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A REVIEW

Microbial groundwater contamination and effective monitoring system

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SUMMARY : Global urbanization and livestock agriculture are responsible for microbial contamination for groundwater aquifer. Presence of pathogenic microbes and hazardous chemicals in the water bodies has deteriorated the water quality and poses a serious threat to public health. The objective of this review is to summarize the microbial groundwater contamination, their transport system into groundwater aquifer, existence, survival rate and various monitoring system. Livestock agriculture as well as urban wastewater is considered as one of the most important causes of bacterial contamination of surface and groundwater. Factors influencing fecal bacteria and enteric virus survival include moisture, soil type, temperature, pH, manure application rate, nutrient availability and competition. Cool, moist environments are considered optimal for bacterial survival. Field scale transport studies have shown significant transport of bacteria and viruses from sewage to groundwater through infiltration. Methods employed for microbial detection represent multitude culture, molecular and chemical techniques. The basic and rapid monitoring tactics for microbial analysis provide the effective tools to control the fundamental source for groundwater contamination.

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The opening of pathogenic bacteria into groundwater sources poses an immense risk to human health. Naturally-occurring bacteria and their activities allow us to interpret some of the roles of bacteria in groundwater environments. These naturally-occurring bacteria maintain the fertility of soil, transform minerals and nutrients in water, sediments and degrade leaf litter while other plant materials producing useful nutrients to other organisms. Although some of the microbes specially bacteria, viruses, fungus and protozoa that may cause associated diseases to humans and other warm-blooded animals (USGS, 2007). These pathogens are transmitted from one organism to another by direct contact, or through contaminated food and water. Alekseev *et al.* (2009). reported the list of pathogenic bacteria that would abundant in groundwater at different concentration. The bacteria that found in groundwater/drinking water generally are *Escherichia coli*, *Clostridium*, *Campylobacter, Rhodococcus coprophilus*, *Enterococci*, *Arcobacter* (an emerging bacterial pathogen), *Fecal streptococci* and sulphite-reducing *Clostridia*. (Angulo *et al.*, 1997; Altekruse *et al.*, 1999) reported the presence of *Entamoeba histolytica, Giardia intestinalis, S. typhimurium* and *Salmonella* in sewage as well as in drinking water sources. In addition various case studies for virus contamination were observed in reference of groundwater aquifer (Abbaszadegan *et al.*, 1999b; Bagdasarjan *et al.*, 1979). A very serious cases for Astrovirus Yuc8, Norovirus, Norwalk-like viruses (Anderson *et al.*, 2003), Coxsackievirus B4, poliovirus 1, Echovirus 1 pathogenic hepatitis A and E viruses, Caliciviruses, Rotaviruses and Astroviruses have been reported on groundwater (Abbaszadegan *et al.*, 1999a; 1999b and 2003c). Echoviruses and Coxsackievirus are enteric viruses associated with human wastewater. There are certain case studies which reported on the groundwater

contamination with Actinomycete fungus, Protozoa like Cryptosporidium and Giardia (Landry and Wolfe, 1999).

Animal manure applications to agricultural lands are cited as major source of pathogenic microorganisms in surface and groundwater system (Reddy *et al*., 1981). Common sources of anthropogenic contaminants include septic tanks and privies; underground storage tanks; areas where fertilizer, pesticides, or herbicides are used or stored; landfills and unauthorized dump sites (Anderson *et al*., 1991). Groundwater contamination/ pollution may also arise from agriculture, unlined drains and illegal waste disposal sites in urban areas.

The factors that control the transport of bacteria through porous media are not well understood while the study of microbial movement in the field under unsaturated flow conditions has received only limited study to date. Landry and Wolfe (1999) stated that the range of disciplines conducting fecal bacteria research and the diverse nature of the literature are obstacles to application and synthesis of existing knowledge by animal waste managers and scientists.

Several factors may influence the survival of enteric pathogens in soil as well as in groundwater. However, attempts to link survival rates of specific enteric pathogens with soil physic chemical and environmental variables are limited (Reddy *et al.*, 1981; Gerba *et al.*, 1975). The survival of microorganisms is affected by a number of environmental factors such as sunlight, rainfall, soil moisture and holding capacity, temperature, soil composition, pH, presence of oxygen and nutrients availability from the organic matter and the antagonism from soil micro flora. The morphological characteristics of bacterial type may also affect the survival of microorganisms (Engelbrecht and Tredoux, 2000).

The major transport modes of pathogens and indicator organisms in soil receiving manure are through movement with infiltrating water and surface runoff and with the movement of sediment and waste particles (Reddy *et al*., 1981). The various research reports revealed that the transport of fecal bacteria under conditions of ideal matrix flow is inversely related to particle size. Therefore, soils more susceptible to shrinking or cracking, such as clays, could be less efficient than sandy soils in terms of limiting bacterial transport (Jamieson, 2002). It also has been shown that bacterial survival is greater in finer grained soils, which have an enhanced ability to retain moisture and nutrients. Factors that affect the dispersion and survival of aerosolized microorganisms include solar radiation, humidity and atmospheric stability (Tania *et al.*, 2005).

Several projects have focused on developing new techniques for detecting, quantifying and monitoring the microorganisms in groundwater and soils. The review on groundwater has shown various physical, chemical and molecular detection techniques and assay to quantify the microbial contamination.PCR-based methods are eliminating the need to culture the microorganisms for monitoring (Hickey, 1998). A second very important method is the Colilert test. Colisure test and E*Colite test simultaneously determine the presence of total coliforms and *Escherichia coli*. The tests are based on the detection of two enzymes, beta-D-galactosidase and beta-D-glucuronidase, that are characteristic of the total coliform group and *Escherichia coli*, respectively (EPA, 2006).

Review designed:

The review has been designed to find out the major and minor causes of microbial groundwater contamination. The studies include various points to determine the microbial-types population which can pollute the groundwater ecosystem. The major objective of the review are (1) Sources of microbial groundwater contamination (2) Major route of transport of bacteria and viruses (3) Factor that affects the bacterial transport and their filtration in groundwater aquifer (4) Dispersion and survival of microorganisms (5) Factors that affect the dispersion and survival of microorganisms (6) Major monitoring and detection method for analysis of microbial groundwater contaminations.

Sources of waterborne pathogenic microbes:

Solid waste contamination with human and liquid excreta may contain various pathogenic microbes like bacteria and viruses are usually disposed off to the landfill and open dumping sites and near unlined drain sites. Harmful chemical, nutrients and pathogenic microbes seep down to groundwater aquifer and pollute the groundwater ecosystem (Francy *et al*., 2000). Major sources of animal fecal contamination that can reach groundwater include:

–Leakage or overflow from manure storage piles or lagoons at animal feeding operations (AFOs) (feedlots) and concentrated animal feeding operations (CAFOs).

–Land application of improperly treated wastewaters associated with food processing or animal slaughter.

–Animal wastes from pets, animal husbandry, or wild animals.

There are many fecal contaminant sources, including both surface (e.g., CAFOs, wastewater lagoons, etc.) and subsurface (e.g., improperly designed, sited, operated, or maintained septic systems) sources. These contaminant sources can infiltrate groundwater for drinking water supplies, which, if inadequately treated, can lead to waterborne disease outbreaks (Canter and Knox, 1984). It is assumed that urban wastewater may be a combination of some or all of the following: Domestic effluent consisting of black water, excreta, urine and associated sludge and grey water (kitchen and bathroom wastewater) water from commercial establishment and institutions including hospital waste,

industrials effluent, stormwater and other urban runoff (Hamdy and Ragab, 2005 and Engelbrecht, 1993). Sewage-polluted water carries numerous sewage micro-flora, some of which pose a public health risk (Gordon and Stuart, 1972; Baross *et al.*, 1975). The public health problems arising from fecal pollution of natural waters have been documented by several workers (Anderson, 1968; Ogedengbe and Adeniyi, 1978; Ekundayo, 1979). The degree of fecal population present into the urban river and the chance for contamination may increase during the dry season (Olayemi, 1994). Jagals, 1994; Pretorius *et al.* (1999) revealed that urban stormwater outfalls contribute greater microbiological pollution to the receiving surface water body than the effluent from sewage works outfalls originating in the same urban area.

Animals that carry human pathogenic bacteria include beavers, migratory birds, muskrats and other rodents and livestock (Hurst and Murphy, 1996). Doran and Linn (1979) monitored surface runoff from grazed and ungrazed pasture land and counts 5 to 10 times' greater fecal coliform in grazed areas than in ungrazed areas; however total coliform (TC) counts differed little. Household wastes that contribute large numbers of microorganisms include: facial tissues, pet feces carrying human pathogenic viruses, soiled disposable diapers, and decaying foods (Pahren, 1987). Commercial solid wastes, such as food waste and food processing waste, may contribute pathogenic organisms to municipal waste landfills. Based on one test, the ratio of fecal coliform to fecal streptococci found in landfill waste suggests that pathogens in municipal waste are predominantly of non-human, warm-blooded animal origin (human feces has a much higher fecal coliform density than most animals) (Pahren, 1987). The Overflow or seepage septic tanks are among the most frequently reported sources of groundwater contamination linked to disease outbreaks (Table 1) in the United States (Table 1) (Yates *et al*., 1985). Wastewater collection systems are constructed from pipe segments that may leak at connection points or simply as a result of pipe deterioration with aged and closed proximity of PWS wells, become a source of groundwater contamination.

Microbial transport system:

Many researchers have reported that that the sewagewater and septic tank as well as animal manure contain rich diversity of various pathogenic microorganisms. The fate and transport of microbes into the subsurface environment are major issues with respect to human exposure to waterborne pathogens. The ability of microorganisms to survive in any environmental condition allows them to be transported with water, food, or personal contact to a human host (Alhajjar *et al.*, 1988). Contamination can reach to groundwater directly by transport through soil openings and through joints, fractures, or fissures in rock. Soils consisting of primarily silt and clay particles are very effective in physically filtering bacterial cells under conditions of ideal

Table 1: Sources of waterborne disease outbreaks in groundwater systems, 1991-2000*

Cause of contamination	Number of outbreaks	Per cent outbreaks	Cases of illness	Per cent illnesses	Cases per outbreak
Community water systems					
Untreated groundwater	5	26%	167	6%	33
Treatment deficiency	7	37%	1,624	58%	232
Distribution system deficiency	5	26%	803	29%	161
Miscellaneous/unknown	2	11%	183	7%	92
Total	19	100%	2,777	100%	146
Noncommunity water systems					
Untreated groundwater	23	47%	4,057	50%	176
Treatment deficiency	19	39%	3,264	40%	172
Distribution system deficiency	6	12%	442	5%	74
Miscellaneous/unknown	1	2%	386	5%	386
Total	49	100%	8,149	100%	166
Combined					
Untreated groundwater	28	41%	4,224	39%	151
Treatment deficiency	26	38%	4,888	45%	188
Distribution system deficiency	11	16%	1,245	11%	113
Miscellaneous/unknown	3	4%	569	5%	190
Total	68	100%	10,926	100%	161

Sources: United States Centers for Disease Control and Prevention (CDC), 1993*;* Kramer *et al.*, 1996*;* Levy *et al.*, 1998, Barwick *et al.*, 2000 *and* Lee *et al.* 2002

matrix flow (Bales *et al*., 1989). Jamieson *et al*. (2000), Beven and German (1982) reported that macropores, or non-matrix flow, is the dominant transport pathway for fecal bacteria. It also has been shown that bacterial survival is greater in finer grained soils, which have an enhanced ability to retain moisture and nutrients. The shape of macropores play important role for transportation and survival to microbes in soil that may varies from planar slits to cylindrical pipes (White, 1985). Most Sewage-borne bacteria and viruses (enteroviruses, Norwalk-like viruses, coliphage) were regularly detected to depths of 60 meter in the unconfined sandstone (vertically) and to a depth of 91 meter (Berge, 1978) in the confined sandstone.

During land application, pathogens may become airborne and transported long distances as "bioaerosols". Research suggests that bioaerosols from municipal waste land application sites can increase the risk of bacterial transportation in water aquifers (Dowd *et al*., 1997).

Qureshi and Dutka (1979) have shown that stormwater runoff may have microbial densities similar to those found in dilute raw wastewater. Advection, dispersion, deposition (clogging) and entrainment (declooging) are all processes that affect transport in noticeable ways. The diversity of bacteria as well as viruses may show the multiplicity distance from surface to groundwater body, viruses can travel 30.48 meter distance in the subsurface, particularly in sand soils but their numbers are greatly reduced (Scandura and Sobsey, 1997; Corapcioglu, 2006; Yates and Yates, 1989). However, Pang *et al.* (2005) showed that in sandy soils 60.96 meter should be enough distance to meet bacterial standards for recreational waters and reduce viruses by greater than 7 logs.

Factors affects the fate and transport of microbes in the subsurface:

The role of hydrology is paramount when determining the transport characteristics of a plume of contaminants. Microbial fate and transport in the subsurface are influenced by numerous factors including, temperature, hydrogeologic conditions, soil properties (including mineral coatings on grains), water, pH, conductance of aquifer material, inorganic ions/salt species, organic matter, virus, microbial activity, Iron content, moisture content, type and degree of aggregation (Yates and Yates, 1988 and Mattle *et al*., 2001). EPA, 2006 has considered that the transport and persistence of microbes in the subsurface, the unsaturated zone (typically consisting of the soil at the top of the uppermost aquifer) and the saturated zone (typically consisting of one or more aquifers) are important factors in the occurrence of microbial contamination. Locally, weather changes may alter the hydrogeologic environment *i.e*. wetter (high precipitation) conditions may result in high water tables in an unconfined aquifer, thereby potentially reducing the distance and time required for microbes to enter shallower aquifer. Precipitation recharge and other hydrogeologic factors that govern the flow of groundwater are much better documented than the factors that govern the transport of microbial contaminants within groundwater. Physical filtration is believed to be the primary process which limits bacteria mobility in soil (Gerba and Bitton, 1984). Adsorption is the main process which limits the movement of smaller microorganisms, Bacteria (0.2 - 5 µm in size) and Virus (30-300 nm in size) (Gerba and Bitton, 1984).Suspended particles, including bacteria which become deposited at the soil surface, can act as a filter trapping for bacteria (Corapcioglu and Haridas, 1984). The paramount concentrations of pathogens are found up to 50 meter from the surface and the mortality of enteric microorganisms is greatest during hot dry conditions (Mattle *et al*., 2001; Teltsch and Katzenelson, 1978).

Dispersion and survival of microorganism and factor that affect on activity:

A rich of information's have been produced within the past 40 years on the survival of various enteric bacterial species in soil and groundwater systems. A review presented by Gerba *et al*. (1975) reported that survival times of enteric bacteria in soil and groundwater ranged from 2 to 4 months. Filip *et al.* (1988) examined the survivability of several organisms in simulated conditions of saturated soil and observed that most organisms tested for, including *Escherichia coli,* survived for over 100 days at 10°C. Kudva *et al.* (1998) found that *Escherichia coli* O157:H7 survived for 630 days. Survival of some enteric viruses may by greater than one year; especially at temperatures below 10. Probable survival times ranged from 20.7 to 23.3 months. The highest concentrations of pathogens may occur up to 50 m from spray source (Sorber *et al*., 1976; Sorber *et al*., 1984; Teltsch and Katzenelson, 1978). In most cases it appears that 60 - 90 days are sufficient for reduction of pathogens to negligible numbers once they have been to the soil, although survival times as long as five years have been reported (Mubiru *et al.* (Agronomy Notes) and JFP Engelbrecht and Murphy, K, O'H (CSIR Report). Sjogren (1994) estimated survival times by extrapolating the die-off curve to zero counts of bacteria. Few studies that have found; indicator organisms appear to survive longer than pathogens (Mubiru *et al*., 2000). Continued land application of organic wastes may modify the soil environment to the point where it is a more hospitable environment for pathogenic organisms (Dazzo *et al*., 1973). Few studies that have been conducted that indicator organisms have a paramount appearance to survive (Table 2) when comply with pathogens (Mubiru *et al*., 2000).

Factors affects survival:

There are numerous factors that may influence the survival of enteric bacteria in soil (Gerba *et al*., 1975). However, attempts to link survival rates of specific enteric

Table 2: Survival time of indicator pathogens

Microorganisms	^{0}C, m.b./l	T_m, day
Typhoid salmonella	10^2	50-65
	10^4	<120
Paratyphoid B Salmonella	10^2	≥220
	10^4	74-400
Dysentery shigellos	10^2	<174
	10^4	>300
Poliomyelitis viruses	10^{3*}	116
Phages Escherichia coli	10^{3*}	400
Colibacillus and *Enterococci*	10^5-10^6	400

* BOE (base of ear)/ml.

pathogens with soil physico-chemical and environmental variables (Reddy *et al*., 1981) are limited. Additionally, few studies have attempted to verify the assumption that mortality rates of indicator organisms accurately reflect the pathogenic bacterial species (Crane *et al*., 1981; Filip *et al*., 1988). The two to seven year time frame should ensure a 7 log reduction of enteric viruses. Pathogen removal is not adequate at depths of 0.6 of the drain field above the water table (Scandura and Sobsey, 1997; Nicosia *et al*., 2001). Thus, the 1.52 m distance provides additional retention needed for virus removal. The increase depth to groundwater is justified with higher percolation rates as the greater the percolation rates the less removal of pathogens (Pang, 2003). Factors that affect the dispersion and survival of aerosolized microorganisms include solar radiation, humidity and atmospheric stability. Temperature and wind velocity did appear to be significant factors affects the fate of the microorganisms (Teltsch and Katzenelson,1978).

Some of the researchers have suggested that the principal factor affects the survival of enteric bacteria in soil systems is the moisture status (Gerba *et al*., 1975; Tate, 1978; Kibbey *et al.*, 1978; Crane *et al*., 1981; Reddy *et al.*, 1981; Faust, 1982; Mubiru *et al*., 2000; Entry *et al*., 2000b and Entry *et al.*, 2000a) suggested that limited moisture availability in the soil reduces the survival rates of enteric bacteria in manure amended soil systems. Tate (1978) observed that the survival of *Escherichia coli* in an organic soil over an 8-day period after manure application was three fold greater than the sandy soil. Chandler *et al.* (1981) was found that topsoil was the most favourable environment for bacteria and the times required for a 90 per cent reduction in initial bacteria concentrations ranged from 7 to 20 days in topsoil. Zhai *et al*. (1995) reported that fecal bacteria have greater survival rates in top soil than the subsoil. Mubiru *et al.* (2000) stated that long as long there is enhancing moisture retention in fine soil particles; bacterial could increase the survival rate because of the increased ability to retain nutrients. The majority of the literature suggested that an inverse relationship appears to exist between temperature and bacterial mortality (Gerba *et al*., 1975; Reddy *et al.*, 1981) reported that higher temperature decrease the survival times of fecal bacteria. Van Donsel *et al.* (1967) found that 90 per cent of coliform bacteria died within 3.3 days of land application in the summer compared to 13.4 days in the winter. The review compiled by Reddy *et al.* (1981) found that die-off rates approximately doubled with a 10°C increase in temperature. Kibbey *et al.* in 1978 reported that FS has optimum survival in cold conditions but freezing and thawing of soil reduces bacterial populations. Filip *et al*. (1988) determined that *Escherichia coli* could survive for over 100 days in water-soil mixtures kept at 10°C. *Escherichia coli* survived longer in sheep and cattle manure at temperatures below 23°C (Kudva *et al.*, 1998). Enteric bacteria have a shorter survival period in soils possessing a low pH (Gerba *et al.*, 1975; Ellis and McCalla, 1976) with pH of 6 to 7 being optimum for bacterial survival (Cuthbert *et al.*, 1955; Reddy *et al*., 1981). Sjogren (1994) found that *Escherichia coli* survived longer at a neutral to alkaline pH soil than the acidic pH soils of similar texture and organic matter content.

Concentrations of indicator and pathogenic organisms in animal waste vary widely depending on animal type, waste storage system and the level of pretreatment prior to land disposal (Reddy *et al*., 1981 and Crane *et al*., 1986). Patni *et al.* (1985) found that long-term storage of manure decreased median counts of *fecal coliform, fecal coliform* and *fecal streptococci* by 99 per cent, however, these reductions did not occur when fresh manure was added to old manure. Competing microorganisms will limit pathogen survival in soil (Reddy *et al.*, 1981). Ellis and McCalla (1976) concluded that indigenous soil organisms were resistant to new micro-organisms in their environment. It also has been found that certain bacteriophage and free-living soil organisms, such as *Bdellovibrio,* can parasitize *Escherichia coli* cells, thus, limiting their survival (Klein and Casida, 1967).

Major monitoring and detection method for analysis of microbial contamination of groundwater:

Monitoring of bacterial contamination:

The monitoring of microbial population present in wastewater and groundwater are the major concern of the review, where it is signify the best detection techniques and method. There are certain methods of monitoring the microbes in a very fine scale, especially molecular technique where the detection limit are under molecular level. Molecular methods targeting nucleic acids, such as DNA/ RNA fingerprinting, hybridization, or PCR, are still more expensive than traditional culture techniques, which may be labour intensive and time consuming and also do not provide definitive information on viability and infectivity (Sobsey *et al.*, 2006). To address these problems, hybrid protocols that

involve a combination of culture-based enrichment and molecular assays have been developed and are now commercially available (BAX assays, Qualicon, DE) (Ricke *et al.,* 1998). These tests measure biochemical changes in media rather than colony formation (*i.e.* the hydrolysis of MUG, 4-methylumbelliferyl b-D- glucuronide, a specific indicator of *Escherichia coli*) (Zhai *et al*., 1995).There is a need for the development of rapid and effective sample processing protocols to detect specific pathogens that may be in low numbers and tightly bound to particulates in waste (Pillai and Ricke, 2002). Van Poucke and Nelisk (2000) evaluated an enzymatic membrane filtrate technique (bacterial cells are treated with reagents to induce the enzyme β-D-glucuronidase) by using a laser-scanning device to reduce the analysis time. Pyle *et al.* (1999) used a combination of IMS and solid phase laser cytometry for the detection of *Escherichia coli* O156:H7 spiked in water. Concentration steps use magnetic beads coated with anti-O157 rabbit serum and a magnetic separation. Culturable cells were counted by membrane filtration and identified by an immunofluorescence assay using a scanning device. Fluorescent *in situ* hybridization (FISH) technique has been used for the detection of *Escherichia coli* in spiked microcosm (Shi *et al*., 1999) and urine, rivers, sewage and food samples (Regnault *et al.*, 2000). The rRNA content of a bacterium does not completely reflect its physiological status because rRNA molecules can remain for a relatively long period after the loss of culturability (McKillip *et al*., 1998). However, FISH is currently considered as a highly specific detection method and relatively easy method to perform (Rompre *et al*., 2002). Deininger (2002) proposed a rapid determination technique for pathogenic bacteria in Surface Waters. The review investigated the feasibility of IMS and ATP bioluminescence to detect *Escherichia coli* in beach samples rapidly. The entire procedure can be done within one hour without an enrichment step.

The following are brief descriptions of methods used in a number of occurrence studies including detection methods for the bacterial indicators found in focally-contaminated groundwater.

–The time from sample collection to initiation of the analysis may not exceed 30 hours. Systems are encouraged, but not required, to hold samples below 10° C during transit.

–Sample volume is 100 mL for all fecal indicators.

Monitoring viral contamination:

Enteric viruses refer to an important, but diverse, group of viruses found in the intestinal tract of humans and animals (Theil, 1990; Wilhelmi *et al*., 2003; Fong and Lipp, 2005). Enteric viruses are the commonest causes of gastroenteritis worldwide. They are most often transmitted via the fecal-oral route, direct human contact or via fomites (common) (Wilhelmi *et al*., 2003; Weitzel *et al.*, 2007) e.g. Astroviridae, Adenoviridae, Caliciviridae, Parvoviridae, Picornaviridae, Reoviridae etc. To determine the public health risk caused by human enteric viruses in water, a reliable, sensitive and practical method for detecting small concentrations of viruses is needed (Sobsey and Jones, 1979). Concentrating viruses in water by adsorption to and subsequent elution from a positively charged membrane is currently considered to be one of the most useful methods (American Public Health Association, 1995). This method has been applied to tap water (Nupen and Bateman, 1985; Sobsey *et al*., 1985), groundwater (Abbaszadegan *et al*., 1993), river water (Logan *et al*., 1980; Logan *et al*., 1981), lake water (Logan *et al*., 1981), secondarily treated sewage (Shri and Gerba, 1983) or marine water. The virus concentrations are determined by conventional plaque

Indicator	Method
Escherichia coli	Colilert test (Standard method 9223B) Chromogenic substrate
	Colisure test (Standard method 9223B) Chromogenic substrate
	Membrane filter method with MI agar (EPA method 1604)
	m-Coli blue24 test
	E*Colite test
	EC-MUG (Standard method 9212F; SMWW, 20th ed.) or the NA-MUG
	(Standard method 9222G, SMWW, 20th ed.) as a confirmation step after
	the multiple-tube fermentation (Standard method 9221A,B,C,D);
	Membrane filter technique (Standard method 9222 A,B,C)
Enterococci	Multiple-tube technique (Standard method 9230B)
	Membrane filter technique (Standard method 9230C/EPA method 1600)
	Enterolert
Coliphage	Two-step enrichment presence-Absence procedure (EPA method 1601)
	Single agar layer procedure (EPA method 1602)

assays (Logan *et al*., 1980; Logan *et al*., 1981; Nupen and Bateman, 1985; Shri and Gerba, 1983; Sobsey and Jones, 1979; Sobsey *et al*., 1985).

The majorities of immunological methods for enteric viruses is based on antigen detection and employ enzyme immunoassay (EIA), latex agglutination, or immune-chromatography technologies (Richards *et al*., 2003). Most enteric viruses, apart from adenoviruses and parvoviruses are RNA viruses and most molecular techniques, therefore, rely on detecting specific viral RNA sequences (Carter, 2005). By focusing on the viral genome, molecular methods can improve specificity and sensitivity significantly (Thermo Scientific Oxoid Expect). A number of nucleic acid hybridization methods have been developed for enteric viruses, including dot-blot (Takiff *et al*., 1985) and sandwich hybridization techniques (Virtanen *et al*., 1983), but their sensitivity is often little better than that of conventional techniques. Techniques that allow the amplification of target nucleic acid sequences have revolutionized that enteric virus detection in clinical and non-clinical samples. The principal amplification technique used for RNA viruses is reverse-transcriptase polymerase-chain reaction (RT-PCR) (Giuseppina *et al*., 2010). Theoretically, this technique is capable of detecting a single virus within 24 hours and detection of the amplified sequence may be done at the reaction endpoint, or by continuous monitoring (real-time PCR) (Li *et al*., 2002). Multiplex PCR methods have been developed that allow the detection of more than one nucleic acid sequence and, therefore, more than one virus type, in the same assay (Thermo Scientific Oxoid Expect).

Other nucleic acid amplification methods like nucleic acid sequence base amplification (NASBA) have also been developed. This technique is also known as isothermal amplification, as it does not require the repeated temperature cycling used in conventional PCR methods (Lok-Ting Lau *et al*., 2004). The advantages of PCR and related methods are considerable. The main benefit is time saving – 24 hours to a result rather than several days or weeks for a cell culture assay (Clem *et al*., 2007; Morteza *et al*., 1999). Pollard (2012) had quantified the viral abundance in water by using SYBR Gold added to wastewater and viral numbers determined with direct counting using epifluorescent microscopy. This technique differentiates the nucleotide size fractions that are stained with SYBR Gold to show only those associated with Viral DNA and RNA. Except all of the above there are some other commercialized detection techniques as described below:

Male-Specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure (EPA Method 1601, EPA 2001d):

This test is a quantitative method, but was validated by EPA only as a qualitative presence-absence test for coliform.

Male-Specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure (EPA Method 1602, EPA 2001e):

This method is a quantitative procedure that uses a single agar layer technique for determining coliphage density in groundwater samples. The quantity of coliphage in a sample is expressed as plaque forming units (PFU/100 mL).

Coliphage Detection (Standard Method 9224, APHA 1999):

This method is not EPA-approved for groundwater rule (GWR) monitoring, but was used in a number of key studies. The number of plaques is used to calculate the coliphage density. Somatic and male-specific coliphage can be differentiated by the type of *Escherichia coli* host used.

Conclusion:

Major sources for the groundwater contamination are animals and urban wastes (Domestic effluent consisting of black water, excreta, urine and associated sludge) that may lead to mix-up of the fecal contamination into water aquifers. Silt and clay particles and municipal waste land application sites are very effective for physically filtering bacterial into the water aquifers. The diversity of bacteria as well as viruses may show the multiplicity distance from surface to groundwater body. Viruses can travel 30 meter distance in the subsurface, while 60 meter should be enough distance to meet bacterial standards for recreational waters and reduce viruses by greater than 7 logs. Studies have indicated that survival of some enteric viruses may by greater than one year, especially at temperatures below 10^0C, while the two year time frame should ensure a 7 log reduction of enteric viruses.

The most sensitive and rapid technique to monitoring the fecal and non-fecal coliform are hybrid protocols that involve a combination of culture-based enrichment and molecular assays have been developed and are commercially available (BAX assays, Qualicon, DE). A latest technique proposed by Deininger (2002) is based on feasibility of IMS and ATP bioluminescence to detect *Escherichia coli* in beach samples rapidly. However, determining infectivity generally require the use of cell-culture, although other alternatives have been suggested such as detection of oxidative damage in the viral capsid-protein (Hamza *et al*., 2011). Molecular methods specifically nucleic acid amplification technique (PCR, Real time PCR) are fast and in contrast to cell culture assays can be used to detect and quantify all known viruses (Mackay *et al*., 2002). A recent study, however, supported that the viral copy number of enterovirus genes can be correlated to the amount of infectious viruses (Donia *et al*., 2010).

Research recommendation:

Developing new technologies that will ultimately help manage ground-water resources more effectively. Work done in aquifers throughout the country suggests that microbes play important roles in remediating aquifers contaminated by specific compounds. However, they also can mobilize other undesirable constituents, such as arsenic, depending upon the geochemistry of the aquifer." By better understanding these processes, the research will be able to advise resource managers about aquifer-remediation techniques and also help regulators determine guidelines for effective ASR (Aquifer Storage and Recovery (ASR) practices. The risk from fecal contamination of recreational and drinking water sources should be acknowledged by both farmers and municipal authorities controlling public water systems.

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