Concentration of Enteric Viruses from Water with Lettuce Extract

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A method for recovering enteroviruses, adenovirus, and reovirus from water with lettuce extract is described. Lettuce extract at pH 8.5 was added to the sample and the pH was reduced stepwise with hydrochloric acid to 4.0 to 4.5. The flocculent lettuce-extract particles, and adsorbed virus, were readily removed from solution by low-speed centrifugation. Electron microscopy suggests that, under conditions suitable for adsorption, virus particles are coated with the lettuce-extract colloid.

A number of methods for the recovery of virus from water have been described recently (1, 4–7). Virus adsorption followed by elution has been the principal approach for virus concentration. In an earlier report (2), we described a method for enterovirus recovery using lettuce floc. The work has been extended to include two other enteric viruses, reovirus 1 and adenovirus 7a.

Lettuce extract was prepared as described previously (2). The extract is a clear amber-colored colloidal suspension at pH 5.5 or above. A floc forms below pH 5.5 and is readily sedimented by low-speed centrifugation at pH 4.0 to 4.5. The dry weight of the floc varied from 1.5 to 3.0 mg/ml depending on the batch. Approximately 50% of the dry weight was protein, as determined by the method of Lowry et al. (3).

Adsorption to lettuce extract of coxsackievirus types B4 and B5, echovirus type 7, poliovirus type 1 (Sabin), reovirus type 1, and adenovirus type 7a was examined as follows. Virus concentrations of 100 or 1,000 plaque-forming units (PFU) in 0.1 ml of phosphate-buffered saline were added individually to samples containing 10 to 1,000 ml of distilled water. Final virus concentrations varied from 0.1 to 100 PFU per ml of sample. A similar inoculum was added to duplicate samples of 2.5 ml of growth medium to serve as virus controls. A 10% volume of lettuce extract was added to the water samples at pH 5.0, 6.0, 7.0, 8.0, or 8.5. Samples were adjusted to pH 4.0 to 4.5 in 0.5 to 1 log steps by dropwise addition of HCl. After centrifugation at 1,000 × g for 10 min, the pellets were dissolved by adding NaOH; 0.05 ml of 1 N NaOH dissolved the 0.4- to 0.8-ml pellet obtained from a 200-ml sample. Water samples larger than 200 ml were divided, centrifuged, and finally recombined after dissolution of the pellets. The sample was diluted to 2.5 ml with concentrated medium 199 to give single strength medium and then was assayed on a single monolayer of cultured cells as described previously (2). HEp-2 cells were used for the coxsackievirus and poliovirus, Vero cells were used for echovirus, and primary African green monkey kidney cells were used for reovirus and adenovirus. Enterovirus plaques were read after 3 days, adenovirus and reovirus after 8 days.

Results of preliminary experiments indicated that virus in the dissolved pellet alone or with added 10% serum would not adsorb to cell monolayers. Incorporation of medium 199, Earle, or saline with or without 10% serum, however, permitted infection of monolayers.

![Graph](image_url)

**FIG. 1.** Effect of pH on virus recovery from water samples treated with lettuce extract. Results of three different experiments were averaged.
FIG. 2. Reovirus treated with lettuce extract. (A) pH 6.0, not coated; (B) pH 8.5, coated. ×187,000.

FIG. 3. Adenovirus treated with lettuce extract. (A) pH 4.5, not coated; and (B) pH 6.0, coated. ×193,000.
Coxsackievirus B4, B5, echovirus 7, poliovirus 1, and adenovirus 7a were efficiently concentrated from water with colloidal lettuce extract at pH 6.0 or higher. Flocculent extract at pH 5.0 was less efficient in concentrating adenovirus than enteroviruses; reovirus required a pH of 8.0 for effective concentration (Fig. 1).

Quantitative recovery of all viruses tested was accomplished by adding lettuce extract at pH 8.5 to the water sample followed by the dropwise addition of HCl in 0.5 to 1 log steps to pH 4.0 to 4.5. Virus inputs varying from 0.1 to 100 PFU per ml of sample were concentrated from water volumes of 10 to 1,000 ml. Concentrates from volumes as great as 500 ml could be assayed in one plastic dish without apparent toxicity to the cell monolayer. Less than 1% of the virus input remained in the supernatant fluid as unadsorbed virus. Mixed as well as unmixed populations of reovirus and adenovirus were adsorbed.

For electron microscopy, viral suspensions were diluted with 2% potassium phosphotungstate at pH 6.8 and spread on pure carbon or carbon-coated Formvar electron microscope grids. The carbon surface was rendered hydrophilic by a brief treatment (ca. 40 s) of exposure to ionized air in a Plasmod unit (Tegal Corp., Richmond, Calif.). The negatively stained viruses were then examined at a magnification of 60,000 to 100,000 in a Siemens Elmiscop 101.

Electron photomicrographs showed that virus was coated with colloidal particles or aggregates of them at optimal pH levels. Capsomers of reovirus were sharply defined at pH 6.0, but were indistinct at pH 8.5 due to adsorbed lettuce-extract colloid (Fig. 2). Adenovirus was well defined at pH 4.5 but hazy at pH 6.0 (Fig. 3). Controls of reovirus and adenovirus without lettuce extract at similar pH measurements were all sharply defined. Colloidal particles were a few nanometers in diameter; some aggregates of the particles were as large as 100 nm.

By following the procedure outlined above, enteroviruses, adenoviruses, and reoviruses are efficiently removed and concentrated from water.

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