Compact Anaerobic Glove Box for Hospitals and Research Laboratories

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An anaerobic glove box designed for moderate-sized hospitals or research laboratories is described. The compact vinyl and metal enclosure becomes anaerobic rapidly after inflation, requires no vacuum pump, and provides incubator space equivalent to 15 standard anaerobic jars.

The initial description by Rosebury and Reynolds (8) of an enclosed stainless steel glove box for cultivating fastidious anaerobes stimulated the development of alternative systems to simplify this procedure (1-4). Aranki et al. utilized transparent vinyl to afford the operator greater visibility, maneuverability, and ease in establishing anaerobic conditions (1, 2). Incubation of cultures was achieved by including an incubator inside the glove box or by heating the entire enclosure. These improved prototypes, however, were too large and too cumbersome for the limited space available in some crowded hospital and research laboratories. This communication describes the design, construction, and performance of a completely self-contained anaerobic system suitable for laboratories with space limitations. Its simplicity permits its use by those with little experience in anaerobic bacteriology.

As shown in Fig. 1, the incubator module measuring 113 by 52 by 104 cm is composed of a transparent 20-mil vinyl canopy secured to a solid stainless steel base (Heinicke Instruments, Inc., Hollywood, Fla). This combination of materials preserves all of the advantages of a vinyl glove box body and has the added durability of a metal base, which is the site where burns and spillage of biologicals most frequently occur and therefore where periodic disinfection is most often required. Neoprene gloves attached to retractable accordion-pleated sleeves are fastened by aluminum O rings to the front of the module. Three prefabricated stainless steel shelves, which are collapsible during storage, can be erected within moments after inflation. Use of these shelves and modest use of adjacent floor space permits simultaneous incubation of 200

standard petri dishes (100 by 15 mm) or the equivalent of 15 anaerobic jars.

Initially anaerobic conditions are rapidly achieved through cyclic inflation and deflation of the vinyl canopy three times with a gas mixture (7% H₂, 5% CO₂, 88% N₂) through valves in the metal base. Within 45 min of assembly the enclosure becomes sufficiently anaerobic to reduce methylene blue indicator strips from Baltimore Biological Laboratory. Resazurin (0.0002%) or indigo carmine (0.00001%) added to Schaedler agar (Baltimore Biological Laboratory) poured into standard petri dishes becomes reduced within 18 h. Steady-state anaerobiosis is maintained by recycling heated chamber atmosphere over palladium catalyst housed in modular trays beneath the shelves. The entire enclosure maintains a steady temperature of 35 ± 1°C by means of a heater with an automatic safety override. The entry module completely detaches from the glove box for more convenient loading and, more importantly, for transportation to any location in the hospital. After specimen(s) are placed in the entry module, oxygen can be displaced from the lock even while en route to the laboratory. Specimen transfer is achieved by docking the Plexiglas transport unit (Fig. 1, TU) to a matching solid frame in the vinyl canopy. No pump is required, as oxygen is displaced by merely flushing the transport unit with a stream of gas mixture for 5 min.

Table 1 illustrates a wide variety of anaerobic bacteria which are commonly isolated in hospital laboratories and which have been routinely cultivated in the unit by the laboratories that have evaluated it to date. Additional strains of strict anaerobes (5-7) have also been successfully cultivated within the unit; these include *Selenomonas ruminantium* ATCC 12561 (GA 192), *Butyvirbio fibrisolvens* ATCC 27208, *Clostridium haemolyticum* ATCC 9650, and Charles

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FIG. 1. Anaerobic glove box with detachable transport unit (TU). A larger circular transport unit is also available that can accommodate a GasPak jar.

TABLE 1. Commonly occurring anaerobic bacteria cultivated in the described glove box

Bacterium	No. of strains tested
<i>Bacteroides fragilis</i>	3
<i>Bacteroides distasonis</i>	1
<i>Bacteroides thetaiotaomicron</i>	1
<i>Bacteroides oralis</i>	1
<i>Bacteroides melaninogenicus</i> group	2
<i>Fusobacterium nucleatum</i>	1
<i>Veillonella</i> ATCC 17744	1
<i>Veillonella</i> ATCC 17745	1
<i>Clostridium perfringens</i>	2
<i>Clostridium ramosum</i>	1
<i>Clostridium sordellii</i>	2
<i>Clostridium sporogenes</i>	1
<i>Clostridium tertium</i>	1
<i>Clostridium butyricum</i>	1
<i>Propionibacterium acnes</i>	1
<i>Eubacterium limosum</i>	1
<i>Peptostreptococcus anaerobius</i> ATCC 27337	1
<i>Peptococcus variabilis</i>	1

River Breeding Laboratories isolates 356 and 521 (mouse cecal fusiform-shaped bacteria, genera presently undefined). The medium used was Schaedler agar supplemented with 5% defibrinated sheep blood. Individual colonies were triple streaked onto medium reduced in the glove box for at least 24 h before inoculation, and plates were read after 3 days. Growth was confluent in the first two quadrants and heavy in the third quadrant, and approximately 15 to 20 colonies appeared in the fourth quadrant. Since previous experiments had shown that isolate 356 was the most oxygen-sensitive organism tested, it was chosen to quantitatively compare the glove box with the GasPak system (Baltimore Biological Laboratory).

All media were reduced for 48 h in the glove box before use. A serial 10-fold dilution was made of an overnight broth culture (Schaedler broth supplemented with 2% sheep serum) of anaerobe 356 in the glove box by using the same broth as diluent. A calibrated 0.01-ml loop (Curtin Matheson Scientific, Inc.) was used to inoc-

ulate the surface of agar plates from each tube. The growth tube and a set of uninoculated dilution tubes and agar plates were then removed from the glove box. The identical procedure was quickly repeated, and the plates were introduced into a Baltimore Biological Laboratory GasPak jar immediately after inoculation. A stopwatch indicated that the total time that elapsed from the moment the materials were removed from the glove box to the moment the lid was secured onto the GasPak jar was 10 min. The jar was then carefully observed to insure that the GasPak system was operating properly in developing anaerobic conditions, as demonstrated by the appearance of water condensation on the walls of the jar and the reduction of the methylene blue indicator strip. A total count of the growth tube contents was then determined in a hemacytometer, which could be used due to the large size of anaerobe 356 (12 to 14 by 2 to 4 μm). The viable count calculated from agar plates inoculated and incubated inside the glove box (5.5×10^7 colony-forming units per ml) equaled the total count observed from the growth tube (5.1×10^7 colony-forming units per ml). However, no growth was observed on any of the plates incubated in the GasPak jar, thereby indicating a reduction in the total number of viable cells by over 5 logs, to less than 10^2 colony-forming units per ml, which was the level of

sensitivity of the test. Consequently, an anaerobe like isolate 356 cannot be manipulated under aerobic conditions for as few as 10 min, even if reduced media are used, when plates are introduced into a GasPak jar. These data indicate that the completely self-contained anaerobic enclosure described here is capable of facilitating anaerobic bacteriology in both clinical and research laboratories.

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