A Simple Culture Tube Closure Method for Prevention of Contamination by Airborne Fungi and Mites

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ABSTRACT

Fungal cultures in tubes closed with metal or plastic caps can be effectively protected against cross-contamination and mites by sealing with strips of plastic film.

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The traditional cotton plug has been largely supplanted in most biological laboratories by metal or plastic closures for culture tubes. The advantages of metal caps over cotton plugs were cited by Morton (1). He claimed that the cap design prevented airborne microorganisms from gaining entrance to culture tubes, but this is contrary to our experience. We have found that contamination occurs frequently during incubation and storage, particularly when sporulating cultures of *Penicillium, Aspergillus,* or *Neurospora* spp. are included in a collection. This was clearly demonstrated on one occasion when a few cultures of *N. crassa* Shear & Dodge were incubated in a cabinet with an assortment of other fungi which had been transferred previously. Characteristic *N. crassa* colonies with orange-colored spores soon developed in almost every tube in the cabinet,

beginning invariably at the upper end of the agar slant. The metal caps did not prevent the aerial transfer of fungal spores from one tube to another. Presumably air currents created by opening and closing the incubator door were involved.

We found that contamination could be eliminated by covering the slots at the sides of metal caps and the gap between metal and glass at the base with strips of either Parafilm® (American Can Company) or Saran Wrap® (Dow Chemical Company). The latter material required an elastic band or cellulose adhesive tape to maintain close contact with the glass. Sterilization of the protective material was not required.

At the time that these materials were being tested on part of our culture collection, a severe infestation of mites developed in the room where the cultures were stored. Only the cultures that were protected with Saran Wrap remained free of mites and fungal contaminants. Parafilm was not an effective barrier against mites. These observations were confirmed experimentally by keeping sealed and unsealed tubes of sterile medium with steel caps in a closed container with mite-infested *Penicillium* cultures. Within a few days the unsealed tubes and those sealed with Parafilm became infested with mites and contaminated with *Penicillium* whereas those sealed with Saran Wrap remained sterile.

For convenience we now use Stretch 'n Seal® (Imperial Oil Limited), a plastic film which adheres securely to both glass and metal. A standard roll is cut into sections 4 cm wide, using a sharp scalpel. Holding a roll of tape in one hand and a culture tube in the other, the end of the tape is affixed in the required position, then the tube is rotated until two layers of stretched tape have been applied. To complete the seal, the tube is rotated again with the taped area grasped firmly with the fingers.

Using this method we have maintained our cultures free of mites and fungal contamination for over 6 years. Regardless of how long they were to be kept, all cultures were sealed as soon after transfer as new growth had become established but before production of airborne spores. Sealed cultures stored at 15 C have remained viable for as long as 3 years, although in practice they are transferred routinely once a year. The plastic films permit adequate exchange of oxygen and carbon dioxide while greatly reducing moisture loss from the medium, as reported by Reynolds and Lock (2). They correctly surmised that plastic film can also serve to protect cultures from mite invasion.

The chief advantage of plastic film over cigarette paper (3) as a mechanical barrier against mites is the simplicity of the procedure which makes individual protection of any number of cultures feasible. Plastic film also serves the useful purpose of conserving moisture, thereby prolonging the viability of cultures.

LITERATURE CITED

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