PEG precipitation

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Concentration of viral suspensions by precipitation techniques is a useful starting point for virus purification.

Advantages over other concentration methods

Precipitation of macromolecular proteins such as viruses by high molecular weight polyethylene glycol–6000 (PEG), pioneered by Yamamoto et al (1970) for bacteriophages, is an effective concentration method because the viruses are slowly precipitated in a cold, high-salt environment which protects them from chemical and physical denaturation.

PEG precipitation is more gentle than physical concentration by ultracentrifugation or molecular sieve filtration. These are also done in the cold, but ulltracentrifugation often packs the virions so tightly, even atop sucrose cushions, that they cannot be resuspended without significant loss of virus, and ultrafiltration requires magnetic mixing to keep the filter cleared and loses a great deal of virus trapped in the filter itself. PEG is also more effective, in our hands, than ammonium sulfate precipitation, although the latter has been used with good results for astroviruses, caliciviruses, coronaviruses, picornaviruses and many others (Ashley and Caul 1982; Tannock 1973; Wadey and Westaway 1981; Minor 1985). The PEG precipitation procedure outlined below has been performed with good results for coronaviruses (Hierholzer 1976; Lanser and Howard 1980), rhabdoviruses (Obijeski et al 1974), parainfluenzaviruses (Hierholzer et al 1993), respiratory syncytial virus (Anderson et al 1984; Cash et al 1977; Hierholzer et al 1994), rubella virus (Fuccillo and Sever 1989), and picornaviruses (Hasegawa and Inouye 1983; Hierholzer et al 1984).

Protocol and follow-up

1. It is best to start with a large-volume virus culture in which calf serum and other protein additives have been withheld from the maintenance medium.
2. At complete CPE, the cells and medium are harvested by scraping with a rubber policeman. (Multiple cycles of freeze–thawing can effectively break up the cells if the virus is stable to such treatment.) The pooled harvest is clarified by large-volume, low-speed centrifugation, such as in a Beckman JA-14 rotor in a J2-21 centrifuge at 10,000 rpm (15,300 *g*) or a J-21 rotor in an L8-70 centrifuge at 12,000 rpm (15,000 *g*), for 20 min at 3°C.
3. Transfer the supernatant to a large beaker in an ice bath on a magnetic stirrer.
4. *Slowly* add NaCl to a final concentration of 2.3%, with constant but gentle stirring.
5. *Slowly* add PEG-6000 to a final concentration of 7.0%, also with constant and gentle stirring. Cover the beaker and stir for about 1 h more to ensure complete solubilization of the PEG. (Others have used higher salt [to 2.7%] and lower and higher PEG (6.0–10.0%) with good results (Fuccillo and Sever 1989; Hasegawa and Inouye 1983; Hierholzer et al 1984; Lanser and Howard 1980; Yamamoto et al 1970).)
6. Transfer the beaker and ice bath to a refrigerator, and allow the virus (and other proteins) to precipitate overnight at 4°C.
7. Collect the precipitate by the same

centrifugation method used for clarification (step 2). Aspirate or drain the centrifuge bottles thoroughly to remove as much PEG as possible.

8. Resuspend the precipitate in a small volume of TES buffer (0.01 M Tris-HCl, pH 7.2, 0.002 M EDTA, 0.15 M NaCl). The buffer should be added at about 2 ml per centrifuge bottle and aspirated thoroughly with a syringe and 22-gauge needle. The suspension is then transferred to a clean tube, and each bottle is rinsed with an additional 1 ml of buffer which is added to the pooled suspension.
9. Finally, the PEG is removed (pelleted) by centrifugation of this pooled suspension at 13,000 *g* for 4 min at 23°C in a Beckman Microfuge or similar device. The supernatant now contains approximately 100–fold concentrated virus in isotonic TES buffer; the virus preparation may be considered enriched, but not purified.