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Escherichia coli: the best biological drinking water indicator for public health protection

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1. SUMMARY

Public health protection requires an indicator of fecal pollution. It is not necessary to analyse drinking water for all pathogens. *Escherichia coli* is found in all mammal faeces at concentrations of 10 log 9^{-1}, but it does not multiply appreciably in the environment. In the 1890s, it was chosen as the biological indicator of water treatment safety. Because of method deficiencies, *E. coli* surrogates such as the 'fecal coliform' and total coliforms tests were developed and became part of drinking water regulations. With the advent of the Defined Substrate Technology in the late 1980s, it became possible to analyse drinking water directly for *E. coli* (and, simultaneously, total coliforms) inexpensively and simply. Accordingly, *E. coli* was re-inserted in the drinking water regulations. *E. coli* survives in drinking water for between 4 and 12 weeks, depending on environmental conditions (temperature, microflora, etc.). Bacteria and viruses are approximately equally oxidant-sensitive, but parasites are less so. Under the conditions in distribution systems, *E. coli* will be much more long-lived. Therefore, under most circumstances it is possible to design a monitoring program that permits public health protection at a modest cost. Drinking water regulations currently require infrequent monitoring which may not adequately detect intermittent contamination events; however, it is cost-effective to markedly increase testing with *E. coli* to better protect the public's health. Comparison with other practical candidate fecal indicators shows that *E. coli* is far superior overall.

2. CHARACTERISTICS OF A BIOLOGICAL INDICATOR

The Italian scientist Fracasoro, analysing the pattern of bubonic plague deaths and other epidemics in and around the Italian Adriatic coastal cities in 1546, concluded that infection was transmitted by four major routes: hand-to-hand, insects, fecal–oral, and airborne particles (Bulloch 1960). In the 1850s, Dr Snow (in London) conducted the first seminal epidemiological investigation which definitively established the waterborne transmission of a microbial disease, i.e. cholera. The death of Prince Consort Albert in 1861 by typhoid fever provided sufficient political motivation for the British government to re-evaluate its sanitary system for sewage and water distribution and treatment and made it public policy to institute protective measures for the public's health to prevent waterborne disease. The invention of the first usable flush toilet in the 1880s by Thomas Crapper (subsequently knighted) provided the ability for individual homes to be connected to the sanitary system (Lechevalier and Solotorovsky 1965).

In the 1890s, sanitary engineers found that treatment of drinking water with chlorine was an inexpensive, effective,

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and relatively simple way to produce biologically potable drinking water. Thus, the widespread use of disinfection and other processes to produce pathogen-free drinking water began. Because any process requires monitoring to ensure continued effectiveness, a wide variety of physical, chemical, and biological analytes were examined to determined their sensitivity and specificity. These great changes in sanitary engineering were occurring at the same time as the 'Golden Age' of Microbiology, during which specific microbes were definitively established as causes of specific diseases. In the brief period between 1866, Koch's demonstration that for the first time a bacterium specifically caused anthrax, and the 1890s, many of the primary human pathogens were identified and categorized. In addition, much was learned about the normal, nonpathogenic microbial content of people and animals (Edberg 1998).

In the 1890s, one of the central questions of public health protection was: should one monitor the safety of drinking water for specific pathogens or indicators? Paradoxically, one hundred years later, with recent knowledge regarding parasitic and viral waterborne disease transmission, the same question is being actively re-evaluated. As in 1900, the answer in the year 2000 is that monitoring for indicators for fecal pollution better protects the public's health than monitoring for specific pathogens. The overriding rationale for this conclusion is a simple one – there are simply too many, and still unknown, waterborne pathogens. Moreover, these pathogens are present in small concentrations. Although methods of identification now exist for many of the waterborne pathogens, they are generally quite expensive, technically demanding, and time-consuming. Accordingly, the decision was made in the 1890s to search for indicators for fecal pollution that would be universally present in the faeces of humans and mammals, be present in large numbers, be readily detected by simple and inexpensive methods, and would not multiply once they left the body and entered water.

3. SELECTION OF *E. COLI* AS THE BEST BIOLOGICAL INDICATOR

Theobald Smith, an active 'microbe hunter' of the Golden Era, first studied *E. coli* as part of the normal flora of mammals and proposed it as the primary drinking water indicator in the 1890s (Prescott and Winslow 1915). Unfortunately, at that time, there was no specific test for *E. coli*. It required several days and a number of subcultures in order to identify the bacterium. Soon, a number of surrogate tests for *E. coli* were developed.

One of the first surrogates to gain popularity took advantage of the observation that *E. coli* was more thermo-tolerant than other lactose-fermenting, enteric, Gram-negative bacteria. In 1904, Eijkman developed what was referred to as the 'fecal coliform test.' (Eijkman 1904). This method was modified in the early 1900s by the elimination of elevated temperature incubation so that the method now encompassed all members of the *Enterobacteriaceae* that were able to ferment lactose with the production of acid and gas. This functional test, which included *E. coli* and other noncolonic bacteria, became known as the 'total coliform' group. In 1914, the U.S. Treasury Department proposed a standard for drinking water safety which codified the utilization of a total coliform test (Clesceri *et al.* 1998). While the total coliform group was criticized, even then, because it included a number of species not of fecal origin (Frost 1915; Fuller 1915), it was generally thought that the ease of performing the test overcame the lack of fecal specificity. It was reasoned, at that time, that most contamination events of drinking water were of fecal origin and that the total coliform method therefore detected a high proportion of *E. coli*, directly implying a public health threat.

Unfortunately, while it was true that most total coliforms were *E. coli* in the early 1900s, it is not true today. With the implementation of modern construction and engineering techniques, the great majority of positive samples of total coliforms from distribution systems do not contain *E. coli*. However, the mistaken association of total coliforms with fecal contamination remains in the minds of many sanitarians today. Therefore, while the coliform group was originally developed as a surrogate for *E. coli*, this association is no longer valid.

The coliform group comprises genera that satisfy a functional definition, i.e. they utilize lactose to produce acid and gas, or possess the enzyme β-D-galactosidase, which is capable of using a chromogenic galactopyranoside substrate for growth (Clesceri *et al.* 1998). The genera that satisfy this definition include *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter* and *Escherichia*. From both the methods and public health protection points of view, it is critically important to understand that, other than *Escherichia*, these genera are widely found in the environment (i.e. source waters, vegetation and soils), and are not associated with fecal contamination. Therefore, these four genera do not imply a health risk. Numerous studies (LeChevallier *et al.* 1987; LeChevallier and McFeters 1990; Edberg *et al.* 1994a) have documented that *Enterobacter* and *Klebsiella* frequently colonize the interior surfaces of water mains and storage tanks (often called 're-growth') growing in biofilms when conditions are favourable, i.e. nutrients, water temperatures, low disinfection concentrations, long residence times, etc. Many studies have clearly shown that *E. coli* is the only coliform that is an undoubted inhabitant of the gastrointestinal tract. While *Klebsiella*, *Citrobacter* and *Enterobacter* have been isolated from human fecal samples, they are in small numbers when present. For example,

Table 1 Relative number of fecal and non-fecal types of coliform bacteria in various substances

Sources	No. of strains observed	Percentage of strains of *Aerobacter* (*Enterobacter*) *aerogenes* type	Percentage of strains of *E. coli* type
Human faeces	2534	5·9	94·1
Animal faeces	1832	7·4	92·6
Water	2137	35·2	64·8
Milk	1382	43·1	56·9
Grain	288	81·7	18·3
Soil	853	88·1	11·9

Taken from Allen and Edberg (1995).

Table 1 shows a study conducted in 1921 at Iowa State University from a number of animal and human sites.

When the IMViC (Indole, Methyl red, Voges-Proskauer, Citrate utilization) test became established in the 1930s and 40s, Geldreich (1966) examined the occurrence of coliform types from a variety of human and other animal fecal samples. As Table 2 demonstrates, from fecal samples a pattern of ++−− represented 97·9% of the total of 1896 strains examined. This pattern was the only one consistent with *E. coli*.

In the 1970s, miniaturized, multibiochemical reactions such as API 20E were developed for the identification of enteric bacteria. Accordingly, it was now possible to differentiate species of the 'total coliform' group with great specificity. Dufour (1977) again examined the distribution of the total coliform group in human and animal faeces, as demonstrated in Table 3.

While separated by 50 years and using different identification methods, all three studies produced remarkably consistent data. Unequivocally, it was shown that *E. coli* was the only true fecal coliform and that other members of the 'total coliform' group were not generally of fecal origin, most likely originating from vegetation and soil.

By the 1970s, it became clear that the majority of total coliform occurrences from drinking water distribution systems were not *E. coli*. While the means to identify *E. coli*

Table 2 Occurrence of coliform types in samples of animal faeces

	43 human samples		32 livestock samples		28 poultry samples		Summary	
Coliform type	No. of strains examined	Percentage occurrence	No. of strains examined	Percentage occurrence	No. of strains examined	Percentage occurrence	No. of strains examined	Percentage occurrence
++−−	3932	87·2	2237	95·6	1857	97·9	8026	91·8
−−++	245	5·4	0	a	1	0·1	246	2·8
+++−	106	2·4	59	2·5	0	a	165	1·9
−+−−	99	2·2	14	0·6	20	1·1	133	1·5
−+−+	50	1·1	1	a	5	0·3	56	0·6
++−+	35	0·8	27	1·2	11	0·6	73	0·8
−+++	21	0·5	0	a	0	a	21	0·2
++++	6	0·1	0	a	0	a	6	0·1
+−++	14	0·3	0	a	0	a	14	0·2
+−−−	2	a	0	a	2	a	4	0·1
−−−+	2	a	0	a	0	a	2	a
−++−	0	a	1	a	0	a	1	a
Total	4512	–	2339	–	1896	–	8747	–
EC positive	4349	96·4	2309	98·7	1765	93·0	8423	96·3
BALB positive	4274	94·7	2307	98·6	1755	92·5	8336	95·3

a, Insufficient number of cultures examined. Taken from Geldreich 1966).

Table 3 Percentage of genera of coliforms in human and animal faeces

Animal (no. examined)	*E. coli*	*Klebsiella* spp.	*Enterobacter/ Citrobacter*
Chicken (11)	90	1	9
Cow (15)	99·9	–	0·1
Sheep (10)	97	–	3
Goat (8)	92	8	–
Pig (15)	83·5	6·8	9·7
Dog (7)	91	–	–
Cat (7)	100	–	–
Horse (3)	100	–	–
Human (26)	96·8	1·5	1·7
Average percentage	94·5		

specifically were available to specialized laboratories, they were still too costly, too cumbersome, and required too high a level of technical expertise to be used for routine drinking water analysis. Therefore, sanitarians began to rely on the 'fecal coliform' test as a surrogate for *E. coli.* However, it soon became evident that the great majority of 'fecal coliforms' isolated from water distribution systems were not *E. coli*, but noncolonic thermotolerant coliforms, primarily members of the genus *Klebsiella*. These *Klebsiella* were associated with vegetation, agricultural products, wood pulp, and paper mill effluence that were not subject to mammalian contamination. Caplenas and Kanarek (1984) reported that 15% of *Klebsiella pneumoniae* were thermotolerant, and Edberg *et al.* (1994b) reported the findings shown in Table 4.

The fecal coliform test must be performed under exacting temperature standards. Incubation temperatures must be rigidly controlled since even minor variations (as small as 0·2 °C) will produce erroneous data. Also, large populations of heterotrophic bacteria interfere with both the liquid (MPN) and membrane filtration (MF) methods. Moreover, many strains of *E. coli* are unable to ferment lactose (Dufour 1977), resulting in false negative reactions. Unfortunately, many regulatory agencies (including the US EPA), while recognizing *E. coli*, also allow 'fecal coliform' testing to be utilized at the discretion of the laboratory. Because of documented, significant false positives and false negatives with the fecal coliform method, it is recommended that it no longer be considered an adequate surrogate for *E. coli* and should be deleted from all regulatory statutes.

Table 4 Fecal coliform thermotolerance methods: species isolated from water

Species	Percentage of all species identified		
		m-FC plate	
	EC broth	Light Blue	Dark Blue
Escherichia coli	81	64	79
Klebsiella pneumoniae	9	13	10
Klebsiella oxytoca	8	18	9
Enterobacter cloacae	1	3	1
Citrobacter diversus	1	2	1

Taken from Allen and Edberg (1995)

By the late 1970s, it was established that *E. coli* was specific and abundant in human and animal faeces at an average of approximately 10^9 g^{-1}. The species was found in sewage, treated effluent, and all natural waters and soils subject to mammalian fecal contamination. Accordingly, the presence of *E. coli* always indicated a public health threat. In the late 1980s, a number of studies (Edberg *et al.* 1988a; Edberg *et al.* 1988b; Rice *et al.* 1990; Covert *et al.* 1992) supported the use of a completely new technology and method to directly detect and identify *E. coli* from drinking water. The Defined Substrate Technology (DST) contained 4-methy-umbellfieryl-β-D-glucuronide (MUG) which can only be metabolized by a constituent of the enzyme system particular to *E. coli.* This enzyme system, β-glucuronidase, is present in more than 95% of all isolates of *E. coli.* The DST methods are now approved in many countries world-wide and was included in the 19th Edition of *Standard Methods for the Examination of Water and Wastewater* (Clesceri *et al.* 1998) as the 'Chromogenic Substrate' method. The one-step, Defined Substrate Technology methods (Colilert and Colisure, Idexx Laboratories, Westbrook, ME) permitted, for the first time, the direct detection and enumeration of as low as one colony-forming unit of *E. coli* (and simultaneously total coliforms) directly from a 100-ml water sample, inexpensively and with no technical expertise required. Subsequently, β-glucuronidase substrates were incorporated in a variety of media to enumerate *E. coli.* The DST technology provided the impetus to re-insert *E. coli* into drinking water regulations.

4. SURVIVAL OF *E. COLI*

Like the porridge in the well-known children's story, 'Goldilocks', the lifespan of the ideal biological indicator would not be either too short or too long, but just right. Unfortunately, 'just right' has not be determined. Theoretically, 'just right' corresponds to the lifespan of

pathogens transmitted by the fecal–oral route in drinking water. There are many variables, however, that affect the lifespan of both indicators and pathogens. While it is beyond the scope of this paper to discuss them in detail, some of the more important variables include: type of pathogen, type of water (such as surface water, subterranean water, treated distribution water), water temperature, other microflora present, and exposure to sunlight (ultraviolet radiation).

Little is known about the precise reasons that cause a microbe to naturally die, although factors such as loss of energy, age, predation, and environmental stress contribute to the process. What is generally accepted is that vegetative microbes have a shorter lifespan than quiescent or nonmetabolizing microbes. Following this reasoning, vegetative bacteria and the trophozoite form of parasites have a shorter lifespan than bacterial spores, parasitic cysts, and viruses. While the literature supports this theory, the primary public health question is, 'What is a useful lifespan for the indicator?' For example, the innate ability of a bacterial spore to potentially survive hundreds to thousands of years, depending upon environmental circumstances, renders it unusable as an indicator. This protracted lifespan does not coincide with those of pathogens. Therefore, if a spore survives much longer than the pathogens, the water is safe to drink despite the presence of the indicator. In effect, the indicator loses its fecal specificity over time.

Water types and quality have a major influence in the survival of both pathogens and indicators. For example, microbes survive better when attached or sequestered in particles than when exposed. The temperature of the water exerts a number of effects on the indicator, pathogens and other microflora. The colder the water, the longer the microbes live. Surface water tends to have a broader array of other microflora than does subterranean water. Both the indicators and the pathogens are part of the eco-system and its food chain. Accordingly, the more likely a microbe is to be eaten by another microbe, the shorter its lifespan will be. For example, although viruses (because they don't metabolize) may innately survive longer than a bacterium in the environment, they may actually have a shorter lifespan because, being smaller than bacteria and without defense mechanisms, they are more readily phagocytized by the other microflora.

Because of the variability of the environment, one cannot state with certainty what the lifespan of *E. coli* will be in water, but we have considerable information from both laboratory and natural conditions that allow us to bracket its survivability. In one of the most extensive studies of the survivability of *E. coli* in both natural and laboratory conditions, Kudryavtseva (1972) started with a concentration of *E. coli* at levels of 10^4 ml^{-1}. [Note: this concentration is five logs less than the *E. coli* concentration in faeces.] In experimental bacterial inoculation of underground waters and fine-grain sands, the author established that *E. coli* was viable for three months. Under laboratory conditions, the same strains of *E. coli* were viable for four months. The likely cause of the difference in viability between natural and laboratory conditions is that there are more microflora under natural than laboratory circumstances. Likewise, Filip *et al.* (1987) reported that *E. coli* survived in groundwater at 10 °C for up to 100 d. Goldshmid *et al.* (1972) found that *E. coli* survived for 63 d in a recharged well. In the river Sowe in the United Kingdom, Filip found that a strain of *E. coli* K12, which expressed naladixic acid resistance, survived for a few days under the worst conditions (temperature 37 °C, unexposed to sunlight with natural microflora present) to as many as 260 d in sterile river water. Under conditions most analogous to those found in water distribution systems, *E. coli* would be expected to survive in the river water for approximately 30 d. Grabow *et al.* (1975), reported that *E. coli* survived for 55 d in dialysis bags suspended in river water at 9–16 °C. Berry *et al.* (1991) examining the phenomenon 'viable, nonculturable' phase of bacteria found that under laboratory conditions, *E. coli* survived at least three months. After three months, a portion of the laboratory's stored suspensions were heat shocked at 35 °C for 20 min and assayed by culture and microscopy. Heat shocking caused nonculturable bacteria to regain the ability to grow in artificial media and implied that the utility of *E. coli* as an indicator of fecal pollution for the water industry would be extended heat-shock recovery. It should be noted that all these studies utilized concentrations of *E. coli* much lower (four to five logs) than those found in faeces. Accordingly, actual, practical survivability should be longer.

Based on the survival study of *E. coli* in the environment, we can bracket its survival in water containing a moderate microflora at a temperature of 15–18 °C of between 4 and 12 weeks.

5. SURVIVAL OF *E. COLI* BY DISINFECTION

Soon after the introduction of calcium hypochlorite for the disinfection of drinking water and the choice of *E. coli* as a primary biological public health indicator, studies were undertaken to determine the disinfectant resistance of the indicator compared to the pathogens. At that time, the pathogens of concern were almost exclusively bacterial. It was found that *E. coli* survived at least as long as all the bacterial pathogens, and generally longer. With the introduction of other forms of chlorine in the 1920s and 1930s, most notably chloramine, it was observed that a considerable number of variables affected the activity of each chlorine type. Heathman *et al.* (1936) stated, 'The apparent lack

of agreement as to the relative killing power of chlorine and chloramine is of yet unexplained. Possibly, it is explainable on the basis of difference in the chemical characteristics of the water used, peculiarities of the organisms involved, and other similar factors.'

Today, the situation is considerably more complex. We not only have additional forms of chlorine, but other oxidants, ultraviolet light, and various combinations of oxidative processes. While it is beyond the scope of this paper to discuss the factors involved in disinfection efficacy in detail (LeChevallier *et al.* 1982), for the purposes of discussion of the efficacy of biological indicators to protect public health, there are several important factors to consider.

Drinking water public health protection occurs at two separate, distinct eco-system situations. The first is the efficacy of the primary treatment train where source water undergoes a number of processes, invariably including some form of oxidant disinfection such as ozone or chlorination. Ozone, being the most energetic, is the most active against microbes, with bacteria and viruses rapidly killed and the cysts of parasites significantly reduced in viability. Chlorination, being less energetic, is generally active against bacteria and viruses (depending upon a number of variables which will be discussed below) and less so against parasitic cysts. The purpose of primary treatment of the water source is to render the water entering the disinfection system safe by utilizing rapidly acting, highly efficient modalities.

The second area of potential public health risk, the distribution system, is much different. Here, the strategy is to prevent cross connection from sewer pipes and intrusion of nonpotable ground water when there is a loss of pressure and to maintain some form of disinfection residual throughout the system. Because of the concern about production of trihalomethanes (THMs) and the limit of THMs in drinking water, more utilities have been changing to chloramination as the primary disinfectant of choice and a means of better maintaining disinfection residual throughout the system. Chloramines are much less energetic, but more stable, than chlorine. Accordingly, microbes may survive for longer time periods when exposed to chloramines. This potential disadvantage is offset by the fact that chloramines penetrate into the biofilm of pipes better than chlorine, which is rapidly dissipated on the organic surface of biofilms.

The primary threat to public health is related to cross connections or back flows and from events that occur in the distribution system. Under these circumstances, sewage may enter the distribution system. Because of considerable nitrogen content in sewage, even in water which has a chlorine residual, large amounts of chloramines are produced. Therefore, under these conditions, one must view the disinfecting power of chlorine residual not as a high-energy chlorine model, but as a shift to low-energy chloramines. This situation has profound public health implications because the survival of all classes of microbes under these conditions will be longer. For example, Rice *et al.* (1999) found that exposure of both wild-type and the pathogenic *E. coli* O157:H7 were rapidly killed by exposure of $1{\cdot}1\ mg\ l^{-1}$ of free chlorine at pH 7·0 at 5 °C. While this situation would be analogous to source water treatment conditions and a 'clean' distribution system, should sufficient sewage mix with the chlorine to produce chlorinamines, one would expect considerably longer survival times. Moreover, as Rice *et al.* (1999) state, 'Dissipation of chlorine residual can readily occur under adverse conditions and exposure to sunlight or organic chlorine-demand substances can greatly diminish chlorine levels. Protection of organisms associated with particular matter, such as faecal material, can also readily decrease the biocidal activity of chlorine.' As the work of LeChevallier *et al.* (1982, 1987, 1990) has demonstrated, disinfection activity is markedly reduced against all types of microbes, including bacteria, when the microbes are associated with particles or sequestered in biofilm.

Therefore, the question is how does disinfection and its variables affect the utility of *E. coli* as a biological indicator? From source water treatment, where the background matrix of microorganisms is relatively low and water quality conditions well controlled, *E. coli* would best track bacterial pathogens and most viruses, but would not be useful for parasitic cysts. In addition to its decreased oxidant susceptibility, *E. coli* from source drinking waters are generally present in very low numbers. Virtually all sources of drinking water are from well-protected environments which should have a low fecal challenge.

From distribution water, *E. coli* is a much more efficacious biological indicator. First, the public health threat comes from sewage intrusion, which will have very high concentrations of *E. coli* ($10^8-10^9\ ml^{-1}$). Moreover, both the absolute concentration of chlorine would be lower compared to the source treatment conditions and the chlorine flora will be modified from free chlorine to chloramines. *E. coli* is much more likely to be associated with particles and to be sequestered within organic matter which will further lessen the activity of the disinfectant.

6. MODE OF ANALYSIS

Until 1992, most drinking water regulations were based on some numerical index of the biological indicator. In the Code of the Federal Register of the United States (1992), the United States Environmental Protection Agency made a profound change in the mode of analysis. It changed from a minimal acceptable average number to the fre-

quency-of-occurrence mode (P/A, Presence/Absence). For many years, Clark (1969) had reported that concentrations of microbial indicators in drinking water, especially at the low concentrations in which they occurred, were not reproducible, and quantitation produced a false sense of security. His investigations found that using the simple Present or Absent (P/A) mode better protected the public's health by permitting a larger number of tests and increasing the simplicity of the procedure. His work was further extended and quantified by Pipes *et al.* (1987), who showed in a number of investigations that coliform concentrations in a drinking water sample were not stable, the cfu ml^{-1} results were not reproducible, and that the indicators tended to travel in clumps rather than in single cells, thereby rendering cfu ml^{-1} values innately highly variable. Pipes *et al.* (1987) found that there were significant changes in microbial quality which occurred week-to-week or month-to-month and that the P/A mode was more efficient at detecting these changes than the membrane filter method. 'The P/A mode detected more significant changes in the microbial quality of the water than did the average "membrane filtration (MF) coliform colony count," wrote Pipes (Pipes *et al.* 1987). Accordingly, the US EPA adopted the P/A mode as part of its regulations in 1992.

The P/A mode is most compatible with analysis of *E. coli*. Utilizing a 100-ml water sample allowed the introduction of inexpensive, simple, easy-to-perform methods that could directly detect *E. coli* from a water sample with no intermediate subculture or enhancement steps. Because liquid-based methods are more sensitive in detecting bacterial indicators than membrane filter methods (Stukel *et al.* 1987), the utility of *E. coli* as a biological indicator was concomitantly enhanced.

7. METHODS

A particular strength of *E. coli* as a biological indicator is the availability of sensitive, specific, inexpensive, easy-to-use methods for its detection directly from water samples. It is currently possible for unskilled individuals to perform an accurate water analysis with a minimum of training, particularly with the Defined Substrate Technology methods. There is no other bacterial, viral, or protozoal marker that even approaches the advantages of the *E. coli* methods.

In the United States, there are three basic types of methods approved for *E. coli*. Likewise, *Standard Methods for the Examination of Water and Wastewater* (Clesceri *et al.* 1998) also recognizes these three basic types. They are: multiple-tube fermentation (both in the MPN and P/A method formats), membrane filtration, and Defined Substrate Technology (also known in *Standard Methods* as the chromogenic substrate method). Both the MTF and MF methods arrive at *E. coli* by first doing a presumptive total coliform enumeration and then a second procedure requiring 24 h. The DST methods detect *E. coli* directly from drinking water, and hence are faster and less expensive.

The MTF methods first perform a standard total coliform analysis. Any single tube in the MPN format or the entire 100 ml sample in the P/A format showing total coliforms would then be tested to determine if that reaction vessel contains *E. coli*. This is done by transferring an aliquot of positive liquid to a second tube containing lactose broth with MUG. After a further 24 h of incubation, positive fluorescence indicating MUG hydrolysis denotes the presence of *E. coli*. Likewise, the MF method arrives at *E. coli* by first doing a total coliform test. Colonies on the plate identified as total coliforms are transferred to either EC MUG or nutrient agar with MUG to determine if they contain the enzyme β-glucuronidase. Those colonies that do are denoted as *E. coli*.

The DST methods work in a much different way from the lactose-based methods. Here, the substrate acts as a food source, and the metabolism of it allows growth of the target microbe at the expense of others. Accordingly, the DST method is the only one which does not require a confirmation step. Direct colour visualization after a maximum of 18–24 h incubation denotes the presence of both total coliforms (one colour produced) and *E. coli* (fluorescence produced). There are currently three DST methods approved by the US Environmental Protection Agency. They are: Colilert (sometimes abbreviated by the US EPA as MMO-MUG), which accepts a drinking water sample directly and requires a maximum of 24 h incubation; Colilert-18, which requires a maximum of 18 h and has a warming step; and Colisure, which requires a maximum of 24 h. With Colilert and Colilert-18, total coliforms are denoted by the development of a yellow colour and *E. coli* by fluorescence; with Colisure, total coliforms are denoted by the development of a red colour and *E. coli* by fluorescence. The DST methods are refractory to Heterotrophic Plate Count (HPC) suppression (false-negatives) whereas the lactose-based methods are not.

Since the introduction of the first DST Colilert method in 1987, there have been a large number and variety of media containing MUG or other substrates developed and marketed. It is beyond the scope of this paper to discuss any other than the three model types, as approved by the US EPA. The reader is referred to *Standard Methods for the Examination of Water and Wastewater* (Clesceri *et al.* 1998) as a guide for ascertaining which other ones have achieved sufficient field use and peer-reviewed publication substantiation. Table 5 summarizes the current US EPA-approved biological indicator systems.

Table 5 Current US EPA-approved coliform/*Escherichia coli* methods

Indicator System	Included species				
	Klebsiella	*Escherichia*	*Enterobacter*	*Citrobacter*	*Serratia*
Total Coliforms	+	+	+	+	+
MPN	+	+	+	+	+
MF	+	+	+	+	+
Colilert	+	+	+	+	+
Fecal Coliforms	+*	+	−	−	−
MPN	+*	+	−	−	−
MF	+*	+	−	−	−
Escherichia coli	−	+	−	−	−
DST†	−	+	−	−	−
EC-MUG	−	+	−	−	−
MF-NA‡with MUG	−	+	−	−	−

*Approximately 15% thermal tolerant. †Includes Colilert, Colilert-18 and Colisure. ‡Nutrient agar. MPN, Most Probable Number; MF, Membrane Filtration; DST, Defined Substrate Technology; EC-MUG, *E. coli* with methyl-umbellfieryl-β-D-glucuronide; MF-NA with MUG, Membrane Filter-Nutrient Agar with methyl-umbellfieryl-β-D-glucuronide.

8. BIOLOGICAL INDICATORS OTHER THAN *E. COLI*

It is not possible to discuss the merits and efficacies of each biological indicator for which data exists. Under the assumption that we are here discussing indicators of practical use in the drinking water utility industry, such potential indicators as enteric virus culture, genetic amplification, nuclear magnetic resonance, etc., will not be reviewed. Here, we will compare and contrast with *E. coli* those indicators which have been proposed for possible use in routine drinking water monitoring. These are *Enterococcus* (or fecal streptococcus), *Clostridium perfringens* spores, somatic coliphages, and male-specific coliphages. Table 6 summarizes the attributes of the candidate practical biological indicators.

8.1. Enterococci

Enterococcus is also known as fecal streptococcus. This group contains a number of species, of which *Ent. faecalis* and *Ent. faecium* are predominant. Virtually all mammals carry this organism in the colon at concentrations of approximately 10^6–$10^7\,g^{-1}$. Accordingly, enterococci are approximately 100- to 1,000-fold less numerous than *E. coli*. A particular characteristic of the *Enterococcus* group is that it is quite salt-resistant, which makes it a good indicator of estuarine and ocean waters. The bacterium has a lifespan which approximates that of *E. coli*. A number of methods exist that are both sensitive and specific for the *Enterococcus* group. These include variations of the Multiple-Tube method (such as azide dextrose broth), MF (various fecal-streptococci media), and DST (Enterolert). Like *E. coli*, a sample volume of no more than 100 ml is required. Currently, the only regulations in which *Enterococcus* appears concern bathing beaches. *Enterococcus* is strongly being considered as an additional test to *E. coli* or drinking water, but not as a replacement. The primary reason for this strategy is that *Enterococcus* is present at lower numbers than *E. coli* in faeces. It is postulated that if a second, highly fecally specific test is performed, public health protection will increase for a modest cost. Reluctance to include an *Enterococcus* test centres on both the added cost and the requirement to put an additional laboratory quality control infrastructure in place. At this time, it is not clear that the additional costs and expertise required would yield sufficient public health protection over and above *E. coli* testing to justify its inclusion.

8.2. *Clostridium perfringens* spores

The spores of *Clostridium perfringens* are extremely long-lived. In fact, their long life is the major impediment to their use as an indicator of fecal contamination. They simply outlive all known pathogens. In soils and biofilms, they remain quiescent for years. Therefore, many soils with no fecal contamination may still have recoverable *Cl. perfringens* spores. The methods and expertise required to identify *Cl. perfringens* spores directly from drinking water have been published but have not yet been widely field-tested. It is expected that the cost of performing a *Clostridium* assay

Table 6 Characteristics of practical biological drinking water indicators

Indicator	Occurrence	Survival	Lab availability	Sample size	Commercial cost
E. coli	$10^9\,l^{-1}$ of raw sewage, found 100% in human stool	A week to months, not very biodegradable	Good	100 ml	$15/sample
Enterococci	$10^7\,l^{-1}$ of raw sewage, found 100% in human stool	Weeks, lasts longer in soils	Good	100 ml	$15/sample
Cl. perfringens	$10^6\,l^{-1}$ of raw sewage, found 100% in humans and animals	Months to years	Good	100 ml	$35/sample
Somatic phages	10^4–$10^6\,l^{-1}$ of raw sewage, found 50% in humans	Weeks	Currently, not doing it	1–15 l	$35–50/sample
Male-specific (F +) phages	10^4–$10^6\,l^{-1}$ of raw sewage, found 1–3% in humans	Weeks	Currently, not doing it	Litres	$35–50/sample

will be significantly higher (two to three times that of *Enterococcus*) because of the enhanced technical skill required, anaerobic incubation conditions, and more difficult quality control. *Cl. perfringens* spores have been shown to be most appealing as indicators of ground water pollution because of their long residency times; however, they have not been adopted by any regulatory body and remain on many lists for discussion with little funding for field work.

8.3. Coliphages

Somatic coliphages are those bacteriophages that infect members of the total coliform group: they are not *E. coli* specific. They are generally found in concentrations from 10^4 to $10^6\,l^{-1}$ of raw sewage. However, in individual people, they are only found 50% of the time. In ground water, somatic coliphages survive for several weeks. Male-specific, or *F*+, coliphages are thought to be *E. coli* specific, although there is some evidence that they may amplify in nonfecal coliform species, such as *Klebsiella pneumoniae* and *Enterobacter cloacae*. *F*+ coliphages are found in concentrations of 10^4–$10^6\,l^{-1}$ of raw sewage; however, they are only found in 1–3% in individual humans. Like somatic coliphages, they can survive for several weeks in ground water.

The basic format for performing all coliphage testing is the same – a bacterial host is swabbed on the surface of an agar plate (or incorporated into the agar) and a large volume (in the 15-litre range) is concentrated and plated on the surface. Each type of coliphage requires a specific host. After an incubation period, one looks for plaques in the lawn of bacterial growth.

Drinking water microbiologists have studied the efficacy of coliphage utilization as markers of fecal contamination for many years. As of this date, they have not been included in drinking water regulations. The primary limitations include the lack of field data, the difficulty of processing the water sample (requiring multiple steps), and the lack of choice of a stable bacterial host. There has also been a lack of association between the detection of bacteriophages in ground water and disease occurrence (Craun *et al.* 1997). Accordingly, the major limitations for acceptance of coliphages for drinking water testing include: lack of a standard method, lack of extensive field testing, lack of correlation with disease occurrence, and lack of a stable host. It is unlikely that somatic coliphages would be utilized for testing of drinking water from distributions systems because these phages may amplify any member of the coliform group and would not be fecal-specific. However, the *F*+ coliphages have appeal, particularly for ground water testing. A number of groups, particularly those of Sobsey and Yates and associates (personal communication) in the United States, are actively working on these hurdles.

9. CONCLUSIONS

The World Health Organization states, 'Water must be examined regularly and frequently because pollution is often intermittent and may not be detected if examination is limited to one or only a small number of samples. For this reason, it is better to examine drinking water frequently by means of a simple test rather than less often by several tests or a more complicated test.' Furthermore, the WHO states, 'Examination for faecal indicator bacteria in drinking water provides a very sensitive method of quality assessment.' *E. coli* best fulfills these conditions. It is present in extremely high numbers in the faeces of all mammals, it does not appreciably multiply in the environment

outside its host, methods to detect it are inexpensive, simple, sensitive, and specific, and it survives long enough under a broad range of drinking water conditions, so that in almost all circumstances a cost-effective sampling protocol can be developed. For example, in the United States and in many countries, drinking water protocols require that only one sample per 1000 population per month need to be analysed from public water systems. This low level of testing would not be permitted in any manufactured food. Frequent testing for *E. coli* provides regulators with the ability to markedly improve public health protection by increasing the frequency of testing to a realistic number at a low cost.

Should *E. coli* be the only biological indicator we use? Some public health professionals have argued that it will provide sufficient public health protection if performed frequently enough, so that other biological indicators need not be employed. While this strategy is applicable to most circumstances, there will be particular situations in which *E. coli* will not provide sufficient public health protection. For example, from surface waters exposed to *Cryptosporidium* contamination (such as rivers in agricultural areas), an indicator other than *E. coli* may be needed as a sentinel for the intrusion of *Cryptosporidium*. Unfortunately, other than the parasite itself, for which there is currently no method amenable to routine, real-time drinking water testing, there is no other useful candidate biological indicator. Another approach to the *Cryptosporidium* sentinel problem is to closely examine the operating parameters of the treatment train using a combination of biological (Bacillus spores), physical, and chemical measurements which together ensure that pathogens are removed during water processing (Rice *et al.* 1996).

Should *E. coli* be the sole biological indicator in ground water? Again, some practitioners feel that if an *E. coli* test is done frequently enough, it will detect any fecal pollution event. Others believe that *E. coli* alone will not provide sufficient public health protection because viruses travel at different rates than bacteria in the subsurface, and a viral test (such as a bacteriophage analysis) should be performed. In an evaluation of outbreaks of gastroenteritis associated with the consumption of ground water, Craun *et al.* (1997) found from routine coliform analyses, which includes *E. coli* as a component (this study did not differentiate the *E. coli* component of the coliform method), that the presence of a positive coliform test correlated very well with the presence of viral gastroenteritis.

Should the total coliform test be abandoned? Some public health practitioners say it should because the mere appearance of a total coliform-positive water test does not indicate a public health threat. Others argue that coliforms should not be found in finished drinking water and that their presence indicates an operating deficit in the system that needs to be addressed, i.e. levels of nutrients capable of supporting regrowth, lower disinfectant residuals, and very long residence times within the distribution/storage network. The latter view has the most merit. Many countries already require both a total coliform and *E. coli* analysis from a drinking water sample. In the United States, a single total coliform positive does not require public health action; however, after 5% of the samples are positive, there must be notification. Hopefully, the current regulation will be changed given the large amount of new scientific studies so that the pejorative nature of coliform-positive water samples will be replaced with the full recognition that this group is an indicator of operating efficiency requiring remediation and that a set percentage triggering public notification of positives is not useful.

At the end of this century, like the end of the last century, *E. coli* is the best single biological indicator of drinking water safety. While it disappeared from the drinking water regulations for 90 of those years, the recent developments of a new technology, justifies the use of *E. coli* as the primary indicator of drinking water potability. As this century closes, it is difficult to find any other biological indicator that is even a close competitor.

10. REFERENCES

Allen, M.J. and Edberg, S.C. (1995) The public health significance of bacterial indicators in drinking water. Special publication, *The Royal Society of Chemistry 1999*. Gateshead, Tyne and Wear, UK: Athenaeum Press.

Berry, C., Lloyd, B.J. and Colbourne, J.S. (1991) Effect of heat shock on recovery of *Escherichia coli* from drinking water. *Water Science and Technology* **24**(2), 85–88.

Bulloch, W. (1960) *The History of Bacteriology*. Oxford: Oxford University Press.

Caplenas, N.R. and Kanarek, M.S. (1984) Thermotolerant non-fecal source of *Klebsiella pneumoniae*: validity of the fecal coliform test in recreational waters. *American Journal of Public Health* **74**(11), 1273–1275.

Clark, J.A. (1969) The detection of various bacteria indications of water pollution by a presence–absence test. *Canadian Journal of Microbiology* **15**, 771–883.

Clesceri, L.S., Greenberg, A.E. and Eaton, A.D. (ed.) (1998) *Standard Methods for the Examination of Water and Wastewater* 20th edn. Washington, DC: APHA, AWWA, and WEF.

Code of the Federal Register of the United States (1992) 40 CFR, Section 141.63(6).

Covert, T.C., Rice, E.W., Johnson, S.A., Berman, D., Johnson, C.H. and Mason, P.J. (1992) Comparing defined-substrate coliform tests for the detection of *Escherichia coli* in water. *Journal of the American Water Works Association* **84**, 98–104.

Craun, G.F., Berger, P.S. and Calderon, R.L. (1997) Coliform bacteria and waterborne disease outbreaks. *Journal of the American Water Works Association* **89** (3), 96–104.

Dufour, P. (1977) *Escherichia coli: The Fecal Coliform.* Special Technical Publication 65, pp. 48–58. American Society for Testing and Materials.

Edberg, S.C. (1998) *The Germ Theory.* New Haven, CT: Medical Microbiology Lectures, Yale University.

Edberg, S.C., Allen, M.J., Smith, B.D. and the National Collaborative Study (1988a) National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: comparison with presence–absence techniques. *Applied and Environmental Microbiology* 54 (6), 1003–1008.

Edberg, S.C., Allen, M.J., Smith, B.D. and the National Collaborative Study (1988b) 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. *Applied and Environmental Microbiology* 54 (6), 1595–1601.

Edberg, S.C., Patterson, J.E. and Smith, D.B. (1994a) Differentiation of distribution systems, source water, and clinical coliforms by DNA analysis. *Journal of Clinical Microbiology* 32, 139–142.

Edberg, S.C., Allen, M.J. and Smith, D.S. (1994b) *Comparison of the Colilert Method and Standard Fecal Coliform Methods.* American Water Works Association Research Foundation.

Eijkman, C. (1904) *Central Blatt fur Bakteriologie, abstract I orig.* 37, 742.

Filip, Z., Kaddu Mulindwa, D. and Milde, G. (1987) Survival and adhesion of some pathogenic and facultative pathogenic microorganisms in ground water. *Water Science and Technology* 19 (7), 1189–1190.

Frost, W.H. (1915) Some considerations in estimating the sanitary quality of water supplies. *Journal of the American Water Works Association* 2, 712.

Fuller, G.W. (1915) Biochemical and engineering aspects of sanitary water supply. *Journal of the Franklin Institute* 180, 17.

Geldreich, E.E. (1966) Sanitary significance of fecal coliforms in the environment. *Water Pollution Control Research Series Publication WP-20–3.* Cincinnati Water Research Laboratory, US Department of the Interior.

Goldshmid, J. et al. (1972) *Effective Dissolved Salts on the Filtration of Coliform Bacteria and Sand Dunes.* Jerusalem, Israel: Sixth International Water Pollution Research Conference.

Grabow, W.O.K., Prozesky, O.W. and Burger, J.S. (1975) Behaviour in a river and dam of coliform bacteria with transferable or non-transferable drug resistance. *Water Research* 9, 777–782.

Heathman, L.S., Pierce, G.O. and Kabler, P. (1936) Resistance of various strains of *E. typhi* and *Coli aerogenes* to chlorine and chloramine. *Journal of Bacteriology* 72, 1367–1387.

Kudryavtseva, B.M. (1972) An experimental approach to the establishment of zones of hygienic protection of underground water sources on the basis sanitary–bacteriological indices. *Journal of Hygience, Epidemiology, Microbiology, and Immunology* 18, 503–511.

LeChevallier, M.W. and McFeters, G.A. (1990) Microbiology of activated carbon. *Drinking Water Microbiology.* New York: Springer-Verlag.

LeChevallier, M.W. and Solotorovsky, M. (1965) *Three Centuries of Microbiology.* McGraw-Hill.

LeChevallier, M.W., Evans, T.M., Seidler, R.J., Daily, O.P., Merrell, B.R., Rollins, D.M. and Joseph, S.W. (1982) *Aeromonas sobria* in chlorinated drinking water supplies. *Microbial Ecology* 8, 325–333.

LeChevallier, M.W., Schiemann, D.A. and McFeters, G.A. (1987) Factors contributing to the reduced invasiveness of chlorine-injured *Yersinia entercolitica. Applied and Environmental Microbiology* 53, 1358–1364.

Pipes, W.O., Mueller, K. and Troy, M. (1987) Frequency-of-occurrence monitoring for coliform bacteria in small water systems. *Journal of the American Water Works Association* 79, 59–63.

Prescott, S.C. and Winslow, C.-E. (1915) *Elements of Water Bacteriology* 3rd edn. New York: John Wiley.

Rice, E.W., Allen, M.J. and Edberg, S.C. (1990) Efficacy of β-glucuronidase assay for the identification of *Escherichia coli* by the defined substrate technology. *Applied and Environmental Microbiology* 56, 1203.

Rice, E.W., Fox, K.R., Miltner, R.J., Lytle, D.A. and Johnson, C.H. (1996) Evaluating plant performance using endospores. *Journal of the American Water Works Association* 88 (9), 122–130.

Rice, E.W., Clark, R.M. and Johnson, C.H. (1999) Chlorine inactivation of *Escherichia coli* O157:H7. *Emerging Infectious Diseases* 5, 461–463.

Stukel, T.A., Reed, F.C., Greenberg, E.R. and Jacobs, N.J. (1987) Comparing three sampling designs for monitoring coliforms in small community water systems. *Journal of the American Water Works Association* 79, 50–54.