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Some Practical Aspects of the Use of Laminar Airflow Systems for Tissue Culture Manipulations

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The use of laminar airflow systems for tissue culture offers real promise in the control of airborne contamination. Some of the theoretical considerations in testing, design, and operation have been presented (1, 2). It is the purpose of this note to compare the advantages of a laminar airflow system with the conventional bench top, and to discuss some of the unique problems of these units in the tissue culture laboratory.

The major advantage ascribed to laminar systems is that the design of the unit provides a particle-free, aseptic environment in which cultures may be manipulated without fear of microbial contamination. A unit received in this laboratory (model no. CVF-RX; Baker Corp., Biddeford, Me.) was subjected to sterility testing to determine whether microorganisms could be recovered by incubating petri dishes with media after exposure to the airflow. Petri dishes (100 mm) containing Trypticase Soy Agar medium were exposed on the laminar flow bench surface and on an adjacent table top in the tissue culture laboratory. Activity of personnel in the room and the duration of exposure of the plates were the principal variables studied.

In the first test, plates were exposed for 16 hr during an overnight period. No entry to the room was permitted.

In the second test, plates were exposed for 90 min during extensive morning activity in the laboratory. No control over entrance or exit was maintained.

The third test was the most severe challenge of all. Installation of electrical fixtures in the room by two workmen was accompanied by the generation of plaster dust and dirt. Plates were exposed during 1 hr of their labors. The results of these comparisons are reported in Table 1.

The laminar flow system prevented contamination of plates exposed within it, and the degree of protection was independent of room activity. Furthermore, operational experience with the unit over a 6-month period has shown a marked reduction in the incidence of contaminated cultures.

At present, there are no commercial laminar airflow units available which have been expressly designed for tissue culture. The existing models have been developed for the manipulation and assembly of solid materials in connection with the electronics and space industries. The unit consists of a cabinet which is enclosed on all sides but the front. A blower directs a stream of air through high-efficiency (HEPA) filters. The effluent air, exiting at a rate of about 100 ft^3 per min, is free from almost all suspended particles greater than 0.3 μ in diameter. In addition, the path of the airstream is parallel or laminar, thus providing a barrier to the entrance of contaminated air from the exterior.

The unit design is of two principal types. The air current may be vertical (i.e., from the cabinet ceiling to its floor) or horizontal (i.e., from the rear wall of the cabinet to the open front). Both designs provide excellent protection for the cultures from contamination from the outside. Unfortunately, the horizontal flow models readily sweep aerosols from culture manipulations directly onto the operator's person and into the culture laboratory. Where infectious agents are being handled the danger is evident. The use of animal sera, antibiotics, and radiochemicals in the medium also provide real hazards to the operator in the form of potential hypersensitization and radioisotope incorporation. In addition, some animal tumors have been passaged by the direct inhalation of droplets of cell suspensions (H. S. N. Greene, *personal communication*). The horizontal flow units, therefore, can be said to be completely harmless for culture manipulations only when the media are free from infectious agents, serum, antibiotics, radiochemicals, and cells.

Vertical flow units, in contrast, can be constructed in a manner that makes the escape of droplet nuclei impossible. In the better-designed models, a perforated work surface is provided

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TABLE 1. *Comparison of laminar airflow and conventional bench top systems for tissue culture*

Test	Room activity	Exposure (hr)	Contaminated plates after exposure on	
			Laminar bench	Open bench
1	None	16.0	0/44[a]	11/35[a]
2	Moderate	1.5	0/41	29/34
3	Heavy	1.0	0/15	15/15

[a] No. of contaminated plates/total plates exposed.

so that the downflow of air passing over the cultures is completely collected. The air may then be passed through additional HEPA filters for recirculation in the unit or exhausted to the exterior of the building where it is quickly diluted in the atmosphere. A drip pan with an external drain located immediately beneath the perforated work surface can simplify routine cleaning and decontamination procedures in the event of an accident. As currently designed, these units are not suitable for the handling of hazardous infectious agents, because the HEPA filters which entrap them cannot be decontaminated. The "Biohazard" unit proposed by Coriell may circumvent these difficulties (Bacteriol. Proc., p. 11, 1968).

The material of which the cabinet is constructed varies with the manufacturer. With cabinets composed of laminated plastics or wood, there are many cracks and corners which are difficult to clean. The best material is stainless steel. This surface is readily cleaned, decontaminated, and even sterilized with chemical agents.

The design features of laminar flow units pose several problems to their use in tissue culture laboratories. Some of the best models are so bulky that they do not readily pass through a conventional door frame, necessitating enlargement of the door or disassembly of the unit. The traditionally cramped quarters of a sterile passage room are further limited by the large cabinet. The noise of operation of the blower and airstream is roughly comparable to that of a large window-mounted air conditioner. The sound is annoying after prolonged exposure. In addition, it is not unusual for a large unit to generate sufficient heat from electrical resistance of motors, lights, and air-friction to raise the temperature of a small sterile passage room by 10 F. A steady supply of cooled air to the room is mandatory.

The rapid airflow is responsible for other problems. When organs or tissues are dissected in the process of preparing cultures, unless care is taken to ensure that they are totally immersed in fluid or covered with moist gauze, drying may be noticeable. Petri dish cultures, because of the large exposed surface area, are especially susceptible to loss of fluid and gaseous atmosphere. This may result in alkaline and slightly hypertonic cultures unless the manipulations are rapid or the numbers of cultures are small.

When a laminar flow unit is received in a culture laboratory, one cannot assume that it is already in a satisfactory operating condition. The filter integrity may have been lost as a result of shipping damage to the seals around the filter plenum. Sterility testing is necessary before risking valuable cultures. Petri dishes of bacteriological agar may be exposed to the airflow on the work suface. An exposure time of more than 5 hr will result in drying of the plates. In this case, they may be rehydrated with sterile distilled water. If the plates are numbered and the original location recorded, a leak in the seals can be localized.

Is it then necessary to continue to add antibiotics to media or to flame the mouths of culture vessels? These procedures can probably be discontinued for most operations but undoubtedly many laboratory workers will continue to use them. Although it is possible to use a bunsen burner in the airflow, the burner should be placed to the rear of the bench, because the hot air rising from the burner does disrupt the laminar pattern and, when near the front of the unit, contaminated air may be drawn into the work space.

The question most often asked about the application of a laminar airflow system to tissue culture is if the improvement in asepsis outbalances the cost and other disadvantages. The savings in time, labor, and anxiety which the decrease in contaminated cultures has meant since our unit was installed, has far outweighed its purchase cost. The other disadvantages described are minor in view of this fact.

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