A REVIEW
Enteric viruses in the aquatic environment

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1. INTRODUCTION

Water quality, and therefore human health, may be significantly affected by the presence of pathogenic enteric microorganisms derived from sewage discharged to surface waters. Knowledge of the role of water in the ecology and transmission of these agents in the environment varies; the part played by enteric viruses is less well understood than that of many bacteria and protozoa, mainly due to the difficulties associated with detecting the agents in the aquatic environment.

Outbreaks of enteric virus disease have been linked to water at various times and to different causes. Pipeline failures account for most incidents, such as those at Bramham, UK, in 1980 when 3000 individuals were affected (Short 1988). Waterborne disease outbreaks with other origins have been reported from many countries; consumption of contaminated ice, borehole or well pollution (Lawson *et al.* 1991; Beller *et al.* 1997; De Serres *et al.* 1999), and contamination of a municipal supply with sewage (Kaplan *et al.* 1982) are typical documented causes. Viruses in the water supply may also cause disease by foodborne transmission through individuals involved with food preparation becoming contaminated (Brugha *et al.* 1999). The list of potentially pathogenic microorganisms transmitted by water increases every year (AAM Report 1996). Waterborne disease may be transmitted by consumption of polluted drinking water, by immersion in recreational water or by contact through skin or inhalation. The health effects caused by microorganisms have been reviewed extensively; Galbraith (1987) and Stanwell-Smith (1994) documented drinking water outbreaks in the UK and Hunter (1997), Dadswell

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(1993) and Prüss (1998) reviewed disease associated with recreational water contact.

Though explosive outbreaks may be well documented epidemiologically, with the exception of hepatitis A virus (HAV), disease transmission through ingesting water has been conclusively demonstrated in very few cases. Virus presence in water is underestimated because those agents which cause most enteric disease cannot be grown by normal culture methods. The driving force for obtaining more information on viruses in the aquatic environment is thus one of a need for increased knowledge contributing to better public health. Greater knowledge of the behaviour of these agents in water will facilitate the provision of appropriate advice to the public and responsible authorities on use and treatment of water. The Bathing Water Directive (1976) and the Drinking Water Directive (1999) are EU instruments which aim to ensure that water is fit for purpose. Compliance with the parameters of the Directives implies that water is monitored regularly and that no indicator organisms are found above the set limits. However, technological advances now permit the direct detection of many enteric pathogens. Accumulation of information also permits better sampling strategies to be devised, and more reliable data to be obtained. This in turn allows more confident risk assessment to be done and the development of models for predicting the risks associated with use of different kinds of water (Macgill *et al*. 2000).

Analysis of water for enteric viruses is usually a two-stage process. Viruses are too few to be detected directly so the sample is processed to *concentrate* them into smaller volumes, usually less than 10 ml. A range of *detection* methods is then available, and if used in a quantitative fashion any virus present may be enumerated. Where necessary, virus may be isolated and identified. This review will focus on the methods for both concentration and detection of enteric virus from water and similar materials. It should be noted that, despite repeated efforts to improve recovery rates for many enteric viruses from water, there is no method which gives consistently high recovery. Indeed, improvements in the detection of enteric viruses, driven by clinical diagnostic needs, have not been matched by advances in concentration techniques to provide reproducible high recovery for different virus types.

2. THE MATRIX

The aquatic environment comprises many water types (matrices), each characterized by its concentration of organic matter and salts. Good quality drinking water, by definition, contains few impurities and no pathogens. Other matrices may contain human viruses if they contain human faecal material; untreated sewage will contain the highest quantity. Treated sewage effluent contains a variable number of viruses depending on the season, the viruses circulating in the population at any one time, and the degree of virus removal during sewage treatment. When sewage effluent is discharged into fresh or marine water, human viruses may therefore be present. Methods to concentrate viruses in sewage and other waters are well established and discussed below. However, methods for processing the highly variable and potentially toxic sewage sludge or biosolids are not yet optimized or of proven efficiency. In view of the increased land disposal of sludge, much current research is directed at improving recovery methods for this matrix (Mignotte *et al*. 1999; UKWIR 2000).

3. ENTERIC VIRUSES IN WATER

Many groups of viruses may inhabit the human intestine and replicate in the epithelial cells which cover the villi. Only some cause sufficient local damage for gastroenteritis or other illness to result. The term 'enteric viruses' encompasses all those groups of viruses which may be present in the gastrointestinal tract; they may cause disease, or infection may be asymptomatic. Being shed in the faeces they will be present in sewage, but as they are obligate intracellular parasites they can not multiply in the environment. They may be divided into two groups according to their growth in cell culture. Most enteroviruses, which form a distinct taxonomic group (including poliovirus, Coxsackievirus and echovirus), grow well in readily available cell cultures of primate origin and are thus well characterized. Other groups that may be grown in cell culture such as some adenovirus serotypes and reoviruses do not cause recognizable gastrointestinal disease.

The remaining enteric viruses form a heterogeneous group which includes the rotaviruses, astroviruses, adenovirus 40/41, human caliciviruses (Norwalk-like viruses and Sapporo-like viruses) and hepatitis A and E. These cannot be grown easily, if at all, in culture. Their importance lies in their causing distinct gastrointestinal disease or hepatitis. The important viruses in the context of water pollution are discussed below.

3.1. Enteroviruses

Enteroviruses are *Picornaviridae* and are naked icosahedral particles approximately 27 nm in diameter with no obvious surface structure. It is of relevance to their environmental survival that the virions are stable within the pH range 3–10, and sensitive to chlorine and UV radiation (Porterfield 1989). They are classified by biomolecular and biochemical characterization, and serological typing which also relates to the species in which they are found. Enteroviruses are present in the gastrointestinal tract of clinically healthy individuals, either as vaccine poliovirus or as nonpolio

enteroviruses, usually of low pathogenicity. Although most infections are asymptomatic, enteroviruses may cause a variety of symptoms, including paralysis, meningitis and cardiomyopathy, and less severe conditions such as colds and fever. They are not associated with gastroenteritis in the absence of wider systemic illness but they are nevertheless a potential public health hazard. More information is available on the occurrence of enteroviruses in the environment than any other virus group because they have been the most readily detectable in the laboratory for many years. They can be concentrated from river water or seawater and can be grown and identified in standard cell culture assays. The numbers shed in faeces are not as high as bacterial indicators; the number of enteroviruses in untreated sewage may reach 10^5 plaque-forming units (pfu) l^{-1} whereas the count of total coliforms is often 10^8/100 ml. Neither are viruses shed consistently throughout the year. Isolations of Coxsackieviruses from river water, for example, showed a seasonal trend which peaked in mid-summer and which matched reports to clinical laboratories (Sellwood 2000).

The majority of studies on enteric viruses in water and associated materials took place between 1975 and 1985 in the UK and the US, and most involved enteroviruses. The consensus of findings was and remains that in the case of polioviruses, serotype 2 predominates, type 3 being the least numerous across all water types (Payment *et al.* 1983; Morris and Sharp 1984). Wild-type strains continue to be identified (Pöyry *et al.* 1988; Divizia *et al.* 1999) although in the UK and more recently in other parts of the world only vaccine strains have been reported (Sellwood *et al.* 1995).

Coxsackievirus B serotypes 1–6 have all been detected in water, although B3, B4 and B5 are the most frequently identified (references as for poliovirus, and Hughes *et al.* 1992). The predominant serotype has been shown to change over time and to reflect the serotype most frequently found in clinical material. Echoviruses have been least frequently identified (Morris and Sharp 1984) unless liquid culture was used when the combined serotypes of echoviruses were the most numerous strain (Sellwood *et al.* 1981).

Geographically the presence of enteroviruses in water has been shown to follow a similar pattern in temperate countries across the world. In addition to surveillance and determination of water quality, data has been used in attempts to assess health risks associated with use of water for drinking or recreational purposes (e.g. Gerba *et al.* 1996).

3.2. Norwalk-like viruses (NLV)

Human caliciviruses are naked single-stranded positive-sense RNA viruses with a single capsid protein and, following sequencing of the genome, are classified into two groups (Jiang *et al.* 1993; Lambden *et al.* 1993). Norwalk-like viruses (NLVs, formerly termed SRSVs in the UK) are divided into genogroups I and II (Pringle 1998; Anon. 2000). The Sapporo-like viruses (SLV), formerly termed 'classic caliciviruses', are also currently divided into two genogroups (Berke *et al.* 1997; Hale *et al.* 1999; Vinjé *et al.* 2000).

Norwalk-like viruses are the principal cause of viral gastroenteritis in adults and were initially defined by a ragged particle outline and poorly defined surface morphology (Fields *et al.* 1996). Up to six antigenic types have been described by immune electron microscopy (Lewis *et al.* 1995). Multiple genotypes and strains are recognized within the two genogroups and are identified by the geographical area where they were initially identified. Norwalk, Southampton and Desert Shield viruses are classified as genogroup I. Lordsdale virus, Mexico virus and Snow Mountain agent are classified within genogroup II (Norcott *et al.* 1994).

Being shed in faeces, NLVs will be transported through sewerage systems and may reach receiving waters. The agent is robust and will therefore survive in the environment, where it may present a hazard to public health, as evidence of shellfish-associated gastroenteritis testifies (e.g. Lees *et al.* 1995). Waterborne outbreaks of NLV infection due to sewage-contaminated drinking water are well documented (e.g. Lawson *et al.* 1991; Kukkula *et al.* 1997, 1999), but reports of illness associated with recreational contact are less common and are based on epidemiological or circumstantial evidence (Gray *et al.* 1997).

Sapporo-like viruses ('classic' human caliciviruses) cause gastroenteritis, mainly in babies under 1 year old, during which time they are excreted in the faeces (Vinjé *et al.* 2000). Morphologically they display a well-defined surface structure. Since young children are commonly infected and adults are immune, their public health significance in relation to potential for waterborne disease remains uncertain.

3.3. Astroviruses

Astroviruses (AV), the only genus in the *Astroviridae,* are 28 nm diameter particles which often bear a five- or six-pointed stellate surface structure and are associated with sporadic cases and occasionally outbreaks of diarrhoea, principally in children, though adults may also be affected (Willcocks *et al.* 1992). They do not grow in commonly used cell cultures, though propagation has been reported in cell lines derived from intestinal tumours (e.g. Willcocks *et al.* 1990; Wyn-Jones and Herring 1991).

Few reports exist on the incidence of astroviruses in the aquatic environment. Detection of astrovirus in environmental samples has been described by Marx *et al.* (1995), and Pintó *et al.* (1996) demonstrated the occurrence of infectious astrovirus in water from an area where a concurrent gastroenteritis outbreak was reported. Abad *et al.* (1997) studied the survival of astroviruses in different

water types. The survival characteristics found in these studies were comparable to those determined for rotavirus and enteric adenoviruses, though astrovirus decay was more pronounced at the higher temperatures.

3.4. Rotavirus

Rotaviruses are the major viral cause of infantile gastroenteritis. They are 75 nm diameter icosahedral virus particles with a triple capsid protein layer (Estes 1996); the virion consists of a core and a shell of intermediate and outer layers from which 60 spikes protrude. The genome consists of 11 segments of double-stranded RNA (dsRNA) that are capable of genetic reassortment. The complete, infectious particle has a smooth appearance by EM which becomes a rough outline when the outer shell is lost. Faeces contains many more rough noninfectious particles, which are environmentally robust, than complete infectious ones. Rotaviruses are classified on the basis of serogroups, serotypes and, most recently, genogroups. Six serogroups, termed Groups A–F (plus a possible seventh, G), are recognized and between each of these groups no cross-reaction occurs using assays based on the inner capsid antigen, VP6. Group A is the most common human rotavirus. Serotypes are based on neutralization assays for outer capsid proteins VP7 and VP4. Genogroups are based on gene 4 (P-types, VP4) and gene 9 (G-types, VP7). Strains circulating in the community can thus be monitored (Iturriza *et al*. 2000). Limited numbers of human or monkey kidney cell culture types, such as CaCo-2 and MA-104 will support rotavirus replication.

Limited numbers of reports of waterborne transmission have been published and those that have are as a result of sewage contaminated drinking water (reviewed by Gerba *et al*. 1996).

3.5. Enteric adenoviruses

Enteric adenoviruses (types 40 and 41) are important aetiological agents of paediatric gastroenteritis, principally in temperate climates (Uhnoo *et al*. 1984). Their role in transmission of disease in water is undetermined. Enriquez and Gerba (1995) found that they were more stable in water than poliovirus 1 or hepatitis A virus. Genthe *et al*. (1995) first reported detection of the genome in a high proportion of water samples in South Africa, and similar results have been found in other countries. Other adenovirus serotypes are found in sewage but they are not associated with gastrointestinal disease (Sellwood *et al*. 1981).

3.6. Hepatitis A and E viruses

Because of its well-defined association with waterborne and, particularly, shellfish-associated disease (Mackoviak *et al*. 1976), there is much information on the role of the environment in the transmission of HAV, and though it is relatively uncommon in countries which have a good standard of hygiene (Maguire *et al*. 1995), raised numbers of cases are still reported every 8–10 years. It is a picornavirus and the only member of the *Hepatovirus* genus. One serotype is recognized, with strain differences. Humans are the only natural host. HAV is relatively resistant to low pH and to heating, so is environmentally robust. Outbreaks have occurred in many residential settings, especially schools where hygiene is poor. Outbreaks in the US have been associated with drinking water drawn from wells (Bloch *et al*. 1990) and after contact with recreational water (Mahoney *et al*. 1992). Contamination of borehole water in Ireland in 1991 (Fogarty *et al*. 1995) resulted in a major outbreak of gastroenteritis but a follow-up study identified no cases of hepatitis. HAV has been detected in well water (De Serres *et al*. 1999), sewage (Jothikumar *et al*. 1998) and in sewage sludge (Graff *et al*. 1993).

Hepatitis E virus is a member of the *Caliciviridae* and is a 30-nm iscosahedral particle without obvious structure by electron microscopy. Hepatitis E virus is not endemic in the UK but cases are imported from Asia, China and Africa. The infection is similar to HAV hepatitis but can be more severe, especially in pregnant women. Large scale waterborne outbreaks have been reported (Jothikumar *et al*. 1993).

4. CONCENTRATION OF ENTERIC VIRUSES

Virus particles exhibit polarity and can adsorb to a wide variety of charged matrices which may be immobilized (such as membranes) or fluid (such as glass powder). Considered as protein, virus particles have a high relative molecular mass ($M_r > 10^6$) and lend themselves to concentration by ultrafiltration and by ultracentrifugation. Based on these general properties, numerous methods have been devised for the concentration of viruses from aquatic matrices.

To be of optimum practical use, any method must fulfil the following criteria (after Block and Schwartzbrod 1989):

(i) be technically easy to accomplish in a short time;
(ii) have a high virus recovery rate;
(iii) concentrate a large range of viruses;
(iv) provide a small volume of concentrate;
(v) not be costly;
(vi) be capable of processing large volumes of water;
(vii) be repeatable (within a laboratory) and be reproducible (between laboratories).

There is no single method which fulfils all these requirements.

There are four principal approaches to virus concentration based on different properties of virus particles; they have been in place for many years, and each technique has numerous variations (Table 1).

Table 1 Summary of concentration techniques for viruses in water and water-related materials

Technique	Method	Water quality	Initial volume (litres)	Relative virus content	Recovery	Capital cost	Revenue cost	2ary concn. required?	Comments
Adsorption/elution	Gauze pads	Sewage or effluent	Large	High	Low to medium	Nil	Very low	No	Not quantitative
	Electronegative membranes	All waters	1–1000	Low to medium	50–60% with practice	Medium	Medium	Yes	High vols. require dosing pumps
	Electropositive membranes	All waters	1–1000	Low to medium	50–60% with practice	Medium	High	Yes	No preconditioning required
	Electronegative cartridges	Any low turbidity	1–50	Low to medium	Variable: higher with clean waters	Low	Low	Yes	May clog more quickly than membranes
	Electropositive cartridges	All waters	1–1000	Low to medium	Variable	Medium	High	Yes	Wide range of viruses
	Glass wool	All waters	1–1000	Low to medium	Variable	Low	Very low	Yes	No preconditioning required
	Glass powder	All waters	< 100	Any	20–60%	Medium	Low	If vol. > 100 l	Special apparatus
Entrapment: ultrafiltration	Alginate membranes	Clean only	Low	High	Good	Low	Low	No	Very slow. Clogs rapidly if turbid
	Single membranes	Clean	Low	Any	Variable	Medium	Low	No	Slow
	Tangential (= cross) flow and hollow fibres	Treated effluents or better	High	Low	Variable	High	Medium	Sometime	Prefilter for turbid waters
	Vortex flow	Treated effluents or better	High	Low	Unknown	High	Medium	Unknown	Undeveloped yet
Hydroextraction	PEG or sucrose	Any	Low	High	Variable (toxicity)	Negligible	Very low	No	High virus loss in wastewaters
Ultra-centrifugation		Clean	Low	High	Medium	High	Medium	No	Wide range, but usually impractical
Other techniques	Ferric oxide floc	All	Low	Any	Variable	Low	Low	No	
	Biphasic partition	All	< 7	Any	Variable	Low	Low	No	Toxic to cells
	Immunoaffinity and magnetic beads	Unknown	Low	Low	High	High	Low	No	New method

Most procedures can be used to concentrate viruses in sample volumes of 1–100 l, and some (mainly those based on cartridges) are suitable for volumes of 1000 l or more. For immobilized filter matrices the maximum volume that can be filtered will be dependent upon the turbidity of the sample. Groundwater and drinking water will contain very few viruses and 100 l or more will need to be processed, while recreational and river waters may contain many more viruses so processing 10 l samples is sufficient. Sewage effluent that has received secondary treatment will have higher levels of virus so one litre samples are sufficient. Viruses in crude sewage may be detectable directly or 100 ml samples may be processed.

Adsorption/elution and some entrapment techniques comprise the first stage (primary concentration) which reduces the initial volume to between 100 and 500 ml in a two-stage concentration process. The second stage concentrates the virus further, usually by acid flocculation and low-speed centrifugation to deposit the virus-containing floc, which is resuspended in 5–10 ml neutral buffer.

4.1. Adsorption/elution

The development of virus adsorption/elution (Viradel) methods stems from the work of Melnick and colleagues in Houston, Texas (e.g. Wallis and Melnick 1967a, b, c). In general, the virus-containing sample is brought into contact with a matrix to which virus will adsorb under specific conditions of pH and ionic strength. Once virus is adsorbed, the water in which it was originally suspended is discarded. Virus is then eluted from the matrix into a smaller volume, though this is still too large to be analysed directly. Choice of adsorbing matrix, eluting fluid and processing conditions will be influenced by the nature of the sample and by experience. Elution of virus from the matrix is commonly done using one of three solutions, beef extract at pH 9·5, skimmed milk at pH 9, or glycine/NaOH at pH 9·5–11·5. In EU laboratories, beef extract or skimmed milk dissolved in glycine buffer is often used. Following elution, the virus is further concentrated in the eluate by acid precipitation (flocculation) and centrifuging the floc to which the virus is adsorbed (Katzenelson *et al.* 1976).

Each concentration method has advantages and disadvantages and it is important to recognize that there is no single method which fulfils all the above criteria. The challenge is to maximize virus recovery whilst minimizing virus loss due to the concentration procedure. Whilst enteroviruses are robust enough to retain infectivity following exposure to pH 3·5 and pH 9·0, this is not the case for other agents, e.g. rotaviruses, where infectivity is substantially reduced below pH 5·0. The principal variations of the adsorption/elution approach are as follows:

4.1.1. Gauze pads. Cotton gauze pads trap suspended particles as water flows through them. Recovery of virus is very variable. They cannot be used in waters low in suspended matter, and they are therefore best applied to sewage and wastewaters, for which they were originally developed (e.g. Manor *et al.* 1999).

4.1.2. Electronegative membranes and cartridges. Concentration of viruses in water using negatively charged microporous filters has been practised for many years and there are many variations of the technique, though little change in the basic process. The popularity of cellulose nitrate membranes lies in their availability in various pore sizes, configurations and compositions. For waters containing particulate material, a prefilter is used upstream of the membrane. The choice of filter will depend partly on the sample; for seawaters, filters of pore diameter 0·45 μm and 1·2 μm are commonly used, and prefilters are employed. By judicious choice of prefilters and adsorbing filters, it is possible to get good recoveries of virus from turbid waters accompanied by good flow rates and a minimum of filter clogging. In addition, many solids-associated virus can be recovered. Virus is bound to the filter by opposing electrostatic forces and is eluted using beef extract or skimmed milk solution. This method is currently the preferred way of recovering viruses from effluent, diluted raw sewage and activated sludge samples, as well as recreational and surface waters in most water virology laboratories in the EU, and is the UK recommended method (SCA 1995). It is also a tentative Standard Method for the recovery of viruses from waters and wastewaters, as published by the American Public Health Association (USEPA/APHA 1984). The final concentrate volume is a practical quantity for cell culture assay but is a large volume for molecular studies. Enteroviruses (Morris and Waite 1980a) and NLVs (Wyn-Jones *et al.* 2000) can be concentrated by this method. Among the few reports of this approach to detect rotaviruses are Smith and Gerba (1982) and Merrett *et al.* (1991).

The water sample is preconditioned to facilitate electrostatic binding of virus to filter matrix by adjustment to pH 3·5 and sometimes addition of $AlCl_3$, though opinion is divided as to whether additional metal ions are needed when using cellulose nitrate membranes.

Negatively charged filters have also been used in epoxy resin-bound glass-fibre tube form. They were originally used for concentration of viruses from tapwater (Jakubowski *et al.* 1974) and have since been employed for concentration of viruses from river water (e.g. Morris and Waite 1980a) and other waters (USEPA 1984). These filters also need the sample to be conditioned for virus particles to adsorb; their recoveries appear at least as good as membrane filters, they are less expensive and can be obtained complete in disposable cartridge form. They are, however, prone to clogging and

cannot be used with even moderately turbid water according to Gerba (1987), though the 8 μm porosity variants as used by Morris (personal communication) and Edwards and Wyn-Jones (1981) gave no problems in this respect. Because of problems of clogging of membrane or tube filters, the processing of seawater samples in this way is limited to a maximum of 20 l before filters have to be changed (Block and Schwartzbrod 1989); nevertheless they remain useful for processing of 10 l EU Bathing Water samples.

Pleated fibreglass membrane cartridge filters were evaluated by Farrah *et al.* (1976). The increased surface area allowed for higher flow rates, and recoveries were better than given by disk membranes. However they are expensive, though they may be regenerated up to five times by soaking in NaOH.

Many modifications to the filtration process have been made but have had little effect on practicability, recovery, or other aspects. Generally, recovery rates are as variable with negatively charged filter media as with any other kind. Block and Schwartzbrod (1989; citing Beytout *et al.* 1977) considered cellulose nitrate membranes relatively efficient insofar as they gave 60% recovery of enterovirus; the same authors recorded glass-fibre filters giving a poor average yield on wastewater but 70% recovery with river water. Payment and Trudel (1979), using glass-fibre filters, reported 38–58% recovery of 10^2–10^6 pfu enterovirus seeded in 100 ml to 1000 l volumes. Few controlled studies have been done on recovery efficiencies from marine waters, though they have been done to evaluate the efficiency of the method using drinking water. Melnick *et al.* (1984) reported considerable variation in the quantity of virus recovered following processing of 100 l tap water samples containing poliovirus. Though the average recovery was 66% (of 350–860 pfu virus), values ranged from 8 to 20% in two laboratories, 49–63% in three laboratories and 198% in one laboratory. Recovery levels were higher and less variable where a higher input level of virus was used, but it must be noted that even the 'low' level of 350–680 pfu is more than is routinely found in northern European bathing water (European Union 2001).

4.1.3. Electropositive cartridges and membranes. Positively charged filters adsorb virus from water and other materials without the need for prior conditioning of the sample with acid or metal cations. They adsorb virus in the pH range 3–6, though at pH values above 7 the adsorption falls off rapidly, so the pH still needs to be monitored. These properties make the use of positively charged filters attractive, not only for the convenience of not having to condition the sample but also because it makes possible the concentration of viruses such as rotavirus and coliphages, which are sensitive to the low pH conditions needed for adsorption to negatively charged media. Further, Keswick *et al.* (1983) reported that type 1 poliovirus and rotavirus SA11 survived at least five weeks on electropositive filters at 4°C, so this may make them useful for extended surveys or transmission through postal systems. Other than not needing to condition the water sample, electropositive filters are used in the same way as electronegative materials. Virus is eluted from the filter and secondary concentration is carried out as for the electronegative types.

Recoveries from positively charged filters are similar to those from negatively charged filters. Sobsey and Jones (1979) reported 22·5% recovery using a two-stage procedure in the concentration of poliovirus from drinking water, and Pina *et al.* (1998) recovered adenovirus, enteroviruses and HAV from seawater, river water and slaughterhouse sewage using Zeta-plus filters; Pintó *et al.* (1996) recovered infectious astrovirus using the same system. Chapron *et al.* (2000), using 1MDS Zetapor cartridges, detected infectious astrovirus, enterovirus and adenovirus type 40 when surface water samples were processed and concentrates analysed by integrated cell culture PCR (ICC-PCR).

The original positively charged material, Zeta-plus Series S, is made of a cellulose/diatomaceous earth/ion-exchange resin mixture. Sobsey and Glass (1980) compared these filters with Filterite (fibreglass) pleated cartridge filters and for recovery of poliovirus from 1000 l tapwater and obtained recoveries of about 30% with both types. Though expensive, especially in cartridge form, these filters have an advantage in the large volumes they can handle without the need for conditioning the sample. Elution from the filter still needs to be carried out at pH 9 or above, which limits their use to viruses stable below that pH. Bosch *et al.* (1988) and Raphael *et al.* (1985) successfully concentrated rotaviruses using positively charged cartridges. Organic materials in the sample, especially fulvic acid, have been reported to interfere more with virus recovery from Virozorb cartridges than from glass-fibre materials (Sobsey and Hickey 1985). A different electropositive material, MK, was developed, which was cheaper but recoveries were reported to be not as good as Virozorb-1MDS in comparative tests (Ma *et al.* 1994). Positively charged filter materials are popular in the US but are not so widely used in Europe.

Advances in membrane technology have resulted in charge-modified nylon membranes being available for concentration of viruses from water. Gilgen *et al.* (1995, 1997) used positively charged nylon membranes coupled with ultrafiltration for the concentration of a variety of enteric viruses prior to detection by RT-PCR. Nylon membranes are made in varying pore sizes and carry a positive surface charge over the pH range 3–10, which promotes strong binding of negatively charged virus particles. Nylon filter material is also manufactured in re-sterilisable cartridge form, which would increase the convenience of use. Few

studies have been done on this material in respect of recovering viruses from bathing waters, and its low cost and ease of use suggest that further evaluative research should be done. Triple-layered PVDF membranes and cartridges have been used in industry for the removal of polio and influenza viruses from pharmaceutical products (AranhaCreado *et al.* 1997), though whether the virus can be recovered from the filter is not known.

4.1.4. Concentration using glass powder. Borosilicate glass beads of 100–200 μm diameter form a good adsorbent for viruses under conditions similar to those used for glassfibre filters. They form a fluidized bed and so have the advantage that the filter matrix cannot become clogged as with glass-fibre systems. Sarrette *et al.* (1977) first developed this technique, which was extended by Schwartzbrod and Lucena-Gutierrez (1978). For low sample volumes (< 100 l) the method produces a low eluate volume which does not need secondary concentration prior to inoculation into cell culture. However, in common with the performance for glassfibre tube filters, the recovery varies widely with the type of sample, from 60% with potable water to 20% with urban wastewater (Joret *et al.* 1980). Bosch *et al.* (1988), using glass powder to concentrate rotavirus from natural and seeded tap water and sewage, obtained approximately 50% recovery from the tap water but only 5% from sewage in seeded samples, though, however, 100% of unseeded sewage samples were positive for rotavirus.

4.1.5. Concentration using glass wool. Glass wool evenly packed in a column at a density of 0·5 g cm^{-3} is an efficient virus adsorbent. Its principal advantages are its low cost and that it will adsorb virus at near neutral pH without the addition of cations. The technique was first applied to the concentration of a range of viruses from surface, drinking and waste waters (Vilaginès *et al.* 1993). Sample sizes ranged from 100 to 1000 l for drinking waters, 30 l for surface waters and 10 l for wastewaters, and the only pretreatment necessary was dechlorination of drinking waters. Virus was eluted from the filter with beef extract solution, and secondary concentration was done by organic flocculation. Recovery efficiency of approximately 10^2 pfu poliovirus seeded into 400 l drinking water averaged 74%. For surface waters the recovery rate was 63% and 57%, respectively. Clogging of the filters was reduced by lowering the flow rate to 50 l h^{-1}.

Since virus concentration on glass wool does not need the sample to be conditioned, the technique lends itself to large sample monitoring (for surface waters) and to continuous monitoring (for drinking waters). Other viruses were also concentrated during field evaluation of the method; adenoviruses and reoviruses were also recovered, though enteroviruses predominated. Vilaginès *et al.* (1993) also reported a survey of two rivers over a 44-month period, and concluded that the technique was robust enough in physical and experimental terms to be used for routine monitoring of surface waters. Glass wool is also very cheap and thus the method is economic.

In a later study Vilaginès *et al.* (1997) conducted a round-robin trial to investigate the use of the method for recovering virus from water sample seeded with poliovirus. Drinking water and seawater samples were used, and high levels of input virus were employed in order to evaluate the recovery efficiencies and the repeatability and reproducibility of the method. About half the laboratories involved in the exercise had had no experience of virological analysis of water. The mean recovery from seawater samples in 39 separate assays was 75%. Results confirmed the simplicity of the virus concentration method by adsorption-elution through glass wool, and demonstrated the ability of nonexpert laboratories to carry out such analysis, though this was limited to the concentration/elution stages, the concentrates being sent back to a central expert laboratory for assay. Glass wool has been used in other laboratories; Hugues *et al.* (1991), when analysing biologically treated wastewaters, found it more sensitive than the glass powder method, both in terms of number of positive samples and in the level of virus detected; Wolfaardt *et al.* (1995) used glass wool to concentrate NLVs from seeded sewage and polluted water samples prior to detection by RT-PCR and Marx *et al.* (1995) used the method to recover astroviruses from a variety of environmental samples.

4.1.6. Secondary concentration. Following binding, any virus is eluted from the filter matrix into a smaller volume of fluid, which is then further concentrated to a final volume of about 10 ml. Virus is most commonly eluted with a protein solution (beef extract or skimmed milk (casein)) at pH 9·5. The pH is then lowered to below the isoelectric point of the protein (usually pH 3·5–4·5), when it flocculates (Katzenelson *et al.* 1976). Virus is adsorbed to the floc which is centrifuged, dissolved in a small volume of neutral phosphate buffer and can be inoculated into cell cultures for assay or analysed by other detection methods. It can also be stored below –20°C if assay is not to be performed immediately. Beef extract batches may vary in their ability to flocculate and the process may be modified by the addition of small quantities of ferric chloride (Payment *et al.* 1984), or replace beef extract with skimmed milk.

4.2. Entrapment techniques

In entrapment, virus in a sample is held in a filter matrix principally by virtue of its molecular size rather than by any charges on the particle.

4.2.1. Ultrafiltration. Early ultrafiltration methods involved passing the water sample under pressure through aluminium/lanthanum alginate filters (Poynter *et al.* 1975), which had the unique advantage that they were soluble in isotonic sodium citrate. However, the flux obtained was too low for all but the cleanest waters unless they had been prefiltered, which precluded analysis of surface waters in volumes over 1 litre.

More recent techniques involve passing the sample through capillaries (e.g. Rotem *et al.* 1979), membranes (e.g. Divizia *et al.* 1989a, b), or hollow fibres (Belfort *et al.* 1982) with pore sizes that permit passage of water and low molecular mass solutes but exclude viruses and macromolecules. Earlier workers used membranes with nominal cut-off levels of 10 kDa, but the majority of systems use cut-off levels of 30–100 kDa. Where the fluid passes directly through the filter, nonfilterable components quickly block the matrix or precipitate at the membrane surface, so this type of filter is only useful for small (1000 ml or less) volumes.

Most ultrafilters employ tangential flow (also called cross-flow), where the sample is repeatedly swept past the membrane surface. Small molecular mass species, including salts and water, pass through into the filtrate, and larger species, including viruses, are withheld in the retentate. The fluid is circulated through the multiple membrane layers or hollow fibre cartridge until the retentate is reduced in volume sufficiently to allow further processing. The minimum 'dead' volume (e.g. 10–15 ml, Divizia *et al.* 1989a) depends on the apparatus. If this is small enough then it may be analysed by detection procedures, or it may have to be further processed by secondary concentration. Soule *et al.* (2000) reported concentration of poliovirus, rotavirus and HAV from tap and seeded distilled water; all three viruses were recovered in seeding experiments, and enteroviruses and rotaviruses were found in five of 90 tapwater samples examined.

Some workers have experienced binding of virus to the membrane rather than just prevention of its passage through it. In these cases the virus was eluted by backwashing with glycine buffer or beef extract and the elute reconcentrated by organic flocculation. Some authors have even reported differences in binding between related viruses. Divizia *et al.* (1989b) noted that HAV was recovered with 100% efficiency; poliovirus on the other hand was recovered very poorly under standard conditions but this improved if the membranes were pretreated with different buffers. Further, recovery was best if the virus was eluted with beef extract at neutral (not high) pH. Ultrafiltration has also been used to reconcentrate viruses recovered from treated wastewater by adsorption/elution.

A variation in ultrafiltration is vortex flow filtration (VFF), where Taylor vortices are established in a VFF device by rotation of one pressurized cylinder inside another and keep the filter surface from clogging. The technique has been used by several authors, e.g. Paul *et al.* (1991), for concentration of T2 bacteriophage from seawater, but there are few significant reports in the literature of its use in the field for concentrating human viruses. One is that of Tsai *et al.* (1994), who used it for inshore waters in Southern California. Fifteen litres of each sample were concentrated to 100 ml using a 100-kDa cut-off membrane.

The advantages of ultrafiltration are principally that the sample requires no preconditioning and that a wide range of viruses can therefore be recovered, including bacteriophages (e.g. Urase *et al.* 1993). Efficiency of recovery is usually good, though as with all methods it is variable. The main constraints upon its use are the high initial cost of the equipment and that, despite the advantages of tangential flow, turbid samples still tend to clog the membrane. Surface water may take a long time to process if they are turbid; Nupen *et al.* (1981) were able to filter 50 l volumes but this took from 40 h to 72 h depending on the sample. In other studies workers have used different parameters (though generally all use 30–100 kDa membranes or hollow fibre cartridges) with differing results. The technique is generally seen as an advance on adsorption/elution (e.g. Grabow *et al.* 1984; Muscillo *et al.* 1997), and recovery efficiencies, though variable, appear to be higher than those obtained using adsorption/elution techniques.

4.2.2. Hydroextraction. Hydroextraction with polyethylene glycol (PEG, M_r 6000–20 000) or sucrose has been employed for concentration of wastewater and sewage (Wellings *et al.* 1976), but its use is limited by volume, the maximum being about 1 l, and some by cytotoxic compounds being co-concentrated with the virus.

4.3. Ultracentrifugation

Ultracentrifugation is a catch-all method since it is capable of concentrating all viruses in a sample, provided sufficient g-force and time are used, and differential ultracentrifugation allows separation of different virus types. A number of studies have been reported, including one where viral numbers in natural waters were as high as $2{\cdot}5 \times 10^8$ ml^{-1}, 10^3–10^7 times as high as had been found by plaque assay (Bergh *et al.* 1989). However, the limited volumes that can be processed, even using continuous flow systems, together with the high capital costs and lack of portability of the equipment limit its usefulness in concentrating viruses directly from natural waters. It does find a use as a secondary concentration method, however; Murphy *et al.* (1983), in an investigation of a gastroenteritis outbreak associated with polluted drinking water, concentrated 5 l samples of bore-

hole water using an ultrafiltration hollow fibre device to 50 ml and followed this by ultracentrifugation to pellet the virus for electron microscopical examination. They were thus able rapidly to detect rotaviruses, adenoviruses and NLVs, as well as enteroviruses, which were confirmed by cell culture.

4.4. Other methods

4.4.1. Two-phase separation. Viruses can be partitioned between the two immiscible phases produced when two different organic polymers are dissolved in water. Lund and Hedstrom (1966) used sodium dextran sulphate and polyethylene glycol 6000 mixture for enterovirus recovery from sewage. By controlling the phases, viruses can be partitioned into one of them and if the virus-containing phase is made small relative to the original volume of sample then concentration is achieved. The method is limited by the occasional toxicity of the polymer for cell culture; it is also limited to a maximum volume of about seven litres. Lodder *et al*. (1999) used a dextran T40/polyethylene glycol mixture as the second stage in concentrating NLVs from sewage.

4.4.2. Immunoaffinity columns and magnetic beads. These are relatively new techniques which have been used in a biochemical or molecular biological context (e.g. Jothikumar *et al*. 1998). They are useful for small volumes but their application to virus concentration from larger volumes has yet to be demonstrated. Loisy *et al*. (2000) used oligonucleotide-treated beads to improve the sensitivity of detection procedures for NLV.

There are several less-used methods and method modifications which it is appropriate to mention in a review for completeness. None appears to offer significant improvement over those already described, which taken together are used by the majority of laboratories engaged in water virology. These methods include iron oxide flocculation (Rao *et al*. 1986), talc-celite adsorption (Ramia and Sattar 1979) and adsorption to bituminous coal (Dahling *et al*. 1985).

5. DETECTION, ENUMERATION AND IDENTIFICATION

The selection of a detection method for enteric viruses in water or other environmental materials largely depends on whether the agent grows in cell culture. For those types that do, such as the enteroviruses, detection by virus replication in cell culture demonstrates infectivity as well as presence, and this is clearly an advantage when assessing whether the water is microbiologically hazardous. Cell culture also allows quantitative assays to be done, though any subsequent virus serotype identification requires immunological or molecular tests.

In the absence of infectivity assays, NLV, rotavirus and the other viruses that are major causes of gastroenteritis must be detected using serological or molecular biological approaches and, while these do not give a direct indication of infectivity nor are they satisfactory in enumerating viruses, they do permit the typing of viruses or genome analysis. The use of PCR as a routine tool has not only permitted virus detection, but has facilitated the refined characterization of many viruses found in water. Used in combination with cell culture as an integrated cell culture-RT-PCR (ICC-RT-PCR), the technique has allowed the detection of viruses which show no evidence of infectivity in any other way. These three main approaches to virus detection are considered below.

5.1. Detection by cell culture

Virus-specific killing of cells (cytopathic effect, c.p.e.) is visible by ordinary light microscopy and allows the presence or absence of virus to be determined. There are many cell lines suitable for growing enteroviruses, including HEp-2, HeLa and VERO, although the Buffalo Green Monkey cell line (BGM, Barron *et al*. 1970; Dahling *et al*. 1974) has been favoured for enumeration of those types which are waterborne. This was reported to give higher plaque assay titres of polio, Coxsackie B, some echo- and reoviruses than obtained in rhesus or grivet monkey kidney cells, though this is not the case with isolation of enteroviruses from clinical specimens. BGM cells are used almost exclusively for the detection and enumeration of waterborne enteroviruses. Morris (1985) examined 10 cell lines for their ability to grow enteroviruses isolated from wastewater effluent. Eighty-two percent of samples were positive in BGM cells, 73% in RD cells and 64% in chimpanzee liver cells. BGM was also the most sensitive in the number of plaques counted. Human rhabdomyosarcoma (RD) cells support plaque production by echoviruses and are therefore sometimes used in conjunction with BGM cells, in which plaque production by echoviruses is variable.

Dahling and Wright (1986) optimized the BGM line in respect of a number of assays for waterborne viruses, work which has become the accepted basis for many standard methods.

Other cell lines, derived from intestinal tissue, have been investigated for their ability to support the growth of enteric viruses. Most of these studies have been directed at growing the more fastidious agents like rotaviruses and astroviruses, but Patel *et al*. (1985) carried out a large survey on the susceptibility of a range of lines to different enteroviruses, including all 31 serotypes of echovirus; they found that two lines, HT-29 and SKCO-1 derived from human colonic carcinoma, had a markedly wider sensitivity for enteroviruses than primary monkey kidney or RD cell cultures.

They require a high seed density and do not grow quickly, however, and perhaps this is why they have not found greater favour in the detection of waterborne enteric viruses. Generally CaCo-2 cells (Fogh 1977) have been used for detection of a number of waterborne viruses, e.g. astroviruses (Pintó *et al*. 1996). Grabow *et al*. (1993) used the PLC/PRF/5 hepatoma cell line for the detection of adenovirus 41 from water.

Cell culture assays may be carried out either in liquid culture or under agar, as plaque assays.

5.1.1. Liquid culture assay. Liquid culture involves the growth of a monolayer of cells attached to a plastic or glass solid phase. Polioviruses, Coxsackieviruses and echoviruses grow well in human amnion, Hep-2, fibroblast cells, in PMK as well as VERO and BGM. Human amnion cells are the most sensitive but the difficulty of availability precludes their more general use. Liquid cell culture systems will detect the widest range of enterovirus serotypes but enumeration can only be done by titration and the calculation of 'most probable number' (MPN) or 'tissue culture dose 50' (TCD50).

5.1.2. Plaque assays. Plaque assays may be used either as a monolayer of cells overlayed with agar or as cells suspended in agar (Cooper 1967, and further adapted for detection of enteroviruses by Morris and Waite 1980b). Dahling and Wright (1988) compared the sensitivity of these two formats and found the suspended cell assay detected five to eight times as many plaques compared with the monolayer method. However, it may not detect slower-growing viruses since suspended cell cultures do not last as long as monolayers. Morris (1985) compared 10 cell lines for their ability to isolate enterovirus from effluent and found BGM cells not only isolated enterovirus from the highest number of samples but also generated the most plaques. The number of actual virions contained in an infectious unit has been estimated at 10–100 or approximately 700 (Schiff *et al*. 1984). The suspended cell plaque assay is the standard technique for enumerating enteroviruses when monitoring 10 l samples of bathing water for the EU Bathing Water Directive in the UK although in other EU countries the monolayer plaque assay is more commonly employed.

5.2. Immunological detection

The serum neutralization test (SNT) is the most common method for enterovirus identification but is technically demanding and time-consuming, and results are not available for three to five days. Immunofluorescence for the identification of many serotypes of enterovirus involves infected cells from culture being transferred to a microscope slide, dried, fixed and stained with one of a range of virus serotype-specific monoclonal antisera followed by antimouse IgG/FITC conjugate. Infected cells emit a bright green granular fluorescence with the specific serotype. This method is very quick (less than two hours) and interpretation easier than SNT. Antisera for polioviruses, Coxsackievirus B and some echoviruses are available (Rigonan *et al*. 1998).

Immunofluorescence was also used to identify rotavirus in MA-104 cells by (amongst others) Smith and Gerba (1982), and Bosch *et al*. (1988), and in CaCo-2 cells by Abad *et al*. (1998). Relatively few studies have been done on the occurrence of rotavirus because of this subjective and laborious method, although Bosch *et al*. (1988) detected rotavirus in most Barcelona sewage samples throughout the year.

Flow cytometry has been used to accelerate IF procedures; Baradi *et al*. (1998) reported the use of flow cytometry for the detection of rotavirus in MA-104 cells, and Abad *et al*. (1998) reported its use in detecting infectious rotavirus in environmental as well as clinical samples.

Environmental samples have not often been tested directly by ELISA for rotavirus as insufficient VP6 antigen is present even after concentration (Guttmann-Bass *et al*. 1987) and VP6-directed ELISA only detects noninfectious particles. Steinmann (1981) published the first report of the identification by ELISA of rotaviruses in 6/24 samples of sewage and Dahling *et al*. (1993) reported rotavirus in three out of 30 sewage samples from Puerto Rico that had been concentrated by positive membrane filtration and were found to be positive by direct ELISA. Genthe *et al*. (1991) found approximately 30% of sewage, fresh and marine water samples were rotavirus-positive after concentration by ultrafiltration.

Immunomagnetic separation has been used as a preliminary stage to RT-PCR in the detection of rotavirus in water (Grinde *et al*. 1995) and for HAV in sewage (Graff *et al*. 1993).

5.3. Detection by molecular biology

The explosion in molecular biology technology has been exploited in water virology as in other fields of biology. Detection based first on probes then using PCR has provided a wealth of information about waterborne viruses which would not otherwise have been available. It has facilitated the detection of pathogenic viruses which do not grow well, if at all, in cell culture, particularly rotaviruses and NLVs, as well as enhancing the information already available on enteroviruses.

Gene probes have been widely used (e.g. Dubrou *et al*. 1991; Enriquez *et al*. 1993). They are easy to prepare and if

used in conjunction with Southern blotting they produce satisfactory results. However, they lack sensitivity and even though they now incorporate digoxigenin instead of radioisotopes they have largely been superseded.

The PCR reaction (Saiki *et al.* 1988) addresses these shortcomings. RT-PCR detection of enteroviruses generally has been extensively described (e.g. Rotbart 1990a, b). Numerous investigations have used RT-PCR to detect enteroviruses in different environmental samples, including river and marine recreational waters (e.g. Kopecka *et al.* 1993; Wyn-Jones *et al.* 1995), ground waters (Abbaszadegan *et al.* 1993), sludge-amended field soils (Straub *et al.* 1995), and shellfish (e.g. Le Guyader *et al.* 1994). The technique has been extended to cover other virus groups present in water, including adenoviruses (Puig *et al.* 1994), HAV (Graff *et al.* 1993), astrovirus (Marx *et al.* 1995) and rotavirus (Gajardo *et al.* 1995). Pallin *et al.* (1997) devised a method for recovering all the virus in a concentrate into a single PCR tube, which allowed direct comparisons of sensitivity with cell culture methods where the whole of the concentrate is assayed.

RT-PCR is not without practical problems. Chief among these is the presence of fulvic and humic acids in the concentrates which inhibit the RT and/or polymerase reactions. Different remedies have been found to remove these but most rely on guanidinium isothiocyanate (GIT) extraction of the RNA and adsorption to silica (e.g. Shieh *et al.* 1995). Ijzerman *et al.* (1997) removed inhibitors with no loss of virus by Ficoll/PEG dialysis, solvent extraction, ultrafiltration and silica adsorption. Lewis *et al.* (2000) analysed the influence of common sample components on poliovirus 2 detection efficiency by RT-PCR and compared this with their influence on detection by cell culture. They found that RT-PCR was 10–100 times more sensitive than cell culture in the absence of contaminants. However, in the presence of contaminants at the higher concentrations tested, plaque assay was more efficient. Both techniques were inhibited by the presence of bentonite clay, presumably due to virus adsorption; this could be reversed by eluting the virus. As reported by many authors, Lewis *et al.* (2000) found that humic acid at a concentration of 25 mg l^{-1} significantly affected the RT-PCR, and suggested this level might well be found in concentrates of environmental samples. It was reduced by filtration of the concentrate through Sephadex. Cell culture detection was not affected by humic acid. Overall these authors found that clays, humic acids and shellfish tissue residues have a greater effect on RT-PCR detection of virus than on cell culture detection.

Refinement of the RT-PCR and restriction enzyme analysis of amplicons has permitted the differentiation of virus types within the enterovirus group. Hughes *et al.* (1993) compared the nucleotide sequences of six Coxsackievirus B4 isolates from the aquatic environment with those of four CB4 isolates from clinical specimens and found that the isolates fell into two distinct groups not related to their origin. Wyn-Jones, Pallin and Lee (unpublished results) devised a method for identifying enterovirus concentrated from bathing waters based on restriction enzyme analysis which assigned any isolate to at least group level and many to serotype, using just four enzymes, the groupings correlating with those determined by immune electron microscopy, and Sellwood *et al.* (1995) reported a system using restriction fragment length polymorphism (RFLP) analysis to discriminate between wild-type and vaccine-like strains of poliovirus. Egger *et al.* (1995) devised a multiplex PCR for the differentiation of polioviruses from nonpolioviruses, which made an important step in the accumulation of public health information.

Integration of cell culture with RT-PCR has permitted the indirect detection of infectivity. Reynolds *et al.* (1996) described a system for detecting infectious enterovirus where sample concentrates were inoculated into BGM cell cultures. RT-PCR of the culture lysates showed positive signals for virus, even where the original inoculum had been negative by cell culture. This approach has been adopted in detecting astroviruses and enteric adenoviruses (e.g. Chapron *et al.* 2000), though there are no reports of infectious NLVs being detected this way.

Further use of the RT-PCR has led to detection of a wider range of viruses. Cho *et al.* (1999) described a multiplex RT-PCR for the detection of adenoviruses and enteroviruses from tapwater and river water, and Bofill-Mas *et al.* (2000) reported the detection of polyomaviruses in sewage using RT-PCR.

The most important virus of epidemic adult gastroenteritis is NLV. Several groups have developed RT-PCR based procedures for the detection of this agent in shellfish (e.g. Lees *et al.* 1995), but there are fewer reports of its presence being demonstrated in water. Lodder *et al.* (1999) reported detection of NLVs in sewage, and seeding studies using seeded untreated potable water have been reported by Myrmel *et al.* (1999). Kukkula *et al.* (1997, 1999) identified NLVs of genogroup II in untreated, treated water and tap water samples during an outbreak of gastroenteritis in a Finnish village, and in the UK Wyn-Jones *et al.* (2000) reported the occurrence of NLVs in a variety of water-related materials including seawater, river water, sewage and sewage effluent. Griffin *et al.* (1999) detected NLVs in water off the Florida Keys.

6. SUMMARY

Enteroviruses in the aquatic environment have been well studied over many years and have demonstrated the distribution and persistence of human enteric viruses. However, the development of molecular biological tech-

niques has enabled the detection of those viral pathogens that cannot be readily cultivated but are major causes of gastrointestinal disease. The detection of NLV in the aquatic environment has been demonstrated. The concentration of these viruses and others is necessary from many types of water samples but the effectiveness of available methods is variable and many are costly.

7. ISSUES THAT MUST BE ADDRESSED

Several issues arise as a consequence of improved ability to concentrate and detect a wider range of enteric viruses in the aquatic environment. The distribution and survival of viruses in biosolids (sludge) is poorly understood, as is their persistence once sludge has been spread on agricultural land, a practice that is likely to increase in the EU following prohibition on the dumping of sewage sludge at sea. Toxicity and variability in composition mean that novel methods have to be devised in order to recover viruses in a reproducible fashion.

Concentration methods require continued development. Viruses are concentrated with different degrees of success; for example, rotaviruses are more susceptible to pH changes in sample processing, and their recovery (especially of infectious virus) is not as good as that of other types.

Molecular biological detection methods need to be reconciled with infectivity if quantitative hazard assessments are to be done on those agents which do not grow in culture, particularly NLV. ICC-RT-PCR is of some use in this respect but does not allow virus quantification.

Refinement of detection methods is now possible by use of genotyping/genogrouping of viruses isolated from water. This should lead to increased understanding of the spread of individual strains and whether some strains are more resistant to environmental breakdown. This in turn should clarify wider public health issues, including assessment of risk associated with recreational water polluted by enteric viruses. Enteroviruses are well characterized in this respect but information on other agents is lacking. It will also be important to determine the role of animal enteric viruses in (i) any transmission to humans by the water route and (ii) whether they produce false positive data in respect of human faecal pollution of water.

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9. REFERENCES

AAM Report (1996) *A Global Decline in Microbiological Safety of Water: A Call for Action.* American Academy of Microbiology.

Abad, F.X., Pintó, R.M., Villena, C., Gajardo, R. and Bosch, A. (1997) Astrovirus survival in drinking water. *Applied and Environmental Microbiology* **63**, 3119–3122.

Abad, F.X., Pintó, R.M. and Bosch, A. (1998) Flow cytometry detection of infectious rotaviruses in environment and clinical samples. *Applied and Environmental Microbiology* **64**, 2392–2396.

Abbaszadegan, M., Huber, M.S., Gerba, C.P. and Pepper, I.L. (1993) Detection of enteroviruses in ground water by PCR. *Applied and Environmental Microbiology* **65**, 444–449.

Anon. (2000) Molecular epidemiology of Norwalk-like viruses (NLVs). *Communicable Disease Report* **10**, 403–404.

AranhaCreado, H., Oshima, K., Jafari, S. and Brandwein, H. (1997) Virus retention by a hydrophilic triple-layer PVDF microporous membrane filter. *PDA Journal of Pharmaceutical Science and Technology* **51**, 119–124.

Baradi, C.R.M., Emslie, K.R., Vesey, G. and Williams, K.L. (1998) Development of a rapid and sensitive quantitative assay for rotavirus based on flow cytometry. *Journal of Virological Methods* **74**, 31–38.

Barron, A.L., Olshevsky, C. and Cohen, M.M. (1970) Characteristics of the BGM line of cells from African green monkey kidney. *Archiv Gesamte Virusforschung* **32**, 389–392.

Bathing Water Directive (1976) Council directive of 8 December 1975 concerning the quality of bathing water (76/440/EEC). *Official Journal of the European Community* **L31**, 1–7.

Belfort, G., Paluszek, A. and Sturman, L.S. (1982) Enterovirus concentration using automated hollow fiber ultrafiltration. *Water Science and Technology* **14**, 257–272.

Beller, M., Ellis, A., Lee, S.H., Drebot, M.A., Jenkerson, S.A., Funk, E., Sobsey, M.D., Simmons, O.D., III, Monroe, S.S., Ando, T., Noel, J.S., Petric, M., Middaugh, J.P. and Spika, J.S. (1997) Outbreak of viral gastroenteritis due to a contaminated well. *Journal of the American Medical Association* **278**, 563–568.

Bergh, O., Borsheim, K.Y., Bratbak, G. and Heldal, M. (1989) High abundance of viruses found in aquatic environment. *Applied and Environmental Microbiology* **340**, 467–468.

Berke, T., Golding, B., Jiang, X., Cubitt, W.D., Wolfaardt, M., Smith, A.W. and Matson, D.O. (1997) Phylogenetic analysis of the caliciviruses. *Journal of Medical Virology* **52**, 419–424.

Bloch, A.B., Stramer, S.L., Smith, J.D., Margolis, H.S., Fields, H.A., McKinley, T.W., Gerba, C.P., Maynard, J.E. and Sikes, R.K. (1990) Recovery of hepatitis A virus from a water supply responsible for a common source outbreak of hepatitis A. *American Journal of Public Health* **80**, 428–430.

Block, J.C. and Schwartzbrod, L. (1989) *Viruses in Water Systems. Detection and Identification*. New York: VCH Publishers.

Bofill-Mas, S., Pina, S. and Girones, R. (2000) Documenting the epidemiological patterns of polyomaviruses in human populations by studying their presence in urban sewage. *Applied and Environmental Microbiology* **66**, 238–245.

Bosch, A., Pintó, R.M., Blanch, A.R. and Jofre, J.T. (1988) Detection of human rotavirus in sewage through two concentration procedures. *Water Research* **22**, 343–348.

Brugha, R., Vipond, I.B., Evans, M.R., Sandifer, Q.D., Roberts, R.J., Salmon, R.L., Caul, E.O. and Mukerjee, A.K. (1999) A community outbreak of food-borne SRSV gastro-enteritis caused by a contaminated water supply. *Epidemiology and Infection* **122**, 145–154.

Chapron, C.D., Ballester, N.A., Fontaine, J.H., Frades, C.N. and Margolin, A.B. (2000) Detection of astroviruses, enteroviruses and adenovirus types 40 and 41 in surface waters collected and evaluated by the information collection rule and an integrated cell culture-nested PCR procedure. *Applied and Environmental Microbiology* **66**, 2520–2525.

Cho, H.B., Lee, S.-H., Cho, J.-C. and Kim, S.-J. (1999) Detection of adenoviruses and enteroviruses in tapwater and river water by reverse transcription multiplex PCR. *Canadian Journal of Microbiology* **46**, 417–424.

Cooper, P.D. (1967) The plaque assay of animal viruses. *Advances in Virus Research* **8**, 319–378.

Dadswell, J.V. (1993) Microbiological quality of coastal waters and its health effects. *International Journal of Environmental Health Research* **3**, 32–46.

Dahling, D.R. and Wright, B.A. (1986) Optimization of the BGM cell line and viral assay procedures for monitoring viruses in the environment. *Applied and Environmental Microbiology* **51**, 790–812.

Dahling, D.R. and Wright, B.A. (1988) Optimisation of suspended cell method and comparison with cell monolayer technique for virus assays. *Journal of Virological Methods* **20**, 169–179.

Dahling, D.R., Berg, G. and Berman, D. (1974) BGM, a continuous cell line more sensitive than primary rhesus and African green kidney cells for the recovery of viruses from water. *Health Laboratory Sciences* **11**, 275–282.

Dahling, D.R., Phirke, P.M., Wright, B.A. and Safferman, R.S. (1985) Use of bituminous coal as an alternative technique for the field concentration of waterborne viruses. *Applied and Environmental Microbiology* **49**, 1222–1225.

Dahling, D.R., Wright, B.A. and Williams, F.P. (1993) Detection of viruses in environmental samples: suitability of commercial rotavirus and adenovirus test kits. *Journal of Virological Methods* **45**, 137–147.

De Serres, G., Cromeans, T.L., Levesque, B., Brassard, N., Barthe, C., Dionne, M., Prud'homme, H., Paradis, D., Shapiro, C.N., Nainan, O.V. and Margolis, H.S. (1999) Molecular confirmation of hepatitis A virus from well water: epidemiology and public health. *Journal of Infectious Diseases* **179**, 37–43.

Divizia, M., de Filippis, P., di Napoli, A., Venuti, A., Perez-Bercoff, R. and Pana, A. (1989a) Isolation of wild-type hepatitis A virus from the environment. *Water Research* **23**, 1155–1160.

Divizia, M., Santi, A.L. and Pana, A. (1989b) Ultrafiltration: an efficient second step for hepatitis A and poliovirus concentration. *Journal of Virological Methods* **23**, 55–62.

Divizia, M., Palombi, L., Buonomo, E., Donia, D., Ruscio, V. et al. (1999) Genomic characterization of human and environmental polioviruses isolated in Albania. *Applied and Environmental Microbiology* **65**, 3534–3539.

Drinking Water Directive (1999) Council directive 98/83/EC of November, 1998 on the quality of water intended for human consumption. *Official Journal of the European Community* **L330/32**, EN.

Dubrou, S., Kopecka, H., Lopez-Pila, J.M., Maréchal, J. and Prèvot, J. (1991) Detection of hepatitis A virus and other enteroviruses in wastewater and surface water samples by gene probe assay. *Water Science and Technology* **24**, 267–272.

Edwards, E.R. and Wyn-Jones, A.P. (1981) Incidence of human enteroviruses in water and wastewater treatment systems associated with the River Wear. In *Viruses and Wastewater Treatment* ed. Butler, M. and Goddard, M. p. 109. Oxford: Pergamon Press.

Egger, D., Pasamontes, L., Ostermayer, M. and Bienz, K. (1995) Reverse transcription multiplex PCR for differentiation between polio- and enteroviruses from clinical and environmental samples. *Journal of Clinical Microbiology* **33**, 1442–1447.

Enriquez, C.E. and Gerba, C.P. (1995) Concentration of enteric adenovirus 40 from tap, sea and waste water. *Water Research* **29**, 2554–2560.

Enriquez, C.E., Abbaszadegan, M., Pepper, I.L., Richardson, K.J. and Gerba, C.P. (1993) Poliovirus detection in water by cell culture and nucleic acid hybridization. *Water Research* **27**, 1113–1118.

Estes, M.K. (1996) Rotaviruses and their replication. In *Fields' Virology* ed. Fields, B.N., Knipe, D.M. and Howley, P.M. pp. 1625–1657. Lippincott-Raven.

European Union (2001) Website http://europa.eu.int/water/

Farrah, S.R., Gerba, C.P., Wallis, C. and Melnick, J.L. (1976) Concentration of viruses from large volumes of tap water using pleated membrane filters. *Applied and Environmental Microbiology* **31**, 221–226.

Fields, B.N. and Knipe, D.M. and Howley, P.M. (ed.) (1996) *Fields' Virology*. 3rd edn. Lippincott-Raven.

Fogarty, J., Thornton, L., Hayes, C. et al. (1995) Illness in a community associated with an episode of water contamination with sewage. *Epidemiology and Infection* **114**, 289–295.

Fogh, J., Fogh, J.M. and Orfeo, T. (1977) One hundred and twenty-seven cultured human tumour cell ines producing tumours in nude mice. *Journal of the National Cancer Institute* **58**, 209–220.

Gajardo, R., Bouchrit, N., Pintó, R.M. and Bosch, A. (1995) Genotyping of rotaviruses isolated from sewage. *Applied and Environmental Microbiology* **61**, 3460–3462.

Galbraith, N.S. (1987) Water and disease after Croydon: a review of waterborne and water associated disease in the UK 1937–86. *Journal of the Institute of Water and Environmental Management* **1**, 7–21.

Genthe, B., Idema, G.K., Jrif, R. and Grabow, W.O.K. (1991) Detection of rotavirus in South African waters: a comparison of a cytoimmunolabelling technique with commercially available immunoassays. *Water Science and Technology* **24**, 241–244.

Genthe, B., Gericke, M., Bateman, B. et al. (1995) Detection of enteric adenoviruses in South African waters using gene probes. *Water Science and Technology* **31**, 345–350.

Gerba, C.P. (1987) Recovering viruses from sewage, effluents and water. In *Methods for Recovering Viruses from the Environment* ed. Berg, G. pp. 1–23. CRC Press.

Gerba, C.P., Rose, J.B., Haas, C.N. and Crabtree, K.D. (1996) Waterborne rotavirus: a risk assessment. *Water Research* **30**, 2929–2940.

Gilgen, M., Wegmuller, B., Burkhalter, P., Buhler, H.-P., Muller, U., Luethy, J. and Candrian, U. (1995) Reverse transcription PCR to detect enteroviruses in surface water. *Applied and Environmental Microbiology* **61**, 1226–1231.

Gilgen, M., Germann, D., Luethy, J. and Huebner, P. (1997) Three-step isolation method for sensitive detection of enterovirus, rotavirus, hepatitis A virus and small round-structured viruses

in water samples. *International Journal of Food Microbiology* **37**, 189–199.

Grabow, W.O.K., Puttergill, D.L. and Bosch, A. (1993) Plaque assay for adenovirus type 41 using the PLC/PRF/5 liver cell line. *Water Science and Technology* **27**, 321–327.

Grabow, W.O.K., Nupen, E.M. and Bateman, B.W. (1984) South African research on enteric viruses in drinking water. *Monographs in Virology* **15**, 146–155.

Graff, J., Ticehurst, J. and Flehmig, B. (1993) Detection of hepatitis A virus in sewage by antigen capture polymerase chain reaction. *Applied and Environmental Microbiology* **59**, 3165–3170.

Gray, J.J., Green, J., Gallimore, C., Lee, J.V., Neal, K. and Brown, D.W.G. (1997) Mixed genotype SRSV infections among a party of canoeists exposed to contaminated recreational water. *Journal of Medical Virology* **52**, 425–429.

Griffin, D.W., Gibson, C.J., III, Lipp, E.K., Riley, K., Paul, J.P. III and Rose, J.B. (1999) Detection of viral pathogens by reverse transcription PCR and of microbial indicators by standard methods in the canals of the Florida Keys. *Applied and Environmental Microbiology* **65**, 4118–4125.

Grinde, B., Jonassen, T.O. and Ushijima, H. (1995) Sensitive detection of group A rotavirus by immunomagnetic separation and RT-PCR. *Journal of Virological Methods* **55**, 327–338.

Guttmann-Bass, N., Tchorsh, Y. and Marva, E. (1987) Comparison of methods for rotavirus detection on water and results of a survey of Jerusalem wastewater. *Applied and Environmental Microbiology* **53**, 761–767.

Hale, A., Crawford, S.E., Ciarlet, M., Green, J., Gallimore, C., Brown, D.W., Jiang, X. and Estes, M.K. (1999) Expression and self-assembly of Grimsby virus: Antigenic distinction from Norwalk and Mexico viruses. *Clin. Diagn. Lab. Immunol.* **6**, 142–145.

Hughes, M.S., Hoey, E.M. and Coyle, P.V. (1992) Enteroviruses in recreational waters of Northern Ireland. *Epidemiology and Infection* **108**, 529–536.

Hughes, M.S., Hoey, E.M. and Coyle, P.V. (1993) A nucleotide sequence comparison of Coxsackievirus B4 isolates from aquatic samples and clinical specimens. *Epidemiology and Infection* **110**, 389–398.

Hugues, B., André, M. and Champsaur, H. (1991) Virus concentration from waste water: glass wool versus glass powder adsorption methods. *Biomedical Letters* **46**, 103–107.

Hunter, P.R. (1997) *Waterborne Disease: Epidemiology and Ecology*. Chichester: John Wiley.

Ijzerman, M.M., Dahling, D.R. and Fout, G.S. (1997) A method to remove environmental inhibitors prior to the detection of waterborne enteric viruses by reverse transcription-polymerase chain reaction. *Journal of Virological Methods* **63**, 145–153.

Iturriza Gomara, M., Green, J., Brown, D.W.G., Ramsay, M., Desselberger, U. and Gray, J. (2000) *Seroepidemiological and Molecular Surveillance of Human Rotavirus Infections in the UK*. London: Public Health Laboratory Service.

Jakubowski, W., Hoff, J.C., Anthony, N.C. and Hill, W.F. (1974) Epoxy-fiberglass adsorbent for concentrating viruses from large volumes of potable water. *Applied Microbiology* **28**, 501.

Jiang, X., Wang, M., Wang, K. and Estes, M.K. (1993) Sequence and genome organisation of Norwalk virus. *Virology* **195**, 51–61.

Joret, J.C., Block, J.C., Lucena-Gutierrez, F., Schwartzbrod, L., Hugues, B. and Plissier, A. (1980) Virus concentration from secondary wastewater: Comparative study between epoxy fibreglass and glass powder adsorbents. *European Journal of Applied Microbiology and Biotechnology* **10**, 245–252.

Jothikumar, N., Aparna, K., Kamatchiammal, S., Paulmurugan, R., Saravanadevi, S. and Khanna, P. (1993) Detection of hepatitis E virus in raw and treated wastewater with the polymerase chain reaction. *Applied and Environmental Microbiology* **59**, 2558–2562.

Jothikumar, N., Cliver, D.O. and Mariam, T.W. (1998) Immunomagnetic capture PCR for rapid concentration of hepatitis A virus from environmental samples. *Applied and Environmental Microbiology* **64**, 504–508.

Kaplan, J.E., Goodman, R.A., Schonbereger, L.B. et al. (1982) Gastroenteritis due to Norwalk virus: an outbreak associated with a municipal water system. *Journal of Infectious Diseases* **146**, 190–193.

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

Keswick, B.H., Pickering, L.K., DuPont, H.L. and Woodward, W.E. (1983) Organic flocculation: an efficient second step concentration method for the detection of viruses in tap water. *Applied and Environmental Microbiology* **46**, 813–816.

Kopecka, H., Dubrou, S., Prèvot, J., Maréchal, J. and Lopez-Pila, J.M. (1993) Detection of naturally-occurring enteroviruses in waters by reverse transcription, polymerase chain reaction, and hybridization. *Applied and Environmental Microbiology* **59**, 1213–1219.

Kukkula, M., Arstila, P., Klossner, M.-L., Maunula, L., von Bonsdorff, C.-H. and Jaatinen, P. (1997) Waterborne outbreak of viral gastro-enteritis. *Scandinavian Journal of Infectious Diseases* **29**, 415–418.

Kukkula, M., Maunula, L., Silvennoinen, E. and von Bonsdorff, C.-H. (1999) Outbreak of viral gastroenteritis due to drinking water contaminated by Norwalk-like viruses. *Journal of Infectious Diseases* **180**, 1771–1776.

Lambden, P.R., Caul, E.O., Ashley, C.R. and Clarke, I.N. (1993) Sequence and genome organisation of a human small round-structured (Norwalk-like) virus. *Science* **259**, 516–519.

Lawson, H.W., Braun, M.M., Glass, R.I., Stine, S.E., Monroe, S.S., Atrash, H.K., Lee, L.E. and Englender, S.J. (1991) Waterborne outbreak of Norwalk virus gastroenteritis at a southwest US resort: role of geological formations in contamination of well water. *Lancet* **337**, 1200–1204.

Le Guyader, F., Dubois, E., Menard, D. and Pommepuy, M. (1994) Detection of hepatitis A virus, rotavirus and enterovirus in naturally contaminated shellfish and sediments by reverse transcription PCR. *Applied and Environmental Microbiology* **60**, 3665–3671.

Lees, D.N., Henshilwood, K., Green, J., Gallimore, C. et al. (1995) Detection of small round structured viruses in shellfish by reverse transcription-PCR. *Applied and Environmental Microbiology* **61**, 4418–4424.

Lewis, D., Ando, T., Humphrey, C.D., Monroe, S.S. and Glass, R.I. (1995) Use of solid-phase immune electron microscopy for classification of Norwalk-like viruses into six antigenic groups from 10

outbreaks of gastroenteritis in the United States. *Journal of Clinical Microbiology* **33**, 633–640.

Lewis, G.D., Molloy, S.L., Greening, G.E. and Dawson, J. (2000) Influence of environmental factors on virus detection by RT-PCR and cell culture. *Journal of Applied Microbiology* **88**, 633–640.

Lodder, W.J., Vinjé, J., van de Heide Roda Husman, A.M., Leenen, E.J.T.M. and Koopmans, M.P.G. (1999) Molecular detection of Norwalk-like caliciviruses in sewage. *Applied and Environmental Microbiology* **65**, 5624–5627.

Loisy, F., Le Cann, P., Pommepuy, M. and Le Guyader, F. (2000) An improved method for the detection of Norwalk-like caliciviruses in environmental samples. *Letters in Applied Microbiology* **31**, 411–415.

Lund, E. and Hedstrom, C.E. (1966) The use of an aqueous polymer phase system for enterovirus isolations from sewage. *American Journal of Epidemiology* **84**, 287–291.

Ma, J.-F., Naranjo, J. and Gerba, C.P. (1994) Evaluation of MK filters for recovery of enteroviruses from tap water. *Applied and Environmental Microbiology* **60**, 1974–1977.

Macgill, S.M., Fewtrell, L. and Kay, D. (2000) Towards quality assurance of assessed waterborne risks. *Water Research* **43**, 1050–1056.

Mackoviak, P.A., Caraway, C.T. and Portnoy, B.J. (1976) Oyster associated hepatitis: lessons from the Louisiana experience. *American Journal of Epidemiology* **103**, 181–191.

Maguire, H.C., Handford, S., Perry, K.R., Nicholas, S., Waight, P., Parry, J.V., O'Mahoney, M. and Begg, N.T. (1995) A collaborative case control study of sporadic hepatitis A in England. *Communicable Disease Review* **5**, R33–R40.

Mahoney, F.L., Farley, T.A., Kelso, K.Y., Wilson, S.A., Horan, J.M. and McFarland, L.M. (1992) An outbreak of hepatitis A associated with swimming in a public pool. *Journal of Infectious Diseases* **165**, 613–618.

Manor, Y., Handsher, R., Halmut, T., Neuman, M., Bobrov, A. et al. (1999) Detection of poliovirus circulation by environmental surveillance in the absence of clinical cases in Israel and the Palestinian authority. *Journal of Clinical Microbiology* **37**, 1670–1675.

Marx, F.E., Taylor, M.B. and Grabow, W.O.K. (1995) Optimization of a PCR method for the detection of astrovirus type 1 in environmental samples. *Water Science and Technology* **31**, 359–362.

Melnick, J.L., Safferman, R., Rao, V.C., Goyal, S., Berg, G., Dahling, D.R. and Wright, B.A. (1984) Round robin investigations of methods for the recovery of poliovirus from drinking water. *Applied and Environmental Microbiology* **47**, 144–150.

Merrett, H., Stackhouse, C. and Cameron, S. (1991) The incidence of rotavirus in the marine environment: a two year study. *Proceedings of the First UK Symposium on Health-Related Water Microbiology* Glasgow, 3–5 September. ed. Morris, R., Alexander, L., Wyn-Jones, A.P. and Sellwood, J. pp. 148–157. London: IAWPRC.

Mignotte, B., Maul, A. and Schwartzbrod, L. (1999) Comparative study of techniques used to recover viruses from residual urban sludge. *Journal of Virological Methods* **78**, 71–80.

Morris, R. (1985) Detection of enteroviruses: an assessment of ten cell lines. *Water Science and Technology* **17**, 81–88.

Morris, R. and Sharp, D.N. (1984) Enteric viruses levels in wastewater effluents and surface waters in the Severn Trent Water Authority 1979–81. *Water Research* **18**, 935–939.

Morris, R. and Waite, W.M. (1980a) Evaluation of procedures for the recovery of viruses from water – I Concentration systems. *Water Research* **14**, 791–793.

Morris, R. and Waite, W.M. (1980b) Evaluation of procedures for recovery of viruses from water – II Detection systems. *Water Research* **14**, 795–798.

Murphy, A.M., Grohmann, G.S. and Sexton, M.F.H. (1983) Infectious gastroenteritis in Norfolk Island and recovery of viruses from drinking water. *Journal of Hygiene* **91**, 139–146.

Muscillo, M., Carducci, A., la Rosa, G., Cantiani, L. and Marianelli, C. (1997) Enteric virus detection in Adriatic seawater by cell culture, polymerase chain reaction and polyacrylamide gel electrophoresis. *Water Research* **31**, 1980–1984.

Myrmel, M., Rimstad, E., Wasteson, Y. and Skjerve, E. (1999) Detection of small round structured viruses in artificially contaminated water using filter adsorption and reverse transcription polymerase chain reaction. *International Journal of Food Microbiology* **49**, 85–94.

Norcott, J.P., Green, J., Lewis, D., Estes, M.K., Barlow, K.I. and Brown, D.W.G. (1994) Genomic diversity of SRSVs in the UK. *Journal of Medical Virology* **44**, 280–286.

Nupen, E.M., Basson, N.C. and Grabow, W.O.K. (1981) Efficiency of ultrafiltration for the isolation of enteric viruses and coliphages from large volumes of water in studies on wastewater reclamation. *Water Pollution Research* **13**, 851–863.

Pallin, R., Place, B.M., Lightfoot, N.F. and Wyn-Jones, A.P. (1997) The detection of enteroviruses in large volume concentrates of recreational waters by the polymerase chain reaction. *Journal of Virological Methods* **57**, 67–77.

Patel, J.R., Daniel, J. and Mathan, V.I. (1985) A comparison of the susceptibility of three human gut tumour-derived differentiated epithelial cell line, primary monkey kidney cells and human rhabdomyosarcoma cell line to 66-prototype strains of human enteroviruses. *Journal of Virological Methods* **12**, 209–216.

Paul, J.H., Jiang, S.C. and Rose, J.B. (1991) Concentration of viruses and dissolved DNA from aquatic environments by vortex flow filtration. *Applied and Environmental Microbiology* **57**, 2197–2204.

Payment, P. and Trudel, M. (1979) Efficiency of several micro-fiber glass filters for recovery of poliovirus from tap water. *Applied and Environmental Microbiology* **38**, 365–368.

Payment, P., Ayache, R., Trudel, M., Schaub, S.A., Bausum, H.T. and Taylor, G.W. (1983) A survey of enteric viruses in domestic sewage. *Canadian Journal of Microbiology* **29**, 111–119.

Payment, P., Fortin, S. and Trudel, M. (1984) Ferric chloride flocculation for non-flocculating beef extract preparations. *Applied and Environmental Microbiology* **47**, 591–592.

Pina, S., Puig, M., Lucena, F., Jofre, J. and Girones, R. (1998) Viral pollution in the environment and in shellfish: human adenovirus detection by PCR as an index of human viruses. *Applied and Environmental Microbiology* **64**, 3376–3382.

Pintó, R.M., Abad, F.X., Gajardo, R. and Bosch, A. (1996) Detection of infectious astroviruses in water. *Applied and Environmental Microbiology* **62**, 1811–1813.

Porterfield, J.S. (1989) Picornaviridae. In *Andrewes' Viruses of Vertebrates*. pp. 120–145. Bailliere Tindall.

Poynter, S.F.B., Jones, H.H. and Slade, J.S. (1975) Virus concentration by means of soluble ultrafilters. In *Methods for Microbiological*

Assay ed. Board, R.G. and Lovelock, D.W. pp. 65–74. London: Academic Press.

Pöyry, T., Stenvik, M. and Hovi, T. (1988) Viruses in sewage waters during and after a poliomyelitis outbreak and subsequent nationwide oral poliovirus vaccination campaign in Finland. *Applied and Environmental Microbiology* **54**, 371–374.

Pringle, C.R. (1998) Virus Taxonomy – San Diego 1998. *Archives of Virology* **143**, 1449–1459.

Prüss, A. (1998) Review of epidemiogical studies on health effects from exposure to recreational water. *International Epidemiology Association* **27**, 1–9.

Puig, M., Jofre, J., Lucena, F., Allard, A., Wadell, G. and Girones, R. (1994) Detection of adenoviruses and enteroviruses in polluted water by nested PCR amplification. *Applied and Environmental Microbiology* **60**, 2963–2970.

Ramia, S. and Sattar, S.A. (1979) Second-step concentration of viruses in drinking and surface waters using polyethylene glycol extraction. *Canadian Journal of Microbiology* **25**, 587.

Rao, V.C., Metcalf, T.G. and Melnick, J.L. (1986) Development of a method for concentration of rotavirus and its application to recovery of rotavirus from estuarine waters. *Applied and Environmental Microbiology* **52**, 484–488.

Raphael, R.A., Sattar, S.A. and Springthorpe, V. (1985) Rotavirus concentration from raw water using positively charged filters. *Journal of Virological Methods* **11**, 131–140.

Reynolds, C.A., Gerba, C.P. and Pepper, I.L. (1996) Detection of infectious enterovirus by an integrated cell culture-PCR procedure. *Applied and Environmental Microbiology* **62**, 1424–1427.

Rigonan, A.S., Mann, L. and Chonmaitree, T. (1998) Use of monoclonal antibodies to identify serotypes of enterovirus isolates. *Journal of Clinical Microbiology* **36**, 1877–1881.

Rotbart, H.A. (1990a) PCR amplification of enteroviruses. In *PCR Protocols: a Guide to Methods and Applications.* pp. 372–377. Academic Press.

Rotbart, H.A. (1990b) Enzymatic RNA amplification of the enteroviruses. *Journal of Clinical Microbiology* **28**, 438–442.

Rotem, Y., Katzenelson, E. and Belfort, G. (1979) Virus concentration by capillary ultrafiltration. *Journal of Environmental Engineering-ASCE* **5**, 401–407.

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K.B. and Erlich, H.A. (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239**, 487–491.

Sarrette, B., Danglot, B. and Vilaginès, R. (1977) A new and simple method for the recuperation of enteroviruses from water. *Water Research* **11**, 355–358.

Schiff, G.M., Stefanovic, G.M., Young, E.C., Sander, D.S., Pennekamp, J.K. and Ward, R.L. (1984) Studies of echovirus 12 in volunteers: determination of minimal infectious dose and the effect of previous infection on infectious dose. *Journal of Infectious Diseases* **150**, 858–866.

Schwartzbrod, L. and Lucena-Gutierrez, F. (1978) Concentration des enterovirus dans les eaux par adsorption sur poudre de verre: proposition d'un appareillage simplifié. *Microbia* **4**, 55–58.

Sellwood, J. (2000) Studies on enteric viruses in water and sewage. PhD Thesis, University of Reading.

Sellwood, J., Dadswell, J.V. and Slade, J.S. (1981) Viruses in sewage as an indicator of their presence in the community. *Journal of Hygiene* **86**, 217–225.

Sellwood, J., Litton, P.A., McDermott, J. and Clewley, J.P. (1995) Studies on wild and vaccine strains of poliovirus isolated from water and sewage. *Water Science and Technology* **31**, 317–321.

Shieh, Y.-S.C., Wait, D., Tai, L. and Sobsey, M.D. (1995) Methods to remove inhibitors in sewage and other faecal wastes for enterovirus detection by the polymerase chain reaction. *Journal of Virological Methods* **54**, 51–66.

Short, C.S. (1988) The Bramham incident, 1980: an outbreak of waterborne infection. *Journal of the Institute of Water and Environmental Management* **2**, 390.

Smith, E.M. and Gerba, C.P. (1982) Development of a method for detection of human rotavirus in water and sewage. *Applied and Environmental Microbiology* **43**, 1440–1450.

Sobsey, M.D. and Glass, J.S. (1980) Poliovirus concentration from tap water with electropositive adsorbent filters. *Applied and Environmental Microbiology* **40**, 201–210.

Sobsey, M.D. and Hickey, A.R. (1985) Effects of humic and fulvic acids on poliovirus concentration from water by microporous filtration. *Applied and Environmental Microbiology* **49**, 259–264.

Sobsey, M.D. and Jones, B.L. (1979) Concentration of poliovirus from tapwater using positively-charged microporous filters. *Applied and Environmental Microbiology* **37**, 588–595.

Soule, H., Genoulaz, O., Gratacap-Cavallier, B., Chevallier, P., Liu, J.-X. and Seigneurin, J.-M. (2000) Ultrafiltration and reverse transcription-polymerase chain reaction: An efficient process for poliovirus, rotavirus and hepatitis A virus detection in water. *Water Research* **34**, 1063–1067.

Standing Committee of Analysts (SCA) (1995) *Methods for the Isolation and Identification of Human Enteric Viruses from Waters and Associated Materials.* London: HMSO.

Stanwell-Smith, R. (1994) Recent trends in the epidemiology of waterborne disease. In *Water and Public Health* ed. Golding, A.M.B. pp. 39–56. London: Smith-Gordon.

Steinmann, J. (1981) Detection of rotavirus in sewage. *Applied and Environmental Microbiology* **41**, 1043–1045.

Straub, T.M., Pepper, I.L. and Gerba, C.P. (1995) Comparison of PCR and cell culture for detection of enteroviruses in sludge-amended field soils and determination of their transport. *Applied and Environmental Microbiology* **61**, 2066–2068.

Tsai, Y.L., Tran, B., Sangermano, L.R. et al. (1994) Detection of poliovirus, hepatitis A virus, and rotavirus from sewage and ocean water by triplex reverse transcriptase PCR. *Applied and Environmental Microbiology* **60**, 2400–2407.

Uhnoo, I., Wadell, G., Svensson, L. and Johansson, M.E. (1984) Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis of infants and young children. *Journal of Clinical Microbiology* **20**, 365–372.

UKWIR (2000) *Methods for the Detection and Enumeration of Pathogens in Biosolids.* Report to the UK Water Industry Research.

United States Environmental Protection Agency (1984) *USEPA/APHA Standard Methods for the Examination of Water and Wastewater.*

Urase, T., Yamamoto, K. and Ohgaki, S. (1993) Evaluation of virus removal in membrane separation processes using coliphage Q beta. In *Development and Water Pollution Control in Asia* ed. Bhamidimarri, R. et al. pp. 9–15. Oxford: Pergamon.

Vilaginès, P., Sarrette, B., Husson, G. and Vilaginès, R. (1993) Glass wool for virus concentration at ambient water pH level. *Water Science and Technology* **27**, 299–306.

Vilaginès, P., Sarrette, B., Vilaginès, R. et al. (1997) Round robin investigation of glass wool method for poliovirus recovery from drinking water and sea water. *Water Science and Technology* **35**, 445–450.

Vinjé, J., Deijl, H., van der Heide, R., Lewis, D., Hedlund, K.-O., Svensson, L. and Koopmans, M.P.G. (2000) Molecular detection and epidemiology of Sapporo-like viruses. *Journal of Clinical Microbiology* **38**, 530–536.

Wallis, C. and Melnick, J.L. (1967a) Concentration of viruses from sewage by adsorption on to Millipore membranes. *Bulletin of the World Health Organisation* **36**, 219–225.

Wallis, C. and Melnick, J.L. (1967b) Concentration of viruses on aluminium and calcium salts. *American Journal of Epidemiology* **85**, 459–468.

Wallis, C. and Melnick, J.L. (1967c) Concentration of viruses on membrane filters. *Journal of Virology* **1**, 472–477.

Wellings, F.M., Lewis, A.L. and Mountain, C.W. (1976) Demonstration of solids-associated virus in wastewater and sludge. *Applied and Environmental Microbiology* **31**, 354–360.

Willcocks, M.M., Carter, M.J., Laidler, F.R. and Madeley, C.R. (1990) Growth and characterisation of human faecal astrovirus in a continuous cell line. *Archives of Virology* **113**, 73–81.

Willcocks, M.M., Carter, M.J. and Madeley, C.R. (1992) Astroviruses. *Reviews in Medical Virology* **2**, 97–106.

Wolfaardt, M., Moe, C.L. and Grabow, W.O.K. (1995) Detection of small round-structured viruses in clinical and environmental samples by polymerase chain reaction. *Water Science and Technology* **31**, 375–382.

Wyn-Jones, A.P. and Herring, A.J. (1991) Growth of clinical isolates of astrovirus in a cell line and preparation of viral RNA. *Water Science and Technology* **24**, 285–290.

Wyn-Jones, A.P., Pallin, R., Sellwood, J. and Tougianidou, D. (1995) Use of the polymerase chain reaction for the detection of enteroviruses in river and marine recreational waters. *Water Science and Technology* **31**, 337–344.

Wyn-Jones, A.P., Pallin, R., Dedoussis, C., Shore, J. and Sellwood, J. (2000) The detection of small round-structured viruses in water and environmental materials. *Journal of Virological Methods* **87**, 99–107.