

Organic flocculation to concentrate virus:

I had some luck using organic flocculation to concentrate the *Ambystoma tigrinum* virus (Iridoviridae: *Ranaviridae*) from water samples in order to test them with antigen-capture ELISA. The method is modified from others designed (e.g., Katzenelson et al 1976) to concentrate virus after elution from adsorbent filters. Recovery was relatively good (~40%), but could be quite variable (4-50%). Organic flocculation seemed to work far better than precipitation with polyethylene glycol, although I have only tried this once. Neither have I tried this on cell culture.

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- 1) Filter or centrifuge water sample to remove particulate matter
- 2) Add 10% beef extract solution (w:v; Sigma B4888, 50g/\$21.60) to a final concentration of 0.1% beef extract. (Others use 1-3% beef extract, but I found 0.1% worked best. Please do experiment.)
- 3) Adjust the pH to 3.5 with 2N HCL while stirring.
- 4) Stir for 30min at 4°C
- 5) Centrifuge at 10,000 x g for 10 minutes (again, a range is found in the literature)
- 6) Carefully pour off the supernatant and resuspend the pellet in PBS with phenol red by vortexing about 30 seconds
- 7) Adjust pH to about neutral (ideally ~7.2. Phenol red helps get close with small samples)

Katzenelson, E., B. Fattal, and T. Hostovesky. 1976. Organic flocculation: an efficient second-step concentration method for the detection of virus in tap water. *Applied and Environmental Microbiology* 32(4):638-639.

Schwab, K.J., R. De Leon, M.D. Sobsey. 1995. Concentration and purification of beef extract mock eluates from water samples for the detection of enteroviruses, Hepatitis A virus, and Norwalk virus by reverse transcription-PCR. *Applied and Environmental Microbiology*. 61(2):531-537.