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* On leave from Department of Botany, B. R. College, Agra University, Agra, India.

30 August 1957

Stainless-Steel Closures for Replacement of Cotton Plugs in Culture Tubes

The science of bacteriology became established on a practical basis following the discovery in 1853 by Schroeder and von Dusch (*1*) that filtering air through cotton removed airborne microorganisms. Pasteur (*2*) demonstrated later that a capillary space sufficiently long and curved accomplished the same purpose. During the intervening century not only have technical improvements sometimes failed to keep abreast of the demands in the ever expanding field of microbiology, but distinct disadvantages in the use of cotton have been observed. Inhibitory substances from cotton have been reported for pneumococci (*3*), tubercle bacilli (*4*), a diphtheroid and *Hemophilus pertussis* (*5*), *Brucella abortus* (*6*), and *Histoplasma capsulatum* (*7*). The closures described in this report eliminate the undesirable features of cotton plugs, screw caps, and caps of glass, plastic, or aluminum, while offering many desirable features.

The closures (*8*) are fabricated of type 305 stainless steel and so have the noncorrosive properties typical of that metal. This type of stainless steel does not present the problem of contamination of the culture medium by stray metallic ions. As shown in Fig. 1, the closures consist of a cylindrical body closed at one end. At the juncture of the end wall and cylindrical body are located three equally spaced indentations whose functions are to insure an air passageway between the end of the tube and the closure and to center the closure over the end of the culture tube. In the cylindrical body there are three equally spaced resilient fingers which are integrally connected to the body of the closure near its open end; their free ends are directed inward and toward the closed end of the closure. These fingers allow for normal variation in the outside diameter of culture tubes of a given size yet grip the tubes sufficiently to prevent the closures from coming off if the tubes are tilted or shaken and to enable the tubes to be picked up by their closures.

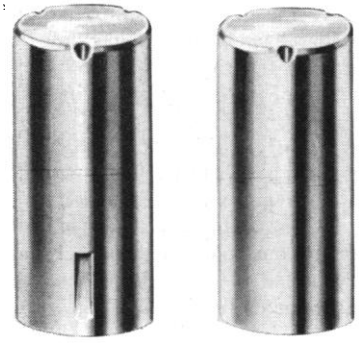

Fig. 1. Stainless steel closures for 16-mm bacteriological culture tubes. Type 16 (left) has fingers for engaging tubes; Type 16A (right) is made without fingers.

While tubes designated as 16 mm O.D. (outside diameter) vary from 16.54 to 15.46 mm (0.651 to 0.608 in.) outside diameter, the type 16 closures will accommodate tubes from 16.54 to 15.3 mm O.D. If it is desirable to check a supply of tubes which has accumulated over many years and from many sources, this may be done with a "go–no-go" gauge with openings of 0.652 and 0.602 in. The length of the closures, 1.5 in., permits easy manipulation with the fingers and creates between the walls of the closure and tube a capillary space which is sufficiently narrow, long, and curved to prevent airborne microorganisms from gaining entrance to the culture tubes. Hundreds of tubes of sterile media maintained their sterility when stored under varying conditions of temperature for periods varying from 5 to 25 weeks.

In contrast to cotton, there is no fire hazard, glassware is not fouled with oily substances during sterilization, the physical and chemical states of the culture medium are not altered by the presence of fibers, and the annoying feature of the irritation of the nostrils of workers with fine cotton dust is eliminated. The closures may be cleaned readily. Nothing projects into the tube; thus the greater portion of the culture tube is usable. Covering the top and a considerable area of the uppermost exterior surface of the culture tube with an impervious metal prevents the collection of dust and microorganisms around the rim of the culture tube and the contamination of cultures by growth of fungi through cotton plugs. The flaming of the open ends of culture tubes before an inoculating needle is inserted—a procedure which has become a ritual with bacteriologists—is now unnecessary.

The loss of culture media by evaporation from tubes with the closures is nearly one-half of that from tubes with cotton plugs. The closures allow an adequate exchange of gasses under aerobic and anaerobic conditions. This was determined by the pigment production of *Serratia marcescens* and *Chromobacterium violaceum*, the growth of *Salmonella typhosa* and *Salmonella schottmuelleri* on and in Russell's medium, the rate of diffusion of oxygen into thioglycollate medium, and the growth of *Clostridium tetani* and *Clostridium botulinum*.

In addition to the many technical advantages, there are several economical features of the stainless steel closures. By being used repeatedly, they save materials and labor. When a closure has been used 25 times it has realized savings equivalent to its original cost. The closures permit empty Vacutainer tubes (*9*) to be used as culture tubes. Culture tubes with closures need only be balanced in the centrifuge trunions before centrifuging; there are no cotton plugs to make secure. Since there are fewer storage problems, the closures are more convenient than cotton plugs on expeditions or field trips. The closures can be color coded readily by placing on their tops a dot of colored ink as used with the Marktex Tech-Pens (*10*). The colored markings withstand repeated sterilizations and washings but may be removed readily with acetone.

The majority of the tests which have been briefly summarized above have been performed with closures (*11*) for test tubes of 16 mm O.D. which are commonly employed in bacteriological work. Closures of similar type have been developed and tested for tubes of 13, 18, 20, 25, and 38 mm O.D.

HARRY E. MORTON
Department of Microbiology, School of Medicine, University of Pennsylvania, Philadelphia

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3 September 1957