An Inexpensive Device for Evacuating and Gassing Anaerobic Systems with In-House Vacuum

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An inexpensive, compact device to aid in establishing an anaerobic environment for bacterial cultures is described. This device is designed for use with an in-house vacuum system and can replace the vacuum pump, "U" tube manometer system commonly used for this purpose.

Systems commonly used to provide a suitable environment for culturing anaerobic bacteria include anaerobic jars, the Hungate roll tube, the roll-streak tube with prereduced anaerobically sterilized media, and anaerobic glove boxes (1). Although each system has its advantages and disadvantages, recent studies have shown that all of the systems are suitable for isolating anaerobes commonly associated with human infections if certain principles of anaerobic bacteriology are followed (2). The commonly used Brewer, Torbal, and GasPak jars rely on the same general principle for the removal of oxygen (1).

Either evacuation-replacement procedures or disposable GasPak gas generators can be used with jars to achieve anaerobic conditions. Both techniques are effective, but the evacuation-replacement method is more economical if the volume of work justifies the purchase of the equipment. A common evacuation-replacement system consists of a gas tank containing the desired gas mixture, a two-stage pressure-reducing gas regulator, the "U" tube manometer, and an adequate vacuum pump (1), all of which occupy a considerable amount of laboratory space.

We have designed an inexpensive device which can be used with an in-house vacuum system. It has replaced the vacuum pump, U tube manometer systems previously used in our laboratory. The Bacteriology Training Branch at the Center for Disease Control also uses these devices in its laboratory course in anaerobic bacteriology. We have received numerous requests for instructions on how to assemble the device from persons who want to use such an apparatus in their laboratory. In this article we describe the apparatus, list the needed parts, and indicate the method of operation. The new system (Fig. 1) is compact (9 by 6 by 7 inch [ca. 22.86 by 15.24 by 17.78 cm] and weighs

approximately 3 lb (48 oz). After the anaerobic jar is evacuated, it is filled with a known gas mixture to allow development of anaerobic conditions (1). This device saves the laboratory the expense and time needed for maintenance of a vacuum pump. It also eliminates the need for a mercury manometer (which is a potential hazard in the laboratory).

A house vacuum of 20 to 24 inch (ca. 50.80 to 60.96 cm) of mercury is needed to evacuate the vessels. The building maintenance engineer can usually adjust the vacuum or an adjustable controller can be installed on the existing house vacuum system (vacuum control part no. DA-31 with 0 to 30 inch [ca. 0 to 76.20 cm] of Hg Var.; the Mercoird Corp., Chicago, Ill.). Figure 1 shows the assembled device connected to a vented GasPak jar. Operating instructions follow. (a) Connect the jar to the lower male hose connector. (b) Open the circle seal valve to the vacuum line and evacuate the jar until the compound gauge indicates -20 inch (ca. -50.80 cm) of vacuum. (c) Close the vacuum valve. (d) Open the circle seal valve from the nitrogen source and refill the jar until the compound gauge indicates 0. (e) Close the nitrogen valve. (f) Repeat steps (b) through (e). (g) Open the vacuum valve and evacuate to -20 inch of vacuum again. (h) Refill the jar for the last time with the gas mixture, for example, 5% CO₂, 10% H₂, and 85% N₂ (1).

Nitrogen gas is less expensive than the anaerobic gas mixture. If nitrogen is not used, a 0.25-inch (0.635-cm) Swagelok plug is used to close off that part of the system. The parts needed for the construction of the system are shown in Fig. 2.

The parts and quantities required are: 1 Ashcroft 2.50-inch (ca. 6.35-cm) compound (1009 series), lower 0.25-inch pipe, 15PSI-30 inch (ca. 76.20-cm) gauge; 3 0.25-inch brass, female circle seal valves (9500 series); 4 0.25-inch brass, close

FIG. 1. Purge and charging system for use with in-house vacuum.

FIG. 2. Parts needed for construction. Legend: G, Ashcroft compound $\pm$ gauge; N, 0.25-inch brass hex nipple; T, 0.25-inch brass tee female; C, 0.25-inch brass cross female; HC, 0.25-inch brass male hose connector; SL, 0.25-inch street L; MC, 0.25-inch pipe to 0.25-inch Swagelok; and V, 0.25-inch brass female circle seal valve.

hex nipples; 1 0.25-inch brass, tee female; 1 0.25-inch brass, cross female; 2 0.25-inch brass, male hose connectors; 2 0.25-inch brass, street elbow; 3 0.25-inch pipe to 0.25-inch Swagelok; and 1 0.25-inch brass, Swagelok plug.

These parts may be purchased at a local hardware or plumbing supply store. If necessary, contact Crawford Fitting Co., (Cleveland, Ohio) for the name of a local sales representative. All threaded portions should be wrapped with Teflon tape to assure a gas-tight connection before assembly. Teflon tape for this purpose may be purchased at a local hardware or

plumbing supply store. This system can be assembled for under \$60.00 using the parts described above.

LITERATURE CITED

1. Dowell, V. R., Jr., and T. M. Hawkins. Laboratory methods in anaerobic bacteriology. 1974. United States Department of Health, Education, and Welfare publication no. 74-8272. Center for Disease Control, Atlanta, Ga.
2. Killgore, G. E., S. E. Starr, V. E. DelBene, D. N. Whaley, and V. R. Dowell, Jr. 1973. Comparison of three anaerobic systems for isolation of anaerobic bacteria from clinical specimens. *Am. J. Clin. Pathol.* 59:552-559.