (i) real differences in unit performance or (ii) sampling artifacts. The product protection of the cabinet may be inherently more resistant to disruption by crossdrafts than is the personnel protection capability. Sampling artifacts may occur due to two causes. First, due to the basic test setup (Fig. 3), most of the generated aerosol could have been blown past the unit, thus not offering a sufficient challenge. Second, the 30-fpm variance could have been a measure of the difference in sampling efficiency between the methods utilized in the two test series. Settling plates must rely on gravity and cabinet airflows to deposit spores onto their agar surfaces. Whether a particle is impacted upon such a surface depends upon its size and the velocity and characteristics of the airstream carrying it. Because of the small size of most spore-bearing particles (1 to 5 μm) and the velocity of the airstreams involved, many organisms entering the unit may have remained in the cabinet airflow and resulting turbulence and thus were not deposited on the work surface plates. By contrast, the air samplers used in the personnel protection test enumerate a high percentage of the total number of viable airborne spore-bearing particles (slit samplers) or individual spores themselves (impingers) in a given air volume. This more aggressive sampling procedure, accompanied by a 100-fold-higher aerosol challenge to the cabinet, might have caused the 30-fpm difference. Settling plates instead of the more sensitive air samplers were used in the product protection testing for two reasons, the first being to make these procedures more easily comparable with the use of similar standardized procedures in other laboratories. The second was due to the size of the sampling apparatus itself. When placed in the cabinet, the sampling outlet of either a slit sampler or an impinger sits 11 to 13 inches (ca. 28 to 32 cm) above the work surface and 3 to 5 inches (ca. 7.6 to 12.7 cm) above the view-screen edge. The zone of turbulence generally created by airflow disturbances and the area where most of the work within the cabinet occurs are much closer to the work surface itself and could not be sampled adequately.

A general rule of thumb should be that, if the crossdraft or other disruptive room airflow exceeds the velocity of the air curtain at the unit face, then problems do exist. Unfortunately, in most laboratories such disruptive room airflows are present to various extents. Drafts from open windows and doors are the most hazardous sources because they can be far in excess of 200 fpm and accompanied by substantial turbulence. Heat and air conditioning vents perhaps pose the greatest threat to the safety cabinet because they are much less obvious and therefore seldom considered. According to the *Handbook of Air Conditioning System Design* (4), the exit velocity from such vents should be from 300 to 750 fpm; the *ASHRAE Guide and Data Book* (2) places this value at 250 to 500 fpm, velocities which are capable of dramatic biological safety cabinet airflow disruption. Even personnel traffic may create velocities in excess of those used by the cabinet. A person walking at an average of 3 miles (4.8 km) per h is walking at 264 fpm and pushing a column of air in front of him at almost that speed.

Within the cabinet itself, engineering difficulties prohibit the increase of airflows much above 100 fpm, the velocity of the front air curtain in many other currently marketed units. Above this velocity the resultant sound level becomes uncomfortable to operators, and the control of airflows becomes more difficult due to increased turbulence at the corners and other airflow "bends" (Fig. 1). It is imperative, then, that all room airflow sources and patterns be considered before laboratory installation of a safety cabinet. Once correctly installed, all windows and doors

should be shut, and personnel traffic should be kept to a minimum while work is in progress within the cabinet. To avoid the false sense of security that many workers acquire once in front of a safety cabinet, they must first be educated in its functions and limitations. When properly operated, the cabinet will provide adequate protection for most procedures, but when it is improperly understood and utilized, the workers, surrounding laboratory, and experimental work are all in danger of contamination.

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