APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1983, p. 501–502
0099-2240/83/080501-02$02.00/0

Vol. 46, No. 2

Survival of Enteric Viruses Adsorbed on Electropositive Filters

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Received 12 November 1982/Accepted 19 April 1983

Three viruses (poliovirus type 1, rotavirus SA-11, and bacteriophage f2) adsorbed on electropositive microporous filters survived at least 5 weeks at 4°C. Poliovirus type 1 and bacteriophage f2 also survived at least 6 weeks at −20°C. Rotavirus SA-11 was not recovered after 1 week at −20°C. The stability of viruses adsorbed on electropositive filters may enable extensive monitoring of viruses in water.

The recent development of electropositive filters (5) has greatly simplified the collection of large volume samples of water for enteric virus detection. Although it is now relatively easy to collect such samples, processing, including elution, culture, and identification of viruses, is restricted to a few laboratories because of the equipment and training required. To make it possible for water to be monitored for viruses on a routine basis, it may be more practical to establish regional laboratories which conduct virus analysis on samples collected on filters and shipped to them from water and wastewater treatment plants throughout the country. For this to be feasible, it is necessary that the viruses survive on the positively charged filters for a sufficient period of time to be eluted and detected by a central laboratory. In this study we have investigated the survival of three viruses on positively charged filter material.

In duplicate experiments, 2.5-cm-square pads of Zeta Plus 50S (AMF CUNO) filter material (gift of Charles P. Gerba) were seeded with 1 ml each of dilutions of rotavirus SA-11, poliovirus type 1, or bacteriophage f2. These three viruses have been used repeatedly as models to demonstrate the efficiency of the filter adsorption elution technique (1–6). Three pads, each containing one of the three viruses, were immediately eluted with 5 ml of 3% beef extract at pH 10 for 2 min. The pads were squeezed dry, and the pH of the eluate was adjusted to 7.5. The sample was infected onto BGM or MA104 cell culture monolayers or *Escherichia coli* Hfr host bacteria cells for quantification by plaque assay. Replicate pads were placed in Whirlpack bags, sealed, and placed in a refrigerator (4°C) or freezer (−20°C) for periods of up to 6 weeks. The 4 and −20°C temperatures represent conditions likely to be found in water treatment plant or sewage treatment plant laboratories. At daily or weekly intervals a pad seeded with each virus was withdrawn and eluted as described above. In the first experiment, refrigerated samples were assayed daily with little resultant loss in poliovirus (43%) or bacteriophage f2 (33%) titer (Fig. 1).

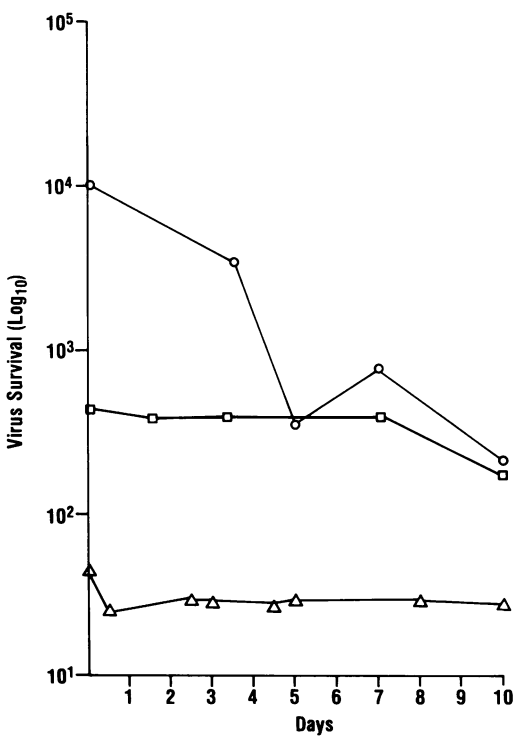

FIG. 1. Effect of storage at 4°C on stability of viruses adsorbed to positively charged filters. Symbols: ○, rotavirus SA-11; □, poliovirus type 1; △, bacteriophage f2.

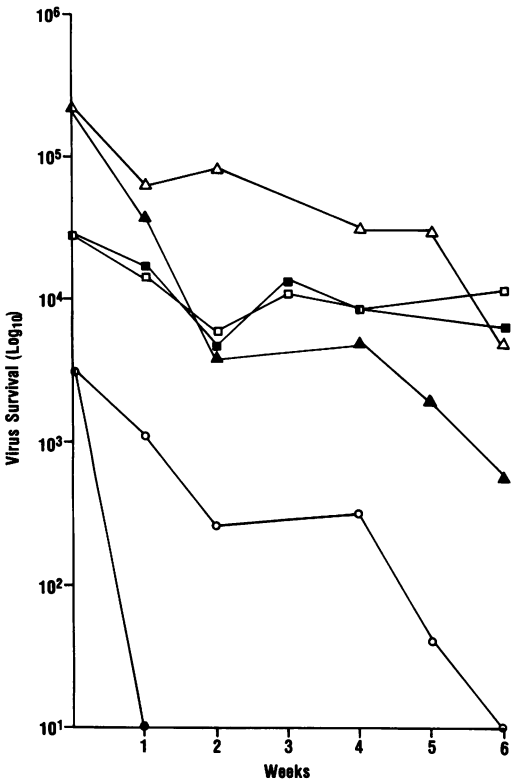

FIG. 2. Comparative effect of storage at 4 and −20°C on the stability of viruses adsorbed to positively charged filters. Rotavirus SA-11, 4°C (○) and −20°C (●); poliovirus type 1, 4°C (□) and −20°C (■); and bacteriophage f2, 4°C (△) and −20°C (▲).

Rotavirus SA-11 was less stable over the 10-day period, with a 98% loss of titer. Since the viruses survived well over a period of days, we examined virus survival over a period of weeks when stored under conditions of refrigeration and freezing to represent possible shipping and storage conditions. Poliovirus type 1 and bacteriophage f2 could be detected after 6 weeks of storage at either 4 or −20°C (Fig. 2). Rotavirus SA-11 survived refrigerator temperatures of 4°C but was not detected after only 1 week of storage at −20°C. This may have been due to inactivation of virus adsorbed to filters during the freezing process or to inability to elute the virus from the filter. Evidence not shown indicates that the virus is inactivated since viral antigen could be detected in eluates by enzyme immunoassay in the absence of infectivity. Furthermore, a recent report indicates that virus infectivity remains stable and that elution efficiency is increased by storage and transport of filters in the presence of eluent (S. Oglesbee, A. Cuenca, and A. Meinhold, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, Q114, p. 278). Increased virus recoveries were reported after 3 days of storage in eluent.

These results indicate that electropositive filters could be used to collect water samples in the field for viral analysis at a central monitoring laboratory. Furthermore, these results extend two previous studies (3, 6) that filters used to sample water in the field can be held at 4°C for at least 300 h and can even be shipped through the mail without appreciable loss of virus titer. Increased monitoring of water supplies should provide valuable information concerning the presence in the environment of important causes of waterborne illness, such as rotaviruses. This information in turn will be valuable in developing detection and monitoring methods for the important waterborne viruses of the Norwalk group and other, as yet undiscovered, waterborne viruses.

The technical assistance of Farhad Kazazi is greatly appreciated.

This work was supported in part by a University of Texas Biomedical Research Support Grant.

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