

Rapid detection of bacteria in drinking water and water contamination case studies

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Abstract Water systems are inherently vulnerable to physical, chemical and biologic threats that might compromise a systems' ability to reliably deliver safe water. The ability of a water supply to provide water to its customers can be compromised by destroying or disrupting key physical elements of the water system. However, contamination is generally viewed as the most serious potential terrorist threat to water systems. Chemical or biologic agents could spread throughout a distribution system and result in sickness or death among the consumers and for some agents the presence of the contaminant might not be known until emergency rooms report an increase in patients with a particular set of symptoms. Even without serious health impacts, just the knowledge that a water system had been breached could seriously undermine consumer confidence in public water supplies. Therefore, the ability to rapidly detect contamination, especially microbiological contamination, is highly desirable. The authors summarize water contamination case studies and discuss a technique for identifying microbiological contamination based on ATP bioluminescence. This assay allows an estimation of bacterial populations within minutes and can be applied using a local platform. Previous ATP-based methods requires one hour, one liter of water, and has a sensitivity of 100000 cells for detection. The improved method discussed here is 100 times more sensitive, requires one-hundredth of the sample volume, and is over 10 times faster than standard method. This technique has a great deal of potential for application in situations in which a water system has been compromised.

Keywords drinking water, bacteria, waste water treatment plants

1 Introduction

Water systems are spatially diverse and are therefore inherently vulnerable to physical, chemical and biologic threats that might compromise a systems' ability to reliably deliver safe water. Community water supplies are designed to deliver water under pressure and generally supply most of the water for fire fighting purposes. A loss of water or a substantial loss of pressure could, therefore, disable fire-fighting capability, interrupt service, and disrupt public confidence. This loss might result from sabotaging pumps that maintain flow and pressure, or disabling electric power sources that might cause long-term disruption. Many of the major pumps and power sources in water systems have custom designed equipment and could take months or longer to repair and/or replace (Clark and Deininger, 2001).

Major areas of vulnerability include:

- Raw water source (surface or groundwater)
- Raw water channels and pipelines
- Raw water reservoirs
- Treatment facilities
- Connections to the distribution systems
- Pump stations and valves
- Finished water tanks and reservoirs.

Each of these system elements present unique challenges to a water utility in safeguarding water supply (Clark and Deininger, 2000) and water systems are vulnerable to both physical and/or contamination.

The ability of a water supply to provide water to its customers can be compromised by destroying or disrupting key physical elements of the water system. These elements include raw water facilities (dams, reservoirs, pipes and channels) treatment facilities, and distribution system elements (transmission lines and pump stations). Physical disruption may result in significant economic cost, inconvenience, and loss of confidence by customers, but

has a limited direct threat to human health. Exceptions to this generalization include: 1) destruction of a dam that causes loss of life and property in the accompanying flood wave; and 2) an explosive release of chlorine gas at a treatment plant. Water utilities should examine their physical assets, determine areas of vulnerability, and increase security accordingly. An example of such an action might be to switch from chlorine gas to liquid hypochlorite, especially in less secure locations which decrease the risk of exposure to poisonous chlorine gas. Redundant system components would provide backup capability in case of accidental or purposeful damage to facilities.

Contamination is generally viewed as the most serious potential terrorist threat to water systems. Chemical or biologic agents could spread throughout a distribution system and result in sickness or death among the consumers and for some agents the presence of the contaminant might not be known until emergency rooms report an increase in patients with a particular set of symptoms. Even without serious health impacts, just the knowledge that a group had breached a water system could seriously undermine consumer confidence in public water supplies (Clark, 2002). Accidental contamination of water systems has resulted in many fatalities. Examples of such outbreaks include cholera contamination in Peru (Clark et al., 1995), *Cryptosporidium* contamination in Milwaukee, Wisconsin (US) (Fox and Lytle, 1996), and *Salmonella* contamination in Gideon, Missouri (US). In Gideon, the likely culprit was identified as pigeons infected with *Salmonella* that had entered a tank's corroded vents and hatches (Clark et al. 1996).

2 Waterborne pathogens

Waterborne pathogens have been recognized as a threat to human public health throughout history, but the development of drinking water treatment techniques has controlled this threat since the beginning of the twentieth century. Although modern drinking water treatment has virtually eradicated waterborne disease from developed countries, drinking water treatment systems have been identified as a potential security vulnerability.

Water-related microbial pathogens can be categorized as water-based or waterborne pathogens. Water-based pathogens spend part of their life cycle to reach and infect a potential host. An excellent example of a water-based pathogen is malaria for which mosquitoes are a vector. Since water-based pathogens are not transmitted totally through water they are not potential agents of bio-terrorism.

However, waterborne pathogens are those transmitted through ingestion of contaminated water primarily through the fecal-oral route. In this case water acts as a passive carrier of infectious agents. Some waterborne pathogens

that can cause problems in drinking water include: *Campylobacter jejuni*, pathogenic *Escherichia coli*, *Yersinia enterocolitica*, enteric viruses such as rotavirus, calicivirus, astrovirus, and parasites such as *Giardia lamblia*, *Cryptosporidium parvum* and Microsporidia. Some species of environmental bacteria have demonstrated the ability to survive in drinking water biofilms and have been identified as opportunistic pathogens including *Legionella* spp., *Aeromonas* spp., *Mycobacterium* spp., and *Pseudomonas aeruginosa* (Geldreich et al., 1992; Rice et al., 1999; Abbaszadegan and Alum, 2004).

Bacterial pathogens can cause gastroenteritis including cramps, diarrhea, nausea, vomiting, chills, and mild fever. Bacterial pathogens are generally sensitive to disinfectants such as chlorine and include (Clark and Deininger, 2000; Clark and Deininger, 2001; Abbaszadegan and Alum, 2004; Field, 2004):

- *Salmonella*
- *Shigella*
- *Escherichia coli* O157:H7
- *Yersinia*
- *Vibrio*
- *Campylobacter*
- *Legionella*.

Viral pathogens can pose a 10 to 10000 fold higher infection risk than bacteria. Important waterborne viral pathogens include:

- *Adenovirus*
- *Astroviruses*
- *Hepatitis A*
- *Hepatitis E*
- *Norovirus*
- *Rotaviruses*.

Parasitic pathogens are a significant threat to drinking water supplies. Nearly 20000 protozoan parasites have been identified of which 20 genera are known to cause disease in humans including:

- *Acanthamoeba*
- *Cryptosporidium parvum*
- *Entamoeba histolytica*
- *Microsporidia*
- *Naegleria*.

In general, the most effective mechanism for controlling these pathogens is disinfection, especially chlorine. In addition to general pathogens of concern in water supplies some pathogens can be categorized as biologic warfare agents. Some of the organisms that have potential use in bio-terrorism are discussed below (Burrows and Renner 1998, Burrows and Renner 1999; Clark and Deininger, 2000; Clark and Deininger, 2001).

3 History of water system contamination

As discussed by Clark (2011) the recorded history of attacks on water systems dates from 4500 years ago

(Gleick, 2006). Urlama, King of Lagash and his son Illater cut off the water supply to Girsu, a city in Umma during the period 2450 to 2400 BC. In New York in 1748 an angry mob burned down a ferry house on the Brooklyn shore of the East River. It is reported that this act was revenge for unfair allocation of East River water rights. Small groups attacked small dams and reservoirs in the 1840s and 1850s in the eastern and central US due to concerns about threats to health and to local water supplies. In the Owens Valley of California between 1907 and 1913 farmers repeatedly dynamited the aqueduct system being built to divert their water to the growing city of Los Angeles.

Bacillus anthracis, a spore-forming bacterium, which has been weaponized for aerosol application was used by the Japanese Army during World War II to contaminate food and water supplies of Chinese cities (Williams and Wallace, 1989; Burrows and Renner, 1999). Abdominal pain, fever, vomiting, bloody diarrhea and shock are the principal manifestations of this form of the disease, which has an incubation period of 2–7 days. Anthrax spores are easily removed by any water treatment filter system with pore size $< 1 \mu\text{m}$. The Japanese Army also used a number of other organisms to contaminate food and water including: the bacterium *Vibrio cholera*; plague a disease of rodents, both wild and domestic, caused by *Yersinia pestis* and transmissible to humans is generally considered to be a threat in water as well; *Salmonella typhimurium* often found in outbreaks of food poisoning; and *Shigella dysenteriae*. Mycotoxins, T-2 toxin is one of several trichothecene mycotoxins isolated from cereal grains infected with *Fusarium* and some other genera of fungi. Russian experience with infected agricultural products indicates that ingested trichothecenes could impose a deadly threat (Burrows and Renner, 1999). Unconfirmed and controversial findings suggest the use of trichothecenes as biological warfare agents in Laos, Cambodia, and Afghanistan, and Iraq has investigated the weaponization of trichothecenes. Other trichothecenes, viz., nivalenol, 4-deoxynivalenol, and diacetoxyscirpenol, may be present in crude preparations; their toxicities are probably similar to but no greater than that of T-2.

In the fall of 1996, we sampled drinking water in Washington DC. A sample of high bacterial level is shown in Fig. 1.

The drinking water supply was overchlorinated but otherwise safe. Tests in other places also showed contamination, but not above the allowable levels in Washington DC (see Figs. 1 and 2).

There have been many examples of contamination and potential water supply contamination in the US including water borne outbreaks. For example, in New York City, low levels of plutonium were found in the drinking water (in the order of 20 femtocuries). The usual background is below 1 femtocurie. However, a person would have to



Fig. 1 Fountain in the center of Washington DC with the capitol in the background



Fig. 2 Analyzing for bacteria levels in front of the Smithsonian Institute

drink several million liters of water to acquire a lethal dose estimated at about 100 microcuries.¹⁾ Another case was the contamination of salad bars in the Dalles, Oregon, by the Rajneeshee religious cult, using vials of *S. typhimurium*, which is a highly toxic bacterium frequently carried by birds. The cult also contaminated a city water supply tank using *Salmonella*. A community outbreak of salmonellosis resulted in which at least 751 cases were documented in a county that typically reports fewer than five cases per year. The cult apparently cultured the organisms in their own laboratories (Clark and Deininger 2000; Gleick, 2006). Monitoring of chlorine residuals is not a universal practice, but it can be done at minimal cost. On July 10th, 1986, the water supply to the presidential rooms of the White House was cut off after a monitor indicated a lack of chlorine. President Reagan got his morning coffee anyhow, using bottled water. The supply to the West Wing was not shut

1) A femtocurie is nine orders of magnitude smaller than a microcurie (Clark and Deininger, 2000)

off, but the staff was warned not to drink the water (New York Times, 1986). Strategically placed monitors in a distribution system provide one solution for the protection of a water supply system.

3.1 Cabool, Missouri case study

Cabool, located in the South-eastern corner of Missouri, experienced a large outbreak of *E. coli* O157:H7 during the winter of 1989–1990. Cabool a town of approximately 2100 people, experienced a waterborne disease outbreak in which 243 cases were reported, with 32 hospitalizations and four deaths. This was the largest waterborne outbreak of *E. coli* O157:H7 that had been reported in the US. At the time of the outbreak, the water source was untreated groundwater. Shortly after the outbreak, the USEPA sent a team to conduct a research study to determine the underlying cause of the outbreak.

Exceptionally cold weather prior to the outbreak contributed to two major water system line breaks and 43 water meter replacements throughout the city area. The sewage collection lines in Cabool were generally located away from the drinking water distribution lines, but did cross or were near water lines in several locations. At the time of the outbreak, storm water drained via open ditches along the sides of the streets and roads. During heavy rainfalls, sewage was observed to overflow manhole covers and flow into streets, parking lots, and residential foundations.

The town's water system (untreated groundwater) was implicated in the outbreak. Two of the town's four wells were operating at the time of the outbreak: one was 305 m deep and the other was 396 m deep. Both wells had protected wellheads, and the monitoring data from the 10 years before the outbreak indicated that no coliforms had been detected in either well. Investigation of the outbreak indicated that the distribution system was not well maintained and was vulnerable to sewage contamination at several points. Approximately 35% of the total flow was lost in the system — suggesting leaks, inaccurate meters, or unmetered connections. The town sewer system was also in poor condition and operating beyond capacity, resulting in regular sewage back-ups and overflows.

A number of risk factors apparently contributed to this outbreak. In mid-December 1989, unusually cold weather caused two large water mains and 45 in-ground water meters to fail. Ten cases of bloody diarrhea were reported to the local health department on January 4th, 1990. A boil-water order was issued on January 5th, and water chlorination was initiated on January 12th. Analyses of the temporal distribution of the cases indicated that the first cases occurred seven days before the first water main break (December 23rd), and the last case occurred three days after the implementation of water chlorination. The early cases may have been due to leaks and holes that developed prior to the main break. There was a small increase in the

incidence of diarrhea after the first main break and a large increase in diarrhea cases about four days after the second main break on December 26th.

Hydraulic and water quality models were applied to examine the movement of water and contaminants in the system. Steady-state scenarios were examined and a dynamic analysis of the movement of water and contaminants associated with meter replacement and the aforementioned breaks was conducted. Typical demand patterns were developed from available meter usage for each service connection and it was found that the water demand was 65% of the average well production, indicating inaccurate meters, unmetered uses, and a high water loss in the system.

The modeling effort revealed that the pattern of illness occurrence was consistent with water movement patterns in the distribution system assuming two water line breaks. It was concluded, therefore, that some disturbance in the system, possibly the two line breaks or 43 m replacements, allowed contamination to enter the water system. Analysis showed that the simulated contaminant movement covered 85% of the infected population.

Replacement of the failed water meters may have contributed to contamination of the distribution system. During the replacement of the meters and main break repairs, the lines were subjected to “limited flushing” but were not disinfected by super-chlorination, and no water samples were tested for microbial indicators to examine the water quality before bringing the lines back into service. Although sewage overflow into the distribution system via the main breaks and intrusion was believed to be responsible for the outbreak, microbial contamination of the distribution system could not be confirmed because water samples from the distribution system were never collected and analyzed. Hydraulic modeling of the system reinforced the evidence that the second main break had the potential to contaminate a greater portion of the distribution system, including the northern part of the town where 36% percent of the cases occurred (Geldreich, et al. 1992).

3.2 Gideon, Missouri case study

In 1993, the town of Gideon, Missouri suffered from an outbreak of salmonellosis that affected more than 650 people and caused seven deaths (Hrudey and Hrudey, 2004). At the time of the outbreak, Gideon was a small town (population 1100) in a rural-agricultural area with an unemployment rate greater than 11%. Twenty five percent of the population was living below the poverty level at the time.

The Missouri Department of Health (MDOH) had identified 31 cases of laboratory-confirmed salmonellosis (Clark et al., 1996). The State Public Health Laboratories identified 21 of these isolates as dulcitol negative *Salmonella enterica* serovar *Typhimurium*. *Salmonella* is a pathogenic bacterium that has been classified into several

serotypes (common set of antigens). *Salmonella* serovar *Typhimurium* is among the most common *Salmonella* serovars causing salmonellosis in the US. Fifteen of the 31 laboratory culture-confirmed patients were hospitalized (including two patients hospitalized for other causes and who developed diarrhea while in the hospital). These 15 patients were admitted to 10 different hospitals. Seven nursing home residents exhibiting diarrheal illness died; four of these patients were culture confirmed (the other three were not cultured). Two of the patients had positive blood cultures. Interviews conducted by the MDOH during this period suggested that there were no food exposures common to a majority of the patients. However, all of the ill persons, including the culture-confirmed patients, had consumed municipal water which supported the association. The MDOH reported their suspicion to the Missouri Department of Natural Resources (MDNR).

The Gideon municipal water system was originally constructed in the mid-1930s and obtained water from two adjacent, 1300-ft-deep wells. The well waters were not disinfected at the time of the outbreak. After the outbreak emergency, chlorination was initiated, and later a permanent chlorination system was installed. The distribution system consisted primarily of small-diameter (2-, 4-, and 6-inch) unlined, steel and cast iron pipe. Tuberculation and corrosion were major problems in the distribution pipes. Raw water temperatures were unusually high for a groundwater supply system (14.4°C), because the system overlies a geologically active fault. Under low flow or static conditions, the water pressure was close to 50 psi. However, under high flow or flushing conditions the pressure dropped dramatically. These sharp pressure drops were evidence of major problems in the Gideon distribution system. The municipal system had two elevated tanks. One tank was a 50000 gallon (gal) tank (referred to as small tank) and the other was a 100000 gal tank (referred to as large tank).

In early November, a cold snap caused a thermal inversion in the water storage tanks that resulted in taste and odor problems. In response, the water system was systematically flushed on November 10th. The first cases of acute gastroenteritis were reported on November 29th and diagnosed as *S. typhimurium*. However, the outbreak investigation later revealed that diarrhea cases in Gideon started around November 12th with a peak incidence around November 20th. By early December, there was a 250% increase in absenteeism in the Gideon schools and a 600% increase in anti-diarrheal medication sales. Over 40% of nursing home residents suffered from diarrhea and seven people died (Angulo et al., 1997). However, the outbreak was not linked to the water system until December 15th when the water system samples were reviewed and investigative water sampling was initiated. A boil-water advisory was issued on December 18th. On December 22nd, emergency chlorination was added to the production well and the two municipal storage tanks were

superchlorinated. The last reported cases occurred on December 28th.

Water samples collected from the distribution system on December 16th, 17th, 20th, and 21st were positive for total coliforms, and the samples from December 20th and 21st were also positive for fecal coliforms. The outbreak strain of *S. typhimurium* was detected in one large volume sample collected from a fire hydrant. Inspection of the water storage tanks suggested that the outbreak was probably caused by contamination by bird feces in one or more of the tanks. The larger of the municipal tanks was in disrepair and had birds roosting on the roof. The private storage tank had an unscreened overflow pipe and a hole at the top of the tank that was large enough for birds to enter. This private tank had been drained on December 30th, but the outbreak strain of *S. typhimurium* was detected in samples of sediment collected on January 5th, 1994. The remaining water on the bottom of the tank was described as black and very turbid, with rust, suspended particles and bird feathers floating on the top. Initially attention was focused on the private tank as the source of the outbreak as reported by Skala (1994). On January 14th, 1994, an EPA field team, in conjunction with the CDC and the State of Missouri, initiated a field investigation that included a sanitary survey and microbiological analyses of samples collected on site. A system evaluation was also conducted in which EPANET was used to develop various scenarios to explain possible contaminant transport in the Gideon system.

Although the private tank was initially suspected as the cause of outbreak an in-depth hydraulic analysis of the Gideon system, conducted as part of the outbreak investigation, raised questions about the possibility of the private tank being the source of the outbreak. A subsequent review of as-built drawings of the Gideon system by MO DNR personnel revealed that the private tank was separated from the municipal system by a backflow prevention valve, but there were conflicting reports about whether this valve was open or closed when the tank was first inspected. To test the integrity of the valve a “fire-pumper” was brought to the site and it was found that the backflow prevention valve held under a pressure of 100 psi. In a subsequent hydraulic analysis the private tank was eliminated as a contamination source for the outbreak. The analysis demonstrated that elimination of the private tank yielded results that were consistent with the behavior of the system as observed during the outbreak scenario. This analysis also pointed to the largest municipal tank as the most likely source of the outbreak. A visual inspection of the large municipal tank revealed broken and rusted hatches, bird parts and feathers on the top of the tank and bird parts and feathers floating on the surface of the tank water. Therefore, the subsequent EPA field investigations and modeling efforts focused on the two municipal tanks as the source of contamination.

The key analysis was focused on a flushing program

conducted earlier by the utility in response to taste and odor complaints. A sequential flushing program was conducted on November 10th, 1993, involving all 50 hydrants in the system. The flushing program was started in the morning and continued through the entire day. Each hydrant was flushed for 15 min at an approximate rate of 750 gallons per minute (gpm). It was observed that the pump at one of the wells was operating at full capacity during the flushing program (approximately 12 h), which would indicate that the municipal tanks were discharging during this period.

During the evaluation, it was hypothesized that the taste and odor problems may have resulted from a thermal inversion that had taken place due to a sharp temperature drop prior to the day of the complaint. If stagnant or contaminated water were floating on the top of a tank, a thermal inversion could have caused this water to be mixed throughout the tank and to be discharged into the system resulting in taste and odor complaints (Fennel et al., 1974). As a consequence, the utility initiated the aforementioned city-wide flushing program. Turbulence in the tank from the flushing program could have stirred up the tank sediments that were subsequently transported into the distribution system. It is likely that the bulk water and/or the sediments were contaminated with *Salmonella* serovar *Typhimurium*. During the EPA field visit, a large number of pigeons (bird droppings are known to contain *Salmonella*) were observed roosting on the roof of the 100000 gal municipal tank.

The EPA study team evaluated the effects of distribution system design and operation, demand, and hydraulic characteristics on the possible propagation of contaminants in the system. Given the evidence from the laboratory samples and the results from the valve inspection of the private tank, it was concluded that the most likely contamination source was bird droppings in the large municipal tank. Therefore, the analysis concentrated on propagation of water from the large municipal tank in conjunction with the flushing program. Other possible sources of contamination, such as cross connections were also studied, but ruled out as a source of the outbreak.

The system layout, demand information, pump characteristic curves, tank geometry, flushing program, and other information needed for the modeling effort were obtained from maps and demographic information and numerous discussions with consulting engineers and city and MDNR officials. EPANET was used to conduct the contaminant propagation study (Rossman et al., 1994).

The EPANET network model was calibrated by simulating flushing at the hydrants assuming a discharge of 750 gpm for 15 min. The “C” factors (pipe roughness) were adjusted until the head loss in the model matched head losses observed in the field. After the calibration, the hydraulic model was simulated for 48 h. Thereafter, the flushing program was simulated starting at 8 a.m. on day 3, by sequentially imposing a 750 gpm demand on each

hydrant for 15 min. Utilizing the TRACE option in EPANET, the percentages of water from both municipal tanks were calculated at each node over a period of 72 h.

During the simulation of the flushing program, the pump at one of the wells was operated (as previously observed) at full capacity, which was over 800 gpm, and then reverted to cyclic operation. The simulation results showed that the tank elevation fluctuated for both municipal tanks, and both the tanks discharged during the flushing program. At the end of the flushing period, nearly 25 % of the water from the large municipal tank passed through the small municipal tank where it was again discharged into the system. The model predicted dramatic pressure drops during the flushing program. Based on the information available, it was felt that these modeling results replicated the conditions that existed during the flushing program closely enough to provide a basis for an analysis of water movement in the system.

Data from the simulation study, the microbiological surveillance data, and the outbreak data were utilized to provide insight into the nature of both general contamination problems in the system and the outbreak itself. The water movement patterns showed the majority of the collected samples that were total coliform and fecal coliform (FC)-positive occurred at points within the zone of influence of the small and large tanks. During both the flushing program and for large parts of normal operation, these areas were predominately served by tank water, which confirmed the belief that the tanks are the source of the fecal contamination since there were positive FC samples prior to chlorination.

3.3 Walkerton, Canada case study

The first documented outbreak of *E. coli* O157:H7/*Campylobacter* spp. gastroenteritis associated with a municipal water supply in Canada occurred in the small rural town of Walkerton, Ontario (population 1261) in May 2000 (The Walkerton Herald Times, 2000; Grayman et al., 2004). At the time of the outbreak, the town's drinking water was supplied by three wells (Wells 5 — 7), which fed a common distribution system. A hydro-geological assessment revealed that two of the three wells (Wells 5 and 6) were under the influence of surface water. A microbiological investigation found *E. coli* O157:H7 and *Campylobacter* spp. on a cattle farm adjacent to Well 5 with identical molecular characteristics to those isolated from outbreak victims. The water supply pumped by generator to the local hospital is seen in Fig. 3.

In order to understand the factors that caused the outbreak a water quality model of the Walkerton water distribution system was developed. Using a “cross-sectional” study, it was demonstrated that during the outbreak, residents living in homes connected to the municipal water supply and consuming Walkerton water were 11.7 times more likely to have developed gastro-



Fig. 3 Water supply pumped by generator to the hospital in Walkerton

enteritis than those not exposed to Walkerton water.

Modeling of the Walkerton water system involved estimating the following parameters for use in WaterCAD (a proprietary water quality model):

- Pipe diameter and length, location, age, and composition of all water pipes
- Size, storage capacity, and active volumes of the two stand pipes (water towers) in the system
- Well pump specifications (including pump curves)
- Pipe friction.

The objective of the water modeling exercise was to recreate the pattern of water flow throughout the town's distribution system immediately before and during the outbreak period. The system was modeled as a network of nodes, which included water sources, tanks and demand (customer) locations. Individualized temporal water demand patterns were assigned to each commercial node and residential users were assigned to the nearest residential node. As residential users in Walkerton were not metered, hourly demand was estimated using the daily volume of water supplied to the system after accounting for commercial users and fire events, and literature based hourly demand patterns. Well pump controls were added to the model and pump on and off times were set in accordance with the historical pump records. Computerized data from the supervisory control and data acquisition (SCADA) system for the water supply containing 15-min pumage rates for the three wells were utilized. Exposure scenarios were created by adding a hypothetical inert contaminant to each well at predetermined times and concentrations and then using the model to follow the movement and relative concentrations of contaminants through the distribution system.

Conditional logistic regression was used to quantitatively evaluate the relationship between exposure to potentially contaminated well water, from each of Walkerton's three wells and the likelihood of experiencing infection. A

median incubation period of 2 to 5 days was assumed for both *E. coli* O157:H7 and *Campylobacter* for water supplied to homes 2 to 5 days prior to the illness onset date and potential exposure scenarios were calculated for each case. This was done for each of six exposure scenarios.

The results of this study clearly supported the hypothesis that Well 5 was the primary, if not the only, well involved in the Walkerton O157:H7/*Campylobacter* waterborne outbreak. The results also suggest that an extreme rainfall event, which occurred just prior to the peak of the outbreak, may have played a significant role in the propagation of the contaminants.

3.4 *Cryptosporidium* outbreak in Milwaukee

Milwaukee's *Cryptosporidium* outbreak started in March and April of 1993 (Fox and Lytle, 1996). Historically it was the largest US waterborne disease outbreak causing illness in more than 400,000 people. Milwaukee was served by two treatment plants, the Linwood Water Treatment Plant primarily served the northern part of the city, and the Howard Avenue Water Treatment Plant (HAWTP) served the southern part of the city. Analysis of the outbreak data indicated that the HAWTP was the source of the outbreak and consequently the plant was closed and a boil-water order was issued. The Linwood plant was able to serve the entire city.

4 Need for development of a rapid detection method

Because of the occurrence of waterborne outbreaks and the potential for intentional contamination in water systems, there is a great deal of interest in development of rapid techniques for the identification of potential water system contamination events (Deininger et al., 1997; Deininger and Lee, 1998; Lee and Deininger, 1999). Although most current microbiological methods focus on a single group of indicator organisms to measure the bacteriological safety of drinking water, there is important information that can be gained from determining the total number of heterotrophic bacteria in water samples. Many opportunistic pathogens are not in the coliform group and a high HPC has been shown to interfere with coliform determination. For example, in the Gideon waterborne outbreak high HPC counts were found in water samples prior to the outbreak and a rapid HPC method would have been helpful (Fig. 4).

The present HPC method using an R2A agar is known to be the most sensitive test for enumerating the bacteria from treated water. The only disadvantage of the test is that it takes seven days to complete and when the results are known, the water has been consumed. A test is needed to determine the total bacterial population in a very short time so that corrective actions can be taken in a timely manner.



Fig. 4 Luminometer and equipment for field application

The ATP bioluminescence assay allows an estimation of bacterial populations within minutes and can be applied using a local platform. The estimation of a bacterial count base on the ATP bioluminescence of the water is not new. Standard Methods (APHA-AWWA-WEF, 1995) indicates that the method requires one hour, one liter of water, and has a sensitivity of 100000 cells. This method is also described and specified in ASTM D 4012.81 (2003). However, the method discussed here is 100 times more sensitive, requires one-hundredth of the sample volume, and is over 10 times faster than the standard method. To validate the method a series of cooperative studies were conducted in collaboration with the Michigan Department of Community Health in Lansing. Several waters were tested and it was found that the method was valid (Michigan Department of Community Health, 2002).

5 Method development

5.1 Sample filtration

To prepare the water samples for testing a Filtravette, which is a combination of a filter and a cuvette with a filtration size of $0.45\ \mu\text{m}$, was placed in a Swinex filter holder (13 mm Millipore Corporation, Bedford, MA). A sterile syringe was used to extract the sample. Water extraction volumes varied between 0.1 and 10 mL, based on the expected number of bacteria in the water sample. The filter holder was screwed onto the syringe and the water sample was forced through the filter. The filtravette was taken from the filter holder and placed on a sterile plotter lying over a sterile plotting paper. The remaining water in the 3 mL syringe was removed with a specially converted 3 mL syringe by applying gentle positive air pressure.

5.2 ATP bioluminescence

A somatic cell releasing agent (New Horizon Diagnostic Corporation, Columbia, MD) was used to lyse all non-bacterial ATP through the filter. This was performed twice. At this stage the viltraette contains all the cell membranes intact on its surface after this procedure. The Filtravette was then inserted into a micro luminometer. At this stage, the filtravette retains the bacteria on its surface, and the bacterial ATP remains within the bacterial cell membranes. A bacterial cell releasing agent was then added to lyse the bacterial cells. Released bacterial cell ATP was then mixed with 50 mL of luciferine/luciferase (NHD, Columbia, MD) and the drawer of the micro-luminometer is closed. The light emissions are evaluated after a 10 s period and the light emission is recorded (RLU). The result was expressed as RLU/mL of by dividing the water volume.

The detection limit and the sensitivity of the luminometer were tested using a serially diluted ATP solution (NHD, Columbia, MD). Distilled de-ionized water was used for the dilution with a pH of 7.8. The activity of the ATP was checked, and it was found that the RLU are proportional to the amount of ATP, and it is proportional equivalent to the amount of viable material.

5.3 Bacterial enumeration: AODC, DVC, and HPC

The total (nonviable and viable) bacterial cells were determined from a formaldehyde fixed (2%, v/v final concentration) sample with the AODC method. Bacterial cells were stained with acridine orange (0.01% w/v, Fluka, Switzerland) after filtration onto a $0.2\ \mu\text{m}$ pore-size black polycarbonate membrane filter (Poretics, Livermore, CA). Cells were enumerated at a magnification of $\times 1000$ with an Olympic Provis epi-fluorescence microscope (Olympus Optical Co., Japan) equipped with a mercury arc lamp and a 400–490 nm excitation filter. The number of bacteria was counted in 10 microscopic fields using three subsamples and was then averaged. The number of bacteria per milliliter of sample was calculated using the equation in Standard Methods (American Public Health Association (APHA)), American Water Works Association (AWWA), Water Environment Federation (WEF), 1995).

The viable cells were counted by the DVC method. The sample were then incubated with yeast extract (0.005% w/v, Difco, Detroit, MI) and nalidixic acid (10mg/L, Sigma, St. Louis, Mo) without dilution for 24 h at 20°C . The modifications were used at a lower concentration of yeast extract and no dilution. After incubation, the fixation, counting and calculations of elongated bacteria was done following the AODC method.

The HPC was determined for each water sample in triplicate using a R2A medium (Difco, Detroit, Mi). The bacterial colonies were counted after an incubation period of 7 days at 28°C .

6 Method validation

The intent of the study was to determine if a rapid ATP assay could estimate bacterial populations in real water samples in a practical and timely manner (Lee and Deininger, 2001; Deininger and Lee, 2007). For quality control purposes and to test the accuracy of both the ATP and HPC test, a direct enumeration of the bacteria in a water sample was done using two epi-fluorescence methods. The AODC method was used to enumerate the total number of bacteria, which includes both the number of viable cells and the nonviable. The other method was the DVC method that selectively enumerates the viable cells.

6.1 Collection of water samples

Water samples were taken from drinking waters fountains or distribution systems in the US and abroad. Samples in the US were taken from locations in California, Colorado, Florida, Georgia, Illinois, Kentucky, Maryland, Michigan, New York, Ohio, Oregon, Tennessee, Texas, Washington, and Washington, DC. Some of the samples were taken from airports (California, Illinois, Kentucky, Maryland, New York, Oregon, Tennessee, Texas, and Washington) and others were obtained from cooperating utilities (Colorado, Florida, Georgia, Michigan, and Ohio).

Samples were also taken from Argentina, Austria, Australia, Brazil, Egypt, France, Germany, Hungary, Japan, Korea, Lithuania, Netherlands, Israel, Panama, Peru, Saudi Arabia, Switzerland, Ukraine, and the UK.

The ATP bioluminometer, a Profile 1 was used from New Horizon Diagnostics. Figure 4 shows the entire instrument. Everything fits on a small table. Samples from the US and the European Capitals are shown in Figs. 5 and 6. The US data and the overseas data show that the relationship can be described by

$$\lg \text{HPC}(\text{CFU}/\text{mL}) = 1.74 \times \lg \text{ATP}(\text{RLU}/\text{mL}). \quad (1)$$

For the countries overseas the relationship is

$$\lg \text{HPC}(\text{CFU}/\text{mL}) = 1.68 \times \lg \text{ATP}(\text{RLU}/\text{mL}). \quad (2)$$

The two relationships are very similar. This means that the US standard operations procedure can be used in any foreign country.

6.2 Public water supplies in France

To illustrate the application of the technique we report on a number of public drinking supplies taken in Paris, France in August of 1999 (Lee and Deininger, 1999). Samples were taken at the Charles de Gaulle Airport, the Eiffel Tower, Hospital Pitie, The Louvre, Notre Dame, Palais Chaillot, Sacre Coeur, and the UNESCO buildings. Data was collected on the metal concentrations and the French method of analysis for ATP was applied to the samples.

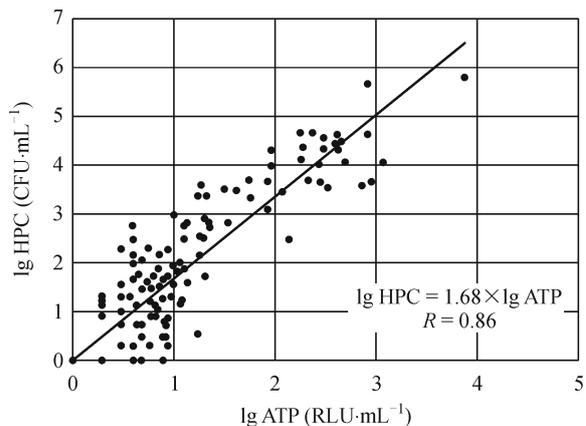


Fig. 5 ATP analysis of foreign water supplies

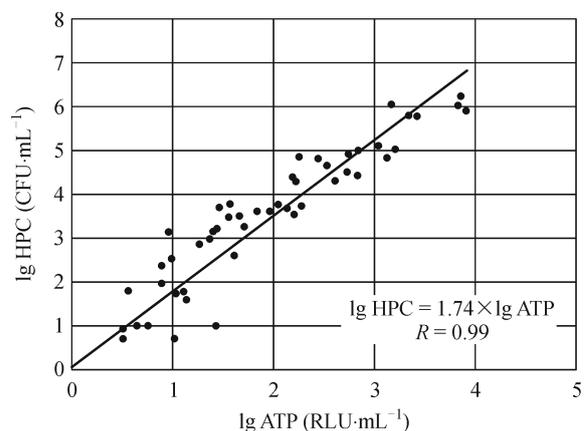


Fig. 6 ATP of domestic water supplies

The water samples were analyzed for their ATP and the metal content using ICP/MS. Table 1 shows the metal concentrations and Table 2 shows bacterial concentrations using three methods. The drinking water samples were analyzed using three methods. We used an ATP assay, the HPC using an R2A agar for 7 days, and a plate count using the French method. The method uses:

- Caseine peptone 5 g
- Yeast extracts 2.5 g
- Glucose 1 g
- 15 g/L of distilled water
- Incubated at 22°C for 3 days.

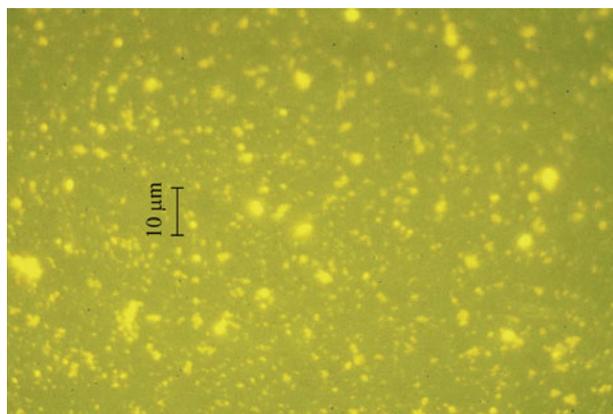
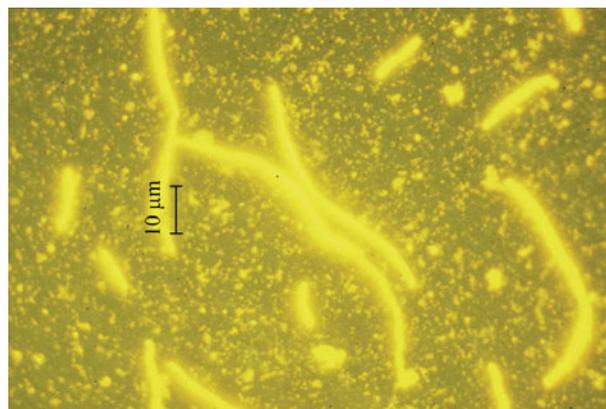
Since the water samples were in transit for about two days one should not place a great emphasis on the absolute number, but rather the relative relationships. The water quality varied significantly from location to the next location, and visual observation showed different patterns of colony size and colors. Figure 7 shows bacterial counts measured with AODC and Fig. 8 shows bacterial counts measured of DVC (elongated ones are living bacteria).

Table 1 Concentration of metals in Paris drinking water/(mg·L⁻¹)

Location	Pb	Mg	Al
Palais Chaillot	0.2	2659	1.1
Eiffel Tower	3.1	1454	1.5
Hotel St. Andre	5	1421	1.2
Notre Dame	3.6	1365	1.2
Parc Viviani	91	1655	2.6
Near Louvre	2.4	1647	1.9
Hospital Pitie	0.6	2400	13
UNESCO	5.5	1930	1.2
Sacre Coeur	5.5	1930	1.2
CDG Airport	0.02	5370	20
Bottled Water	0.02	16,000	0.7
Water Dispenser	0.1	4781	0.8

Table 2 Bacteria in Paris drinking water/(CFU·mL⁻¹)

Vocation	ATP/(RLU·mL ⁻¹)	R2A/(CFU·mL ⁻¹)	French Method/ (CFU·mL ⁻¹)
Eiffel Tower	435	10920	2300
Hotel St. Andre	952	45000	18200
Notre Dame	4	65	10
Parc Viviani	296	16310	1000
Near Louvre	10	80	15
Hopital Pitie	539	52000	3610
UNESCO	47600	176000	2000
Sacre Coeur	27	33	5
CDG Airport	173	4,670	170
Bottled Water	13	108	2
Water Dispenser	813	57400	4350

**Fig. 7** Bacterial count measured with AODC**Fig. 8** Bacterial count measured with DVC

6.3 Results and discussion

The detection limit of ATP was determined with high accuracy. It showed that the micro-luminometer was able to determine ATP concentrations as low as 0.2 picograms. It is known that the average ATP concentration in one bacterial cell is about 10×10^{-15} g (i.e. 1 femtogram). Thus the 0.2 picograms correspond to about 200 bacterial cells.

As a follow-up about 120 water samples were analyzed with ATP bioluminescence, HPC, DVC, and AODC methods, in triplicate. The correlation coefficient between ATP and HPC was 0.84, and the correlation coefficient between ATP and DVC was 0.8 which was statistically highly significant. The prediction of HPC can be accomplished by the following equation. $HPC (CFU/mL) = RLU \exp 1.47$.

7 Conclusions

A later publication from Delahaye et al. (2003) showed that water for Paris came from many different sources and origins.

There are other accounts of bacterial analysis in metal working fluids. In a study of the microbial hazards in metal working fluids showed that an ATP analysis can show the microbial risk (Webster et al., 2005).

ATP analysis was used for determining the bacterial biofilm buildup in automated rodent watering systems (Meier et al., 2008). Together with immunomagnetic separation, ATP analysis can measure fecal bacteria in water and wastewater (Lee and Deininger 2001, 2010; Bushon et al. 2009; Lee et al. 2010).

In summary, the ATP method is fast (under 5 min), simple and can be done onsite. It can be used as a good alternative. By using it on a regular basis, it can serve as an early warning detection system and a first line of protection of public health in regards of microbial quality of water.

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