

Ferric Chloride Flocculation for Nonflocculating Beef Extract Preparations

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The addition of 2.5 mM ferric chloride to 0.5% beef extract solution at pH 3.5 was found to be highly efficient in the recovery of seeded poliovirus type 1 (Sabin) or indigenous viruses from environmental samples. This method was extremely useful to reconcentrate viruses from beef extract solutions that did not flocculate at pH 3.5.

Since its description by Kalzenelson et al. in 1976 (1), beef extract flocculation has been widely used to reconcentrate viruses from environmental samples. This method takes advantage of the property of organic material to precipitate when the pH of the solution is lowered near the isoelectric point of the substances. Viruses are effectively adsorbed to the floc formed at acid pH values and can thus be concentrated. This method has been used in our laboratory during several studies on the distribution of human enteric viruses in the aquatic environment (2-5). However, recent lots of beef extract from our supplier did not flocculate, as was also experienced by others. Experiments with seeded poliovirus type 1 (Sabin) have shown that viruses were effectively eluted from virus-adsorbing filters by this type of beef extract but that they were not recovered in the light precipitate formed at pH 3.5. Beef extract in dry form or paste obtained from different suppliers had similar properties. A 1.5% solution of beef extract was clear and usually lightly colored. Upon adjustment to pH 3.5 with 1.2 N hydrochloric acid, the solution remained unchanged or slightly turbid. When these solutions were centrifuged at $2,000 \times g$, a light precipitate was recovered. Under similar conditions, preparations obtained a year ago or more were strongly colored, and adjustment to pH 3.5 provoked the formation of a large amount of precipitate easily recovered by low-speed centrifugation. When beef extract solutions were artificially seeded with poliovirus type 1 (Sabin), high recoveries (about 85%) were obtained with older preparations whereas recent batches were totally inefficient (less than 5% recovered).

To overcome the problem associated with the nonflocculating preparations, we modified the method to include a nontoxic flocculating agent, ferric chloride (F.2877; Sigma Chemical Co., St. Louis, Mo.). A 0.5 M stock solution prepared in distilled water (13.5 g/100 ml) and filter sterilized on a 0.22- μ m filter after prefiltering on an AP-25 prefilter (Millipore Corp., Bedford, Mass.). After preliminary experiments and taking into account the amount of floc as well as viral recovery, the following optimal conditions were determined: 0.5% beef extract, 2.5 mM ferric chloride, pH 3.5. A higher concentration of beef extract may result in the formation of a toxic precipitate with some preparations. The ferric chloride concentration can be within 1.0 and 5.0 mM without major effects on the reconcentration procedure.

The efficiency of viral recovery under these conditions is

illustrated in Table 1. Poliovirus type 1 was experimentally seeded in eluates obtained after filtering 100 to 1,000 liters of water as previously described (2). Recoveries after reconcentration by organic flocculation ranged from 56 to 100% of seeded virus, recoveries similar to the one reported with flocculating beef extract (3). The addition of ferric chloride thus appears an excellent alternative to reconcentrate viruses from environmental samples when the beef extract solution is not flocculating. To confirm that other viruses were effectively reconcentrated, we also compared the recovery of indigenous viruses from raw domestic sewage, using both methods. Similar recoveries were observed with flocculating beef extract and with nonflocculating beef extract plus 2.5 mM ferric chloride, whereas with nonflocculating beef extract alone less than 5% of the viruses were recovered.

We are now using this method for the reconcentration of eluates from large-volume samplings (100 to 1,000 liters) of surface or treated waters or raw sewage samplings (20 liters). We have encountered very little toxicity to the cell cultures currently used in our laboratories (Vero, BGM, or BS-C-1 cell lines) using this method, and the number of viruses isolated has been increased.

Ferric chloride flocculation for nonflocculating beef extracts appears to be an efficient alternative for beef extract preparations that fail to flocculate.

TABLE 1. Poliovirus type 1 recovery from experimentally seeded eluates, using ferric chloride flocculation of beef extract^a

Expt no.	% Recovery	Origin of eluate (liters)
1	79	Tap water (1,000)
2	56	Tap water (1,000)
3	88	Tap water (100)
4	100	Tap water (1,000)
5	70	River water (100)
6	100	River water (100)

^a Samples of eluates (100 ml) were obtained from environmental concentrates (100 to 1,000 liters of water; pH 3.5, 1.5 mM aluminum chloride, Duo Fine 0.25- μ m filters; elution with 2.0 liters of 0.5% Difco beef extract, pH 9.75). Poliovirus type 1 (Sabin) (10^6 PFU) was added to the eluate at pH 7.0, the pH was lowered to pH 3.5, and 2.5 mM of ferric chloride was added. After 20 min at room temperature, the precipitate was centrifuged at $1,000 \times g$ for 15 min and recovered in 2 ml of 0.1 M glycine buffer (pH 9). The eluate was neutralized to pH 7.0 with 1.0 N NaOH. The amount of virus recovered was measured by plaque assay on Vero cells.

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