

Glass Capping of Bacterial Culture Flasks

Mayilsamy Muniaraj*, Rajaiah Paramasivan and Natarajan Arunachalam

*Centre for Research in Medical Entomology (Indian Council of Medical Research),
Chinna Chokkikulam, Madurai, India*

The use of cotton plug as closure of a bacterial culture flask had been reported to have many disadvantages such as inhibitory nature of cotton to certain microbes, chances of contamination during handling and accumulation of used cotton as biological waste. To overcome the disadvantages of cotton plugs, we have developed a new method of capping bacterial culture flasks. In the present study, three sets of experiments were conducted, one was to find out the efficiency of bacterial growth in culture flasks closed by either glass caps or cotton plugs and the second set was to find out the chances of getting contamination of sterile broth closed by either glass caps or cotton plugs and the third set was to find out the evaporation of water in conical flasks closed by glass caps or cotton plug. The results showed that the bacterial cultures closed by glass caps showed better growth with less chance of contamination and evaporation of the culture media. By this method, the bacterial culture work is made very simpler than using cotton plug.

Key Words: Glass caps, Cotton plug, Bacterial culture, Conical flask, Contamination

INTRODUCTION

The science of Microbiology became established on a practical basis following the discovery by Schroeder and von Dusch (1) that filtering air through cotton removed airborne microorganisms. The cotton plugs are capable of mechanically screening out the contaminating microbes, and the contaminant-free atmosphere can provide the necessary environment for growth of any organisms present in the culture medium. Pasteur (2) demonstrated that a capillary space sufficiently long and curved, accomplished the same purpose. Although there have been revolutionary developments in the field of micro & molecular biology, allowing very small samples to be investigated in detail,

much research are still depends on the availability of large quantities of pure culture of microbial cells (3). Despite the several disadvantages listed below, the cotton plugs are continuously in usage for centuries without any major change. Inhibitory substances from cotton have been reported for pneumococci (4), tubercle bacilli (5), diphtheroid and *Hemophilus pertussis* (6), *Brucella abortus* (7), and *Histoplasma capsulatum* (8). The oxygen diffusion through cotton plug decreases as the density of cotton plug increases (9). During initial inoculation and subsequent inoculations, the cotton plugs are prone to contamination by atmospheric microorganisms, even if the cotton plug is exposed to the air for only a very short time. As the cotton plugs cannot be directly sterilized by the flame and the plug directly touches the inner wall of the flask, the probability that the culture gets contaminated will increase with the number of transfers (10). Although several reports available about different caps, none was found suitable in replacing the cotton plug. Morton (11) reported that his stainless steel caps are superior to the caps made of plastic and aluminium. However, later, Sutter (12) found that, the chance of contamination was

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*Corresponding author: Mayilsamy Muniaraj. Centre for Research in Medical Entomology (Indian Council of Medical Research), No. 4, Sarojini Street, Chinna Chokkikulam, Madurai - 625002, India.
Phone: +91-452-2525131, Fax: +91-452-2530660
e-mail: mmuniaraj@gmail.com

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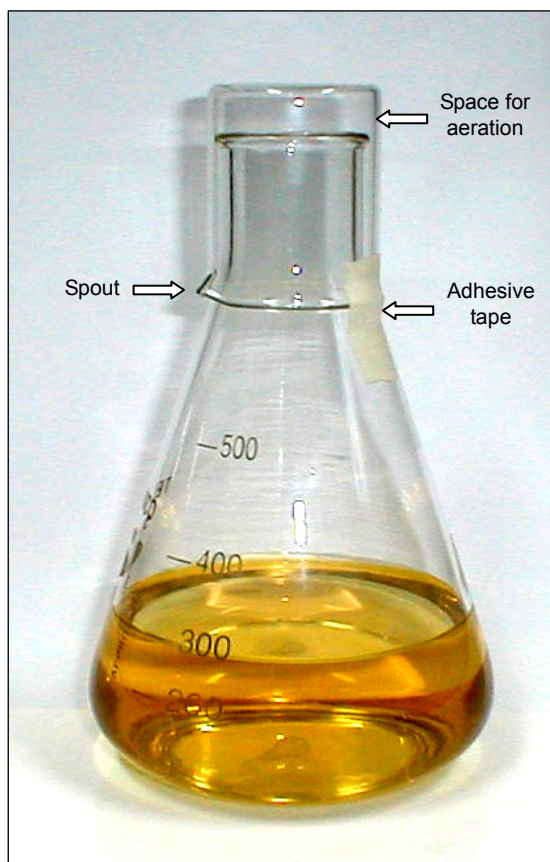


Figure 1. A glass cap similar to ungraduated beaker is used here as a cap for nutrient broth medium. The spout of the beaker and the free space between mouth of the flask and the top, inner portion of the beaker ensures free gaseous exchange. The adhesive tape shown is used to hold the beaker with flask during autoclaving and shaking.

more in stainless steel closures / caps when comparing with cotton plugs, which later discouraged the use of stainless steel closures. Today, despite all the listed, disadvantages, laboratories throughout the world employ non-absorbent cotton plug as a standard procedure for stoppering conical culture flasks and more than 90% of small scale microbial cultivations are performed with cotton plugs (13, 14). To overcome the disadvantages of cotton plugs, we have developed a new method of using glass caps as the replacement of cotton plugs in the cultivation of bacteria in conical flasks. The glass caps used in this study were much similar to the un-graduated beakers with a spout. When the glass cap is placed in inverted condition over the mouth of the conical flask, the cap will rest on the neck, leaving a

little space between the mouth of the flask and the bottom (inverted) of the glass cap. The spout of the glass cap provides free aeration and the gap between the mouth of the flask and the bottom of the glass cap ensures free gaseous movement (Fig. 1). We are successfully using this method in our laboratory for a quite long period for bacterial and protozoan (*Leishmania donovani* promastigotes) culture works. In the present study, we report the suitability of the glass capping as a replacement of cotton plugs.

MATERIALS AND METHODS

The glass caps were selected in such a way that, it has to receive the entire neck of the conical flask freely, and at the same time it should not be loose. The bacterial cultures used were *Bacillus subtilis* (obligate aerobe), *Escherichia coli* and *Streptococcus pyogenes* (facultative anaerobes). Cultures stored in nutrient agar slants were separately sub-cultured in 10 ml nutrient broth incubated at 37°C for 24 hrs in a mechanical shaker. The cell number was counted in Neubauer counting chamber. The number of bacteria in five fields was determined using the 40× objective lens with bright-field illumination. The mean bacterial load of *B. subtilis*, *E. coli* and *S. pyogenes* per millilitre was then calculated and the number of bacterial cells was found to be $3 \times 10^7/\text{ml}$, $2 \times 10^7/\text{ml}$ and $8 \times 10^6/\text{ml}$ respectively. These cultures were used as stock. Three sets of experiments were conducted, one was to find out the efficiency of bacterial growth in culture flasks closed by either sterile glass caps or cotton plug and the second set was to find out the chances of getting contamination of sterile broth kept in culture flasks closed by either sterile glass caps or cotton plugs and the third set was to find out the evaporation of water in conical flasks closed by glass caps or cotton plug. The caps and the flasks were affixed with a piece of autoclavable adhesive tape (approximately 3 cm in length) to hold the glass cap with flask for one set before autoclaving wherever described and the other set was sterilized with cotton plug. One side of the adhesive tape, probably the cap part of the tape can be removed to open the flask, before using the medium for inoculation. In the first experiment,

the stock cultures of *B. subtilis*, *E. coli* and *S. pyogenes* were separately inoculated in two sets of conical flasks, each of having three 100, 250, and 500 ml capacity conical flasks holding 75, 150, and 300 ml nutrient broth. The mouth of one set of conical flasks was closed either by 25/50 ml capacity sterile glass caps and the other set was closed by cotton plug. About 7.5, 15, and 30 μ l stock cultures of *B. subtilis*, *E. coli* and *S. pyogenes* were separately inoculated in 75, 150, and 300 ml nutrient broth. The fixed adhesive tape during autoclaving was reattached to hold the cap with conical flask to avoid wiggling while the cultures were incubated in a mechanical shaker. After the incubation, the number of bacteria from each flask was determined as described before. Two un-inoculated controls of nutrient broth, one was closed by glass caps and the other by cotton plugs, were maintained throughout the experiment. The total 56 conical flasks were incubated under 37°C for 24 hrs in a mechanical shaker. Secondly, a mock experiment was also conducted to determine the chances of contamination when using the cotton plugs and glass caps for bacterial culture. Two sets, each of having three 250 ml capacity conical flasks, holding 150 ml nutrient broth were prepared. The mouth of the conical flasks of one set was closed by 50 ml capacity glass caps and the other set was closed by cotton plugs. After sterilization, the cotton plugs / glass caps were removed in front of Bunsen burner flame, in a laminar air flow chamber, for 30 seconds to mimic the culture transfer procedure, then the closures were closed and the medium in the conical flask was kept under incubation at

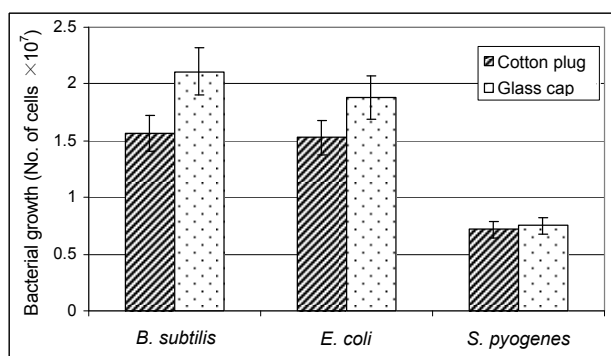


Figure 2. Growth of three different bacterial cultures in culture flask closed by cotton plug or glass cap, Error bars = 10%.

37°C. This same procedure was carried out each day for up to 10 days. The culture flask once found contaminated was recorded and removed from the experiment. Thirdly, the amount of water evaporated from the conical flask was determined in two sets of conical flasks; each set was having three 250 ml conical flasks with 150 ml distilled water. One set was closed by cotton plug and the other was closed by glass cap. The weight of the conical flask along with 150 ml distilled and cotton plug/glass cap was measured on every 3rd day from the day 0 to day 15. The flasks were kept at room temperature (28~31°C). The weight obtained was deducted from the previous value to find out the actual amount of water evaporated on every three days. The mean value was obtained for the triplicate flasks and the values were plotted in a bar diagram. The glasswares used were purchased from Borosil, Mumbai, India and the media used were purchased from Hi Media, Mumbai, India.

RESULTS

The direct microscopic counting of cultures of *B. subtilis*, *E. coli* and *S. pyogenes* grown in conical flasks, closed by either cotton plugs or glass caps are shown Fig. 2. The growth of the aerobic organism, *B. subtilis* and facultative anaerobic organism, *E. coli* was comparatively higher in the culture flask closed by glass caps. Although the difference

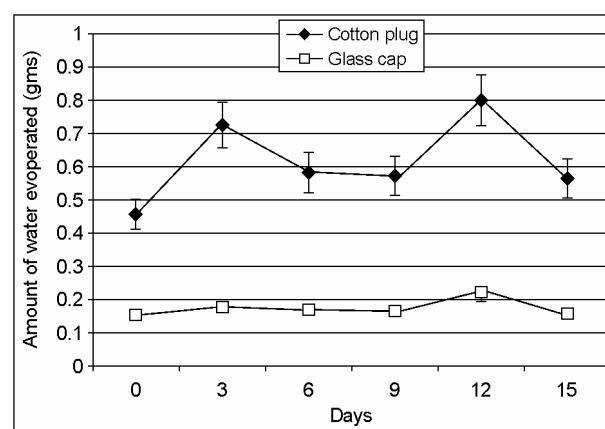


Figure 3. Evaporation of water in conical flasks closed by cotton plug or glass cap, Error bars = 10%.

was less, the other facultative anaerobic organism used, *S. pyogenes* showed better growth in the cap closed flasks when comparing with cotton plug closed flasks (Fig. 2). In the determination of susceptibility for contamination, one culture flask closed by cotton plug was found contaminated on 6th day and another found contaminated on 9th day. The culture flasks closed by glass caps were found virtually free from any contamination up to 10th day of incubation. Cotton plugs closed culture flasks were found to be losing water comparatively faster than the flasks closed by glass caps (Fig. 3).

DISCUSSION

This study prescribes a better but simple alternative to the practice of using cotton plug closures as a standard procedure in the laboratory cultivation of microbial cells. This method is based on the principle of swan necked flasks used by Louis Pasteur (2) to disprove the theory of spontaneous generation. The loss of culture media by evaporation from the flasks with glass caps is 4.6 times lesser than the flasks with cotton plugs. In addition to many technical advantages, there are several economical features of the glass caps. In contrast to cotton plugs, use of the glass caps have several advantages such as, there is no fire hazard, glassware is not fouled with oily substances during sterilization, the physical and chemical states of the medium are not altered by the presence of fibres and the annoying features of the irritation of the nostrils of workers with fine cotton dust is eliminated. The glass caps can be cleaned readily and is transparent; the possibilities for the presence of hidden dusts are less. Covering a considerable area of the uppermost exterior surface of the culture flask with glass caps prevents the collection of dusts and microorganisms around the rim of the culture flask and the contamination of culture by growth of fungi through cotton plugs. Since nothing projects into the culture flask, the chances of introducing contaminants into the flask, which happens when using cotton plug, is averted. Since, the glass cap covers the external area near the mouth of the flask, the flaming of the open end of the flask before and after

inoculation - a procedure which has become a ritual with bacteriologists - is now unnecessary. By being used repeatedly, they save materials and labour. Accumulation of used cotton, after microbial culture work as biological waste is another burden when using cotton plugs.

The glass capping was successfully used in our laboratory as a better alternative to cotton plugs and has reduced the chances of contamination, showed better growth of tested bacterial species and reduced the accumulation of used cotton as biological waste. The glass capping could be used in the place of cotton plugs with better performance and fewer problems in the culture work of not only bacteria, but also other microbes such as cyanobacteria, algae, fungi, protozoans and also for tissue culture works and could be a potential future alternative of cotton plugs in microbial culture works.

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