Improved Dispenser for Use in Preparing Prereduced, Anaerobically Sterilized Medium

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An improved, all glass, gravity-fed quantitative device for dispensing media during preparation of prereduced, anaerobically sterilized media with minimal loss due to oxidation of media is described.

One of the most important aspects of the production of prereduced, anaerobically sterilized medium (PRAS) (3) is the development and maintenance of a low oxidation-reduction potential in the medium just prior to and after addition of reducing agents, usually cysteine (1, 2, 3). This results because the added reducing agents are used to further poise the oxidation-reduction potential rather than to remove oxygen from the medium. In the PRAS technique (1), the medium is first boiled to drive off oxygen and then cooled to room temperature under a stream of oxygen-free carbon dioxide in an ice bath. In our laboratory, to provide an additional margin of safety against reoxidation of the medium, we follow the practice of Hungate (2) by both boiling and cooling under oxygen-free gas. Prior to adding the reducing agents, oxidized compounds in the medium are reduced during the boiling step by other compounds in the medium, most probably the reducing sugars added separately or with the yeast extract component (2). Any oxidation of the medium after addition of the reducing agent will render it less effective for isolation and cultivation of obligately anaerobic bacteria (1). We have unpublished data showing that colony-forming unit counts of obligately anaerobic bacteria from an oral cavity are halved by momentary oxidation of PRAS medium during inoculation despite subsequent "re-reduction" of the medium by the added reducing agents in it.

In our hands, as well as in some others, the presently described devices (e.g., 1, 3) for dispensing PRAS medium present some difficulties. Medium may oxidize as it is drafted into syringes, pumps, etc., and then dispensed through plastic or rubber tubing.

We have found useful an all glass, gravity-fed dispenser. This device can dispense accurately measured amounts of medium. Our dispenser, suggested by that of Williams (4), omits the condenser, adds a pipette as a measuring

FIG. 1. All glass PRAS medium dispenser. A, Oxygen-free gas inlet; B, three-way glass stopcock; C, three-way glass stopcock; D, medium outlet; E, 10-ml pipette fused into system; F, vent, made up of rubber tubing with pinch clamp; and G, ball and socket joint (optional).

device, and rearranges the geometry to allow gravity feed. All parts of the device the PRAS medium contacts are made of glass. The stopper connecting the flask to the dispenser contains three holes. The glass tubing passes through two of these. The third hole serves as a vent through which excess oxygen-free gas escapes.

Follow these directions to use the dispenser (Fig. 1): (i) Make PRAS medium according to directions (1). (ii) Thoroughly flush medium dispenser with oxygen-free gas before attaching medium flask. Be sure that both stopcocks are in position for three-way flow and that side arm F has been thoroughly flushed by opening the pinch clamp for 15 s. (iii) Readjust stopcock C so that two-way gas flow, from left to right (through pipette E, stopcock C, and through siphon tube), is established. (iv) Carefully place flask of PRAS medium to be dispensed on the apparatus, removing the flask-gassing cannula just as the stopper seats. Support the flask. (v) Turn stopcock B so that gas flows from A through stopcock into flask only. (vi) Simultaneously open pinch clamp F and cover vent (3rd hole) in flask stopper to cause medium to flow into siphon tube, through stopcock C, and into pipette E. Uncover vent as soon as siphon action is established. (vii) As soon as pipette is filled to desired level, quickly close stopcock C to no-flow in any direction. (viii) Immediately turn stopcock B to three-way flow and close

pinch clamp F. No further manipulation of these is required as siphon action is established in steps (v)–(vii). (ix) Empty pipette into medium tube by turning stopcock C so that medium flow is from pipette E, through stopcock C, exiting via D. Turn stopcock C to no-flow position when medium level in pipette E reaches the bottom graduation mark. The receiving tube must be flushed continuously with oxygen-free gas during dispensing. Seal tube with stopper according to directions (1). (x) Turn stopcock C to two-way flow from right to left so that medium siphons from flask into pipette until desired volume is reached. Quickly close stopcock C. Dispense as in (ix).

The first one or two portions of medium may oxidize and should be discarded. After this, in a properly constructed and properly used dispenser, no further oxidation will occur.

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