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Evaluation of Laminar Flow Microbiological Safety Cabinets

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The microbiological control efficiency of two class 100 laminar down-flow hoods was determined by using aerosols of *Bacillus subtilis* var. *niger* spores. The first unit challenged utilized a slanted eyelid to partially enclose the front work opening. This hood showed nearly perfect control of ambient organisms in the work area. It also gave a 10^6 or greater drop in the number of organisms passing out of the exhaust system. However, when the interior work area of the hood was challenged, significant numbers of spores penetrated the air barrier and escaped into the ambient air. A redesigned laminar flow hood was built incorporating a vertical eyelid and a reduced opening to the work area. This hood showed the same excellent characteristics for controlling ambient contamination. Exhaust system leakage was also extremely low. Air barrier efficiency for the newer hood was increased with lower amounts of spore penetration into the ambient air.

Since the development of the laminar airflow principle for controlling micron-sized particles, many laminar flow devices have been designed. One application is the laminar down-flow fumehood (7). Extensive testing has shown general laminar flow clean rooms to be extremely efficient in controlling nearly any type of airborne particulate matter, including bacteria and fungi (2, 4, 6). However, the fume hood differs in one major characteristic from laminar flow rooms. It has an open access to ambient air and thus a chance for turbulence to occur. Thus, contamination associated with ambient air may be introduced into the clean environment at the interface of the two air systems.

The mixing of the "dirty" air with the filtered air can be reduced by enclosing the front and working with gloves sealed in portholes converting the hood into a miniature clean room. A second method is to increase the airflow at the front interface of the fume hood, thus creating an air curtain barrier. The air curtain barrier allows a technician nearly complete freedom of movement within the hood work area. The degree of worker protection from organisms moving through the air barrier originating from inside the hood and the amount of the protection to the materials within the hood from ambient contamination are still undefined.

To more clearly define the practical limits of such laminar flow devices for microbiological applications, we chose units with solid stainless-steel floors utilizing exhaust slots at both the front and back edge of the floor. These Class 100 (Fed. Std. No. 209a, G.S.A., Business Service Center, Washington, D.C., 1966.) units had airflow rates of 70 to 90 ft (21 to 27 m)/min through the work space and 200 ft (61 m)/min at the developing air barrier. The exhausts were equipped with high efficiency particulate air (HEPA) filters (3) and were not of the recycling type.

MATERIALS AND METHODS

All challenges were made with *Bacillus subtilis* var. *niger* spores prepared according to Beakley, Whitfield, and Mashburn (2). These were used at a concentration of 10^7 spores per ml unless otherwise noted.

The hoods tested were produced by Envirco, Inc., (Albuquerque, N. M.). The units tested were a Balanced Air Clean Bench model no. DF-400 with an exhaust HEPA filter attached (Fig. 1), and an Enviromedic DF-600-F hood modified for the University of New Mexico according to our recommendations (Fig. 2). The modifications included a perpendicular 0.25-inch (0.64 cm) plexiglass eyelid extending down to within 15 cm of the work surface, and a solenoid-activated, electronically controlled damper used in airflow balance adjustments, i.e., to effect either a positive or negative pressure relative to the operator. The modified hood also had a recessed floor creating a splash lip at the edges protecting the exhaust system from spillage. All positive pressure plenums were sealed with butyl caulking compound in addition to the normal gaskets applied to all seams. Aerosols were produced by using a no. 40 nebulizer (De Vilbis Co., Somerset, Pa.).

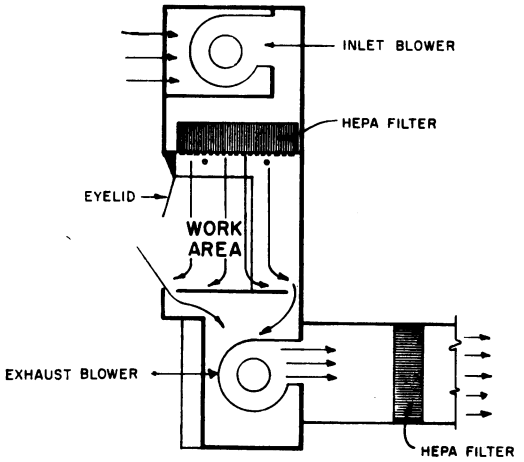

FIG. 1. *Cross section of the DF-400 safety cabinet indicating airflow patterns, exhaust filter adaption, and a slanted eyelid.*

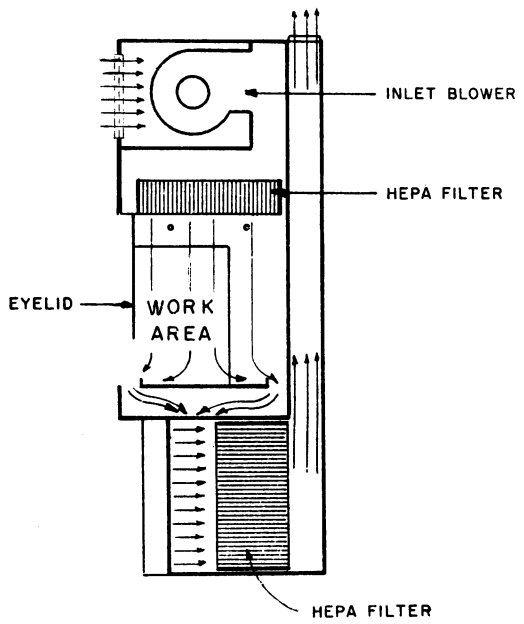

FIG. 2. *Cross section of the DF-600-F safety cabinet indicating airflow patterns, the modified vertical eyelid, and recessed work surface.*

All ambient air sampling was done with Andersen samplers (1). Sampling inside the hood was done with open settling plates and sterile stainless-steel strips, 1 × 3 × 1/16 inches (2.54 × 7.6 × 0.16 cm). Tryptic Soy Agar (Difco) was used as the culture medium in all experiments. Results were recorded as the number of colonies per plate showing the typical *B. subtilis* var. *niger* morphology after incubation at 35 C for 48 to 72 hr. Colony counts on Andersen plates were all corrected according to Andersen's "positive-hole" technique.

Challenge of the DF-400 hood. For the interior challenges, the nebulizer was centered inside the DF-400 hood and directed toward the front (perpendicular to the front of the cabinet). It was placed 5 cm from the front of the cabinet for the first experiment and then moved toward the rear of the hood in 2.5-cm increments for each succeeding experiment. The nebulizer was placed at two heights for each experiment: 15 cm above the stainless-steel floor (normal working height) and at 30 cm above the floor (equal to the level of the lower edge of the eyelid). The Andersen sampler was centered exterior to the hood with the orifice at the hood floor level. The sampler was started just before the release of the aerosol and run for 10 min. The spore suspension was dispensed from the nebulizer in 1-ml samples, with nitrogen flowing at 10 ft^3/hr with a resulting exit velocity from the nebulizer orifice of approximately 100 ft/min.

A second series of experiments reduced the spore velocity capable of barrier penetration to zero by placing the nebulizer parallel to and 12 and 20 cm from the cabinet front.

Surface impingement and migration of the aerosol were studied with stainless-steel strips and settling plates placed on the floor of the hood. The aerosol was released at various places in the hood at 12 to 15 sample sites each time. At the conclusion of an experiment, the petri dishes were covered, or the stainless-steel strips were removed, placed in petri dishes, and overlaid with Tryptic Soy Agar.

Leakage of the exhaust filter system was determined with Andersen samplers placed in the exhaust duct of the DF-400 hood and run concurrently with the interior challenge.

In the exterior challenge, the nebulizer was directed at the front of the hood from distances of 15 and 30 cm from the hood. Three elevations were chosen: work surface, 30 cm above the work surface (eyelid level), and 76 cm above the work surface (level with the prefilters). Penetration into the hood was determined by settling plates arranged so that the front of the work surface of the hood was covered with fewer plates extending farther back into the hood.

Challenge of the DF-600-F hood. The modified hood was challenged with parallel and perpendicular aerosols at heights of 15 and 30 cm above the work surface. Leakage around the ends of the eyelid was determined by directing the aerosol toward the front corners. Interior surface impingement and migration of the aerosol were studied by using the same methods used for the DF-400 hood.

The exhaust duct on the DF-600-F was inaccessable for sample work; however, Andersen samplers were placed next to the seams of the lower positive pressure plenum. The samplers were started and 10^9 spores were released into the rear exhaust slot.

The exterior challenge for the DF-600-F hood was identical with that of the DF-400, except that a single nebulizer height of 30 cm above the work surface was used.

RESULTS AND DISCUSSION

Table 1 lists the corrected Andersen plate counts for the internal challenge directed per-

TABLE 1. *Total corrected colony count of B. subtilis var. niger from Andersen samplers[a] for the interior challenge with the nebulizer perpendicular to the front of the DF-400 hood*

Distance (cm) from front of hood	Colony count at	
	30 cm above work surface	15 cm above work surface
5	+3[b]	+3
7.6	+3	+3
10	+3	+3
12.7	+3	+1
15	+2	+2
17.7	+2	382
20	+2	2,488
23	2,443	120
25	+2	1,707
28	133	1,264
30	91	67
33	13	82
35.5	11	229
38	22	39
40.6	4	10
43	2	3
45.7	2	7

[a] In addition to the Andersen correction, the normal background of *B. subtilis* var. *niger* was subtracted from the first-stage plate count.

[b] The number of plates with completely filled rosette patterns.

pendicular to the front of the DF-400 hood. Both heights show an initial reduction at 13 to 15 cm inside the hood. Progressing into the hood, leakage at the 15-cm height dropped down to a low level much quicker than at the 30-cm height. The spore velocity is one reason for penetration; however, complete spore elimination was not seen for either height until the dividing line for the airflow to the two exhausts was reached (approximately midway between the front and rear of the hood). This and the continued high level of penetration for the 30-cm height indicated more turbulence at the front than was previously believed.

Marsh et al. (5) indicated any obstruction in the path of the laminar flow air will cause turbulence below the obstruction. The slanted eyelid represents such an obstruction. The laminar airflow becomes increasingly isotropic with greater turbulence at the lower eyelid edge as it contacts the ambient air and curls around the eyelid surface escaping the exhaust system. The turbulence in this area is also enhanced by the "cross grain" flow of nitrogen from the nebulizer.

Parallel challenge results for the DF-400 unit (Table 2) showed a very marked reduction in the

TABLE 2. *Total corrected colony counts of B. subtilis var. niger from the Andersen samplers[a] for the interior challenge with the nebulizer parallel to the front of the DF-400 hood*

Distance (cm) from front of hood	Colony count	
	30 cm above work surface	15 cm above work surface
12.7	7	12
20	1	4

[a] See footnote *a*, Table 1.

TABLE 3. *Total corrected colony count of B. subtilis var. niger from Andersen samplers[a] for the interior challenge with the nebulizer perpendicular to the front of the DF-600-F hood[b]*

Distance (cm) from front of hood	Colony count at	
	30 cm above work surface	15 cm above work surface
5	36	44
10	73	69
15	50	48
20	10	6
25	3	21
30	27	10

[a] See footnote *a*, Table 1.

[b] The balance control was set for a negative pressure.

barrier penetration when compared to the previous results; however, leakage was noted particularly at the upper level. The supply airflow was recalibrated by Envirco technicians to match the exhaust airflow more closely, but rebalancing the hood had no effect on the results.

With these results in mind, we asked to have the hood eyelid on the DF-600-F hood constructed so that it would be flush with the upper surfaces and perpendicular to the filter bank. The lower edge extended to within 15 cm of the exhaust slot rather than the 30 cm of the first unit. The new eyelid caused the developing air barrier to have an increased flow rate when it contacted the ambient air, thus lessening the chance for the air to double back on itself and release contaminants to the ambient air. Tables 3 and 4 list the results from internal challenges on the DF-600-F hood. When titanium tetrachloride acid smoke was released next to the interior surface of the eyelid, a small amount of leakage could be seen. This also was apparent from the spore challenges directed perpendicular to the eyelid. The control of the spores released 5 cm from the interior surface of the eyelid and with no outward

velocity was nearly completely controlled with at least a 10^6 drop in count across the barrier; however, leakage around the ends of the eyelid was observed. Similar results were obtained for settings of the balance control damper in a neutral position as well as in a negative pressure position.

Migration of the bacteria on the work surface was seen in a completely random pattern for both hoods. The aerosol would generally impinge on the stainless-steel strips directly below the source and extend out about 30 cm from the nebulizer. At various times, the bacteria were carried along the bottom of the hood similar to the smoke pattern in Fig. 3. This drift seemed to be completely random and was more evident in the depressed floor model than in the flat floor model. This contamination does not escape out to the ambient air but is drawn into the exhaust slots.

Table 5 lists the results of the external challenge for both hoods. There was almost no detectable penetration in any case either through the filter media or through the air barrier. The colonies reported may be due to incidental contamination while opening or closing the plates, because of the high concentration of spores used in the challenge studies.

TABLE 4. *Total corrected colony counts of B. subtilis var. niger from Andersen samplers[a] for the parallel interior challenge of the DF-600-F vertical eyelid hood.*

Hood air pressure relative to ambient air	Height above work surface	Colony counts at		
		5 cm from front of hood	10 cm from front of hood	15 cm from front of hood
Negative	30 cm	7	5	3
	15 cm	1	1	1
Neutral	30 cm	1	3	0

[a] See footnote *a*, Table 1.

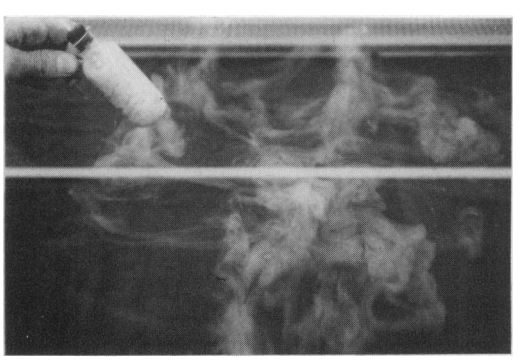

FIG. 3. *Smoke studies showing the migration patterns on the floor of a laminar flow safety cabinet.*

TABLE 5. *Total colony counts of B. subtilis var. niger on settling plates for the exterior challenge with the aerosol directed toward the hood[a]*

Hood	Height of nebulizer above work surface (cm)	Colony counts at	
		15 cm from front of hood	30 cm from front of hood
DF-400	0	0	3
	30	1	1
	76	1	2
DF-600-F	30[b]	2	0

[a] The balance control was set for a negative pressure.

The exhaust filter and plenum sealing results also show nearly complete control of bacteria with only 3 typical colonies recorded from a total of 12 experiments (approximately 10^8 spores) on the exhaust filter, and 7 typical colonies from the plenum seal experiments (approximately 10^{10} spores).

In practical application experiments, such as running a blender filled with a dense spore suspension and emptying a spore-filled hypodermic syringe into an open petri dish, we detected no leakage of bacteria from either the DF-400 or DF-600-F hood. This suggests our challenge tests were more severe than conditions normally encountered in the laboratory.

Smoke studies indicated that, when the technician places his arms into the hood work area, some contamination travels up the arms until it reaches the exhaust area and is sucked away from the arms. Laboratory coat sleeves and cuffs or similar wearing apparel can trap the contamination and prevent it from entering the exhaust system.

To obtain optimal efficiency of this type of hood for microbiological applications, we recommend that any material to be kept "clean" should be placed to one side of the manipulation being performed and elevated slightly above the work surface. The material should also be placed in the back half of the hood to avoid any possible contamination that is sloughed off from the worker. When controlling internal contamination, we recommend that work next to the eyelid be avoided. Whenever possible, any manipulation producing an excessive aerosol should be performed in the rear of the hood.

Laminar flow safety cabinets of the DF-600-F type are extremely efficient in preventing high bacterial levels produced in the ambient air from

reaching the work surface and also in controlling bacterial contamination after it is sucked into the exhaust system. Even though the modified air barrier performs very efficiently, it is not an absolute barrier to contamination, and use of an aseptic technique should be employed when working in these hoods.

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