APPLIED MICROBIOLOGY, Mar. 1974, p. 618-619
Vol. 27, No. 3

Printed in U.S.A.

Use of Plastic Bags to Maintain a Humidified Atmosphere for Animal Cell Cultures

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Received for publication 16 November 1973

A technique is described which allows incubation of petri dish tissue cultures in a dry incubator.

Humidified CO_2 incubators that are widely used in the culture of animal cells have some serious drawbacks. Condensation of water on and around the inner doors is unavoidable. Even if the doors and gaskets are wiped daily with disinfectants, it is possible to isolate bacteria, notably *Pseudomonas*, and various species of fungi from the door gaskets of the incubators. The growth of these organisms in the incubator presents a constant danger to the sterility of tissue cultures, especially if the cultures are handled frequently and incubated over prolonged times. In addition, frequent opening and closing of the incubators during the day invariably reduces the humidity of the chambers and accelerates drying out of the cultures. These difficulties have prompted us to look for an alternative to the humidified incubator, and we have devised the following procedure which prevents dehydration of cultures and at the same time minimizes microbial contamination.

We use Wedco model 2-17H incubators with a chamber size of 21 inches wide by 16 inches deep by 29 inches (ca. 53 by 41 by 74 cm); however, any other incubator which can be gassed with CO_2 is satisfactory. The incubator is operated *without* humidifier. Petri dishes with cell cultures are kept on stainless-steel trays, 15.5 by 17.5 inches (ca. 39 by 44 cm). These trays are sealed in flat, commercial, polyethylene tubing 18 inches wide by 0.004 inches (ca. 46 cm by 0.1 mm) thick and may be stacked in the incubator chamber on the shelves supplied with the incubator. Sealing of the polyethylene tubing is accomplished with an impulse Audion heat sealer (model 580-5A). This device has a 23-inch (ca. 33-cm)-wide sealing element and produces a water-tight seal on 0.004-inch (0.1-mm) commercial polyethylene tubing in approximately 4 s (8 s per bag). The sealing bar can be hand operated, but sealing units which are pneumatically actuated with a foot pedal allow the operator the use of both hands for aligning the tray in the plastic tubing. An integral knife is provided with the sealer to cut the tubing after it is sealed. Polyethylene tubing (0.004 inch [ca. 0.01 cm] thick) is available in rolls of 50 to 70 lbs (ca. 22 to 31 kg) representing 900 to 1,250 ft (ca. 274 to 435 m). The bags do not require a separate humidifying system. Only a small amount of water needs to evaporate from the plates to saturate the atmosphere as the following consideration shows: the volume of the atmosphere held in a plastic bag containing a 15.5- by 17.5-inch (ca. 39- by 44-cm) tissue culture tray is about 4.5 liters. Under ideal conditions (no condensation on the bag and complete impermeability of the plastic for water) it would take about 0.2 ml of water to bring the relative humidity in this atmosphere to 100% (1). However, because there is some condensation on the bag and also some escape of water through the plastic, the actual evaporation of water from the tissue cultures is higher. We found that, on a tray with 12 60-mm plates and 5 ml of medium per plate, the average loss of water was 0.48 ml per plate if kept for 1 week in a 0.1-mm polyethylene bag at 37 C in a dry incubator.

Polyethylene is permeable to CO_2, and our incubators are gassed with a mixture of 5% CO_2 and air which maintains a pH of approximately 7.2 by using medium 199 or F10. Alternatively it is possible to use bags of laminated paper, polyethylene, and aluminum foil which are impermeable to both water and CO_2. Laminated bags are fabricated from 25-lb machine-glazed Kraft paper, 0.001-inch (ca. 0.25-cm) polyethylene, 0.0005-inch (ca. 0.1-μm) aluminum foil, and 0.002-inch (ca. 0.5-μm) low-density polyethylene as the interior material. (Richmond Corp., Redlands, Calif.) Such bags are available in individually precut sizes with one end already sealed. They can also be heat-sealed;

however, before sealing a measured amount of CO_2 must be added to the bag through a metering valve. In this case a simple 37 C incubator without CO_2 may be used.

We have adopted sealing in polyethylene bags for all of our cell culture work and have used this procedure successfully for 2 years. The laminated bags have been used less extensively, but with equally satisfactory results. They offer the additional advantage that the incubators need not be gassed with CO_2. The only precaution which is necessary is that all tissue culture trays be wrapped in Kraft paper or in merchandise bags[5] and autoclaved before use; otherwise fungus spores trapped on the trays can cause contamination. Merchandise bags, 17 by 4 by 24 inches (ca. 43 by 10 by 61 cm), are manufactured by Crown Zellerbach Corp. The sealing of tissue culture trays in polyethylene bags has several advantages: (i) dehydration of cultures is reduced. (ii) Accidental contamination with bacteria and fungi is minimized. (iii) Opening and closing of incubator doors does not lead to a sudden loss of CO_2 from the atmosphere surrounding the tissue culture plates. (iv) The bag and the trapped atmosphere in the bag also have an insulating effect for the tissue cultures. Drops of temperature during opening and closing of the incubator are reduced. (v) In working with several viruses the chances of cross-contamination between different trays are minimized. (vi) If accidental spillage of radioactive material from tissue culture plates occurs, the spillage is contained and can be cleaned easily.

This study was supported by Public Health Service research grant no. CA-13213 and contract no. NIH-NCI-72-2032 (under the Virus Cancer Program) from the National Cancer Institute.

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