



Development and Evaluation of a Colorimetric Coliphage Assay Detection System

M. Marian Ijzerman, Charles Hagedorn III, R.B. Reneau, Jr.



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M. Marian Ijzerman
Charles Hagedorn III
R.B. Reneau, Jr.

Department of Crop and Soil Environmental Sciences
Virginia Polytechnic Institute and State University

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Abstract

A colorimetric coliphage assay detection system (CCADS), composed of a liquid colorimetric presence-absence (LCPA) method and a colorimetric agar-based (CAB) method, was developed to overcome the limitations imposed by the *Standard Methods for the Examination of Water and Wastewater* agar-based coliphage method (APHA method). Both CCADS methods are based on the induction of β -galactosidase in *Escherichia coli* and the release of the enzyme through a lytic cell infection. The released enzyme then cleaves a chromogenic substrate, which produces a colored reaction product.

The CCADS was evaluated against the APHA method under laboratory conditions using a common sewage coliphage strain as a model (American Type Culture Collection-13706-B2), and under field conditions using water samples collected from four different sources. During the laboratory evaluation, both the LCPA and CAB methods were found to be superior to the APHA method in coliphage detection because: 1) the LCPA and CAB methods were easier to read and interpret than the APHA method, 2) the LCPA and CAB methods were not subject to false positive results, 3) the LCPA method theoretically detected fewer coliphage particles than the APHA method, and 4) the CAB method detected roughly twice the number of coliphage particles detected with the APHA method. During the field evaluation, the results indicated: 1) the LCPA method was as reliable as either the CAB or APHA method in coliphage detection; 2) the LCPA and CAB methods were easier to read and interpret than the APHA method; 3) neither the LCPA method nor the CAB method were subject to false positive results; 4) the CAB method detected more coliphages than the APHA method under conditions of high fecal pollution, but both methods performed equally well in coliphage detection under conditions of low fecal contamination; and 5) the LCPA and CAB methods were equally as sensitive in coliphage detection as the APHA method.

Finally, the coliphage group proved to be a useful indicator of fecal pollution in nonpotable water supplies exhibiting a high degree of fecal pollution, whereas they were not shown to be useful indicators in potable water supplies exhibiting low levels of fecal contamination. The lack of coliphage detection sensitivity under conditions of low fecal contamination does not appear to be method-limited, but the result of inefficiencies in processing environmental samples using the concentration methods currently available.

Keywords: coliphage detection, fecal contamination, *Escherichia coli*

1 Introduction

1.1 Research Goals

The primary objective of this research was to develop and evaluate a colorimetric coliphage assay detection system (CCADS) that would overcome the limitations imposed by the APHA single-agar-layer plaque method currently in use. The CCADS consists of two parts: a liquid colorimetric presence-absence (LCPA) method, and a colorimetric agar-based (CAB) method. Both methods are based on the phage-induced lysis and release of induced β -galactosidase from *Escherichia coli* strain C. Once the enzyme is released, it hydrolyzes β -galactosides coupled to chromogenic molecules, which results in the release of the chromogen and the formation of a unique color product that indicates the presence of coliphage in the sample.

The secondary objective was to use the CCADS to evaluate the potential of coliphages to serve as suitable indicators of fecal pollution by comparison testing against the currently accepted standard, the coliform bacterial group.

1.2 Enteric Viruses and Waterborne Disease

Many microorganisms and viruses excreted in the feces and urine of man and other warm-blooded animals are pathogens that can cause disease when consumed from water supplies that have been contaminated by fecal material (Berg, 1978). These pathogens include strains of *Salmonella*, *Shigella*, *Leptospira*, enteropathogenic *Escherichia coli*, *Pasturella*, *Vibrio*, *Mycobacterium*, human enteric viruses, protozoa, and intestinal worms (Dutka, 1973; Geldrich, 1972). While water can be directly analyzed for pathogens, such testing entails the isolation and identification of many different microorganisms, is an expensive and time-consuming approach, and is further limited by insensitive methodology that limits detection of some waterborne disease agents (Berg, 1978). The most common approach involves the detection of an indicator organism, and the interpretation that the presence of the indicator implies that a contamination route exists that also could carry pathogens.

In 1914, the United States Public Health Service adopted the coliform bacterial group (total and fecal) as the accepted indicator of fecal pollution in drinking water (Gerba, 1987). Although many of the bacteria included in the coliform group can be of nonfecal origin (plant and/or soil) and, thus, not indicators of fecal pollution, the coliform group remains the accepted standard for potable water (Berg, 1978). The total coliform

group is composed of *E. coli* (the target indicator bacterium), all other fecal coliforms, and many nonfecal lactose-fermenting gram-negative bacilli (Berg, 1978).

Although the occurrence of waterborne bacterial disease outbreaks in the United States is low because of high treatment standards that are directed by routine measurements of coliform indicators, it is apparent that the coliform group does not adequately demonstrate the presence or absence of pathogenic viruses in water (Dutka, 1973; Fattal et al., 1984; Gerba, 1987; Mahdy, 1979; Marzouk et al., 1980; Sproul, 1983). Coliforms are not considered good indicators of enteric viruses because studies have shown that most enteric viruses are more resistant than *E. coli* to a variety of environmental conditions and water treatment processes (including chlorination) primarily due to their highly resistant protein coat, and, as a result, viruses tend to persist longer in aquatic environments than do bacteria (Berg et al., 1978; Committee report, 1981, Hurst, 1989; Melnick, 1984; Scarpino et al., 1972). Rose et al. (1986) reported that enteroviruses or rotaviruses were detected in 24% of the finished water samples that had >0.2 mg free chlorine l^{-1} and that were below acceptable coliform limits. Keswick et al. (1985) reported that 3 of 26 finished water samples that met acceptable total coliform limits were positive for rotaviruses. Gerba et al. (1979) reported that enteroviruses were detected in 43% of the samples obtained from recreational waters considered safe based on the total coliform standard, and in 44% of the water samples considered safe based on the fecal coliform standard. Furthermore, enteroviruses were detected in 35% of the samples obtained from waters that were below acceptable coliform limits for shell-fish harvesting. The detection of enteric viruses in bacteriologically safe waters also was reported by Bosh et al., 1991; Fattal et al., 1983; Goyal et al., 1978; Grabow, 1986; Hejkal et al., 1982; Marzouk et al., 1980; Vaughn and Metcalf, 1975; and Wellings et al., 1975. The frequent detection of enteric viruses in waters found safe based on current bacteriological standards indicates that such indicator standards of total and fecal coliforms do not accurately reflect the presence of enteric viruses in water (Gerba et al., 1979).

More than 120 viruses are known to be excreted in human feces and urine (Melnick, 1984), and most of these are transmitted from person to person by the fecal-oral route (Haas, 1986; Hurst, 1989; Hurst, 1991). Viruses in human waste include the entero-, adeno-, reo-, cytomegalo-, rota-, and Norwalk viruses, and cause a variety of illnesses: paralysis, meningitis, encephalitis, infectious hepatitis, respiratory illnesses, congenital heart anomalies, diarrhea, fever, and skin rashes (Rao and Melnick, 1986). Viral pathogens found in raw sewage are a greater

health problem than bacterial pathogens because the dose to cause illness is much less (Gerba and Haas, 1988; Prins and Lustig, 1988). Ward and Akin (1984) reviewed the literature on minimum infectious dose of human viruses and found that as few as one or two plaque-forming units (PFU) were capable of causing infections. Payment (1984) reported that the 50% infective dose of poliovirus and echovirus 12 was only 20-30 viral particles, and Schiff et al. (1984) reported that the 1% human-infectious dose of echovirus 12 was 17 PFU.

Enteric viruses have been detected in concentrations as high as 10^{10} virus particles per g of feces and as high as 5×10^5 virus particles per l in raw sewage (Melnick et al., 1978; Melnick, 1984). Unlike coliforms, which are excreted in numbers of approximately 1.3×10^7 organisms g^{-1} of feces by all individuals, enteric viruses are not a normal part of the fecal microflora of humans (Berg, 1978; Geldrich, 1978; Mahdy, 1979; Safferman, 1982), and their presence in raw sewage varies depending on such factors as the hygienic and socioeconomic level of the community and the time of year (APHA, 1992; Buras, 1976; Melnick et al., 1978). Enteric viruses are excreted only by infected individuals, mainly infants and young children, and in numbers ranging from 10^2 - 10^8 PFU g^{-1} of feces during the infectious period, which can range from a few days to as long as 6 weeks (Geldreich, 1978; Grabow, 1986).

Enteric viruses have been detected in all types of aquatic environments, including marine, recreational, and potable waters and groundwater. The presence of enteric viruses in marine waters is attributed to direct discharge of domestic sewage or via sewage-contaminated rivers and streams (Gerba, 1988; Goyal et al., 1978). Many viruses find their way into groundwater sources due to overflow from septic tanks and cesspools (Keswick and Gerba, 1980; Yates, 1985), from septic systems in soils with limited renovation capacity (Cogger, 1988; Reneau et al., 1989), from use of slow-rate sewage irrigation of cropland (Goyal et al., 1984), or during groundwater recharge with tertiary-treated wastewater (Vaughn et al., 1978; Vaughn et al., 1981). The incomplete treatment of water containing human waste by sewage treatment plants has resulted in the detection of enteric viruses in water used for recreational activities (Seyfried et al., 1984) and for consumption purposes (Keswick et al., 1985; Melnick, 1984). Water treatment plant deficiencies are responsible for the presence of enteric viruses in potable water (Payment and Armon, 1989; Rose et al., 1986).

Of the viruses present in human excreta, only the hepatitis type A virus (HAV), rotavirus, and Norwalk-like viruses have been implicated as etiological agents responsible for waterborne disease outbreaks (Rao and

Melnick, 1986). Hepatitis type A virus was responsible for the largest waterborne outbreak of hepatitis in New Delhi, India, where 30,000 cases of hepatitis type A were reported (Dennis, 1959). More recently, 21 cases of hepatitis type A were reported in a military camp in Spain (Bosh et al., 1991), and 33 cases in Sweden (Andersson and Stenstrom, 1986). Rotavirus has been identified as the major pathogen responsible for nonbacterial diarrhea in children throughout the world (Rao and Melnick, 1986), and was recently reported as the agent responsible for 3200 cases of such illness in a waterborne outbreak in Sweden (Andersson and Stenstrom, 1986). Norwalk virus and related agents were identified as major causes of waterborne nonbacterial gastroenteritis outbreaks in Pennsylvania, Washington, California, Georgia, Maryland, North Carolina, and New York (Gerba, 1984). Two types of non-A and non-B hepatitis viruses also have been implicated as etiological agents responsible for waterborne outbreaks of hepatitis, and two types of adenoviruses, AD40 and AD41, have been recognized as agents responsible for cases of acute infantile gastroenteritis (Rao and Melnick, 1986). Although the most studied of the enteric viruses, poliovirus, coxsackievirus A and B, and echovirus have not yet been proven to be directly responsible for waterborne disease outbreaks (Rao and Melnick, 1986).

Waterborne disease outbreaks caused by enteric viruses are not easily recognized, and most go unreported because present epidemiological methods are not sensitive enough to detect low-level transmission of these viruses through water (Melnick, 1984; Rao and Melnick, 1986). Concentration methods are only 60% efficient in the recovery of enteric viruses from aquatic systems, and cell culture techniques, used to enumerate the enteric viruses recovered, are capable of detecting only a small portion of the viruses that inhabit the gastrointestinal tract of warm-blooded animals. As a result, only a fraction of the total number of enteric viruses present in aquatic systems are detected (Berg, 1984; Feachem et al., 1983; Melnick, 1984; Payment and Armon, 1989; Sobsey, 1982). Also, many enteric viruses cause a wide spectrum of diseases, and cases of acute illness usually have symptoms too varied to be attributed to a single etiological agent (Melnick, 1984). All of these factors may account for the fact that, of the 26 outbreaks of waterborne disease reported between 1989 and 1990 in the United States, in 16 (54%) of these cases, the etiology of acute gastrointestinal illness could not be determined, although individuals in many of these outbreaks had symptoms suggestive of viral causes (Herwaldt, 1992).

Enteric viruses are obligate parasites that can multiply only inside living cells. Thus, live organisms such as animals, chick embryos, or tissue

culture must be used for their isolation. For enteric viruses, cell cultures of two types commonly are used: primary cell culture using monkey kidney cells (PMK), and continuous cell lines using either buffalo green monkey (BGM) cells or human rhabdomyosarcoma (RD) cells (Rao and Melnick, 1986). Enteric viral detection using cell culture is time consuming, requiring 3 days to 6 weeks, depending on the virus type and the host cells used (Sobsey, 1982). There is no universal host system to detect all enteric viruses, and many enteric viruses, such as calicivirus, coronavirus, hepatitis non-A, non-B, Norwalk and Norwalk-like agents, will not grow and form plaques in cell culture (Dahling, 1991). Further, there is the potential of false negative results using cell lines that experience a decrease in sensitivity to viruses with age (Dahling et al., 1984). Cell culture is expensive, costing as much as \$500 per sample for processing in one cell line, and \$1200 per sample for processing in two cell lines along with testing in suckling mice (Sproul, 1983). Finally, enteric viral testing requires sophisticated facilities and highly-skilled personnel (Grabow, 1986). All of these factors contribute to the lack of methods and regulations that specify routine examination of water sources for enteric viruses (Kott, 1981).

1.3 Coliphages as Indicators of Enteric Viruses

Coliphages are bacteriophages that infect strains of *E. coli* (Snowdon and Cliver, 1989). Coliphages frequently are suggested as enteric viral indicators in contaminated waters because they more closely resemble animal viruses in structure and composition, and persist longer in the aquatic environment than do bacteria (Grabow et al., 1984; Kott et al., 1974; Shah and McCamish, 1972; Stetler, 1984). To be a perfect indicator, Kott (1984) stated that coliphages should meet the following criteria: 1) coliphages should be found wherever human enteric viruses are found; 2) coliphage numbers recovered should be the same or greater than those of enteric virus numbers recovered, 3) coliphages should be as resistant to adverse environmental conditions and water treatment processes as enteric viruses, and 4) coliphage isolation and enumeration should be quicker and cheaper than the isolation and enumeration of enteric viruses.

Coliphages, like enteric viruses, consist of a nucleic acid molecule (genome) surrounded by a protein coat or capsid (IAWPRC Study Group on Water Virology, 1991). Six major families of bacteriophages are currently recognized: Myoviridae (T-even phages), Styloviridae (λ -phage group), and Podoviridae (T7 and related phage groups) are composed of a capsid of cubic symmetry containing double-stranded DNA and a helical tail; Microviridae (X174 group) and Leviviridae are composed of a capsid

of cubic symmetry that contains single-stranded DNA and RNA, respectively; Inoviridae comprise the filamentous bacteriophages that contain single-stranded DNA (IAWPRC Study Group on Water Virology, 1991). The coliphages belonging to the Leviviridae family closely resemble enteroviruses, although they are smaller in size and molecular weight (Hurst, 1991; Snowdon and Cliver, 1989).

Infection of susceptible bacterial hosts by bacteriophages occurs by adsorption to receptors that may be located either on the cell surface (somatic phages) or on extracellular appendages (e.g., pilus-dependent phages) (Snowdon and Cliver, 1989). Generally, with few exceptions, bacteriophages in the families Styloviridae, Myoviridae, Podoviridae, and Microviridae comprise the somatic phages, while bacteriophage in the families Leviviridae and Inoviridae are pilus-dependent (Dimmock and Primrose, 1987; Snowdon and Cliver, 1989). The host range of somatic phages usually is restricted to a few bacterial species due to the highly specific nature of cell surface receptors. The host range of pilus-dependent phages is broader, and includes any bacterial strain that contains and expresses (either extrachromosomal or in the chromosome) a pilus-encoding plasmid (Dimmock and Primrose, 1987; IAWPRC Study Group on Water Virology, 1991; Snowdon and Cliver, 1989).

The detection and enumeration of coliphages from feces, sewage, and sewage-polluted waters is highly dependent on the *E. coli* host strain. Wild-type *E. coli* strains are not considered good hosts for detecting and enumerating coliphages found in sewage and sewage-polluted waters because they have a complete O-antigen that masks most of the phage receptor sites found on the core polysaccharide (Bell, 1976; Havelaar and Hogeboom, 1983). Rough or semi-rough mutant *E. coli* strains (incapable of completing either the O-antigen polymer or the core polysaccharide) are most often used for analysis of coliphages from environmental samples (Havelaar and Hogeboom, 1983). The most commonly used hosts for the isolation and enumeration of somatic coliphages are *E. coli* strain C (Furuse et al., 1983; Havelaar and Hogeboom, 1983; Ignazzitto et al., 1980; Isbister et al., 1983; Stetler, 1984), *E. coli* strain B (Bell, 1976; Kott et al., 1978; Stetler, 1984), and derivatives of *E. coli* strain K-12 (Dhillon et al., 1970). Commonly used plasmid-carrying bacterial host strains used for the detection of pilus-dependent phages are derivatives of *E. coli* strain K-12 (e.g. F+ or Hfr strains; Dhillon et al., 1970; Havelaar and Hogeboom, 1984; Kott et al., 1978), and *E. coli* strain HS (pFamp)R, which has ampicillin on the Famp plasmid that also encodes for pilus production (Debartolomeis and Cabelli, 1991).

Coliphages are ubiquitous in the feces of man and other warm-blooded animals in sewage and sewage-polluted waters where enteric viruses also may be found (Gerba, 1987; Kott, 1981; Scarpino, 1978). Coliphages have been isolated from the feces of man, horses, cows, pigs, sheep, chickens, and birds (Dhillon et al., 1976; Furse et al., 1983; Havelaar et al., 1986; Osawa et al., 1981). Coliphages have been isolated from raw sewage (Dhillon et al., 1970; Snowdon and Cliver, 1989; Ignazzitto et al., 1980; Kennedy et al., 1985), finished waters (Rose et al., 1986) and fecal-contaminated rivers (Borrego et al., 1987; Morinigo et al., 1992; Ratto et al., 1989; Simkova and Cervenka, 1981), streams (Toranzos et al., 1988), lakes (Palmateer et al., 1991), estuaries (O'Keefe and Green, 1989; Vaughn and Metcalf, 1975), sea water (Borrego et al., 1990; Morinigo et al., 1992), and groundwater (El-Abagy et al., 1988; Ratto et al., 1989).

Coliphage numbers have been found to be equal to or greater than those of enteric viruses in sewage, sewage sludge, natural waters, and throughout the various processes in wastewater treatment facilities. Grabow et al. (1984) reported that, in sewage, the ratio of coliphages to enteric viruses ranged from 476:1 to 1524:1, depending on the coliphage host strain used. Williams and Hurst (1988) reported a coliphage to enterovirus ratio of 100:1 to 1000:1 in primary sewage sludge. In creek and stream water samples, Kott et al. (1978) reported that the ratio of coliphages to enteroviruses ranged from 1:1 to 10:1, while, in oxidation pond effluent, the ratio was as high as 1000:1. Stetler (1984) observed the average enterovirus to coliphage ratio along the conventional wastewater treatment process to be 1:3500 in raw water, 1:5700 in water after sedimentation, and 1:14,600 in water after rapid sand filtration.

Coliphages appear to have a greater survival potential than enteric viruses in natural waters, soil systems, and estuarine waters. In a comparison of poliovirus and coliphage survival in natural waters, Kott et al. (1978) reported that poliovirus I strain was not detected after 100 days, while coliphage f2 survived more than 300 days. The authors also reported that, in dry sand, poliovirus was not detected beyond 217 days, while f2 phage was found at levels greater than 1,000 PFU l⁻¹. Vaughn and Metcalf (1975), in a study on survival of coliphage and coxsackievirus in estuarine waters, reported that coxsackievirus was not detected after 42 days, while bacteriophage T4 was still active and detectable after 58 days.

Coliphages appear to be both more resistant to chlorination and ultraviolet (UV) radiation than enteric viruses, but less resistant to

ozonation. Kott et al. (1978) reported that f2 bacteriophage were found in concentrations of 1.7×10^4 PFU l⁻¹, when no enteric viruses were recoverable, in a secondary effluent that had been exposed to 20 mg l⁻¹ of chlorine, stored for 49 days in a holding pond, and followed by a second exposure of 15 mg l⁻¹ chlorine and a 2-h contact period. Shah and McCamish (1972) and Snead et al. (1980) also report a greater resistance of f2 bacteriophage to chlorination than poliovirus. Bosch et al. (1989) reported that coliphage f2 was more resistant to UV irradiation than poliovirus type 1 (LSc2ab) and simian rotavirus SA11 during the water treatment process. However, Evison (1978) reported that, during ozone disinfection, coliphage 185 (intestinal T-phage) and the RNA coliphage M52 were less resistant than two strains of coxsackievirus (B3 and B5) and two strains of poliovirus (strains 2 and 3).

Finally, coliphage detection and enumeration, and use as a water pollution indicator, is an inexpensive technique, does not require skilled operators, and yields results in less than 24 hours, thus making it a desirable method for determining the possible presence of enteric viruses in water (Gerba, 1987; Kott, 1984; Shah and McCamish, 1972).

1.4 Methods Available for Coliphage Detection

A plaque count technique using an agar medium is the only method currently available for the detection and enumeration of coliphages from environmental samples (APHA, 1992). Simply, a culture of *E. coli* in logarithmic phase is added to molten agar (50°C) along with a water sample that may or may not contain coliphages (Adams, 1959). There are many variations of this basic agar assay procedure that involve both single or multiple agar layers, the addition of cations, a variety of bacterial hosts, and special media formulations that are all designed to enhance phage infection of the host and to improve plaque visibility (Grabow and Coubrough, 1986; Havelaar and Hogeboom, 1983; Havelaar and Hogeboom, 1984; Ibsister et al., 1983; Kennedy et al., 1985; Kott, 1966; Kott et al., 1978; Wentsel et al., 1982). However, only one method is currently proposed by the American Public Health Association (APHA, 1992) for the detection and enumeration of coliphages from groundwater and surface waters. The APHA recommends the use of *E. coli* strain C (ATCC 13706) as the host because it lacks restriction and modification systems and is sensitive to a broad spectrum of sewage phages (Havelaar and Hogeboom, 1983), and the addition of 2,3,5-triphenyl tetrazolium chloride (TPTZ) that is added to the agar to aid in counting plaques. As non-infected *E. coli* cells grow, the TPTZ is reduced and lends a pale pink color to the agar, while plaques (infected and killed *E. coli*) remain colorless.

There are three main limitations associated with the use of the APHA single-agar-layer plaque method for the detection and enumeration of coliphages from groundwater and surface waters. The first is the difficulty in accurately determining a coliphage count due to the lack of color contrast on an agar plate—colorless plaques are surrounded by a pale pink background and are difficult to observe by the untrained eye. The second is the inability to detect low numbers of coliphages with the APHA method (Kott, 1966). The APHA recommends their method only when there are greater than 5 PFU/100 ml of sample. The third problem, when used with environmental samples, is the appearance of plaque-like cytotoxic areas in the agar, caused by nonviral toxic material, that can be interpreted as a false positive result (Sobsey, 1982).

2 Materials and Methods

2.1 An Improved Agar-Based Method of Coliphage Detection and Enumeration

2.1.1 Overview

An improved method for coliphage detection based on the induction of β -galactosidase in *E. coli* is described. Upon infection by coliphages, the cells are lysed and a stable indolyl product that is dark blue becomes visible within each plaque. The improved method is compared to the proposed coliphage detection procedure described in *Standard Methods for the Examination of Water and Wastewater*.

Our improved method is based on the inducible lactose operon system found in *E. coli*. β -galactosidase, encoded by the *lacZ* gene of the *lac* operon, is an essential enzyme in the metabolism of lactose. β -galactosidase can be induced by a number of chemically synthesized analogs that mimic lactose. The most powerful inducer is isopropyl- β -D-thiogalactoside (IPTG). In the absence of an inducer, β -galactosidase levels have been found to be fewer than 10 molecules per cell, while, in the presence of IPTG, β -galactosidase levels are increased 1000 fold (Beckwith, 1987). To conduct our procedure, a water sample containing a predetermined level of phage is added to molten agar along with IPTG and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal), which is a substrate for β -galactosidase, along with a predetermined quantity of host. Upon infection by coliphages in the water sample during the incubation period, the cells are lysed, and a stable indolyl product that is dark blue becomes visible within each plaque. Individual viral plaques then are easily detected and enumerated by the distinct blue circle that develops within each plaque.

2.1.2 Laboratory Studies

Two methods were compared in this study. The first is the proposed procedure for coliphage detection outlined in the 17th edition of *Standard Methods for the Examination of Water and Wastewater* (APHA, 1989), which, for simplicity, will be referred to as the TPTZ method. This procedure was altered as follows: the assay volume was tripled for ease of handling and divided between two 100x15-mm petri plates, and the average plaque count was recorded.

The second method, referred to as the IPTG/X-gal method, involves the use of *E. coli* strain C (ATCC 13706) inoculated from a stock Luria-

Bertani agar (LB) slant into 10 ml sterile LB broth plus 10% (v/v) glycerol, shaken at 240 osc min^{-1} and incubated at 37°C overnight. One-half ml of this culture was transferred to 25 ml sterile LB broth plus 10% (v/v) glycerol incubated at 37°C and 240 osc min^{-1} until an O.D. (Milton Roy Spectronic 20D) of 0.5 at 520 nm was obtained (equivalent to approximately 1×10^9 *E. coli* C cell ml^{-1}). The culture was maintained at -20°C in a nonfrost freezer no longer than 30 days to reduce the loss of host culture viability.

To conduct the IPTG/X-gal assay, the frozen culture of *E. coli* strain C was thawed in a 44°C water bath. One-half ml of host was added to 18 ml of modified LB agar at 50°C (to the ingredients of LB broth add ammonium nitrate NH_4NO_3 , 1.60 g l^{-1} ; strontium nitrate, $\text{Sr}(\text{NO}_3)_2$, 0.21 g/l ; and agar, 15 g/l). Fifteen ml of water sample containing bacteriophage (ATCC 13706-B2) (a common sewage coliphage) was added along with $66 \mu\text{l}$ IPTG (200 mg ml^{-1}) and $360 \mu\text{l}$ X-gal (10 mg ml^{-1} dissolved in dimethylformamide; Gold Biotechnology, Inc. St. Louis, Mo). The solution was swirled and evenly distributed into two $100 \times 15\text{-mm}$ petri dishes and, after the agar hardened, the plates were inverted and incubated at 35°C . Pale blue plaques begin to appear after 4 hours, and the color intensifies as the plaques develop after 7-8 hours. The blue color will further intensify if the plates are stored for a few hours at 4°C . The average plaque count was recorded.

Proof that the blue circles were actually coliphage plaques was obtained by aseptically removing circles from the agar and using these as samples to perform a single-agar-layer plaque assay. Results demonstrated that each blue circle contained thousands of coliphage particles (data not shown). Final confirmation was obtained by using a Zeiss 10CA transmission electron microscope to observe thin sections of the blue circles removed from the agar plates ($120\text{-}150 \times 10^3$ magnification). The electron micrographs demonstrated visible phage particles within the material prepared from the blue circles (micrographs not shown).

2.2 A Liquid Colorimetric Presence-Absence Coliphage Detection Method

2.2.1 Overview

A liquid, colorimetric presence-absence coliphage detection method based on the induction of β -galactosidase by *E. coli* is described. The release of β -galactosidase in the medium due to lytic cell infections by coliphages permits the hydrolysis of a yellow chromogenic substrate that develops into a distinct red coliphage-positive sample, while a coliphage-negative

sample remains yellow. This method has proven to be rapid, simpler to perform than an agar medium assay, easy to read and interpret, inexpensive, and highly sensitive.

The liquid, colorimetric presence-absence coliphage detection method was developed to overcome the limitations imposed by the APHA method. The first of its kind to be developed in the field of public health virology, the liquid method provides an alternative to traditional plaque counting techniques. We propose that the liquid method be used alone or in combination with an agar-based coliphage detection method, such as either the APHA method or the improved agar-based method developed by Ijzerman and Hagedorn (1992). The liquid method can provide a quick and easy way to evaluate large numbers of water samples and provide a presence-absence decision for those samples that may need to be further evaluated with an agar-based method to estimate coliphage density.

The liquid, colorimetric coliphage detection assay is based on the phage-induced lysis and release of induced β -galactosidase from *E. coli* strain C. β -galactosidase, encoded by the *lacZ* gene of the lactose operon, is an essential enzyme in the metabolism of lactose, and can be induced by a number of chemically synthesized analogs that mimic lactose, the most powerful inducer being isopropyl- β -D-thiogalactoside (IPTG). In the absence of an inducer, β -galactosidase levels have been detected to be fewer than 10 molecules per cell, while in the presence of IPTG, β -galactosidase levels are increased 1000-fold (Beckwith, 1987). Due to a lytic infection by coliphages present in the water sample, β -galactosidase is released from the cell. The released β -galactosidase hydrolyzes β -galactosides coupled to chromogen molecules that results in the release of the chromogen and the formation of a unique color. The chromogenic substrate, chlorophenol red β -D-galactopyranoside (CPRG), was used in the liquid assay because CPRG is a water-soluble substrate for the detection of β -galactosidase; CPRG is 10-times more sensitive than 2-nitrophenyl- β -D-galactopyranoside (ONPG) in the kinetic assay of β -galactosidase; and a distinct chlorophenol red color appears when hydrolyzed by the enzyme (Eustice et al., 1991).

2.2.2 Laboratory Studies

The liquid, colorimetric coliphage detection method was developed with *E. coli* strain C (ATCC-13706), an F⁻ (recipient) that lacks restriction and modification systems and is sensitive to a broad spectrum of sewage phages (Havelaar and Hogeboom, 1983). The common sewage

coliphage (ATCC-13706-B2) was used to determine the sensitivity of the method.

E. coli strain C was grown (24 h at 37°C) and stored no longer than 30 days at 4°C on Luria-Bertani (LB) agar (Sambrook et al., 1989). A single colony was aseptically transferred into 10 ml LB broth supplemented with 5 mmol $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Fisher Scientific, NJ) after autoclaving, and 1.25 mmol $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Sigma Chemical Co., St. Louis, MO) prior to autoclaving. The culture was incubated on a rotary shaker (Lab-line Orbit Environ-Shaker) at 37°C and 200 revolutions per minute (rpm) for 12 hours.

One hundred and twenty-five μl of the 12-h *E. coli* strain C culture (O.D. 1.80 at a wavelength of 520 nm; Milton Roy Spectronic 20D) was aseptically transferred into 25 ml (20-25°C) LB broth supplemented with 5 mmol $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ after autoclaving and 1.25 mmol $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ prior to autoclaving. The culture was incubated at 37°C and 200 rpm for 1 hour. After 1 hour, the culture was aseptically inoculated with 25 μl of IPTG (Boehringer Mannheim Biochemicals, Indianapolis, IN; 0.1 M stock) and incubated at 37°C and 200 rpm for an additional 30 min. At the end of 30 min, a concentrated water sample (SM buffer (Sambrook et al., 1989), seeded with coliphage) no greater than 1.25 ml, was added to the culture. Sample concentration was first performed using the CH2 Ultrafiltration System equipped with a 100,000 molecular weight (MW) cut-off spiral-wound membrane cartridge (S1Y100; Amicon, Inc., MA) and second using the Centriprep-100 concentrator (Amicon, Inc., MA) to further concentrate the sample to 1.25 ml. The culture was swirled and maintained at room temperature without shaking for 10-15 min, and then returned to the 37°C shaker for an additional 105 min at 200 rpm.

The culture was added to a sterile 50-ml centrifuge tube (Perfector Scientific, Atascadero, CA) and centrifuged (Beckman Model TJ-6) at 6°C, 5000 rpm, for 15 min. The supernatant was vacuum-filtered through a 0.2 μm low protein-binding filter (Supor-200, Gelman Sciences, Ann Arbor, MI) into a sterile 20x150-mm test tube. To 9 ml of Z buffer (Miller, 1977), 1 ml of filtrate was added plus 100 μl of CPRG (Boehringer Mannheim Biochemicals, Indianapolis, IN; 5 mmol stock) and incubated at 37°C for 30 min.

2.3 Field Evaluation of a Colorimetric Coliphage Assay Detection System

2.3.1 Overview

A CCADS, composed of a LCPA method and a CAB method, was developed to overcome the limitation imposed by the *Standard Methods for the Examination of Water and Wastewater* agar-based coliphage method. Both CCADS methods are based on the induction of β -galactosidase in *E. coli* and the release of the enzyme through a lytic cell infection. The released enzyme then cleaves a chromogenic substrate, which produces a colored reaction product. The LCPA and CAB methods were tested against the APHA method by comparing a total of 90 split water samples from four different sources. A total of 52 samples were positive by the LCPA method, 52 by the CAB method, and 53 by the APHA method. Results indicated that: 1) the LCPA method was not subject to false positive or false negative results, 2) the LCPA method was as reliable as either of the two agar-based methods in coliphage detection, 3) the LCPA method was easier to perform, and both the LCPA and CAB methods were easier to read and interpret than the APHA method, 4) the CAB method detected more coliphages than the APHA method in two of the four water sources, 5) the CAB method was not subject to false positive results, 6) the LCPA and CAB methods were as sensitive in coliphage detection as the APHA method, and 7) the CAB and LCPA methods were well suited to handle the range of water conditions present in the samples during the evaluation.

2.3.2 Laboratory and Field Studies

2.3.2.1 Samples. Ninety water samples were collected from four sources within and around Blacksburg, Virginia, over a 2-month period (February-March 1993). The four sample sources were chosen from a variety of potable and nonpotable sources to provide a wide range of water testing conditions. Sample sources included tap water obtained from 1) a private residence located at the end of the public water distribution line in the town of Blacksburg, 2) spring water obtained from a private residence in Giles County, Virginia, 3) creek water obtained below the dairy pastures at Virginia Tech, and 4) a wastewater sample obtained from the sedimentation basin at the Blacksburg-Virginia Tech Sanitation Authority Stroubles Creek Wastewater Treatment Plant. Samples were collected in presterilized polypropylene or glass containers, transported to the laboratory, and analyzed within 2 hours of collection. Sodium thiosulfate was added to a final concentration of 100 mg l^{-1} to dechlorinate the tap water samples.

2.3.2.2 Water Sample Analyses. Selected water characteristics were determined for the four water sources. Potassium (K), calcium (Ca), magnesium (Mg), manganese (Mn), iron (Fe), sodium (Na), zinc (Zn), and copper (Cu) were determined using an inductively coupled plasma atomic emission spectrometer (ICP-AES) system equipped with a simultaneous spectrometer (Jarrell-Ash ICAP 9000). Cadmium (Cd), chromium (Cr), lead (Pb), and nickel (Ni) were determined using the ICP-AES system equipped with a sequential (scanning) spectrometer (Jarrell-Ash Atomscan 2400). pH was determined with an Orion Research digital pH/mv meter 611, and the 5-day biological oxygen demand (BOD₅) was determined for each water source as outlined by *Standard Methods for the Examination of Water and Wastewater*.

2.3.2.3 Coliphage Methods. Three coliphage detection methods were used in this study. The APHA method for coliphage detection, was altered in the following way: the assay volume was tripled for ease of handling and divided between two 100 x 15-mm petri plates and the average plaque count recorded. The CAB method (Ijzerman and Hagedorn, 1992) was performed as previously described, except that 1.0 ml of host *E. coli* strain C was used instead of the 0.5 ml originally stipulated, and the incubation time shortened to 4-6 hours to coincide with the incubation time of the APHA method. The LCPA method was performed as previously described (Ijzerman et al., 1993).

2.3.2.4 Experimental Design. A sufficient volume of water was collected from each source to perform a simultaneous split sample analyses by the three coliphage methods. All tests were performed using triplicate unconcentrated 15-ml water samples except in the case of tap and spring water, where low coliphage levels were expected and sample concentration was necessary. For the tap and spring water sources, 1.5-l and 3.5-l samples, respectively, were concentrated to approximately 200 ml and then split equally among the three coliphage detection methods. Sample concentration was performed using an ultrafiltration system equipped with a 100,000 molecular weight (MW) cut-off spiral-wound membrane cartridge (Amicon, Inc., Model CH2 ultrafiltration system equipped with an S1Y100 spiral-wound cartridge). The spiral-wound membrane is a non-ionic, slightly hydrophilic, cellulose-based YM membrane with exceptionally low nonspecific protein binding properties that provides maximum solute recovery (Amicon, Inc., personal communication). For the CAB and APHA methods, the mean coliphage number from the three replicate analysis was recorded for each sample. For the LCPA method, a positive in any of the three replicates was recorded as an overall positive result for that sample.

2.3.2.5 Statistical Analyses. The comparison of the results obtained by the three detection methods was complicated due to the fact that the agar-based methods yielded quantitative results and the liquid method yielded qualitative results. As suggested by Lewis and Mak (1989), this difficulty was overcome by treating the agar-based data as presence-absence data (i.e., samples that had mean coliphage values $>0/15$ ml were considered as coliphage present, and samples that had mean coliphage numbers equal to $0/15$ ml were considered as coliphage absent).

The data was analyzed using several nonparametric statistical tests. The McNemar chi-square test was used to compare the overall detection rate of positive samples obtained from the three methods (Conover, 1971). To study patterns of agreement between methods, the index of agreement was computed; i.e., the proportion of all samples for which there was agreement (both presence or both absence) along with the kappa statistic which is a chance-corrected adjustment to the index of agreement. Kappa values range from -1 to $+1$, where $+1$ indicates perfect agreement, 0 indicates no agreement, and negative values suggest less agreement than expected due to chance. The statistical significance of kappa was determined by computing the z statistic (Fleiss, 1981).

The Wilcoxon signed rank test (Zar, 1984) was used to determine whether differences existed in the number of coliphages detected in each source by the CAB and APHA methods. This test uses the sign and magnitude of the rank of the differences between the pairs of sample variances to determine the statistical significance. To determine whether there were differences in the number of positive tests in the three replicate analysis among all three methods, method precision was examined by comparing the sample variability for each method and sample using the Wilcoxon signed rank test (Zar, 1984). Both analyses using the Wilcoxon signed rank test were done using Number Cruncher Statistical Software (Kaysville, UT). All statistical tests were performed at the $\alpha = 0.05$ level of significance.

2.4 Comparison of Coliphage and Coliforms as Indicators of Fecal Pollution in Potable and Nonpotable Water Sources

2.4.1 Overview

Ninety water samples were simultaneously analyzed for coliphage and coliforms (total and fecal) from two potable and two nonpotable sources that exhibited low and high fecal pollution, respectively. Under condi-

tions of low fecal pollution, there was no correlation between levels of coliphage and coliforms; whereas, under high levels of fecal pollution, there was a significant correlation among levels of coliphage and coliforms. In the potable water samples, 16 were positive for total coliforms and negative for coliphage, 13 samples were positive for fecal coliforms and negative for coliphage, and 2 samples were positive for coliphage and negative for total and fecal coliforms. In the nonpotable sources, all samples were positive for coliphage and coliforms. It appears that coliphages may be a useful indicator of fecal contamination in nonpotable water supplies that exhibit a high level of fecal pollution, whereas their usefulness as indicators in potable water supplies, which exhibit low fecal contamination, may be hampered by inefficiencies in processing environmental samples using the concentration methods currently available.

The objective of this study was to determine whether coliphages could be used as reliable indicators of fecal pollution when compared to the classic indicator of fecal pollution, the fecal coliform group, under conditions of low and high contamination found in potable and nonpotable water sources.

3 Results and Discussion

3.1 An Improved Agar-Based Method of Coliphage Detection and Enumeration

The IPTG/X-gal method was superior in coliphage detection as compared to the TPTZ method (table 1). The mean separation between treatments was determined by Duncan's multiple range test when the overall F-test was significant at $P < 0.01$. The IPTG/X-gal method consistently detected twice as many plaques as compared to the TPTZ method proposed by the APHA (1989 and 1992).

Higher counts detected by the IPTG/X-gal method could be partially explained by the ease of detection of the IPTG/X-gal plaques. A dark blue circle formed within the plaque, creating a distinguishable mark that aided in counting. The blue circle is permanent and allows plaque detection even if overgrowth of the plaques should occur. This is a major advantage over the TPTZ method that produces clear plaques that are difficult to detect and count. If overgrowth of the TPTZ plates should occur, accurate plaque counts cannot be obtained.

Higher counts detected by the IPTG/X-gal method also could be explained by the medium used. Laboratory tests showed that, when the TPTZ method proposed by the APHA was followed using modified LB agar instead of the recommended medium, modified TSB agar, higher plaque counts were always obtained (data not shown). Thus, the combination of modified LB agar plus the addition of IPTG and X-gal to the medium produced coliphage counts that were superior to those detected by the TPTZ method proposed by the APHA (1989 and 1992).

The only disadvantage afforded by this procedure was the cost of one of the ingredients, X-gal. When this study was initiated, the cost of X-gal was \$192 gm^{-1} . At the present time, the price has decreased to an acceptable level of \$80 gm^{-1} , which would translate to approximately \$0.28 sample^{-1} . This is an acceptable cost when considering the ease of plaque detection that this method offers.

This study indicated that approximately 50% of the coliphages in the prepared samples would have escaped detection had the proposed APHA coliphage detection procedure been used. Therefore, it is recommended that the APHA (1989 and 1992) procedure be further compared to the IPTG/X-gal method using environmental samples.

3.2 A Liquid Colorimetric Presence-Absence Coliphage Detection Method

A positive coliphage test can be observed by the immediate development of red, in an initially yellow solution, that intensifies over a 15-min period and develops into a uniform purple-red color by 30 min. A negative coliphage test is observed by a uniform yellow throughout the test tube at the end of the 30-min incubation period. Sometimes, due to carry-over of small amounts of enzyme in the filtration apparatus, a slight red band of color will appear at the meniscus of the liquid in an otherwise yellow sample. Such a sample should still be regarded as a negative result, since only a uniform red throughout the tube is positive.

The liquid, colorimetric presence-absence coliphage detection method can be completed and the results obtained in 4.5 hours. The liquid method is simpler to perform than an agar medium assay primarily due to the absence of agar and the difficulties associated with rapid hardening. The results obtained by the liquid method are easy to read and interpret, with a uniform red indicating a positive coliphage test and a uniform yellow indicating a negative coliphage test. The final results in this method are obtained without the need of a spectrophotometer. The estimated cost to perform the liquid method, based on the price of the two main ingredients, IPTG and CPRG, is \$0.07 per 1.25 ml concentrated water sample.

Theoretically, the liquid, colorimetric presence-absence coliphage detection method can detect as few as 2 PFU l⁻¹ (table 2), which is an improvement over the APHA agar-based method, which can only detect as few as 5 PFU/100 ml. At no time throughout the entire study did the liquid method produce a false positive result, and the incidence of false negative results became apparent only at extremely low coliphage titers (table 2). To reduce the rate of false negative results under conditions of low coliphage titers (0-10 PFU), it is advised that 2 ml of filtrate be added to 8 ml of Z buffer, and the incubation time extended for up to 2 hours. It is also important to note that, due to the high sensitivity of the assay, all materials used in this method, including hands, must be thoroughly cleaned of any residual coliphage particles that could possibly lead to a false positive test. In all experiments, positive and negative controls should be used to ensure proper functioning of the liquid colorimetric detection method. Although this method was developed using *E. coli* strain C as the host, this procedure is perfectly suitable for use with other coliphage host strains, including donor *E. coli* strains used for the detection of F⁺ specific bacteriophage strains.

The liquid, colorimetric presence-absence coliphage detection method can be proportioned to accommodate a wide range of water sample volumes by keeping all chemicals in ratio to the 1.25-ml concentrated water sample volume described here. For example, if the final volume of water sample after concentration is approximately 5 ml but no greater, inoculate 100 ml LB broth with 500 μ l of the 12-h *E. coli* strain C culture, and add 100 μ l IPTG. Keep the CPRG and Z buffer volumes and all incubation times the same as described. If water concentration is not feasible, unconcentrated water samples can be analyzed by proportioning the assay to fit a larger sample volume, or by using multiple replication of a smaller volume, thereby ensuring proper representation of the entire volume of sample collected.

The liquid coliphage detection method is not intended to replace an agar medium coliphage detection assay. Its purpose is to test for the presence or absence of coliphages in surface water and groundwater samples much in the same way as the Autoanalysis Colilert Test (EnviroNetics, Inc., CT; Edberg et al., 1988) is used for detecting the presence or absence of coliform bacteria in water samples. A plaque assay using an agar medium is still required to quantify coliphage numbers.

Our liquid coliphage detection method would allow public and private water quality laboratories to more thoroughly and accurately test for the presence of enteric viruses through the use of a coliphage indicator. The increased detection sensitivity afforded by the liquid assay would reduce the potential of human infection by enteric viruses in contaminated water, thereby increasing the overall safety of potable water supplies.

3.3 Field Evaluation of a Colorimetric Coliphage Assay Detection System

3.3.1 Analyses of Selected Water Quality Parameters

The four water sources varied in the water quality parameters measured, thus creating different conditions for field evaluation of the APHA, LCPA, and CAB methods (table 3). In all four water sources, the levels of Cd, Cr, Pb, and Ni were below detectable limits.

3.3.2 Precision Among the Three Coliphage Methods

No differences in method precision were detected between the LCPA method and the APHA method ($P = 1.00$), the CAB method and the APHA method ($P = 0.69$), or the LCPA method and the CAB method

($P = 0.72$). In the tap and spring water sources, all three coliphage methods were positive in 1 of the 3 replicates, except for 1 positive sample detected by the LCPA method that was positive in 2 of the 3 replicates. In the creek and wastewater sources, all three coliphage methods were positive in all three replicates (data not shown).

3.3.3 APHA Method versus the LCPA and CAB Methods

Of the 90 split water samples analyzed, 53 (72% of all positives, 59% of the total number of samples) were coliphage positive by the APHA (1989 and 1992) method and 52 (70% of all positives, and 58% of the total number of samples) were coliphage positive by the LCPA method (table 4) and the CAB method (table 5). There was no difference in the number of positive samples detected by either method in each water source or the entire study when analyzed by McNemar's chi-square test (tables 4 and 5).

To evaluate the rate of agreement between the APHA and LCPA methods, the data was further divided into those samples that were either coliphage negative or coliphage positive in both methods. Both methods were simultaneously coliphage positive in 50 samples, and both were simultaneously coliphage negative in 35 samples, thus agreeing 94% of the time. The kappa statistic indicated an excellent level of agreement between the two methods ($\text{kappa} = 0.88$), and the large value of the z statistic ($z = 8.8$) indicated that the observed agreement reflects reliability beyond chance agreement (Conover, 1971). The 6% disagreement rate between the two coliphage methods always occurred under conditions where the coliphage density was low (mean values ranged from 0-3 PFU l^{-1} and 0-1 PFU l^{-1} in the tap and spring water sources, respectively; table 4).

The APHA and CAB methods were simultaneously coliphage positive in 50 samples, and both were simultaneously coliphage negative in 35 samples, thus agreeing 94% of the time. The kappa statistic ($\text{kappa} = 0.88$) indicated that excellent agreement existed between the two methods. The large statistical value computed for the kappa statistic ($z = 8.8$) indicated that there was more agreement than chance alone could explain. The 6% disagreement rate between the two coliphage methods always occurred when the coliphage density was low (mean values ranged from 0-3 PFU l^{-1} and 0-1 PFU l^{-1} in the tap and spring water samples, respectively; table 5).

The number of coliphages detected by the CAB method compared to the APHA method was only statistically different ($P < 0.001$) in two of the

four sources (creek and wastewater samples, table 6). The larger median coliphage values detected using the CAB method were under conditions of high coliphage densities, whereas both methods were equal in coliphage detection under lower coliphage densities (tap and spring water, table 6).

3.3.4 CAB Method versus the LCPA Method

Of the 90 split samples analyzed, 52 were coliphage positive by the CAB method and 52 were positive by the LCPA method. There was no difference in the number of positive samples detected by either method in each water source or the entire study when analyzed by McNemar's chi-square test (table 7). Both methods were simultaneously positive in 50 samples, and both were simultaneously negative in 36 samples, thus agreeing 96% of the time. The kappa statistic ($\kappa = 0.92$) indicated an excellent level of agreement between the two methods, and the large value computed for the z statistic ($z = 9.2$) indicated that there was more agreement than chance alone could explain. The 4% disagreement rate between the two methods always occurred under conditions of low coliphage densities (mean values ranged from 0-3 PFU l^{-1} in the tap water source; table 7).

3.3.5 Summary and Interpretation of Results

At no time throughout this study, using water sources that varied in their chemical and physical characteristics, did the LCPA method produce a false positive coliphage result (all positive samples were confirmed). The 3% false negative coliphage rate obtained by the LCPA method during the evaluation of the tap and spring water samples (based on the unproven assumption that APHA method gave the correct conclusion of coliphage presence in a water source) was obtained under conditions of low coliphage numbers (mean values ranged from 0-3 PFU l^{-1} and 0-1 PFU l^{-1} in the tap and spring water samples, respectively). Interestingly, the 6% disagreement rate found between the CAB method and the APHA method also occurred with the tap and spring water samples, thus indicating that the error in coliphage detection by the LCPA method was not due to problems with the method, but rather to the high degree of error associated with splitting water samples that contained low numbers of coliphages. Under such conditions, coliphage testing becomes a hit-or-miss situation by all three methods. This conclusion is further supported by the fact that all 3 coliphage methods were coliphage positive at least once while evaluating the 40 tap and spring water samples, yet all 3 methods never produced positive results from the same sample.

The excellent agreement rate found between the LCPA method and the two agar-based methods (tables 4 and 7) indicates that the LCPA method is as reliable as either of the two agar-based methods in coliphage detection. This means that, if the LCPA method is used alone, it would reliably (or as reliably as an agar-based method) indicate the presence or absence of coliphages in a given sample. The development of the LCPA method should be an advantage to the water quality industry, which currently has at its disposal only one technique to detect coliphages from groundwater and surface waters (APHA, 1992). To date, investigators have had to perform the more cumbersome agar-based plaque technique even if coliphage presence or absence were all that was desired in a particular water sample. The LCPA method is not intended to replace the traditional plaque count technique, but rather to screen samples and test for the presence or absence of coliphages in the same fashion as the Autoanalysis Colilert Test (EnviroNetics Inc., MA) provides in screening water samples for the presence or absence of the coliform bacteria group (Lewis and Mak, 1989). A plaque technique using an agar medium is still required to quantify coliphage numbers.

Under the laboratory evaluation of the LCPA method, it was found that the liquid method could be completed and the results obtained in 4.5 hours (Ijzerman et al., 1993). We did not detect any false positive results using the LCPA method, and the rate of false negative results became apparent only at extremely low viral concentrations (2 PFU per concentrated sample). The LCPA method was simpler to perform than an agar-based method due to the absence of agar and the difficulties associated with rapid hardening. The results obtained by the liquid method are easy to read and interpret, with a uniform red indicating a positive coliphage test and a uniform yellow indicating a negative coliphage test, and the final results are obtained without the need of a spectrophotometer. Theoretically, the LCPA method can detect 2 PFU l^{-1} , although, in this study, the LCPA method detected 3 PFU l^{-1} , thus being a highly sensitive method for coliphage detection. The cost to perform the LCPA method based on the price of the two main ingredients, isopropyl- β -D-thiogalactoside (IPTG) and chlorophenyl red β -D-galactopyranoside (CPRG), was estimated at \$0.07 per 1.25 ml concentrated water sample (Ijzerman et al., 1993). Thus, the LCPA method can provide a quick, easy, highly sensitive, cost effective, and highly reliable method for coliphage detection.

Although the CAB method was not found to be more sensitive than the APHA method in the number of positive coliphage samples detected throughout this study, the CAB method did detect more coliphage particles under conditions of high coliphage densities (creek and

wastewater samples) than the APHA method (table 6). This was also found in the laboratory portion of the evaluation of the two agar-based methods, where the CAB method detected approximately twice as many coliphage particles as the APHA method (6). The first advantage in the use of the CAB method is in the ease of plaque detection. A dark blue circle forms within each plaque creating a distinguishable mark that aids in counting, unlike the APHA method where plaques are seen as clearings surrounded by a pale pink background. The second advantage is the absence of false positive results due to the appearance of plaque-like cytotoxic areas in the agar caused by nonviral toxic material with the CAB method. Our laboratory studies showed that the blue circle that formed within each plaque contained thousands of coliphage particles. The only disadvantage afforded by the CAB method was the cost of one of the main ingredients, 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-gal). At the present time the price is \$80 g⁻¹, which would translate to approximately \$0.28 sample⁻¹ (Ijzerman and Hagedorn, 1992). This is an acceptable cost when considering the ease of plaque detection that the method offers.

The LCPA, CAB, and APHA methods were evaluated under four different water sample conditions. The results showed the usefulness of the CAB and LCPA methods as coliphage detection methods in the water quality industry. Both methods possess qualities that will enhance coliphage detection in groundwater and surface waters that are presently lacking in the APHA method. Although the LCPA method was not found to detect fewer coliphage particles than the APHA method in this study (spring water samples), the LCPA method still proved to be an extremely sensitive coliphage detection method. The LCPA method was easier to perform, read, and interpret than the APHA method and, most importantly, it has proven itself to be a highly reliable presence-absence method under field conditions. On the other hand, the CAB method, although as cumbersome to perform as the APHA method, was more sensitive in determining the coliphage density at high coliphage numbers than the APHA method (table 6), and there was no possibility of false positive results using the CAB method under environmental testing conditions.

The use of the LCPA method and the CAB method, either combined or as independent coliphage detection methods, will better provide the means to more effectively test for the presence of enteric viruses in water through the use of coliphage as indicators of fecal pollution.

3.4 Comparison of Coliphages and Coliforms as Indicators of Fecal Pollution in Potable and Nonpotable Water Sources

3.4.1 Water Sample Analyses

The water characteristics determined for each water source are listed in table 3. For all water sources, Cd, Cr, Pb, and Ni were below the detection limits of the equipment used.

3.4.2 Potable Water Supplies

Low levels of fecal contamination were detected in the tap and spring water samples, where the fecal coliform levels were <28.3 colony forming units (CFU)/100 ml, (table 8). Under these conditions, there was a low and nonsignificant correlation between levels of coliforms (total and fecal) and coliphage using the APHA and CAB methods (table 9). Of the 40 potable samples analyzed, 16 were positive for total coliforms and negative for all three coliphage methods, 13 were positive for fecal coliforms and negative for all three coliphage methods, and 2 were positive for coliphage (all three methods) and negative for total and fecal coliforms (tables 10, 11, and 12).

3.4.3 Nonpotable Water Supplies

High levels of fecal contamination were detected in the creek and wastewater samples, where the fecal coliform levels were >350 and >300 CFU/100 ml, respectively (table 8). Under these conditions, there was a highly significant level of correlation between the levels of coliforms (total and fecal) and coliphage using the APHA and CAB methods (table 9). All 50 of the nonpotable samples analyzed were positive for total coliforms, fecal coliforms, and coliphage by all three methods (tables 10, 11, and 12).

3.4.4 Summary and Interpretation of Results

In a review of the use of bacteriophage as indicators of fecal contamination of aquatic systems, Gerba (1987) reported that, in some cases, the levels of coliphage correlated with levels of coliform bacteria; whereas, in other cases, no correlation existed. In our study, there was a significant correlation between levels of coliforms (total and fecal) and coliphage under conditions of high levels of pollution (fecal coliforms >300 CFU/100 ml); whereas, under low levels of pollution (fecal coliforms <28.3 CFU/100 ml), no correlation was demonstrable (table 9). These findings are in agreement with other published reports from

Morinigo et al. (1992), O'Keefe and Green (1989), and Palmateer et al. (1991). The lack of correlation between levels of coliforms (total and fecal) and coliphage in the potable water supplies was most likely the result of low levels of fecal pollution found in the potable water samples, which also has been suggested as the reason for the lack of correlation between coliforms and coliphages in the study by Palmateer et al. (1991).

Although it is well established that viruses are more resistant than *E. coli* to a variety of environmental stresses and water treatment processes, and, therefore, persist longer in aquatic environments than do bacteria (Berg et al., 1978; Committee report, 1981; Melnick, 1984), in our study, coliforms (total and fecal) were detected to a greater degree in the absence of coliphages in the 40 potable water samples. Our findings conflict with findings reported by El-Abagy et al. (1988) and Ratto et al. (1989), who reported that coliphages were detected to a greater degree in the absence of coliform (total and fecal) in potable water supplies. Although coliphages were detected in the absence of coliforms in roughly the same proportion (2 samples) as coliforms, coliforms (total and fecal) were detected in the absence of coliphage (2 and 3 samples, respectively) in the chlorinated tapwater samples (tables 10, 11, and 12), thus suggesting that coliphages were as resistant to chlorination as coliform bacteria, the absence of coliphages in the presence of coliforms in the nonchlorinated spring water samples (tables 10, 11, and 12) suggests that coliphages were not as resistant to environmental stresses as the coliform bacteria. Thus, greater survival and the eventual subsurface movement of coliforms as a result of surface run-off from grass and wooded lands under saturated conditions (samples were usually obtained after a heavy rainfall) may account for the greater occurrence of coliform bacteria than coliphage in the spring-fed source.

The higher incidence of coliforms (total and fecal) in the absence of coliphage in potable water also may be the result of viral sample concentration. In the potable water samples, it was necessary to concentrate samples because coliphage levels were expected to be low. Primrose et al. (1980) demonstrated that most methods used to concentrate phages from low-pollution waters also have low and highly variable recovery efficiencies. In a review of viral concentration methods, Gerba (1987b) reported that concentration via ultrafiltration (used in this study) had a 30-100% viral recovery efficiency depending on the type of membrane and system used. Some authors report an increase in virus recovery when a protein wash with beef extract was used, or if backwashing of the membrane with a phosphate-buffered saline was used after the initial concentration step (Jansons and Bucens, 1986a;

Jansons and Bucens, 1986b). Although elution or backwashing of the membrane was not performed in this study because of the low-protein binding nature of the cellulose-based YM membrane used, elution may have improved viral recoveries, especially in the spring water samples, where turbidity occasionally was a problem. Amicon, Inc., recommends the spiral membrane only for processing dilute, particle-free solutions (personal communication). The entrapment of particulate matter (clay or colloidal organic matter) on the membrane, and the possible adsorption of coliphages to the particulate matter either through cation bridging (Carlson et al., 1968) or Van der Waals forces (Schaub et al., 1974), may have resulted in the loss of coliphages from the final concentrate.

Thus, coliphages may be a useful indicator of fecal contamination in nonpotable water supplies, where they were found to correlate with levels of coliform bacteria. However, their usefulness as indicators in potable water supplies, where fecal pollution is expected to be low, may be hampered by the inefficiencies in processing environmental samples using the concentration methods currently available.

4 Conclusions

Under laboratory conditions, the CAB method proved superior to the APHA method because: 1) the CAB method was easier to read and interpret than the APHA method due to the formation of a dark blue circle within each plaque, which created a distinguishable mark that aided in counting, 2) the CAB method detected roughly twice the number of coliphage particles than the APHA method, and 3) there were no false positive results using the CAB method, for it was determined that the blue circle that formed within each plaque contained thousands of coliphage particles.

Under laboratory conditions, the LCPA method proved superior to the APHA method because: 1) the LCPA method was easier to read and interpret than the APHA method, with a uniform red indicating a positive coliphage test and a uniform yellow indicating a negative coliphage test; 2) the LCPA method theoretically detected 2 plaque forming units (PFU) l^{-1} , which was an improvement over the APHA method, which is only recommended when the coliphage density is greater than 5 PFU/100 ml; and 3) there were no false positive results using the LCPA method, and the rate of false negative results only became apparent at extremely low viral concentrations (2 PFU per concentrated sample).

In the field portion of the evaluation comparing the CAB and LCPA methods to the APHA method, the results indicated: 1) the LCPA was not subject to false positive or false negative results; 2) the LCPA method was as reliable as either the CAB or APHA methods in coliphage detection; 3) the LCPA and CAB methods were both easier to read and interpret than the APHA method; 4) the CAB method detected more coliphages than the APHA method under conditions of high fecal pollution, whereas both methods performed equally well in coliphage detection under conditions of low fecal pollution; 5) the CAB method was not subject to false positive results; and 6) the LCPA and CAB methods were equally as sensitive in coliphage detection as the APHA method.

The LCPA and CAB methods both possess qualities that will improve coliphage detection in groundwater and surface waters over the level of detection achieved with the APHA method. If used alone or in combination with each other, the LCPA and CAB methods would better provide the means to test for the possible presence of enteric viruses through the use of the coliphage indicator, thereby reducing the potential of human viral infections due to the consumption of contaminated water.

The coliphage group may be a useful indicator of fecal contamination in nonpotable water supplies that exhibit a high level of fecal pollution, whereas their usefulness as indicators in potable water supplies, which exhibit low fecal contamination, may be hampered by inefficiencies in processing environmental samples using the concentration methods currently available.

References

Adams, M.H. 1959. *Bacteriophages*. N.Y.: John Wiley & Sons.

American Public Health Association. 1989. *Standard Methods for the Examination of Water and Wastewater*. 17th ed. Washington, D.C.: American Public Health Assn.

American Public Health Association. 1992. *Standard Methods for the Examination of Water and Wastewater*. 18th ed. Washington, D.C.: American Public Health Assn.

Andersson, Y., and T.A. Stenstrom. 1986. Waterborne outbreaks in Sweden—Causes and etiology. *Water Sci. Tech.* 18:185-190.

Beckwith, J. 1987. The lactose operon. In: *Escherichia Coli and Salmonella Typhimurium: Cellular and Molecular Biology*. Vol. 2. eds. F.C. Neidhardt, J.L. Ingraham, K.B. Low, B. Magasanik, B. Schaechter, and H.E. Umbarger. 1444-1452. Washington, D.C.: Amer. Soc. for Micro.

Bell, R.G. 1976. The limitations of the ratio of fecal coliforms to total coliphage as a water pollution index. *Water Res.* 10:745-748.

Berg, G. 1978. The indicator system. In *Indicators of Viruses in Water and Food*. ed. G. Berg. 1-14. Ann Arbor, Mich.: Ann Arbor Sci.

Berg, G. 1984. Monitoring for viruses disseminated in water. In: *Enteric Viruses in Water*. ed. J.L. Melnick. 17-29. Basel, Switzerland: Karger.

Berg, G., D.R. Dahling, G.A. Brown, and D. Berman. 1978. Validity of fecal coliforms, total coliforms, and fecal streptococci as indicators of viruses in chlorinated primary sewage effluents. *Appl. Env. Micro.* 36:880-884.

Bitton, G. 1980. *Introduction to Environmental Virology*. N.Y.: John Wiley & Sons.

Borrego, J.J., R. Cornax, M.A. Morinigo, E. Martinez-Manzanares, and P. Romero. 1990. Coliphages as an indicator of faecal pollution in water—Their survival and productive infectivity in natural aquatic environments. *Water Res.* 24:111-116.

Borrego, J.J., M.A. Morinigo, A. DeVicente, R. Cornax, and P. Romero. 1987. Coliphages as an indicator of faecal pollution in water—Its relationship with indicator and pathogenic microorganisms. *Water Res.* 21:1473-1480.

Bosh, A., F. Lucena, J.M. Diez, R. Gajardo, M. Blasi, and J. Jofre. 1991. Waterborne viruses associated with hepatitis outbreaks. *J. Amer. Water Works Assn.* 83:80-83.

Bosh, A., C. Tartera, R. Gajardo, J.M. Diezv, and J. Jofe. 1989. Comparative resistance of bacteriophages active against bacteroides fragiles to inactivation by chlorination or ultraviolet radiation. *Water Sci. Tech.* 21:221-226.

Buras, N. 1976. Concentration of enteric viruses in wastewater and effluent: A two-year survey. *Water Res.* 10:295-298.

Carlson G.F., Jr., F.E. Woodard, D.F. Wentworth, and O.J. Sproul. 1968. Virus inactivation on clay particles in natural waters. *J. Water Poll. Cont. Fed.* 40:89-106.

Cogger, C. 1988. On-site septic systems: The risk of groundwater contamination. *J. Env. Health.* 51:12-16.

Conover, W.J. 1971. *Practical Nonparametric Statistics*. N.Y.: John Wiley & Sons.

Dahling, D.R. 1991. Detection and enumeration of enteric viruses in cell culture. *CRC Crit. Rev. Env. Cont.* 21:237-263.

Dahling, D.R., R.S. Safferman, and B.A. Wright. 1984. Results of a survey of BGM cell culture practices. *Env. Intern.* 10, 309-319.

Debartolomeis, J., and V.J. Cabelli. 1991. Evaluation of an *Escherichia coli* host strain for enumeration of F male-specific bacteriophages. *Appl. Env. Micro.* 57:1301-1305.

Dennis, J.M. 1959. 1955-56 infectious hepatitis epidemic in Delhi, India. *J. Amer. Water Works Assn.* 51:1288-1298.

Dhillon, T.S., Y.S. Chan, S.M. Sun, and W.S. Chau. 1970. Distribution of coliphages in Hong Kong sewage. *Appl. Micro.* 20:187-191.

Dhillon, T.S., K.S. Dhillon, H.C. Chau, W.K. Li, and H.C. Tsang. 1976. Studies on bacteriophage distribution: Virulent and temperate bacteriophage content of mammalian feces. *Appl. Env. Micro.* 32:68-74.

Dimmock, N.J., and S.B. Primrose. 1987. *Introduction to Modern Virology*. Vol. 2. London: Blackwell Scientific Pubs.

Dutka, B.J. 1973. Coliforms are an inadequate index of water quality. *J. Env. Health.* 36:39-46.

Edberg, S.C., M.J. Allen, D.B. Smith, and the National Collaborative Study. 1988. National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: Comparison with the standard multiple tube fermentation method. *Appl. Env. Micro.* 54:1595-1601.

El-Abagy, M.M., B.J. Dutka, and M. Kamel. 1988. Incidence of coliphage in potable water supplies. *Appl. Env. Micro.* 54:1632-1633.

Eustice, D.C., P.A. Feldman, A.M. Colberg-Poley, R.M. Buckery, and R.H. Neubauer. 1991. A sensitive method for the detection of B-galactosidase in transfected mammalian cells. *BioTechniques.* 11:739-743.

Evison, L.M. 1978. Inactivation of enteroviruses and coliphages with ozone in waters and wastewaters. *Prog. Water Tech.* 10:365-374.

Fattal, B., E. Katzenelson, N. Guttman-Bass, and A. Sadovski. 1984. Relative survival rates of enteric viruses and bacterial indicators in water, soil, and air. In *Enteric Viruses in Water*. ed. J.L. Melnick. 184-192. Basel, Switzerland: Karger.

Fattal, B., R.J. Vasl, E. Katzenelson, and H.I. Shuval. 1983. Survival of bacterial indicator organisms and enteric viruses in the mediterranean costal waters off Tel-Aviv. *Water Res.* 17:397-402.

Feachem, R.G., D.J. Bradley, H. Garelick, and D.D. Mara. 1983. *Sanitation and Disease: Health Aspects of Excreta and Wastewater Managements*. N.Y.: John Wiley & Sons.

Fleiss, J.L. 1981. *Statistical Methods for Rates and Proportions*. Second ed. N.Y.: John Wiley & Sons.

Furuse, K., S. Osawa, J. Kawashiro, R. Tanaka, A. Ozawa, S. Sawamura, Y. Yanagawa, T. Nagao, and I. Watanabe. 1983. Bacteriophage

distribution in human faeces: Continuous survey of healthy subjects and patients with internal and Leukaemic diseases. *J. Gen. Virol.* 64:2039-2043.

Geldreich, E.E. 1972. Water-borne pathogens. In: *Water Pollution Microbiology*. Vol. 1. ed. R. Mitchell. 207-241. N.Y.: John Wiley & Sons.

Geldreich, E.E. 1978. Bacterial populations and indicator concepts in feces, sewage, stormwater, and solid wastes. In: *Indicators of Viruses in Water and Food*. ed. G. Berg. 51-97. Ann Arbor, MI: Ann Arbor Science Pubs.

Gerba, C.P. 1984. Waterborne gastroenteritis and viral hepatitis. *CRC Crit. Rev. Env. Cont.* 15:213-235.

Gerba, C.P. 1987(a). Phage as indicators of fecal pollution. In: *Phage Ecology*. eds. S.M. Goyal, C.P. Gerba, and G. Bitton. 197-209. N.Y.: John Wiley & Sons.

Gerba C.P. 1987(b). Recovering viruses from sewage, effluents, and water. In *Methods for Recovering Viruses from the Environment*. ed. G. Berg. Boca Raton, FL: CRC Press.

Gerba, C.P. 1988. Viral disease transmission by seafoods. *Food Tech.* 42:99-103.

Gerba, C.P., S.M. Goyal, R.L. Labelle, I. Cech, and G.F. Bodgan. 1979. Failure of indicator bacteria to reflect the occurrence of enteroviruses in marine waters. *Amer. J. Pub. Health* 69:1116-1129.

Gerba, C.P., and C.N. Haas. 1988. *Assessment of Risks Associated with Enteric Viruses in Contaminated Drinking Water*. Spec. Tech. Pub. 976-1988. U.S. Public Health Svc., Washington, D.C.

Gerba, C.P., C. Wallis, and J.L. Melnick, J.L. 1975. Viruses in water: The problems, some solutions. *Env. Sci. Tech.* 9:1122-1126.

Goyal, S.M., C.P. Gerba, and J.L. Melnick. 1978. Prevalence of human enteric viruses in coastal canal communities. *J. Water Poll. Cont. Fed.* 50:2247-2255.

Goyal, S.M., B.H. Keswick, and C.P. Gerba. 1984. Viruses in ground-water beneath sewage irrigated cropland. *Water Res.* 18:299-302.

Grabow, W.O.K. 1986. Indicator systems for assessment of the virological safety of treated drinking water. *Water Sci. Tech.* 18:159-165.

Grabow, W.O.K., and P. Coubrough. 1986. Practical direct plaque assay for coliphages in 100-ml samples of drinking water. *Appl. Env. Micro.* 52:430-433.

Grabow, W.O.K., E.M. Nupen, and B.W. Bateman. 1984. South African research on enteric viruses in drinking water. In: *Enteric Viruses in Water*. ed. J.L. Melnick. 146-155. Basel, Switzerland: Karger.

Haas, C.N. 1986. Wastewater disinfection and infectious disease risks. *CRC Crit. Rev. Env. Cont.* 17:1-20.

Havelaar, A.H., K. Furuse, and W.M. Hogeboom. 1986. Bacteriophages and indicator bacteria in human and animal feces. *J. Appl. Bacteriol.* 60:255-262.

Havelaar, A.H., and W.M. Hogeboom. 1983. Factors affecting the enumeration of coliphages in sewage and sewage-polluted water. *Antonie van Leeuwenhoek.* 49:387-397.

Havelaar, A.H., and W.M. Hogeboom. 1984. A method for the enumeration of male-specific bacteriophages in sewage. *J. Appl. Bacteriol.* 56:439-447.

Hejkal, T.W., B. Keswick, R.L. Labelle, C.P. Gerba, Y. Sanchez, G. Dreesman, B. Hafkin, and J.L. Melnick. 1982. Viruses in a community water supply associated with an outbreak of gastroenteritis and infectious hepatitis. *J. Amer. Water Works Assn.* 74:318-321.

Herwaldt, B.L., G.F. Craun, S.L. Stokes, and D.D. Juranek. 1992. Outbreaks of waterborne disease in the United States: 1989-90. *J. Amer. Water Works Assn.* 84:129-134.

Hurst, C.J. 1989. Fate of viruses during wastewater sludge treatment process. *CRC Crit. Rev. Env. Cont.* 18:317-343.

Hurst, C.J. 1991. Presence of enteric viruses in freshwater and their removal by the conventional drinking water treatment process. *Bull. World Health Org.* 69:113-119.

- IAWPRC Study Group on Water Virology. 1991. Bacteriophages as model viruses in water quality. *Water Res.* 25:529-545.
- Ignazzitto, G., L. Volterra, F.A. Aulicino, and A.M. D'angelo. 1980. Coliphages as indicators in a treatment plant. *Water Air Soil Poll.* 13:391-398.
- Ijzerman, M.M., J.O. Falkinham, and C. Hagedorn. 1993. A liquid, colorimetric presence-absence coliphage detection assay. *J. Virol. Methods*, in press.
- Ijzerman, M.M., and C. Hagedorn. 1992. Improved method of coliphage detection based on -galactosidase induction. *J. Virol. Methods.* 40:31-36.
- Ijzerman M.M., R.B. Reneau, Jr. R.B., and C. Hagedorn. 1993(b). Field evaluation of a colorimetric coliphage assay detection system: Comparison with the coliphage detection method described in standard methods. *Appl. Env. Micro.*, submitted for publication.
- Isbister, J.D., J.A. Simmons, W.M. Scott, and J.F. Kitchens. 1983. A simplified method for coliphage detection in natural waters. *Acta Micro. Pol.* 32:197-206.
- Jansons, J., and M.R. Bucens. 1986(a). Concentration of rotavirus by ultrafiltration. *Water Res.* 20:79-83.
- Jansons, J., and M.R. Bucens. 1986(b). Virus detection in water by ultrafiltration. *Water Res.* 20:1603-1606.
- Kennedy, J.E., G. Bitton, and J.L. Oblinger J.L. 1985. Comparison of selective media of coliphages in sewage effluent and lake water. *Appl. Env. Micro.* 49:33-36.
- Keswick, B.H., and C.P. Gerba. 1980. Viruses in groundwater. *Env. Sci. Tech.* 14:1290-1297.
- Keswick, B.H., C.P. Gerba, J.B. Rose, and G.A. Toranzos. 1985. Detection of rotavirus in treated drinking water. *Water Sci. Tech.* 17:1-6.
- Kott, Y. 1966. Estimation of low numbers of *Escherichia coli* bacteriophage by use of the most probable number method. *Appl. Micro.* 14:141-144.

- Kott, Y. 1984. Coliphages as reliable enteric virus indicators. In *Enteric Viruses in the Water Environment: An Underestimated Problem*. ed. J.L. Melnick, J. Am. Water Works Assn, 71:445-449.
- Kott, Y. 1981. Viruses and bacteriophages. *Sci. Total Env.* 18:13-23.
- Kott, Y., H. Ben-Ari, and L. Vinokur. 1978. Coliphages survival as viral indicator in various wastewater quality effluents. *Prog. Water Tech.* 10:337-346.
- Kott, Y., N. Roze, S. Sperber, and N. Betzer. 1974. Bacteriophages as viral pollution indicators. *Water Res.* 8:165-171.
- Lewis, C.M., and J.L. Mak. 1989. Comparison of membrane filtration and autoanalysis colilert presence-absence techniques for analysis of total coliforms and *Escherichia coli* in drinking water samples. *Appl. Env. Micro.* 55:3091-3094.
- Mahdy, M.S. 1979. Viruses in the water environment: An underestimated problem. *J. Am. Water Works Assn.* 71:445-449.
- Marzouk, Y., S.M. Goyal, and C.P. Gerba. 1980. Relationship of viruses and indicator bacteria in water and wastewater of Israel. *Water Res.* 14:1585-1590.
- Melnick, J.L. 1984. Etiologic agents and their potential for causing waterborne virus diseases. In *Enteric Viruses in Water*. ed. J.L. Melnick. 1-16. Basel, Switzerland: Karger.
- Melnick, J.L., C.P. Gerba, and C. Wallis. 1978. Viruses in water. *Bull. World Health Org.* 56:499-508.
- Miller, J.H. 1977. Assay of β -galactosidase. In *Experiments in Molecular Genetics*. ed. J.H. Miller. 352-355. Cold Spring Harbour, N.Y.: Cold Spring Harbour Lab.
- Morinigo, M.A., D. Wheeler, C. Berry, C. Jones, M.A. Munoz, R. Cornax, and J.J. Borrego. 1992. Evaluation of different bacteriophage groups as faecal indicators in contaminated natural waters in southern England. *Water Res.* 26:267-271.
- O'Keefe, B., and J. Green. 1989. Coliphages as indicators of faecal pollution at three recreational beaches on the firth of forth. *Water Res.* 23:1027-1030.

- Osawa, S., K. Furuse, and I. Watanabe. 1981. Distribution of ribonucleic acid coliphages in animals. *Appl. Env. Micro.* 41:164-168.
- Palmateer G.A., B.J. Dutka, E.M. Janzen, S.M. Meissner, and M.G. Sakellaris. 1991. Coliphage and bacteriophage as indicators of recreational water quality. *Water Res.* 25:335-357.
- Payment, P. 1984. Viruses and bathing beach quality. *Can. J. Pub. Health.* 75:43-48.
- Payment, P., and R. Armon. 1989. Virus removal by drinking water treatment processes. *CRC Crit. Rev. Env. Micro.* 19:15-31.
- Primrose S.B., N.D. Seeley, and K. Logan. 1980. The recovery of viruses from water: Methods and applications. In *Viruses and Wastewater Treatment.* eds. M. Godard and M. Butler. 211-233. Oxford: Pergamon Press.
- Prins, C.J., and K.W. Lustig. 1988. Innovative septic system management. *J. Water Poll. Cont. Fed.* 60:614-620.
- Rao, V.C., and J.L. Melnick. 1986. *Environmental Virology.* Washington, D.C.: Amer. Soc. for Micro.
- Ratto, A., B.J. Dutka, C. Vega, C. Lopez, and A. El-Shaarawi. 1989. Potable water safety assessed by coliphage and bacterial tests. *Water Res.* 23:253-255.
- Reneau, R.B., C. Hagedorn, and M.I. Degen. 1989. Fate and transport of biological and inorganic contaminants from on-site disposal of domestic wastewater. *J. Env. Qual.* 18:135-144.
- Rose, J.B., C.P. Gerba, S.N. Singh, G.A. Toranzos, and B. Keswick. 1986. Isolating viruses from finished water. *J. Amer. Water Works Assn.* 78:56-61.
- Safferman, R.S. 1982. Viruses in wastewater. *Env. Intern.* 7:15-20.
- Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular Cloning: A Laboratory Manual.* Vol. 3. Plainview, N.Y.: Cold Spring Harbour Lab. Press.

Scarpino, P.V. 1978. Bacteriophage indicators. In *Indicators of Viruses in Water and Food*. ed. A.G. Berg. 201-227. Ann Arbor, MI: Ann Arbor Sci.

Scarpino, P.V., G. Berg, S.L. Chang, D. Dahling, and M. Lucas. 1972. A comparative study of the inactivation of viruses in water by chlorine. *Water Res.* 6:959-965.

Schaub S.A., C.A. Sorber, and G.W. Taylor G.W. 1974. The association of enteric viruses from with natural turbidity in the aquatic environment. In *Virus Survival in Water and Wastewater Systems*. eds. J.F. Malina and B.P. Sagik. 71-83. Austin: Univ. of Texas Ctr for Research in Water Res.

Schiff, G.M., G.M. Stefanovic, E.C. Young, D.S. Sander, J.K. Pennekamp, and R.L. Ward. 1984. Studies of echovirus-12 in volunteers: Determination of minimal infectious dose and the effect of previous infection on infectious dose. *J. Inf. Disease.* 150:858-866.

Seyfried, P.L., N.E. Brown, C.L. Cherwinsky, F.D. Jenkins, D.A. Cotter, J.M. Winner, and R.S. Tobin. 1984. Impact of sewage treatment plants on surface waters. *Can. J. Pub. Health.* 75:25-31.

Shah, P.C., and J. McCamish. 1972. Relative chlorine resistance of poliovirus I and coliphages f2 and T2 in water. *Appl. Micro.* 24:658-659.

Simkova, A., and J. Cervenka. 1981. Coliphages as ecological indicators of enteroviruses in various water systems. *Bull. World Health Org.* 59:611-618.

Snead, M.C., V.P. Olivier, K. Kawata, and C.W. Kruse. 1980. The effectiveness of chlorine residuals in inactivation of bacteria and viruses introduced by post-treatment contamination. *Water Res.* 14:403-408.

Snowdon, J.A., and D.O. Cliver. 1989. Coliphages as indicators of human enteric viruses in groundwater. *CRC Crit. Rev. Env. Cont.* 19:231-249.

Sobsey, M.D. 1982. Quality of currently available methodology for monitoring viruses in the environment. *Env. Intern.* 7:39-51.

Sproul, O.J. 1983. Public health, financial and practical considerations of virological monitoring and quality limits. *Water Sci. Tech.* 15:33-41.

- Stetler, R.E. 1984. Coliphages as indicators of enteroviruses. *Appl. Env. Micro.* 48:668-670.
- Toranzos, G.A., C.P. Gerba, and H. Hanssen. 1988. Enteric viruses and coliphages in Latin America. *Tox. Assess.* 3:491-510.
- Vaughn, J.M., and T.G. Metcalf. 1975. Coliphages as indicators of enteric viruses in shellfish and shellfish raising estuarine waters. *Water Res.* 9:613-616.
- Vaughn, J.M., E.F. Landry, L.J. Baranosky, C.A. Beckwith, M.C. Dahl, and N.C. Delibas. 1978. Survey of human virus occurrence in wastewater-recharged groundwater on Long Island. *Appl. Env. Micro.* 36:47-51.
- Vaughn, J.M., E.F. Landry, C.A. Beckwith, and M.Z. Thomas. 1981. Virus removal during groundwater recharge—Effects of infiltration rate on adsorption of poliovirus to soil. *Appl. Env. Micro.* 41:139-147.
- Viruses in drinking water committee report. 1981. *J. Amer. Water Works Assn.* 71:441-444.
- Ward, R.L., and E.W. Akin. 1984. Minimum infectious dose of animal viruses. *CRC Crit. Rev. Env. Cont.* 14:297-310.
- Wellings, F.M., A.L. Lewis, C.W. Mountain, and L.V. Pierce. 1975. Demonstration of virus in groundwater after effluent discharge onto soil. *Appl. Micro.* 29:751-757.
- Wentsel, R.S., P.E. O'Neill, and J.F. Kitchens. 1982. Evaluation of coliphage detection as a rapid indicator of water quality. *Appl. Env. Micro.* 43:430-434.
- Williams Jr., F.P., and C.J. Hurst. 1988. Detection of environmental viruses in sludge: enhancement of enterovirus plaque assay titer with 5-iodo'-2'-deoxyuridine and the comparison to adenovirus and coliphage titers. *Water Res.* 22:847-851.
- Yates, M.V. 1985. Septic tank density and groundwater contamination. *Groundwater.* 23:586-591.
- Zar, J.H. 1984. *Biostatistical Analysis*. Second ed. Englewood Cliffs, N.J.: Prentice-Hall.

Tables

Table 1.
Mean coliphage counts of five separate experiments* comparing the
IPTG/X-GAL method with the TPTZ method proposed by the APHA.

Experiment	Mean Coliphage Count† (Plaque forming units, ml ⁻¹)	
	TPTZ	IPTG/X-gal
1	1.9 x 10 ⁷ B	3.4 x 10 ⁷ A
2	1.3 x 10 ⁷ B	3.1 x 10 ⁷ A
3	1.4 x 10 ⁷ B	2.9 x 10 ⁷ A
4	1.8 x 10 ⁹ B	3.4 x 10 ⁹ A
5	1.6 x 10 ⁹ B	3.8 x 10 ⁹ A

* Each experiment consisted of six replications for a total of thirty tests per method.

† Means in each experiment followed by the same letter are not significantly different using Duncan's multiple range test ($p < 0.01$).

Table 2.
The detection sensitivity of the liquid, colorimetric presence-absence
coliphage detection method using *E. coli* strain C as host.

Number (PFU)	Positive Samples (30 min)	Total No. of Samples
60	3	3
30	23	23
22	16	16
11	15	15
6*	15	15
4*	12(15)†	15
2*	8(11)‡	15
0	0	39

* Two ml of filtrate was added to 8 ml of Z buffer, while 1 ml of filtrate plus 9 ml of Z buffer was used for titers >10 PFU, and negative controls (0 PFU) were done both ways.

† An additional three samples turned positive 1.5 h after the end of the designated 30 min incubation period.

‡ An additional three samples turned positive 1.5 h after the end of the designated 30 min incubation period.

Table 3.
Selected water quality parameters of the four
experimental water sources.

Water Source*	Parameter (mg/l in solution)									
	pH	BOD ₅	K	Ca	Mg	Mn	Fe	Na	Zn	Cu
Tap	6.65	7.90	1.27	10.54	4.16	N.D [†]	N.D	5.81	0.63	0.15
Spring	6.84	6.40	0.76	66.62	3.00	0.01	0.53	1.50	0.02	0.01
Creek	7.04	7.30	5.88	57.97	20.94	0.04	0.82	21.50	0.02	N.D
W. water	7.47	9.30	8.47	30.97	12.02	0.02	0.08	32.40	0.06	0.01

* Tapwater obtained from a private residence located at the end of the water distribution system in the City of Blacksburg, VA; Spring water obtained from a private residence in Giles Co., VA, Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Wastewater sample collected from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

[†] N.D= not detectable below analytical range; Mn < 0.001, Fe < 0.005, and Cu < 0.002.

Table 4.
Comparison of coliphage detection by the APHA* method and the
LCPA method.

Water Source†	No. of samples					McNemar <i>P</i> value‡	Kappa (Overall)**
	Total	APHA positive	LCPA positive	Both positive	Both negative		
Tap	25	2	2	0	21	0.69	-§
Spring	15	1	0	0	14	0.50	-
Creek	25	25	25	25	0	1.00	-
W. water	25	25	25	25	0	1.00	-
Combined	90	53	52	50	35	0.50	0.88

* APHA= American Public Health Association; LCPA= Liquid Colorimetric Presence-Absence.

† Tap water was obtained from a private residence at the end of the water distribution system in the City of Blacksburg, VA; Spring water was obtained from a private residence in Giles Co. VA; Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Wastewater was obtained from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

‡ $P < 0.05$ is significantly different.

** A +1 indicates perfect agreement, 0 indicates no agreement, and negative values suggest less agreement than expected due to chance.

§ (-) = not determined.

Table 5.
Comparison of coliphage detection by the APHA* method and the
CAB method.

Water Source†	No. of samples					McNemar <i>P</i> value‡	Kappa (Overall)**
	Total	APHA positive	CAB positive	Both positive	Both negative		
Tap	25	2	2	0	21	0.69	-§
Spring	15	1	0	0	14	0.50	-
Creek	25	25	25	25	0	1.00	-
W. water	25	25	25	25	0	1.00	-
Combined	90	53	52	50	35	0.50	0.88

* APHA= American Public Health Association; CAB= Colorimetric Agar-Based.

† Tap water was obtained from a private residence located at the end of the water distribution system in the City of Blacksburg, VA; Spring water was obtained from a private residence in Giles Co. VA; Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Wastewater was obtained from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

‡ $P < 0.05$ is significantly different.

** A +1 indicates perfect agreement, 0 indicates no agreement, and negative values suggest less agreement than expected due to chance.

§ (-) = not determined.

Table 6.
Median coliphage counts of four different water sources comparing the
CAB* method and the APHA method.

Water Source [†]	Median coliphage count [‡] (Plaque forming units (PFU)/15 ml)	
	CAB	APHA
Tap	0.2A	0.2A
Spring	0.0A	0.2A
Creek	283.4A	257.4B
Wastewater	62.0A	48.0B

* CAB= Colorimetric Agar-Based; APHA= American Public Health Association.

† Tap water samples were obtained from a private residence located at the end of the water distribution system in the City of Blacksburg, VA; Spring water was obtained from a private residence in Giles, Co. VA; Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Wastewater was obtained from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

‡ Median values obtained by each method within a water source followed by the same letter are not significantly different ($P < 0.001$).

Table 7.
Comparison of coliphage detection by the CAB* method and the LCPA method.

Water Source†	No. of samples					McNemar <i>P</i> value‡	Kappa (Overall)**
	Total	CAB positive	LCPA positive	Both positive	Both negative		
Tap	25	2	2	0	21	0.69	-§
Spring	15	0	0	0	15	1.00	-
Creek	25	25	25	25	0	1.00	-
W. water	25	25	25	25	0	1.00	-
Combined	90	52	52	50	36	0.69	0.92

* CAB= Colorimetric Agar-Based; LCPA= Liquid Colorimetric Presence-Absence.

† Tap water was obtained from a private residence at the end of the water distribution system in the City of Blacksburg, VA; Spring water was obtained from a private residence in Giles Co. VA; Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Wastewater was obtained from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

‡ $P < 0.05$ is significantly different.

** A +1 indicates perfect agreement, 0 indicates no agreement, and negative values suggest less agreement than expected due to chance.

§ (-) = not determined.

Table 8.
Median values and population ranges detected for total coliform, fecal coliform, and coliphage in all four water sources.

Water source*	Total coliform (counts/100 ml)		Fecal coliform (counts/100ml)		APHA† (counts/100ml)		CAB‡ (counts/100ml)	
	Median	Range of values	Median	Range of values	Median	Range of values	Median	Range of values
Tap	0.9	0-2.3	0.7	0-1.3	1.0	0-2	1.0	0-2
Spring	42.3	3.3-430	2.0	0-28.3	1.0	0-2	0.0	0
Creek	4,133.0	537-5,967	1,867.0	350-4,433	1,716.0	269-2,849	1,889.0	471-2,782
Waste-water	116,000.0	9,000-310,000	3,813.0	300-61,333	320.0	158-1,373	413.0	271-1,910

* Tap water was collected from a private residence at the end of the water distribution system in the City of Blacksburg; Spring water was collected from a private residence in Giles Co., VA, Creek water was collected below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Wastewater was collected from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

† American Public Health Association method.

‡ Colorimetric Agar-Based method.

Table 9.

Pearson's product-moment correlation coefficient (r) and the significance value (P) calculated between the coliform group (total and fecal) and the coliphage group as determined by the APHA* method and the CAB method.

Water Source†	<u>TC vs APHA</u>		<u>TC vs CAB</u>		<u>FC vs APHA</u>		<u>FC vs CAB</u>	
	r	P ‡	r	P	r	P	r	P
Tap	-0.07	0.747	-0.07	0.747	-0.09	0.653	-0.09	0.653
Spring	-0.17	0.534	-**	-	-0.10	0.732	-	-
Creek	0.78	0.001	0.80	0.001	0.53	0.006	0.59	0.002
W. water	0.41	0.040	0.50	0.011	0.73	0.001	0.79	0.001

* Abbreviations: APHA= American Public Health Association; CAB= Colorimetric Agar-Based; TC=Total Coliform; FC=Fecal Coliform.

† Tap water was obtained from a private residence at the end of the water distribution system in the City of Blacksburg; Spring water was collected from a private residence in Giles, Co., VA; Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Wastewater was collected from the sedimentation basin at the Blacksburg-VPI &SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

‡ P value < 0.05 is significant.

** (-) = not determined because all the values obtained using the Colorimetric Agar-Based were zeros.

Table 10.
Incidence of occurrence of the coliform indicators (total and fecal) and
coliphages using the APHA* method.

Water Source†	Total no of samples	TC+ coliphage -	coliphage + TC -	FC + coliphage -	coliphage + FC -
Tap	25	2	2	3	2
Spring	15	14	0	10	0
Creek	25	0	0	0	0
W. water	25	0	0	0	0

* Abbreviations: APHA=American Public Health Association; TC=Total Coliform; FC=Fecal Coliform.

† Tap water was obtained from a private residence located at the end of the water distribution system in the City of Blacksburg, VA; Spring water was obtained from a private residence in Giles Co., VA; Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Waste-water was collected from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

Table 11.
Incidence of occurrence of the coliform indicators (total and fecal) and
coliphages using the CAB* method.

Water Source†	Total no of samples	TC+ coliphage -	coliphage + TC -	FC + coliphage -	coliphage + FC -
Tap	25	2	2	3	2
Spring	15	15	0	11	0
Creek	25	0	0	0	0
W. water	25	0	0	0	0

* Abbreviations: CAB=Colorimetric Agar-Based; TC=Total Coliform; FC=Fecal Coliform.

† Tap water was obtained from a private residence located at the end of the water distribution system in the City of Blacksburg, VA; Spring water was obtained from a private residence in Giles Co., VA; Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Waste-water was collected from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

Table 12.
Incidence of occurrence of the coliform indicators (total and fecal) and
coliphages using the LCPA* method.

Water Source†	Total no of samples	TC+ coliphage -	coliphage + TC -	FC + coliphage -	coliphage + FC -
Tap	25	2	2	3	2
Spring	15	15	0	11	0
Creek	25	0	0	0	0
W. water	25	0	0	0	0

* Abbreviations: LCPA=Liquid Colorimetric Presence-Absence; TC=Total Coliform; FC=Fecal Coliform.

† Tap water was obtained from a private residence located at the end of the water distribution system in the City of Blacksburg, VA; Spring water was obtained from a private residence in Giles Co., VA; Creek water was obtained below the dairy pastures at Virginia Polytechnic Institute and State University (VPI&SU); Waste-water was collected from the sedimentation basin at the Blacksburg-VPI&SU Sanitation Authority Stroubles Creek Wastewater Treatment Plant.

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**Virginia Water Resources Research Center
Virginia Polytechnic Institute and State University
617 North Main Street
Blacksburg, Virginia 24060-3397
Phone (703)231-5624**