Evaluation of the Edgegard Laminar Flow Hood
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In a horizontal back-to-front flow high-efficiency particulate air-filtered laminar hood, it is shown that a Blake bottle obstruction to the air flow causes a downstream cone of turbulent air which can draw microbial contamination into the work area of the hood. In controlled experiments, contamination with T3 coliphage was reduced by a series of perforations around the open edge of the hood which eliminates the cone of turbulent air. The average reduction in phage counts was 90.75, 86.79, 91.12, and 98.92%, depending upon the site of nebulization. The phage counts were reduced in 48 of the 51 tests.

Many medical applications have been found for filtered laminar airflow first developed by Whitfield (7). Laminar airflow and modifications of it have been shown to reduce airborne microbial counts in medical research laboratories (3), surgical amphitheaters (4), intensive care units (1), and animal colonies (5); a laminar flow hood for highly infectious materials gives better than 99.9% containment (2). In these studies, performance has been documented by low and high volume bacterial air sampling, persistence of concentrated artificially generated aerosols, and frequency of contamination in daily use, i.e., number of contaminations or infections in a laminar flow facility compared to the number in the same area with conventional ventilation.

The best criterion of efficacy of laminar flow installations is the absence of contamination during routine use. This assumes proper techniques are used, because neglect of maintenance, misuse, or both, can reduce the efficiency of any system. Holes or breaks in the high-efficiency particulate air (HEPA) filter or air leaks in the system may allow dirty air to enter. Poor execution of aseptic procedures can negate the effect of laminar flow benches. For example, any object placed in the airstream of laminar airflow will create downstream turbulence. The larger the object, the larger the area of turbulence. If a sufficiently large object is located near the front of a horizontal laminar flow cabinet, contaminants from the room or the operator can be drawn into the work area behind the obstruction. In a recent review (8), Whitfield and Lindell stated that the area of turbulence extends downstream approximately three times the width of the obstruction. This holds true when the airstream travels around both sides of the obstruction. If the airstream travels around only one side of an obstruction, downstream turbulence will extend approximately six times the width of the obstruction.

The purpose of this report is to record the effect of the turbulent cone of air created by an obstruction in a laminar flow airstream on aerosols of T3 bacteriophage, delivered just outside the hood across the tip of the turbulent cone, and to study the effect on this of a series of perforations around the front edge of the hood through which air is drawn by the fan that drives the air through the filter. It has been claimed that these perforations reduce turbulence of the airstream after it passes over an object (6).

MATERIALS AND METHODS

Laminar flow hood. A horizontal laminar flow hood (Edgegard Hood, Baker Co., Inc., Biddeford, Me.) was used in these studies. The working area of this hood was 3 ft wide, 2 ft deep, and 22 inches high (91.44 by 60.96 by 55.88 cm). Air passed from back to front through a HEPA filter which removed 99.97% of dioctyl phthalate particles with a diameter of 0.3 μm. Air left the filter at an average velocity of 100 ft (30.48 m)/min and traveled horizontally through the hood with minimum turbulence, or "laminar" air movement. Air exited at the front of the hood, toward the operator. In the hood under study, some of the exit air was drawn in through perforations located across the bottom and two sides of the hood. These perforations were 0.25 inch wide by 1 inch long (0.635 by 2.54 cm) and numbered 16 per linear ft. Additional make-up air was drawn in through a grill beneath the hood in front of the knees of the seated operator, pre-filtered through a coarse filter, and mixed with the air drawn through the perforations before it passed through the HEPA filter. During the present studies, air in the center of the working area of the hood moved at 85 ± 5 ft/min (25.9 ± 1.5 m/min) with the perforations open. With the perforations covered with masking tape, air speeds were 80 ± 5 (24.38 ±
1.5 m/min). Observations on air turbulence and contamination were made first with the perforations open and then repeated with the perforations covered with masking tape. All other conditions were kept identical.

**Measurement of airflow.** The direction and turbulence of airstreams inside the hood were determined by observing smoke. Smoke was generated by exposing swabs dipped in titanium tetrachloride to air. Air velocities were measured with a hot wire thermoanemometer (Alnor Corp., Chicago, Ill.).

**Test procedure.** To evaluate the effectiveness of the perforations around the open edges of the hood, T3 bacteriophage was nebulized in a DeVilbiss no. 40 nebulizer at various sites near the open edge of the hood, and air samples were collected in all glass impingers (AGI-4), which sampled 0.43 cubic ft (12.5 liters) of air per min (9). Phage was assayed by plaque counts as previously reported (2).

A Blake bottle measuring 12 by 4 by 2.5 inches (30.48 by 10.16 by 6.35 cm) was placed in the airstream (Fig. 1). Two locations of the nebulizer and the location of the impinger are illustrated. Experiments were performed with the nebulizer 6, 9, and 12 inches (15.24, 22.86, and 30.48 cm) above the work surface at location 1. Each experiment consisted of nebulizing the phage for 1 min at a prescribed height with the Blake bottle in place and with the perforations open. The impinger was replaced with a fresh one in precisely the same location, all perforations were covered with tape, and the nebulization and collection were repeated. Generally, two experiments were performed per day. For experiments releasing phage outside the hood, the nebulizer was positioned at location 2, 1 inch outside the hood, 5 inches (12.7 cm) to the right of, and 6 inches (15.24 cm) above the impinger. The Blake bottle was 8 inches (20.32 cm) inside the hood. In control studies without the Blake bottle, no phage particles were detected, regardless of whether the perforations were open or covered with masking tape. These controls were included for each experiment recorded below.

Studies with a thermoanemometer indicated that covering the perforations with masking tape did reduce the average air speed slightly, from 85 ft/min (25.9 m/min) to 80 ft/min (24.38 m/min) in the center of the hood. Control studies with no obstruction showed that the slightly lower velocity with the perforations covered did not account for the aspiration of phage particles into the sampler.

**RESULTS**

Airflow patterns can be illustrated by the path of smoke from a swab with the perforations open and taped (Fig. 2). On the left of Fig. 2, the perforations are open and smoke from a swab

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**Fig. 1.** Diagram showing location of nebulizer, impinger, and Blake bottle in Tables 1, 2, 3, and 4. The cone of turbulent air downstream from the Blake bottle and paths of nebulized phage are shaded. The location of the impinger within this zone is shown by a black circle.
streams directly out of the hood in a laminar pattern. Placing a tissue culture flask obstruction upstream of the swab has no effect on the direction of the smoke when the perforations are open as shown in the center; however, when the perforations are covered with masking tape, the smoke can be observed flowing into the hood toward the obstruction as shown on the right.

To determine if this control of turbulence behind an obstruction by the perforations would produce more efficient protection against microbiological contamination, T3 coliphage was released at various locations near the open edge of the hood with samples obtained inside the hood at a location where routine laboratory work would be performed. The sites of phage release and sampling outlined above were selected to simulate contamination that might be present at the front of the hood from room air or from the clothing, arms, and hands of the operator. The number of phage particles released was many logs greater than expected normal contamination to permit quantitative recoveries and reproducibility. The results of nebulizing phage at heights of 6, 9, and 12 inches (15.24, 22.86, and 30.48 cm) above the work surface are presented in Tables 1, 2, and 3, respectively. These tests were repeated 34 times: 13 times with the nebulizer positioned 6 inches (15.24 cm) above the work surface, 14 times at 9 inches (22.86 cm), and 7 times at 12 inches (30.48 cm) above the work surface. The results with the nebulizer setting at 6 and 9 inches were similar, i.e., the number of phage particles recovered in the impinger was markedly reduced in the 27 tests when the perforations were open. In 16 (59%) of these tests, the reduction was greater than 99%, and in 23 tests (85%), the reduction was greater than 90%. The average reduction was 98.92% with the nebulizer 6 inches above the work surface (Table 1); with the nebulizer 9 inches above the work surface, the average reduction was 91.12% (Table 2). When the nebulizer was placed 12 inches above the work surface, a reduction of phage particles recovered in the impinger was observed in six out of seven experiments, and the average reduction in the six tests was 86.79% (Table 3). At the latter nebulizer setting (12 inches above the work surface), the
TABLE 1. No. of bacteriophage particles (PFU) recovered per cubic foot of air sampled in the lee of a Blake bottle obstruction in an Edgeguard Hood with the perforations open or closed with tape.

| Expt | No. of PFU/ft³ with perforation | Reduction a | % |
|------|---------------------------------|-------------|---|
|      | Taped                           | Open        |    |
| 1    | 8,150                           | <50         | 99.39 |
| 2    | 11,985                          | <50         | 99.58 |
| 3    | 15,650                          | <50         | 99.68 |
| 4    | 69,500                          | <50         | 99.93 |
| 5    | 100,000                         | 50          | 99.95 |
| 6    | 100,000                         | <50         | 99.95 |
| 7    | 100,000                         | <50         | 99.95 |
| 8    | 72,150                          | 950         | 98.68 |
| 9    | 245,000                         | 450         | 99.82 |
| 10   | 102,500                         | <50         | 99.95 |
| 11   | 50,000                          | 50          | 99.90 |
| 12   | 613,500                         | 54,350      | 91.20 |
| 13   | 465,000                         | 9,400       | 98.00 |

a Nebulizer 6 inches (15.24 cm) above work surface (position 1 on Fig. 1).

b In experiments 1 and 2, the impingers were located 4 inches inside the hood. All other studies were done with impingers 6.5 inches inside the hood.

c Reduction calculated: 100 – (open/taped). Average reduction was 98.92%.

TABLE 2. No. of bacteriophage particles (PFU) recovered per cubic foot of air sampled in the lee of a Blake bottle obstruction in an Edgeguard Hood with the perforations open or closed with tape.

| Expt | No. of PFU/ft³ with perforations | Reduction b | % |
|------|---------------------------------|-------------|---|
|      | Taped                           | Open        |    |
| 14   | 581,500                         | 3,815       | 99.34 |
| 15   | 446,500                         | <50         | 99.99 |
| 16   | 7,315                           | <50         | 99.32 |
| 17   | 6,315                           | <50         | 99.21 |
| 18   | 4,875                           | 150         | 96.92 |
| 19   | 3,365                           | 115         | 96.59 |
| 20   | 865                             | <50         | 94.22 |
| 21   | 1,765                           | 250         | 85.90 |
| 22   | 4,535                           | 100         | 97.80 |
| 23   | 3,215                           | 1,285       | 60.03 |
| 24   | 6,100                           | 1,465       | 75.98 |
| 25   | 7,825                           | <50         | 99.36 |
| 26   | 5,900                           | 365         | 93.81 |
| 27   | 1,750                           | 400         | 77.14 |

a Nebulizer 9 inches (22.86 cm) above the work surface (position 1 on Fig. 1).

b Reduction calculated: 100 – (open/taped). Average reduction was 91.12%.

cloud of phage particles is in approximately the geographic center of the open face of the hood, largely above the turbulent air behind the Blake bottle; therefore, the perforations have a decreased but still perceptible effect.

TABLE 3. No. of bacteriophage particles (PFU) recovered per cubic foot of air sampled in the lee of a Blake bottle obstruction in an Edgeguard Hood with the perforations open or closed with tape.

| Expt | No. of PFU/ft³ with perforations | Reduction b | % |
|------|---------------------------------|-------------|---|
|      | Taped                           | Open        |    |
| 28   | 1,900                           | <50         | 97.36 |
| 29   | 16,850                          | 3,585       | 78.79 |
| 30   | 35,650                          | 2,900       | 91.87 |
| 31   | 4,500                           | 6,700       | (+150)c |
| 32   | 2,350                           | 650         | 72.35 |
| 33   | 37,150                          | 3,665       | 90.14 |
| 34   | 66,750                          | 6,515       | 90.24 |

a Nebulizer 12 inches (30.48 cm) above work surface (position 1 on Fig. 1).

b Reduction calculated: 100 – (open/taped). Average reduction was 86.79%.

c Not included in average.

TABLE 4. No. of bacteriophage particles (PFU) recovered per cubic foot of air sampled in the lee of a Blake bottle obstruction in an Edgeward Hood with the perforations open or closed with tape.

| Expt | No. of PFU/ft³ with perforations | Reduction b | % |
|------|---------------------------------|-------------|---|
|      | Taped                           | Open        |    |
| 35   | 24,000                          | <50         | 99.79 |
| 36   | <50                             | <50         | 97.45 |
| 37   | 1,985                           | <50         | 98.15 |
| 38   | 3,685                           | 485         | 95.60 |
| 39   | 4,535                           | 1,187       | 73.83 |
| 40   | 2,700                           | <50         | 98.40 |
| 41   | 1,135                           | <50         | 98.40 |
| 42   | 11,350                          | 365         | 86.84 |
| 43   | 3,165                           | <50         | 86.84 |
| 44   | 3,950                           | 1,475       | 62.70 |
| 45   | 13,000                          | 1,415       | 89.10 |
| 46   | 18,350                          | 50          | 99.73 |
| 47   | 18,850                          | <50         | 99.73 |
| 48   | 15,500                          | <50         | 99.73 |
| 49   | 315                             | <50         | 84.13 |
| 50   | 250                             | <50         | 80.00 |
| 51   | <50                             | <50         | 0 |

a Nebulizer outside the hood, 5 inches (12.7 cm) to the right and 6 inches (15.24 cm) above the impinger (position 2 on Fig. 1).

b Reduction calculated: 100 – (open/taped). Average reduction was 90.75%.
Experiments presented in Table 4 simulate the generation of contaminants in the room air, or from the clothing of the operator, because the nebulizer was placed 1 inch (2.54 cm) outside the hood and 6 inches (15.24 cm) above the work surface. With the perforations taped and the impinger placed behind an obstruction, phage particles were recovered in the impinger behind an obstruction in 15 out of 17 experiments, and with the perforations open the number of phage particles recovered was reduced by 62.70 to 99.79%, with an average reduction of 90.75%.

When all conditions were kept the same as recorded in Table 4 except for moving the nebulizer an additional 5 inches to the right, i.e., 10 inches to the right of the sampler, no phage could be detected in any test with the perforations open. With the perforations closed, phage particles were detected in each test as follows: 135, 650, 1,285, 250, and 50 particles/ft² of air.

DISCUSSION

It is easy to observe the airflow patterns in a laminar flow hood by following the distribution of smoke released at various points in the hood. It is also easy to see the cone of turbulent air on the downwind side of an obstruction in the airstream and to observe that smoke moves in a circular pattern within this turbulent area which extends downwind three to six times the width of the obstruction (8). By means of an automatic dust particle counter (Dynac Corp., Portland, Me.), it can readily be shown that this circular eddy of turbulent air aspirates dust particles into the hood if an obstruction is placed so that the cone of turbulence extends outside the front of the hood. These observations made with smoke and dust particles are significant, but they do not show whether viable organisms such as bacteria or viruses could also be aspirated into the hood behind an obstruction. In continuous use, the air in the hood and in the room is almost free of viable organisms, so it is very difficult to get a statistically reliable test for the efficacy of the perforations. However, in routine use, small numbers of organisms are constantly shed by the operator immediately outside the hood and by his manipulations inside the hood. It is, therefore, desirable to know whether the perforations confer some protection against accidental infection in a horizontal laminar flow hood and whether this can be demonstrated with viable organisms in a controlled experiment which is reproducible, although an exaggeration of what might occur in routine use of the hood.

Other attempts have been made to control creation of turbulence in the lee of obstructions in horizontal laminar flow cabinets. Converging horizontal flow has been recommended to reduce turbulence and provide eddy-free purging behind obstructions. We have not evaluated converging flow units and, to the best of our knowledge, their microbiological significance has not been quantitatively determined.

T3 coliphage was used in these experiments because it is relatively stable in an aerosol, can be quantitatively recovered from the air, and is non-pathogenic for personnel. In each of the experiments reported here, the tests were carried out with the hood in operation and the filtered air moving from back to front of the hood. A technician was seated in front of the hood in all tests. The nebulizer was placed so the cloud of aerosolized phage was carried across the tip of the cone of turbulent air outside the hood. The experiments were designed to determine whether the phage particles were drawn back into the hood and, if so, whether this could be reduced by the perforations. The phage studies show that the number of phage particles recovered was reduced in 48 of 51 tests when the perforations were open. In one test, the number was increased (test 31), and in two tests no difference was detected (tests 36 and 51). The average reduction shown in Tables 1 to 4 ranged from 84.10 to 98.92%, depending on the conditions of the test. It should be noted that with the perforations open, the least contamination was observed when the phage was nebulized 6 inches above the work surface. The usual work area of a hood is 6.5 inches inside the hood and 4 to 8 inches above the surface. The present studies provide evidence that the perforations are desirable under the conditions of the tests. Obstructions of other sizes, shapes, and locations were not tested, but the consistency of the results obtained suggests to us that the perforations are a good addition to any horizontal flow microbiological hood. If a hood without perforations is used, it is important to reduce obstructions to a minimum and to have no obstructions between the surface of the filter and the site of aseptic procedures.

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