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A low cost adaptation of the t-butyl alcohol freeze-drying method

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Resumen: se construyó y probó un aparato de bajo costo adaptado del equipo para secado de especímenes congelados en t-butanol (VFD-20). El equipo fue construido con un desecador al vacío, un bloque de bronce de 1 Kg, una trampa para vacío y una bomba de vacío. Los especímenes procesados (intestino delgado de ratones infectados naturalmente con parásitos) fueron fijados, deshidratados y colocados en t-butanol y secados con el equipo descrito. Al microscopio electrónico de rastreo no se observaron deformaciones ni daño en los especímenes.

Key words: Freeze drying method, Scanning electron microscopy, ultrastructure.

A new freeze-drying method using ter-butyl alcohol to prepare biological specimens for their scanning electron microscope analysis was developed recently (Inoue and Osatake 1988). The method is quicker than critical point drying and the specimens show less alterations. It has been improved with a vacuum freeze drying device (VFD-20) developed by Akahori et al. (1988), or with the apparatus developed by Inoué and Osatake (1989) and sold as the Eiko ID-2. The principle of the former instrument is vacuum sublimation and subsequent solidification of butyl alcohol on a metal block cooled in liquid nitrogen. Using the same principle, we developed a low cost device to dry biological specimens using a common pyrex vacuum desiccator connected to a vacuum pump.

Intestine tisues from adult mice naturally infected with *Eimeria falciformis*, *Giardia muris*, or *Cryptosporidium* sp. were fixed with 2.5 % glutaraldehyde, post fixed with 1 % osmium tetraoxide, dehydrated by a graded series of ethanol (30 to 100 %) and immersed in t-butyl alcohol for three changes. Then, each specimen was placed, with *ca.* 0.5 ml of t-butyl alcohol, in a container made from two Beem capsules, as described by Eisenback (1985). Briefly, to make the containers, the conical portion of one Beem capsule was cut-off and covered with a nylon screen and the top of another capsule, in which a 4mm in diameter hole was perforated. The containers with the samples immersed in t-butyl alcohol were kept in the refrigerator (4 °C) for 30 min. Then, the copper block used for freeze fracture (Hitachi HFZ-1) was cooled in liquid nitrogen. When the t-butyl alcohol with the specimen was frozen, the container and the cooled block were transferred to a vacuum desiccator and evacuated until total sublimation of the t-butyl alcohol had occurred. A glass vacuum trap, immersed in liquid nitrogen or in ice-water, was intercalated between the desiccator and the vacuum pump, connected by a rubber vacuum tube (Fig. 1).

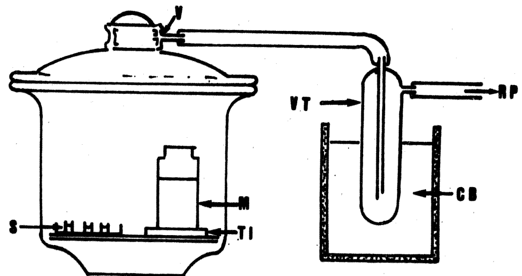

Fig. 1: Low cost modification of the device designed to the t-butyl alcohol freeze-drying method. VD., valve; M., metal block; Th.I., Thermal isolator; S., sample containers; RP., rotary pump. VT., vacuum trap.

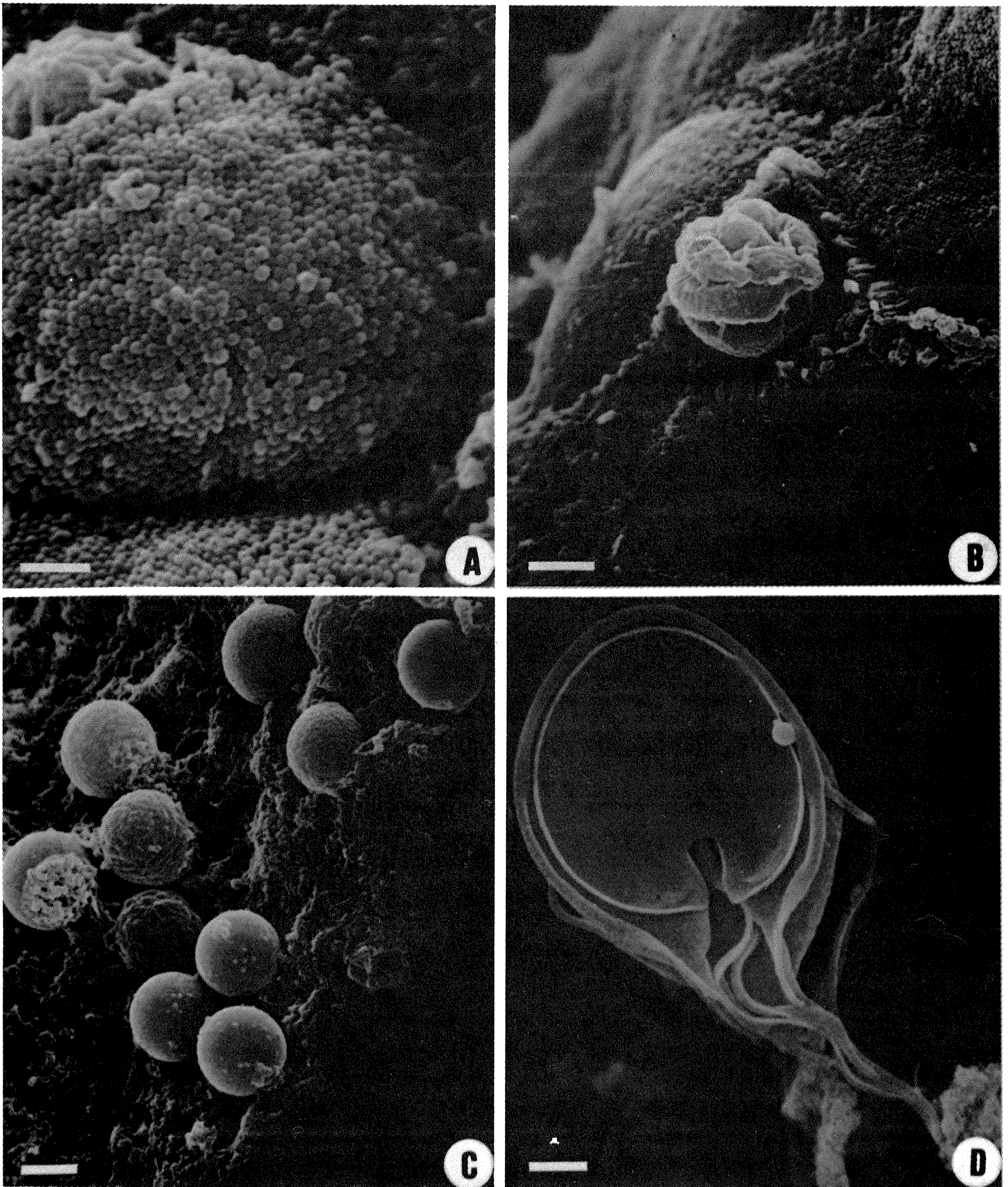

Fig. 2. External appearence of several biological samples dried with t-butyl alcohol in the apparatus propoused in this paper.
A: Mouse intestinal epithelium, shown the dome-shaped end of the microvilli (Bar = 0.5 μm).
B: Oocyst of *Cryptosporidium* sp. attached on a proximal small intestine villi of mouse (Bar = 1 μm)
C: *Eimeria falciformis* oocysts on mucus layer of small intestine of mouse (Bar = 5 μm)
D: *Giardia muris* trophozoite on small intestinal of mouse (Bar = 5 μm)

The sublimated t-butyl alcohol froze on the surface of the metal block, as a white powder. The t-butyl alcohol that was not frozen on the block was captured in the vacuum trap. The required time depended on the amount of t-butyl alcohol and the number of containers placed in the desiccator. We usually dried 3 to 5 specimens together in about 1 hour. Nevertheless, the figures shown in this paper refer to 15 samples dried together in about 3 hours.

Figure 2 shows different biological specimens procesed by this method. Fig. 2A shows the dome-shaped end of intestinal microvilli. Figs. 2B to 2D correspond to: *Cryptosporidium* sp, *E. falciformis* and *G. muris*, respectively. These specimens are well preserved and do not present evident shrinkage or deformations.

This adaptation of the t-butyl alcohol freeze drying method has a low cost. Also, it uses common apparatus that usually are present in any electron microscopy laboratory (a vacuum desiccator, a vacuum pump, and a metal block). Also, depending of the size of the samples its containers could be a small vial, beaker, or a petri dish, and including aluminium-foil containers moulded with the boton of a vial. Besides, it produces good results, even with delicate specimens such as protozoans.

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