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NOTE

Percoll: An Effective Medium for Cleaning Microsporidian Spores[1]

The use of Ludox (a colloidal suspension of 40% silica in NaOH solution, *p*H 9.8, density 1.30 g/ml; DuPont, Wilmington, Dela.) as a density gradient centrifugation medium for cleaning microsporidian (*Nosema algerae*) spore suspensions was described by A. H. Undeen and N. E. Alger (*J. Invertebr. Pathol.* 18, 419–420, 1971). In this procedure, tissue homogenate is strained through 80-mesh copper screen, layered on a preformed continuous gradient of Ludox, and centrifuged at 16,300 *g* for 20 min at 4°C in a swinging bucket head. Mature spores form a band which may be removed with a pipette or syringe.

A modified Ludox centrifugation technique was developed by J. F. Kelly and J. D. Knell (*J. Invertebr. Pathol.* 33, 252, 1979) to eliminate the necessity for preliminary triangulation (R. J. Cole, *J. Invertebr. Pathol.* 15, 193–195, 1970) in the separation of spores from large numbers of mosquitoes. Also, Kelly and Knell found that 100% of the spores of an *Amblyospora* sp. and lesser numbers of spores of their strains of *N. algerae* extruded their polar filaments in unbuffered Ludox. Their modified technique consists of centrifuging strained tissue homogenates at 400 *g* for 30 min through 10% Ludox buffered to *p*H 6.7. Their method is simple, quick, provides spores sufficiently pure for per os treatment of hosts, and eliminates the need for a high-speed centrifuge.

I have adopted the use of Percoll, a colloidal suspension of silica coated with polyvinylpyrrolidone (PVP) (density 1.30, *p*H 8.8; Pharmacia Fine Chemicals, Piscataway, N.J.), for cleaning spores of *Burenella dimorpha*. A quick cleanup, satisfactory for most purposes, is accomplished in the manner of Kelly and Knell. Infected ant pupae are homogenized in an equal volume of distilled water with a glass tissue grinder, strained, and centrifuged at 750 *g* for 20 min through 50% Percoll in water. Highly purified suspensions of mature spores may be obtained by centrifugation of a strained or unstrained homogenate at 10,000 *g* for 20 min through a discontinuous gradient consisting of layers of 25, 50, 75, and 100% Percoll. (Preformed continuous gradients may also be made by centrifugation of Percoll.)

An important advantage of Percoll is its availability in laboratory quantities, whereas Ludox is a component of floor polishes and is not commercially available in less than 55-gal (208 liter) drum quantities. In addition, Percoll is purported to be biologically inert due to the PVP coating, and has a *p*H of 8.8 as compared to 9.8 for Ludox. Percoll can be buffered with triethanolamine–HCl or citrate, but I have not found this necessary. Over 97% of *B. dimorpha* spores freshly cleaned with unbuffered Percoll extrude their polar filaments when ingested by ant larvae (determined by microscopic examination of meconia). Those that do not extrude their polar filaments appear to be not quite mature. Percoll is supplied sterile, but both Ludox and Percoll may be autoclaved.

KEY WORDS: Microsporidia; Percoll.

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