Rapid Entry Port for an Anaerobic Glove Box

VEE J. GILL,^{1*} HAROLD W. TIPTON,² AND SYLVIA M. GERSCH¹

Microbiology Service, Department of Clinical Pathology,¹ and Biomedical Engineering and Instrumentation Branch, Division of Research Services,² National Institutes of Health, Bethesda, Maryland 20014

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A rapid entry mechanism for an anaerobic glove box is described. The entry port is practical for entry or removal of one or two items and requires only seconds to operate. No deleterious effect on isolation or growth of anaerobes has been encountered since installation of the rapid entry port 3.5 years ago. Details for its construction are given.

Anaerobic bacteriology performed in an anaerobic glove box permits the use of traditional bacteriological techniques for the isolation and identification of fastidious anaerobes. The glove box used by our laboratory (Coy Laboratory Products Inc.) has a cylindrical entry lock (12 by 18 inches [ca. 30.48 by 45.72 cm]) that requires repetitive evacuating and refilling with an oxygen-free gas mixture to pass materials into and out of the chamber. Our first 2 years of experience with this glove box suggested that an additional, less complicated and less time-consuming way of entering or removing one or two items at a time would be a desirable feature. This report describes a slide mechanism which permits rapid entry or removal of a single plate, tray, or tube. The design allows for construction of a variety of different carriers, the choice of which would depend upon the needs of a particular laboratory. At present, we use one carrier that fits standard size glass petri dishes, and a second carrier designed to fit microtitration trays which are used for antibiotic sensitivity testing.

MATERIALS AND METHODS

Anaerobic glove box. The rapid entry port was attached to a vinyl anaerobic glove box from Coy Manufacturing, Ann Arbor, Michigan (model B, 84 by 32 by 40 inches [ca. 213.36 by 81.28 by 101.6 cm]).

Rapid entry port. Figure 1 shows the location of the rapid entry port. The port can be attached elsewhere, but for our purposes this location keeps it easily accessible yet in an otherwise unutilized space. This location is initially clumsy to work with, since the operator's left hand must be used, but facility with this position is readily developed.

Figures 2 and 3 are more detailed diagrams which show the construction and operation of the entry port and petri dish carrier. The entire mechanism consists of three separate pieces; an entry gate, a carrier, and a locking closure. Detailed engineering blueprints are available upon request.

Entry gate. The gate body (H) is a block of clear

acrylic plastic (ca. 20 by 28 by 2 cm). A rectangular slot (14 by 3 cm) has been cut into the gate body (Fig. 2). The cutaway view (B-B) of the gate shows the hinged door assembly which consists of the door (D), the hinge block (C), and hinges. The hinge block itself is firmly secured with screws to the gate body. The door gasket (J) is made from a piece of low-durometer gum rubber which has been cut to fit into a groove surrounding the slot. Figure 2 shows the door in its locked position, whereas Fig. 3 shows how the door opens when a carrier is inserted.

The gate body (H) is attached to the glove box by cutting an appropriately sized rectangular hole in the glove box, inserting the gate body into position, and catching the free edges of the glove box vinyl so that it is sandwiched between the outer clamping ring (F) and the chamber gasket (I). An inner clamping ring (E) is inserted; screws then tighten the outer clamping ring, the glove box vinyl, the chamber gaskets, and the inner clamping ring directly against the gate body. This provides a leakproof seal. The chamber gasket is made from a closed-cell neoprene rubber, whereas the clamping rings and screws are aluminum.

The door (D) was initially constructed of a clear acrylic plastic like the gate body. After a year of use, however, a slight curving of the door was noted. This prevented good closure, so a new door constructed of a solid aluminum block (1.5 cm thick) was substituted. No further problems have developed.

Carriers. Figure 3 shows the design of the petri dish carrier (L). The microtitration tray carrier is essentially the same design modified to fit the microtitration trays. The carriers are made of Delrin (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) Each carrier has a handhold on one end and a notch on the other end. The notch permits easy placement and removal of the dish or tray from the carrier. Each carrier also has a carrier stop (M) to limit the distance that the carrier can advance into the glove box, allowing the carrier to be slid in quickly yet without proceeding too far. Figure 3 illustrates the carrier in position; the carrier is inserted into the entry gate slot and pushed into the glove box quickly and smoothly until it is stopped by the carrier stop. Upon removal of the carrier, the hinged door swings immediately back to the closed position. The carrier is attached with light chain or twine to the glove box frame next

FIG. 1. Diagram of glove box showing location of rapid entry port and attachment of carrier.

FIG. 2. Detailed diagram of rapid entry port and locking closure. See text for explanation of parts.

FIG. 3. Detailed diagram of rapid entry port and petri dish carrier. See text for explanation of parts.

to the entry port. Upon removal, it can be dropped or hung nearby and thus be conveniently available.

Locking closure. The locking closure (Fig. 2, cutaway A-A) is necessary to ensure that no leakage will occur through the port when the port is not in use. As shown in the diagram, the wing-headed bolt threads directly into the door itself so that, as it is tightened, the door becomes firmly sealed against the door gasket and gate body. The locking closure is made of a solid acrylic plastic fitted with a stainless-steel wing-headed bolt. Like the carrier, the locking closure is attached to the glove box frame so that it is always readily available.

RESULTS AND DISCUSSION

The rapid entry port has been in use on our present anaerobic chamber for the past 3.5 years. As indicated in the preceding section, a change was made in the door construction from a solid acrylic plastic to a solid aluminum block to prevent further warping. The locking closure was also added at the same time to ensure a good seal. These modifications were done a year after initial construction of the entry port, and since that time we have had uninterrupted use of the port with no operational difficulties.

During operation of the entry port, whenever

the slot is opened, as when a carrier is introduced, an outward surge of gas flow occurs due to the positive pressure within the chamber. This outward flow helps to minimize the entry of room air into the chamber. Since the carriers slide in and out smoothly, less than a second is needed either to push in or pull out the carrier; gas loss from the chamber is thus negligible. Air trapped within a petri dish or within a microtitration tray is carried in, however.

Since installation of the rapid entry port, resazurin indicator media kept in the glove box have remained reduced (colorless). The resazurin is prepared in fluid thioglycollate broth at a concentration of 0.001 g/liter. The broth is dispensed in tubes, loosely capped, and placed in different areas of the glove box. Insufficient reduction of the indicator is obvious, due to the pink color of unreduced resazurin.

Anaerobes in our stock culture collection which are transferred and kept in the glove box have not shown any loss in viability. These organisms include two strains each of *Bacteroides fragilis*, *B. melaninogenicus*, *Fusobacterium nucleatum*, *F. necrophorum*, *Peptostreptococcus anaerobius*, *Peptococcus asaccharolyticus*, and one strain each of *F. varium*, *F. gonidiaformans*, *P. micros*, *P. saccharolyticus*, *P. prevotii*, *Eubacterium limosum*, *E. lentum*, and *Clostridium novyi* type B (ATCC 27606). These are being subcultured once a week for other studies.

Since our specimens are inoculated and evaluated in the glove box, there should be minimal need for transporting plates in and out except when resupplying the chamber with media. This is generally done by using the large entry lock. The rapid entry or removal of a petri dish is thus used mainly when a single item is needed; for example, to remove a subculture plate to see if an organism will grow aerobically or to take in an extra plate of media that is not routinely kept in the glove box.

Entry or removal of microtitration trays is usually done through the rapid entry port, since the number of anaerobic susceptibilities that are done at a given time is low. These susceptibility trays are inoculated outside of the glove box and must then be rapidly placed into the glove box as soon as they have been inoculated. The rapid entry port is much more efficient for this purpose than the large entry lock. In addition, the rapid entry port obviates the problem of bubbling and overflow that occurs when the large entry lock is evacuated.

Use of the rapid entry port for the purposes described above has an added advantage in that less experienced personnel can easily use the mechanism, whereas repetitive training and in-

struction is required for personnel other than the Anaerobe Laboratory technologists to operate the large entry lock. At present, to enter or remove an item, it requires approximately 1.5 min to evacuate and refill the large entry lock three times. Using the rapid entry port requires approximately 5 to 10 s to either introduce or remove an item; this is achieved without expenditure of significant amounts of gas mixture.

An additional practical feature of the rapid entry port is that it can be removed and reinstalled onto a new glove box if necessary.

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