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BDRL, D/A ltr, 29 Sep 1971

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TECHNICAL MANUSCRIPT 542

EVALUATION OF A VERTICAL AIRFLOW MICROBIOLOGICAL CABINET

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JULY 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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EVALUATION OF A VERTICAL AIRFLOW MICROBIOLOGICAL CABINET

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Project 1B662706A072

July 1969

ACKNOWLEDGMENT

The laboratory assistance of Choo Dip Chin and the technical assistance of Robert D. Boyer are appreciated.

ABSTRACT

A prototype vertical airflow cabinet was evaluated to determine adequacy of design, construction features, and operator and product protection. The study reveals both physical and microbiological test parameters. The data indicate that both product protection and operator protection are achieved. There are no restrictive physical barriers to the operator as presently required on existing cabinet systems.

I. INTRODUCTION*

The growing field of cancer research and other research requiring tissue culture with infectious agents has created a need for an enclosure that protects the operator and also provides a contamination-free atmosphere for the manipulation and inoculation of antibiotic-free tissue cultures. The purpose of this study is to evaluate such an enclosure physically and microbiologically to see if it provides both product and operator protection.

Tests of a modified vertical laminar-flow cabinet were conducted under both static and simulated laboratory operating conditions. The results indicate that the cabinet offers adequate protection to both the operator and the product in infectious studies of moderate risk. The cabinet can be installed with relative ease.

The concept of laminar airflow was introduced in 1962 by Whitfield[1] and his associates of the Sandia Corporation of Albuquerque, N.M., to secure an ultraclean atmosphere for the manufacture of spacecraft components and sensitive electronic equipment. The principle of moving air of uniform velocity through high-efficiency filters and across the entire work space was used in clean rooms and work stations. The prime concern was protection for the product; protection for the operator was unnecessary.

One of the first uses of laminar airflow for both product and operator protection was a unit designed for the Chas. Pfizer Cancer Research Unit in 1964. This cabinet is a vertical downflow unit that has its own conditioned air supply, which is exhausted through a steam evacuator mounted on the outside of the building. Although the system performs satisfactorily, it is impractical for most research establishments.

A safety cabinet is needed that will provide the necessary protection and be commercially available, self-contained, and easily installed in an existing facility. It also should be movable through laboratory doors without a major disassembly. Several manufacturers began work on such models in the period 1966 to 1968.

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II. MATERIALS, METHODS, AND RESULTS

A. CABINET SPECIFICATIONS

One of the first prototypes to meet most of the above criteria was purchased from the Baker Company, Biddeford, Maine, and was evaluated in our laboratory. Smaller units were also evaluated by Corriell and McGarrity[2] and Kreider.[3] The Baker stainless steel unit is 85.5 inches tall, 74 inches wide and 34 inches deep (Fig. 1). It has a 14-degree sloped, single-pane, safety glass window, which was later replaced with two panes of clear acrylic plastic. A removable glove panel for using the cabinet with attached rubber gloves and a solid panel to close the cabinet for gaseous decontamination were furnished. The work surface could be either a solid, removable stainless steel pan (60 by 27.5 by 0.5 inches) or a perforated pan of the same dimensions. The air filter system consisted of two cylindrical exhaust filters 10 inches long by 9.5 inches in diameter and a cabinet filter (72 by 24 by 6 inches) for air recirculation. All three were HEPA filters that provided 99.97% efficiency in removing particles 0.3 μ and larger. Air was circulated by a ½-hp, variable-speed motor and a centrifugal blower. Light was provided by four, 5-foot-long, instant-start fluorescent lights located across the top of the work area just under the cabinet filter. The illumination at the work surface was between 250 and 300 ft-c.

B. AIRFLOW

Figure 2 represents the airflow pattern of the cabinet with the solid work surface in place. Approximately 200 ft^3/minute of makeup room air was drawn in through the perforated front 6 inches of the work surface floor, of which 4.5 inches were unobstructed. Observation of smoke patterns indicated that all of the room makeup air was drawn into the front exhaust grill. The recirculating air, which entered the cabinet work area through the cabinet HEPA filter, divided at the center of the cabinet 2 or 3 inches above the floor. Approximately half of this air exited downward through the front 6-inch-wide perforated exhaust grill and the remainder exited through the open 5-inch vertical perforated exhaust grill across the back wall. The exhaust air was then drawn into the chamber in the base of the cabinet, through the blower, and up through the back plenum into the filter plenum, where approximately 16% of it was exhausted, and the remainder was recirculated down through the cabinet filter.

Physical tests were carried out with dioctyl phthalate (DOP) particles with a TD Associates Model 2A detector, smoke candles and tubes, a G.E. Model H2 halogen leak detector, a General Radio Strabotac rpm measuring device, an Alnor thermoanemometer, a Cinco revolving vane anemometer, and a G.E. light meter. The DOP detector revealed several leaks in and around the cabinet HEPA filter and indicated leakage through the electrical utility outlet and the light fixtures and along the corner wall seams. The halogen leak test indicated gas leakage from around the top seam of the cabinet, from the electrical fixtures, and around the filter closure panel. The filter

FIGURE 1. Baker Stainless Steel Cabinet.

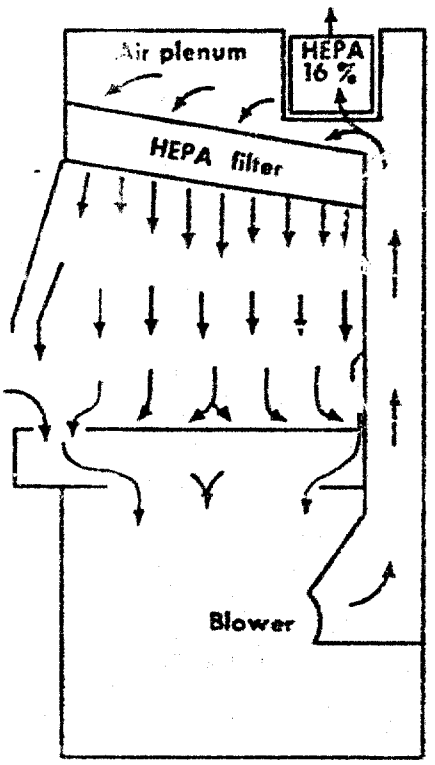

FIGURE 2. Air Flow Pattern.

leakage was corrected by replacing the 72-inch filter with three 24- by 24- by 6-inch HEPA filters mounted on an angle-iron frame that was sealed to the cabinet with silicone sealant. The leakage around the top seam of the cabinet and along the sides was eliminated by removing these sections and resealing with a polysulfide sealant. The electric utility outlet was removed and the opening was covered with a plate and sealed with silicone. The light fixtures were rewired with mineralite cable and sealed with a silicone sealant. The leakage around the filter closure panel was eliminated by installing a new neoprene gasket. This account of these difficulties and modifications is included to indicate potential sources of air leakage that need inspection in any model. The manufacturer of this particular cabinet is making appropriate design changes.

Airflow velocity profiles as obtained with a revolving vane anemometer are summarized in Table 1.

TABLE 1. AIR FLOW VOLUME MEASUREMENTS IN THE BAKER BIOHAZARD HOOD[a]

Measurement Location	Volume of Air Circulated, ft^3/minute	
	1,550 rpm[b]	1,780 rpm[c]
Front return grill	900	1,100
Rear return grill	510	590
Exhaust filter discharge	195	205
Recirculation, %	86	88

a. Tests conducted and data calculated by personnel of Technical Engineering Division, Fort Detrick, Frederick, Maryland.
b. Normal operating blower speed.
c. Maximum blower speed.

C. MICROBIOLOGICAL TESTS

A 24-hour broth culture of *Serratia marcescens* was used as the test microorganism. Aerosols were generated by a Vaponefrin® nebulizer that created particles of 0.5 to 1 μ in diameter. Air was sampled by agar settling plates, AGI-30 liquid impingers, a Reyniers slit sampler, and Fort Detrick slit samplers.[4,5]

D. PRODUCT AND OPERATOR PROTECTION

In the first series of tests, product protection and operator protection were determined under static conditions, i.e., minimum activity in the laboratory. In the second series of tests operator protection was determined under simulated operating conditions.

1. Product Protection

Figure 3 shows the experimental arrangements. The nebulizer was mounted in the room 12 inches from the center of the cabinet front. An AGI-30 air sampler was placed to the side of the sampler pointing into the aerosol cloud. A second AGI-30 sampler sampled the air in the air plenum above the filter bank. Forty-three nutrient agar settling plates were placed in the cabinet, 34 on the solid work surface and nine for positive controls under the front perforated grill on the drain pan. The aerosol was generated for 10 minutes at 0.5 ml/minute. The plates were opened at the start of the experiment and remained open for an additional 5 minutes after the aerosol generator was turned off. The plates were incubated for 24 hours at 30 C and S. marcescens colonies were counted. Serial dilutions were made of the AGI-30 collecting fluid, 0.1-ml amounts were plated on nutrient agar, and these were incubated for 24 hours at 30 C and counted. Similar tests were run replacing the solid work surface with the perforated pan and either operating the cabinet with the back vertical grill open or taped closed. Each test was performed five times. The average results under the three conditions are shown in Table 2.

2. Operator Protection

Because the solid pan appeared to offer the best combination of operating ease and product protection, the operator protection tests were carried out with that work surface. To test operator protection, the nebulizer was mounted inside the cabinet, on the extreme left, 11 inches above the work surface and 13.5 inches in from the front of the cabinet. The aerosol was directed across the cabinet, parallel to the cabinet face. The aerosol was generated for 2 minutes during each 10-minute test, but air sampling was continuous for 35 minutes. Control air samples were obtained by starting the slit samplers 5 minutes before generating the aerosol. Each test was performed five times and each test was carried out on a different day.

To obtain a control count of the aerosol concentration, an AGI-30 air sampler was placed behind the nebulizer and directed into the aerosol cloud. During the static tests, a second AGI-30 air sampler was used to draw air from the filter plenum above the filters. However, during the simulated operating conditions, this second sampler was eliminated to prevent possible leakage of contaminated air into the laboratory. In the latter case, the second AGI-30 was positioned in the room about 24 inches from the front of the cabinet. The Reyniers and Fort Detrick slit samplers were positioned outside the cabinet to the right and left of the face, one at 48 inches from the floor and one at 37 inches from the floor on each side.

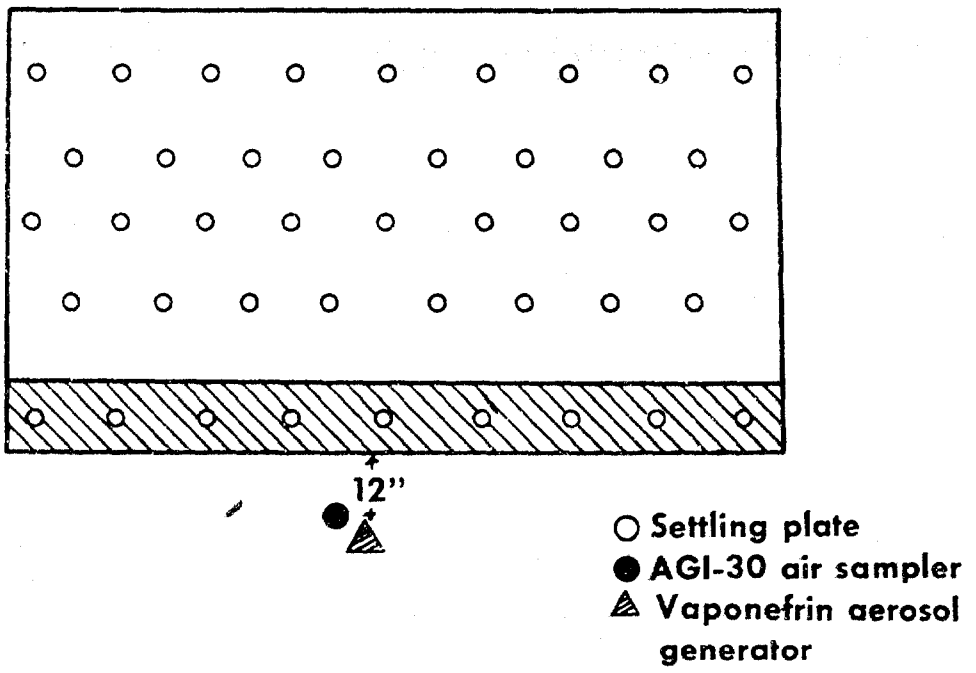

FIGURE 3. Test Arrangement to Check Product Protection.

TABLE 2. ORGANISMS RECOVERED IN THE CABINET FROM AEROSOL GENERATED 12 INCHES FROM THE CENTER FRONT OF THE CABINET

Sampler Location	Type of Sampler	Average Number of Organisms Recovered per ft^3 of Air Sampled per Location[a]		
			Perforated Work Surface	
		Solid Work Surface	Back Vertical Grill Open	Back Vertical Grill Closed
At nebulizer	AGI-30	2.2×10^7	2.0×10^8	3.4×10^6
Above filter	AGI-30	1.8×10^5	1.9×10^6	1.0×10^5
Front grill	Settling plates	TNTC[b]	TNTC	TNTC[c]
In cabinet	Settling plates	0	0 to TNTC[d]	0

a. Aerosol generated from 5 ml of 1×10^9 organisms/ml at rate of 0.5 ml/minute.
b. Too numerous to count - greater than 300 organisms per plate.
c. End plates were uncontaminated.
d. The center three plates of the first row of sampling locations were TNTC, all others were sterile.

Table 3 shows results obtained under static working conditions in the vertical downflow cabinet and compares them with results obtained under similar conditions by Barbeito and Taylor[6] in a Class I cabinet, in which room air sweeps in over the work surface and is exhausted at the back through a filter and ducts to the building air exhaust system.

Simulated working conditions consisted of: (i) moving the arms in and out, (ii) waving the arms back and forth in front of the cabinet to simulate a current that might be caused by walking in front of the cabinet, and (iii) opening and closing the laboratory door.

In the first working condition mentioned the arm movements consisted of thrusting first one arm and then the other into the cabinet, moving each one 45 degrees to the side and back, then up 45 degrees and back down, then out of the cabinet. This comprised 12 movements and was repeated about once every 15 seconds during each aerosol generation period. In the second working condition the arm movement was accomplished by swinging the arms back and forth in front of the cabinet face at a rate of about 50 times a minute during each aerosol generation period. In the third working condition the laboratory door was opened and closed every 15 seconds during each aerosol generation period.

The results of these tests are shown in Table 4, which also shows the comparison with the Class I cabinet.

TABLE 3. OPERATOR PROTECTION DURING STATIC WORKING CONDITIONS PROVIDED BY THE DOWNFLOW CABINET COMPARED WITH A CLASS I CABINET

Sampler Location	Average Number of Organisms Collected per 5 ft^3 Air Sampled per Location		
	Downflow Cabinet[a]	Class I Cabinet[b,c]	
		Panel Off	Panel On, Ports Open
In filter housing[d]	9.3×10^3	NA[e]	NA
Right, simulated mouth level of man sitting[f]	3.0	38.0	0
Right, level at panel opening[f]	1.5	168.8	0
Left, simulated mouth level of man sitting[f]	1.0	33.6	0
Left, level at panel opening[f]	0.7	4.2	0.1

a. Aerosol generated using Vaponefrin nebulizer. Concentration calculated to be 5.3×10^5 organisms/ft^3 air sampled.
b. Data from Barbeito and Taylor.[6]
c. Aerosol generated using pneumatic nozzle. Concentration calculated to be 1.0×10^5 organisms/ft^3 of cabinet space.
d. Sampled from 6 inches above the 12 ft^2 of filter in top of cabinet.
e. NA - not available.
f. Each about 6 inches in front of the cabinet.

III. DISCUSSION

We conclude from the results of these tests that the principle involved in the design of this modified vertical laminar-flow system offers advantages over the conventional Class I cabinet in which room air sweeps over the work surface and then is exhausted through a filter, blower, and ducts to the building air exhaust system. The vertical laminar-flow cabinet not only provides a microbiologically clean work space, but it also protects the operator during studies involving moderate infectious risk. This cabinet is adequate to handle Class 3 and 4 agents[7] if dried or aerosolized (liquid or dried) microorganisms are not used. For instance, antibiotic-free tissue cultures can in all probability be handled without contamination. The Class I

TABLE 4. OPERATOR PROTECTION DURING SIMULATED WORKING CONDITIONS PROVIDED BY THE DOWNFLOW CABINET COMPARED WITH A CLASS I CABINET

Working Condition	Location[a] of Sampler Inlet Port		Average Number of Organisms Collected per 5 ft^3 of Air Sampled		
	Sitting[e]	Panel[f]	Downflow Cabinet[b]	Class I Cabinet[c,d] Panel Off	Panel On, Ports Open
Moving arms in and out of cabinet[g]	Right		0.08	64.0	0
		Right	0.04	104.4	0
	Left		0.25	178.6	0
		Left	0.21	371.4	0
Walking or waving arms in front of cabinet[h]	Right		0.13	113.8	0
		Right	0.23	300+	0.2
	Left		0.16	100.8	0
		Left	0.46	300+	0
Opening and closing laboratory door[h]	Right		0.56	283.6	0
		Right	0.06	300+	0
	Left		0	174.2	0
		Left	0.03	300+	0

a. Each about 6 inches in front of cabinet.
b. Aerosol generated using Vaponefrin nebulizer. Concentration calculated to be 1.5×10^7 organisms/ft^3 air sampled.
c. From Barbeito and Taylor.[6]
d. Aerosol generated using pneumatic nozzle. Concentration calculated to be 1.0×10^5 to 1.0×10^6 organisms/ft^3 of cabinet space.
e. Simulated mouth level of man sitting.
f. Level at panel opening.
g. Average of four tests.
h. Average of five tests.

cabinet with its glove port panel on, without attached rubber gloves, offers equal or slightly better operator protection, but the interior of the cabinet has the same nonspecific microbial contamination as the room air. Our laboratories have not found this background contamination to be of consequence in the usual work with flasks, tubes, and plates of nonviable culture media.

An additional advantage of the downflow cabinet is that the recirculating air system permits installation in any existing facility without the expense of an additional exhaust system. However, whether or nor the air is exhausted to outside the building, it is essential that the cabinet air filters be tested before operation to insure absence of leakage of air through or around the filters.

Work of high hazard to the operator such as with dried or aerosolized microorganisms or Class 5 agents[7] will continue to require a closed gastight cabinet[2] with attached rubber gloves.

Modification of the prototype design incorporating the corrective features applied by this laboratory and reducing the size to permit moving the cabinet through a standard door opening should provide laboratories with a commercially available, self-contained, easily installed cabinet at a reasonable cost.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D
(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
EVALUATION OF A VERTICAL AIRFLOW MICROBIOLOGICAL CABINET

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)
H. Ellen Kukla

6. REPORT DATE
July 1969

7a. TOTAL NO. OF PAGES
17

7b. NO. OF REFS
8

8a. CONTRACT OR GRANT NO.

b. PROJECT NO. 1B662706A072

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
Technical Manuscript 542

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)

10. DISTRIBUTION STATEMENT
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11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

A prototype vertical airflow cabinet was evaluated to determine adequacy of design, construction features, and operator and product protection. The study reveals both physical and microbiological test parameters. The data indicate that both product protection and operator protection are achieved. There are no restrictive physical barriers to the operator as presently required on existing cabinet systems.

14. Key Words
Safety cabinets
Air-flow
Laminar flow
Tissue culture
Sterility

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Security Classification