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TECHNICAL MANUSCRIPT 466

CONTAINMENT OF MICROBIAL AEROSOLS IN A MICROBIOLOGICAL SAFETY CABINET

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DEPARTMENT OF THE ARMY
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TECHNICAL MANUSCRIPT 466

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INDUSTRIAL HEALTH AND SAFETY OFFICE

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ABSTRACT

A microbiological safety cabinet was evaluated to determine conditions under which microorganisms might escape. Tests were conducted under three cabinet-closure conditions, various air flow velocities, and different laboratory operations, when 1.0×10^5, 1.1×10^5, or 1.0×10^6 microorganisms per cubic foot of cabinet space were released per minute for 5 minutes.

Data revealed that: (i) escape of a human infectious dose is possible when a cabinet is used with the glove panel off; (ii) the number of organisms that escaped from the cabinet increased with a decrease in air velocity; and (iii) an increase in laboratory operations increased organism escape. Thus, when the glove panel is off, the cabinet is safe only for operations that release few microorganisms into the cabinet, whereas the cabinet is safe for operations of significantly greater hazard when used with the glove panel on but without gloves attached.

I. INTRODUCTION

For many years chemists have used fume hoods to prevent escape of noxious gases during laboratory and industrial operations. These hoods are designed with large movable windows and adjustable vents at the rear and ceiling of the hoods. Air flow across the open face may vary from 50 to 200 linear ft/min. Until recently, air flow patterns through a fume hood usually were based on the observation of smoke tests. A quantitative approach using propane gas for establishing face velocities of fume hoods has been reported.[1] With this technique, gaseous emission rates from a fume hood were determined at different face velocities, window closures, and laboratory operations. The findings showed that (i) the smaller the face opening, the more effectively the gas was contained and (ii) a man walking past the hood produced a tenfold increase in leakage. Although there are basic similarities between a chemical fume hood and a microbiological safety cabinet,[2] it has not been established that leakage of a gas parallels leakage of microorganisms.

In 1953, the escape of test organisms from a smaller cabinet than that presently used in our laboratories was reported.[3] Specifications of that cabinet were: two glove ports, each 0.12 ft^2 in area; glove panel opening, 1.9 ft^2; cabinet width, 45 inches; and an inward air flow of 50 linear ft/min with the glove panel off. The room housing this cabinet had no air exhaust other than the exhaust through the cabinet. In the tests, 57 to 230 ml of a broth culture of *Serratia indica* containing 1.4×10^5 bacteria per ml were nebulized inside the cabinet in 31 to 96 min, while air samples were taken outside 1 inch from the cabinet surface. Results were as follows:

Closure Conditions	Recovery
Glove port panel off	3[a]
Glove port panel on	0
Glove port panel on with operator moving hands in and out of cabinet	0
Glove port panel off with operator moving hands in and out of cabinet	0

a. Total organisms recovered from 510 ft^3 of air sampled.

Failure to recover organisms outside the cabinet, with the glove port panel off, with an operator moving his hands in and out of the cabinet, is unexplainable.

The objective of the present tests reported here was to determine (i) to what extent microorganisms escape from the type of microbiological cabinet now in use, (ii) the resultant hazard to operating personnel, and (iii) cabinet closure conditions necessary for operations of various degrees of hazard.

II. MATERIALS AND METHODS

A. CABINET SPECIFICATIONS

The interior of the present stainless steel cabinet is 32 inches deep, 6 ft wide, and 3 ft high. The cabinet (Fig. 1) has these major features: sloped glass viewing panel hinged at the top; perpendicular removable panel equipped with four oval glove ports, each with an area of 0.25 ft^2; attached ultraviolet (UV) irradiated double-door air lock (pass-through box); external fluorescent and internal UV lights; air; vacuum; electrical outlet; hot and cold water; drain connected to the central biological decontamination facility; high-efficiency exhaust air filter; and a 300 ft^3/min exhaust blower with a 1,735 rpm fan.* The exhaust from the cabinet is connected to the building exhaust system, where it is refiltered through high-efficiency filters before release to the atmosphere. The cabinet is operated at a reduced pressure of 0.7 inch water gauge when the gloves are attached, and at a minimum air flow of 50 linear ft/min with the glove panel off (open area, 4.6 ft^2). The ventilation of the room in which the cabinet is housed provides six air changes per hour; the room is under negative pressure in relation to atmosphere.

B. TEST PROTOCOL

To determine the organisms released to the room, a liquid suspension of Serratia marcescens was disseminated in the cabinet at a calculated concentration of 1.0×10^5, 1.1×10^5, or 1.0×10^6 organisms per cubic foot (org/ft^3) of cabinet space. The disseminator used was a pneumatic nozzle (liquid-siphon pick-up type**). The nozzle was attached to the cabinet air supply through an air regulating valve.*** Air was supplied to the nozzle at 13 pounds per square inch gauge. The nozzle disseminated

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* Clarage Fan Co., Kalamazoo, Michigan.
** Spraying Systems Co., 3201 Randolph St., Belwood, Illinois.
*** Perfecting Service Co., Charlotte, N.C.

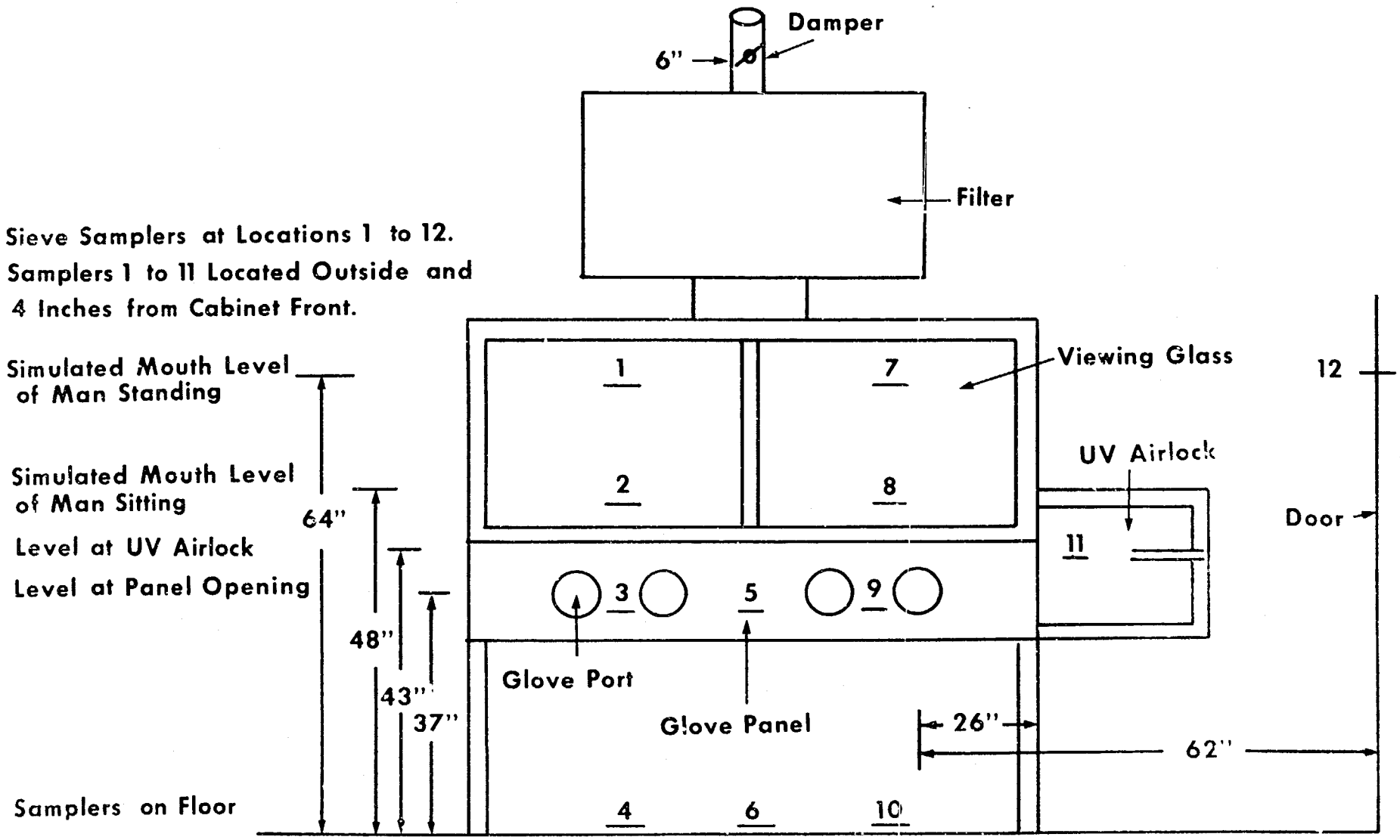

Figure 1. Sieve Sampler Locations for Recovery of Microorganisms Escaping from Cabinet.

9.2 ml of organism suspension per minute. Total dissemination time was 5 min. The nozzle was positioned at a 45 degree angle pointing away from the front of the hood, 16 inches above the cabinet floor level, and 12 inches from the cabinet front. The test parameters were:

Cabinet Closure Conditions	Air Flow (linear ft/min)
Glove panel off	30 and 50
Glove panel on, gloves off	100 and 115
Glove panel on, gloves on	145 and 400[a]

a. Measured in the 6-inch-diameter cabinet exhaust pipe (air leakage of 29 and 80 ft^3/min into the cabinet).

The cabinet leaks in various velocities around gaskets, gloves, and utility penetrations. Air flow was measured with an Alnor velometer.* With each of the three cabinet closure conditions, laboratory operations tested throughout the 5-min culture dissemination were: (i) static, no laboratory manipulations, (ii) one man performing routine microbiological techniques inside cabinet, (iii) one man walking past and parallel to the cabinet within 2 ft of it at 1, 2, 3, and 4 min from starting culture dissemination, (iv) opening laboratory door, which was 3 ft from the cabinet, at 1, 2, 3, and 4 min from starting culture dissemination, and (v) a combination study of operations (ii), (iii), and (iv).

Air samples for recovery of the test organism were taken at 12 locations in the room (Fig. 1). A sterile sieve sampler[4] housing a plastic nutrient agar plate was used at each location, and room air was sampled at a rate of 1 ft^3/min per sampler. The vacuum source for each sampler was a Gast** vacuum pump, and the pumps were synchronized from an electrical control panel. All samplers were run for the 5-min culture dissemination period. Before each test, 5-min control samples were taken at each sampling location; they recovered no test bacteria.

Between tests, a 30-min interval elapsed, during which there were, by calculation, three changes of the room air and between 18 and 143 changes of air in the cabinet depending on closure condition and air flow rate. Simultaneously, the cabinet was irradiated with the installed UV lights; a high-intensity UV light*** which irradiated in all directions, was placed in the center of the room.

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* Illinois Testing Laboratories, Inc., 420 N. LaSalle St., Chicago, Illinois 60600.

** Gast Mfg. Corporation, Benton Harbor, Michigan.

*** 1,200 watts, Hanovia Chemical and Manufacturing Co., Newark, New Jersey.

III. RESULTS

Recoveries of S. marcescens at the 12 sampling locations are shown in Table 1. An estimate of the hazard caused by organisms released to the room was made by converting the number of organisms recovered in 5 min into human infective dose (HID) for a highly infectious microorganism such as the etiologic agents of Q fever, tularemia, or Venezuelan equine encephalitis (see Appendix). This conversion was made on the assumption that 10 infectious organisms constitute an HID.[6] HID was calculated only for the cabinet closure condition with the panel off.

As one would anticipate, the increasing order of microbiological aerosol hazard while working in the cabinet with various closure conditions is: (i) glove panel on, gloves installed; (ii) glove panel on, gloves off; (iii) glove panel off. Microorganisms are not released to the room with the glove panel on, gloves attached, and an air flow of 80 ft^3/min into the cabinet.

The most significant finding of the study for persons concerned with establishing safe working conditions with minimum impediment of motion was that when the glove panel was installed without attached gloves there was essentially no escape of organisms from the cabinet. Under these conditions, organisms were recovered at only six sampling locations of 300 samples taken with a minimum air flow of 50 linear ft/min. The number of organisms recovered per location averaged less than one per sampling station (Table 1).

When the glove panel was removed, organisms were recovered at all 12 locations. HID were produced during all types of activities conducted in the laboratory room. The data showed that the HID increased with increased operations. The most hazardous condition occurred while one person was working at the cabinet and another entered the laboratory, walked past the cabinet, and then left the room. Under these conditions, 3.00 to 13.1^+ HID were recovered.

With no activity in the laboratory but with inward cabinet air flow reduced from 50 to 30 linear ft/min, the total rate of organism escape from the cabinet increased about twofold (Table 1).

TABLE 1. RECOVERY OUTSIDE THE CABINET OF BACTERIA AEROSOLIZED WITHIN THE CABINET[a]

Cabinet Condition	Air Flow: Linear ft/min	Air Flow: ft^3/min	Laboratory Operation	Test Concn Organisms per ft^3 of Cab Space	Average Number of Colonies Recovered per Sampler Position Outside Cabinet in 5 Min: 1	2	3	4	5	6	7	8	9	10	11	12
Panel on, gloves on	145	29	No activity[b]	5.5×10^5	0	0	0	0	0.1	0	0	0.1	0.1	0	0.1	0
Panel on, gloves off	100	100		1.1×10^5	0.1	0	0.1	0	0	0	0.2	0	0.1	0	0	0
Panel off	30	137		1.1×10^5	16.4	73.7	180.3	8.2	179.1	19.8	37.3	108.7	93.7	48.4	67.9	21.0
Human infectious dose[c]					0.72	3.22	7.9	0.36	7.82	0.86	1.63	4.8	4.1	2.11	2.96	0.92
Panel on, gloves on	400	80	No activity[d]	1.0×10^5	0	0	0	0	0	0	0	0	0	0	0	0
Panel on, gloves off	115	115		1.0×10^5	0	0	0	0	0	0	0	0	0	0	0	0
Panel off	50	228.5		1.0×10^5	1.0	38.0	158.8	0.2	130.8	5.6	27.2	33.6	4.2	4.2	4.0	0.6
Human infectious dose					0.04	1.66	7.36	0.01	5.72	0.24	1.19	1.47	0.18	0.18	0.17	0.03
Panel on, gloves on	400	80	Working in cabinet[d]	1.0×10^5	0	0	0	0	0	0	0	0	0	0	0	0
Panel on, gloves off	115	115		1.0×10^5	0	0	0	0	0.2	0	0.4	0	0	0	0	0.2
Panel off	50	228.5		1.0×10^5	3.4	178.6	371.4	1.2	229.6	2.4	11.4	64.0	104.4	14.6	12.6	1.6
Human infectious dose					0.15	7.79	16.20	0.05	10.01	0.11	0.49	2.79	4.56	0.64	0.55	0.07
Panel on, gloves on	400	80	Walking past cabinet each minute during sampling[d]	1.0×10^6	0	0	0	0	0	0	0	0	0	0	0	0
Panel on, gloves off	115	115		1.0×10^6	0	0	0	0	0	0	0.2	0	0.2	0	0	0
Panel off	50	228.5		1.0×10^6	14.6	100.8	300+	13.8	300+	31.8	36.6	113.8	300+	99.6	31.2	29.6
Human infectious dose					0.64	4.40	13.1+	0.62	13.1+	1.39	1.59	4.96	13.1+	4.35	1.36	1.29
Panel on, gloves on	400	80	Opening lab door each minute during sampling[d]	1.0×10^6	0	0	0	0	0	0	0	0	0	0	0	0
Panel on, gloves off	115	115		1.0×10^6	0	0	0	0	0	0	0	0	0	0	0	0
Panel off	50	228.5		1.0×10^6	19	174.2	300+	35.8	300+	53.2	51.8	283.6	300+	229.8	65.8	62.6
Human infectious dose					0.83	7.61	13.1+	1.56	13.1+	2.31	2.26	12.36	13.1+	10.01	2.87	2.73
Panel on, gloves on	400	80	Working in cab, walking past cab, opening lab door each minute during sampling[d]	1.0×10^6	0	0	0	0	0	0	0	0	0	0	0	0
Panel on, gloves off	115	115		1.0×10^6	0	0	0	0.2	0	0	0	0	0	0	0	0
Panel off	50	228.5		1.0×10^6	72.4	203.2	300+	68.8	300+	95.8	103	300+	300+	227.6	120.8	93.4
Human infectious dose					3.16	8.76	13.1+	3.00	13.1+	4.18	4.50	13.1+	13.1+	9.94	5.59	4.07

a. Temperature range inside cabinet, 69.8 to 77.4 F (21 to 25.2 C); laboratory room, 71.6 to 78.4 F (22 to 25.8 C).
Relative humidity range inside cabinet, 27.4 to 80.4%; laboratory room, 30 to 66.4%.
b. Average from 10 tests for each cabinet condition.
c. The indicated human infectious dose refers only to operations when the glove panel was off (see Appendix for sample calculation).
d. Average from five tests for each cabinet condition.

IV. DISCUSSION

Since the development, acceptance, and use of microbiological safety cabinets, greater concern has been shown for the confinement of organisms and protection of the laboratory worker. Because the source of laboratory-acquired infections is unknown in 39 to 86% of the cases,[8] our policy has been to evaluate equipment and techniques in an attempt to eliminate any potential cause. Our present studies show that the hazard increases when persons walk about in the room while others work at the cabinet, when the glove port panel is removed. Results of tests reported by Wedum[3] and present tests do not appear to be in agreement. These differences are not readily explainable. However, there were certain differences in cabinet design and test conditions:

Condition	Old Cabinet	New Cabinet
Width	45 inches	72 inches
Rear air baffle	None	Present
Room housing cabinet	Non-ventilated, operated at atmospheric pressure	Ventilated (six air changes per hour), operated at about 0.02 inch water gauge negative pressure
Glove panel opening	1.9 ft^2	4.6 ft^2
Disseminator position	45 degree angle from floor of hood pointing to rear	45 degree angle to side of hood
Disseminator type	DeVilbiss*	Pneumatic atomizer

*DeVilbiss Co., Somerset, Penna.

In many laboratories a false sense of security exists among personnel who assume that an air flow through a cabinet with open front confines any aerosol to the cabinet at all times. Our tests show that organisms will escape from an open-front cabinet operated at reduced air flow. If one considers the number of organisms aerosolized by common laboratory techniques or accidents as reported by several investigators[5,7-8] and the number of organisms that escaped to the room during the tests reported herein, one must conclude that, for protection of the laboratory worker engaged in infectious work, the cabinet is best operated with the glove port panel in place, with or without attached gloves, depending upon the situation.

The HID should not be relied upon as a final criterion of the level of hazard one could tolerate while engaging in work with infectious agents. Other factors to be considered are: degree of protection by vaccination or prior subclinical infection, state of general health, availability and effectiveness of specific antibiotic treatment, virulence of the disease, chronicity or fatality rate, epidemiological aspects, and public relations. Another important point is that the number of HID that escaped in 5 min into the laboratory room is greater than that recorded as recovered because the efficiency of the sieve sampler is 73% at best,[3,4] and a total of only 1 ft^3/min of air was sampled for 5 min during each test run at each location.

Our conclusions are: (i) when the glove panel is removed and the normal minimum air flow is present, the cabinet is suitable for safe working with microorganisms of moderate pathogenicity; (ii) complete protection is provided when the cabinet is used with the glove panel installed and the gloves attached; (iii) a very useful combination of safety and ease of operation is achieved when the cabinet is operated with the glove port panel installed but without gloves attached.

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APPENDIX

CALCULATION OF HUMAN INFECTIOUS DOSE (HID)

One HID is herein defined as 10 microorganisms inhaled in 5 min when breathing at 12.5 liters per min. Thus:

$$\text{One HID} = \frac{10 \text{ org}}{\text{breathing rate of man}} \times \frac{\text{volume of air sampled}}{\text{sampling time}}$$

$$= \frac{10 \text{ org}}{12.5 \text{ liter/min}} \times \frac{\text{liter}}{0.035 \text{ ft}^3} \times \frac{5 \text{ ft}^3}{5 \text{ min}}$$

$$= 22.9 \text{ org}$$

Therefore, the number of HID recovered in 5 min per sampling position equals the number of organisms recovered divided by 22.9 organisms.

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13. ABSTRACT

A microbiological safety cabinet was evaluated to determine conditions under which microorganisms might escape. Tests were conducted under three cabinet-closure conditions, various air flow velocities, and different laboratory operations, when 1.0×10^5, 1.1×10^5, or 1.0×10^6 microorganisms per cubic foot of cabinet space were released per minute for 5 minutes.

Data revealed that: (i) escape of a human infectious dose is possible when a cabinet is used with the glove panel off; (ii) the number of organisms that escaped from the cabinet increased with a decrease in air velocity; and (iii) an increase in laboratory operations increased organism escape. Thus, when the glove panel is off, the cabinet is safe only for operations that release few microorganisms into the cabinet, whereas the cabinet is safe for operations of significantly greater hazard when used with the glove panel on but without gloves attached.

14. Key Words

*Safety cabinets
Hazards
Microorganisms
*Safety

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