

THE JOURNEY OF MICROBIOLOGICAL MONITORING FOR ENSURING SAFE DRINKING WATER*

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INTRODUCTION

The provision of a safe and sustainable drinking water supply is one of the hallmarks of a successful society. Without safe drinking water, public health is always at risk and the economic well being of the community cannot be realized. Since the discovery by Lord Snow in 1854 that diseases can be attributed to contaminated drinking water, scientists, engineers and public health officials have endeavored to determine which water treatment processes will produce safe drinking water and how best to monitor these processes. This paper describes the evolution or “journey” of microbial monitoring practices for ensuring safe drinking water. It is not the intent to provide an exhaustive review of the literature, but a “roadmap” on the evolution of microbial monitoring practices and other related microbial drinking water issues.

PURPOSE OF WATER TREATMENT

The purposes of treating drinking water are multi-fold and, in general, include: 1) physical removal or inactivation of human pathogens (protozoa, bacteria, viruses)—not to produce sterile water; 2) reduce suspended particles, minimize organics and disinfection by-products, taste, odor, color, etc., 3) comply with applicable regulations that set specific maximum contaminant concentrations based, whenever feasible, on health based epidemiological data; and 4) provide esthetically-acceptable drinking water. Current practices for ensuring that drinking water is free of infectious human pathogens include: 1) watershed management to reduce the source of pathogens from human sewage and livestock; 2) surface water impoundments that provide retention time for pathogens to die-off or settle out of the water; 3) filtration processes—conventional media, membranes, and 4) disinfection (ozone, ultraviolet irradiation, chlorine-based compounds) Together these processes produce safe drinking water as defined by the appropriate health agency, and drinking water that is consumer-acceptable.

THE BEGINNING AND REALITY OF MICROBIOLOGICAL MONITORING

Microbiological monitoring to ascertain the safety of drinking water has been practiced for over 100 years since the link between contaminated drinking water and disease was thoroughly documented (McGuire 2006). Once this correlation was scientifically established, there was an on-going need to determine whether drinking water was contaminated with human pathogens to prevent waterborne disease outbreaks.

In the 1800's, it was realized that monitoring for all known human pathogens, and many of today's pathogens were unknown at that time, e.g., viruses, was an impossibility and that an alternative approach for routine monitoring of the microbial safety of drinking water was necessary. Frankland & Frankland (1894) stated "The longer the bacteriological examination of water is practiced the more evident does it become that in searching for pathogenic organisms special methods must be devised and adopted according to the nature of the particular microbe of which we are in quest, and that only any possibility of such pathogenic bacteria being found in the course of ordinary plate cultivations made with natural waters, the colonies of the common water-bacteria almost invariably so predominating as to exclude all others present in small numbers." It was also noted the need for "...larger volumes of water, as in this manner the chance of discovery is correspondingly increased."

And still today, pathogen monitoring of drinking water to make timely and correct public health decisions is not realistic, although some continue to believe that such monitoring may be possible.

Allen et al (2002) and Payment and Pintar (2006) described the major issues why pathogen monitoring of drinking water is not currently practical. These include:

- The wide diversity of human pathogens, i.e. protozoa, bacteria, viruses, that may be present in drinking water, and therefore the multitude of complex standardized methods that would be necessary to successfully monitor for each type of pathogens.
- The lack of standard methods that provide credible data, i.e. specificity, sensitivity, and reproducibility, to validate the effectiveness of the in-place treatment process and to make correct and timely public health decisions.
- The relatively low numbers of pathogens in surface waters and wastewaters in comparison to the entire total microbial flora in these waters.
- The inability of methods to determine whether pathogens are infectious, viable, or human or non-human infectious strains. Also, "molecular methods" that monitor for nucleic acids of human pathogens fail on this point.
- The need to examine large volumes (>1.0L) of water to have any reasonability of detecting the targeted pathogen.
- The desentization of any method involved with concentrating large volumes for subsequent analysis.
- The very few water laboratories that are capable of attempting pathogen monitoring.
- The inability to simultaneously monitor for all human pathogens that may be present in inadequately treated drinking water, and
- The days to weeks required for test "result" to make valid public health decisions based on the monitoring data.

THE INDICATOR CONCEPT

Yale Symposium

Since some of the above issues were realized in the 1800's, public health scientists have believed an "indicator" of recent fecal contamination that would signal the probability of human pathogens present in drinking water would be the most plausible approach for protecting against the ingestion of pathogen-laden drinking water. In 1885, Theodore Escherich published a paper stating that *Bacillus coli* (later renamed *Escherichia coli*) was the most appropriate indicator of fecal contamination (Escherich 1885, Edberg et al. 1988, 2000). Unfortunately at that time there were no easy and practical methods to isolate and speciate *E.coli* and thus other microbial indicators were used throughout the 19th and 20th centuries.

The attributes of bacterial indicators of recent fecal contamination and thus the potential presence of human pathogens are:

- Indicator organism always present in animal and fecal material
- Indicator organism present in high numbers to better facilitate detection
- Indicator organism persistence in the environment similar to human pathogens
- Detection methods relatively simple, rapid, and inexpensive.

Today the most common indicators used for drinking water include:

- Total coliforms
- Fecal coliforms
- *Escherichia coli*

The functional definition of total coliforms per *Standard Methods for the Examination of Water and Wastewater* is:

- Metabolize lactose to produce acid and gas, and
- Possesses the enzyme β – glucuronidase (only *E. coli*)

The coliform genera and their sources that meet either of these definitions are:

- *Escherichia* – human and animal feces
- *Enterobacter** - environment, feces,
- *Klebsiella** – environment, feces,
- *Citrobacter* – environment
- *Serratia** – environment

* Often found colonized in drinking water distribution and storage systems

As shown above, only *E.coli* is exclusively found in fecal matter and thus best supports its preference as an indicator of pathogens in drinking water. The following tables support the exclusivity of *E.coli* as the indicator of fecal origin.

TABLE 1: Relative Number of Fecal and Nonfecal Types of Coliform Bacteria in Various Substances (Source: Iowa State College Engineering Experiment Station Bulletin, 62 (1921), pg.79)

Source	Number of Strains Observed	Percentage of Strains of <i>Aerobacter aerogenes</i> *	Percentage of Strains <i>Escherichia coli</i>
Human feces	2534	5.9	94.1
Animal feces	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

*Renamed *Enterobacter aerogenes*

TABLE 2: Percentage of Genera of Coliforms in Human and Animal Feces (Source: *Escherichia coli: The Fecal Coliform*. A.P. Dufour. Special Technical Pub. 65, ASTM, pp. 48-58, 1977.)

Animal (Number Examined)	<i>E.coli</i> (%)	<i>Klebsiella spp.</i> (%)	<i>Enterobacter/Citrobacter</i> (%)
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 (26)	100	-	-
Human (26)	96.8	1.5	1.7
Average	94.5		

In both studies spanning 56 years, the percentage of the *E.coli* population in fecal matter is consistently greater than 93 percent (93.4 and 94.5). In particular the 1997 study shows that the percentage of *Klebsiella spp.* and *Enterobacter/Citrobacter spp.* in fecal matter to be insignificant making these species not a relevant indicator of fecal contamination. Despite the overwhelming evidence that *E. coli* is the best indicator of health risk, i.e. presence of pathogens, total coliform monitoring for drinking water continues to be considered as a suitable indicator of possible health risks by some health agencies and public health officials. There still are regulations that specify total coliform monitoring

although there is movement from coliforms to *E.coli*, and eliminating “fecal coliforms” as a valid indicator of water quality.

Coliforms, fecal coliforms, and heterotrophic plate count (HPC organisms) are not unique to drinking water but are the normal flora found in the environment including vegetables that are consumed uncooked without adverse health effects. Table 3 provides such information.

TABLE 3: Bacterial Populations in Uncooked Vegetables*

VEGETABLE	NUMBER TESTED	log/gram			REFERENCE
		TC	FC	HPC	
Alfalfa sprouts	2	ND	ND	8.2	Callister & Agger
	3	>4.0	0.6->3.0	8.8	Reina et al.
Broccoli	1	ND	ND	4.5	Callister & Agger
	36	6.3	ND	6.6	Albrecht et al.
Cabbage	8	ND	2.4	ND	Monge & Chinchilla
	12	ND	ND	5.8	Garg et al.
Carrots	1	ND	ND	7.3	Beuchat Brackett
Cauliflower	9	ND	ND	4.8	Garg et al.
	36	4.9	ND	5.2	Albrecht et al.
Celery	2	ND	ND	6.0	Callister & Agger
	4	ND	ND	4.5	Garg et al.
Cucumber	4	5.2	ND	5.9	Reina et al.
	8	ND	4.2	ND	Monge & Chinchilla
Lettuce	4	ND	ND	5.2	Garg et al.
	7	ND	ND	6.2	Callister & Agger
	8	ND	5.4	ND	Monge & Chinchilla
	36	5.4	ND	5.7	Albrecht et al.
Mixed Salad	1	ND	ND	5.9	Garg et al.
	3	6.1	5.5	7.1	Vescovo et al.
	12	6.2	ND	8.3	Beuchat & Brackett
Radish	8	ND	5.6	ND	Monge & Chinchilla
Spinach	16	ND	ND	5.9	Garg et al.
Tomato	8	ND	2.6	ND	Monge & Chincilla
	36	5.2	ND	5.5	Albrecht et al.

* Provided by Eugene W. Rice

DEVELOPMENT OF FECAL COLIFORM CONCEPT

Microbiologists were aware that some genera of the coliform group (*Klebsiella*, *Enterobacter*) were not necessarily of fecal origin, but still were enumerated by the then existing 35C coliform methods. This resulted in “false positives”, i.e. positive test produced by non-fecal coliforms. To address this problem, it was theorized that by incubating at a higher temperature, 44.5C rather than 35C, the non-fecal coliforms (those

from predominantly environmental sources) would not grow and produce false positive results. This assumption was not entirely valid since research (Edberg 1994) has shown that a significant percentage (~15 percent) of *Klebsiella* isolates did grow at 44.5C, and 10-15 percent of *E.coli* did not grow at 44.5C, possibly due to stress. There were also problems with being able to maintain water incubators within the critical temperature range ($\pm 0.2C$). Another major problem was the mislabeling of thermo-tolerant coliforms as “fecal coliforms.” This misnomer has caused considerable angst with public health officials, the media, and consumers. The term “thermotolerant coliforms” is being used more often than “fecal coliforms,” but the stigma of the name still persists.

In the early 20th century when wastewater was untreated and discharged directly into surface waters that served as downstream drinking water source, the assumption that “fecal coliforms” were a good approximation of *E.coli* populations might have been more valid than today since wastewaters are subject to a higher levels of treatment prior to discharge. With *E.coli* methods now available, the fecal coliform test should be discontinued for all waters.

Regarding *Klebsiella spp.*, some erroneously believe that that this organism poses a public health risk when present in drinking water. In his 1988 review of *Klebsiella* infections, Duncan (1988) found no waterborne disease was ever associated with this organism in drinking water – all were associated with hospital-acquired infections. His conclusion was “...*Klebsiella* in water supplies should therefore not be considered a hazard to human health.”

EARLY COLIFORM/FECAL COLIFORM METHODS DEVELOPMENT

Because there were no reliable tests to speciate for *E.coli*, the scientific community developed coliform detection and enumeration methods for coliforms and fecal coliforms to help in making correct and timely public health decisions.

Standardized methods for coliforms and fecal coliforms were developed using either a liquid-based most probable number (MPN) or the media-based membrane filtration (MF) method; both types of tests are included in *Standard Methods for the Examination of Water and Wastewater* (1998). While the liquid-based (MPN) method was known to have a high recovery rate (thought to be better able to recover stressed organisms), the MF method has been the preferred method since it was thought to accurately enumerate either coliforms or fecal coliforms in water samples although this assumption is without statistical basis (see Table 9222.II. 95% - Confidence Limits for Membrane Filter Coliform Results Using 100mL Sample in *Standard Methods*). *Standard Methods* also contains Table 9221.IV. MPN Index 95% - Confidence Limits (CL) for Various Combinations of Positive Results When Five Tubes are Used per Dilution (10mL, 1.0mL, 0.1mL).

As examples, 10 CFUs on a membrane have a CL of 4.7 to 18.4 CFUs while an MPN combination of 3-0-0 positives has a mean MPN index/100mL of 8 CFUs with a CL of 3.0 to 24 CFUs. With such wide limits by either method and other statistical evidence as

shown in Table 4 (Christian & Pipes 1988), the concept of presence/absence (P/A) for drinking water for compliance monitoring for assessing the safety of drinking water was adopted by U.S. Environmental Protection Agency (USEPA) and other national agencies rather than a absolute number, e.g. <10 CFUs/100mL. Thus, only the presence or absence of *E. coli* is most relevant as a credible health alert, not the NUMBER of *E. coli*, fecal coliforms, or coliforms.

TABLE 4: Variability of An Individual Well-Mixed Water Sample (Christian & Pipes, 1983)

In a random dispersion of 36 100mL of water containing coliforms, the probability of the sample having a bacterium or more was:

- 37 percent one bacterium
- 3 percent no bacteria
- 18 percent two bacteria
- 7 percent > two bacteria

DEVELOPMENT OF CHROMOGENIC ENZYME (CE) METHODS

In the late 1980's, a new media drawing from clinical technologies was developed based on chromogenic enzymes (CE) that simultaneously measured by presence/absence, both total coliforms and specifically, *E. coli* (Edberg et al 1988).

Major drivers in the development of a CE medium included:

- Unique enzyme only found in *E. coli* – β -glucuronidase
- Enzyme found in 95 percent of *E.coli*
- $10^8 - 10^9$ *E. coli* per gram feces
- Metabolize 4-methyl – β – umbeliferyl glucuronide (MUG) for *E.coli* detection
- Rapid, simple, easy to use, inexpensive compared to MPN and MF methods.

After receiving approval by the USEPA compliance monitoring, the media was commercialized as the Colilert® test. Unlike the liquid-based Most Probable Number (MPN) or membrane filtration methods requiring up to 72 hour to complete, the CE test provided “completed” results in 18-24 for making public health decisions, and even sooner when higher numbers of coliforms/*E.coli* are present in the water samples. The test was approved by the USEPA for compliance monitoring for drinking water in the Total Coliform Rule (TCR) and the Colilert method for total coliforms and *E. coli* was included in *Standard Methods*. Similar media based on the same concept have been commercialized and are now approved and available for monitoring drinking water; see Table 5.

Chromogenic enzyme (CE) methods as used in a presence/absence format have several attributes that are attractive for drinking water testing and compliance monitoring, such as:

- Simultaneous detection of coliforms and *E. coli*
- Results are unequivocal and obtained in 24 hours or less enabling timely public health decisions
- Equal or better sensitivity, specificity, reproducibility than either the MF or MPN methods
- Not subject to interference by heterotrophic bacteria (false negatives) as can occur with lactose-based MPN and MF methods
- Availability as dehydrated media with a long shelf life and does not require refrigeration
- Less expensive when compared to the total costs of MF and MPN methods (media preparation, filtration apparatus, incubator, trained analyst, sample shipping to certified laboratory, etc.)
- Approved by the USEPA and other national agencies
- Included in *Standard Methods for the Examination of Water and Wastewater*
- Require minimal training and laboratory equipment to perform the analysis, and
- Can be confidently used by large to small water utilities to reduce the time required from sampling to results.

While both the MPN and MF methods have certainly protected public health by identifying fecally-contaminated drinking water, both methods required up to 72 hours before “completed” data are available to make informed public health decisions. A positive acid-gas reaction using the MPN method or green-metallic sheen colonies when using lactose-based MF media is only the first step of a three-step analysis specified in *Standard Methods for the Examination of Water and Wastewater*; these are “presumptive” results. Both methods need to perform “confirmed” and “completed” tests each requiring up to and additional 24 hours. Both the MPN and MF methods were believed to accurately provide quantifiable data, i.e. numbers of coliforms present in the water sample. However, both methods result in wide variability in numbers as described above and were subject to interferences from heterotrophic bacteria (described later). The very nature of the MF method (bacterial cells entrained on a solid membrane) resulted in lower recovery rates as compared to liquid-based methods.

With there no longer being a defensible rationale to quantify the number of coliforms or *E.coli* in treated drinking water owing to the science- and health-based Presence/Absence (P/A) concept, use of methods (MPN, MF) that supposedly quantified the number of bacteria in drinking water is not necessary. With CE methods now available, the use of MPN and MF methods for drinking water should be questioned if the goal for analysis is to make public health decision as quickly as possible (<24 hours) not 72 hours when using MPN and MF methods. In USEPA’s current discussions for updating the Total Coliform Rule, it would be prudent to stipulate the use of the more rapid microbial monitoring methods that are “completed” in the shortest time.

TABLE 5: Summary of Approved and Accepted Chromogenic Enzyme Methods for EPA Total Coliform Rule Monitoring.*

Medium	Method Designation
Total Coliforms	
Colilert® ² , Colilert – 18® ^{2\}	SM 9223 ¹
Colisure Test ²	SM 9223
Readycult® ³	See footnote 3
E*Colite® Test ⁴	See footnote 4
Colitag® ⁵	See footnote 5
<i>E. coli</i>	
Colilert® or Colilert-18®	SM 9223
Colisure Test ²	SM 9223
Colitag® ⁵	see footnote 5
E*Colite™ Test ⁴	see footnote 4
Readycult® ³	see footnote 3

Footnotes:

¹ *Standard Methods for the Examination of Water and Wastewater*, 18th edition (1992), 19th edition (1995), or 20th edition (1998). American Public Health Association, 1015 Fifteenth Street, NW, Washington, DC 20005. The cited methods published in any of these three editions may be used.

² A description of the Colilert, Colilert-18 and Colisure Tests, may be obtained from IDEXX Laboratories, Inc., One IDEXX Drive, Westbrook, ME 04092. www.idexx.com/water

³ The Readycult® Coliforms 100 Presence/Absence Test is described in the document, "Readycult® Coliforms 100 Presence/Absence Test for Detection and Identification of Coliform Bacteria and *Escherichia coli* in Finished Waters," November 2000, Version 1.0, available from EM Science (an affiliate of Merck KGaA, Darmstadt Germany), 480 S. Democrat Road, Gibbstown, NJ 08027-1297, telephone 800-222-0342. www.merck.com

⁴ A description of the E*Colite® Test, "Presence/Absence for Coliforms and *E. Coli* in Water," Dec 21, 1997, is available from Charm Sciences, Inc., 36 Franklin Street, Malden, MA 02148-4210. www.charm.com

⁵ Colitag® product for the determination of the presence/absence of total coliforms and *E. coli* is described in "Colitag® Product as a Test for Detection and Identification of Coliforms and *E. coli* Bacteria in Drinking Water and Source Water as Required in National Primary Drinking Water Regulations," August 2001, available from CPI International, Inc., 5580 Skylane Blvd., Santa Rosa, CA 95403, telephone 800-878-7654, Fax 707-545-7901, Internet address <http://www.cpiinternational.com>.

*From J. Clancy

While CE media are widely-used for monitor drinking water for the presence of total coliforms and *E.coli* in drinking water, i.e. public water systems, military installations,

commercial airlines, disasters such as Hurricane Katrina, developing countries, etc., their use, especially by small water systems for compliance monitoring, is limited at this time by regulation and the thought by some health agencies and their staff that still believe and require that only fully certified laboratories can and should perform the microbiological testing. While water utilities may be permitted to “screen” their drinking waters, these analyses do not satisfy the compliance requirements thus requiring the utility to send official samples to a certified laboratory.

SIGNIFICANCE OF HETEROTROPHIC PLATE COUNT (HPC) BACTERIA IN DRINKING WATER

While the universal occurrence of heterotrophic plate count (HPC) bacteria in soils, foods (Wadhawa et al. 2002), air, and all sources of water is fully documented, there is a lingering question by some as to whether this group of organisms may signal increased health risk when elevated populations are present in drinking water. In a review (Allen et al. 2004) on the significance of HPC bacteria in drinking water, the following literature- and epidemiological-based conclusions were made:

- There is insufficient evidence to conclude that HPC bacteria in drinking water pose a health risk and, therefore, it is not possible to establish any health-based standards for HPC bacteria in drinking water.
- The various methods used to enumerate HPC bacteria differ significantly in the number and genera detected, and HPC data from different methods is not comparable.
- HPC populations greater than 500-1000 CFU/mL in drinking water can interfere with coliform/*E.coli* analysis by lactose-based methods, which includes the MF method.
- *Klebsiella*, *Pseudomonas*, and *Aeromonas* cannot be considered opportunistic pathogens when found in drinking water, since there is no clinical or epidemiological evidence to support this designation.
- HPC determinations can be a useful tool to monitor the efficacy of drinking water treatment processes and undesirable changes in bacterial water quality during storage and distribution, but not because of health-risk reasons.

Hardalo and Edberg (1997) in their publication “*Pseudomonas aeruginosa*: Assessment of Risk from Drinking Water” concluded it was not practical to eliminate *P. aeruginosa* from food or drinking water and “attempts to regulate *P. aeruginosa* in drinking water would not yield public health protection benefits and could, in fact, be counterproductive in this regard.”

With regard to concern with the cytotoxicity and invasiveness of HPC bacteria isolated from drinking water, Edberg et al. (1997) reported HPC bacteria “did not possess significant virulence characteristics associated with human health threats.”

In a later publication, Allen et al.(2004), noted that HPC analysis in drinking water is a useful tool for:

- Monitoring the efficacy of water treatment processes, including disinfection;
- Obtaining data on HPC populations that may interfere with coliform analysis when using lactose-based media
- Assess changes in treated water quality during distribution and storage
- Assessing microbial growth on materials used in the construction of potable water treatment and distribution systems
- Measuring bacterial regrowth potential in treated drinking water; and
- Monitoring bacterial population changes following treatment modification such as ozone or change in chlorine-based disinfectants.

As noted above, bacterial regrowth can and does occur in water distribution and storage following appropriate treatment. Allen et al. (1980) showed with scanning electron microscopy (SEM) images (see Figures 1 & 2) that water main tubercles (encrustations) attached to pipe walls of water mains contained large numbers of bacteria that appeared in good physiological condition and had colonized at the surface or near-surface of the tubercles. Further studies confirmed that bacteria can colonize on or within tubercles despite the presence of disinfectants.

The source of these bacteria can include survival through treatment processes, shedding of bacteria from the filter media, presence on new or replaced water mains, etc. Research has confirmed that regrowth in water distribution systems is a common phenomenon and is promoted by nutrients, e.g., ($>10\mu\text{g/L}$ assimilable organic carbon (AOC) for unchlorinated systems, $> 100 \mu\text{g/L}$ AOC for disinfected systems), warmer water temperatures ($>15\text{C}$) as found during the summer months, long residence time of the water within the distribution system/storage reservoirs (days to weeks), and low disinfectant residuals especially at the far reaches of the distribution system. While regrowth can be minimized to some extent, it cannot be totally eliminated from distribution systems.

Unfortunately two genera that frequently colonize on or within tubercles are *Klebsiella* and *Enterobacter*, both coliforms. During routine compliance sampling with subsequent analysis for coliforms, these colonized bacteria can detach (e.g. abrupt flow changes or flow reversals) and result in a positive coliform sample. When this happens the “wheels are set in motion” for extensive re-sampling at a cost of resources to the water utility and health agencies. If follow-up samples are positive for coliforms, there is a possibility that the health agency will require the water utility to issue a “health advisory” to boil water before drinking even if it has been determined that there was no failure in treatment or breach of the integrity of the distribution system. If *E.coli* was exclusively used as the appropriate indicator of increased health risk, there would be far fewer coliform-based health advisories.

Public health advisories are quite costly since advisories impact schools, hospitals, hotels, restaurants, medical and dental offices, tourism, business establishments, and the local economy in general. Often there is no definitive end-point when the drinking water is

deemed safe to drink and the consumer now doubts the safety of their drinking water. With such dire economic consequences and interrupting day-to-day activities of residents, health advisories should not be issued unless there is a preponderance of evidence that a substantial health risk exists, i.e. treatment failure, loss of water pressure, catastrophic failure of a water transmission main, and not just a precautionary measure to set an health authority's mind at ease. The repeat occurrence of coliforms without the presence of *E.coli* does not rise to a level for a health advisory!

ANTICIPATED CHANGES TO THE USEPA COLIFORM RULE

In the past few years the USEPA, as required by statute, is reviewing and revising the Total Coliform Rule, i.e Revised TCR. In the multi-stakeholder Agreement in Principle (AIP); see: http://www.epa.gov/safewater/disinfection/tcr/pdfs/tcrdsac/agreement_inprinciple_tcrdsac_2008-09-18.pdf, signed September 9, 2008, by the Total Coliform Rule/Distribution System Advisory Committee (TCRDSAC) included ten criteria for consideration during revisions to the current Total Coliform Rule.

These are:

1. Meeting the objectives of the current rule,
2. Maintaining or enhancing public health protection,
3. Reducing burden
4. Being cost-effective
5. Being simpler to implement
6. Considering implications and linkages to other rules
7. Reflecting variations in system size and type
8. Recognizing the value of effective operators
9. Using the optimal indicator for each purpose or objective, and
10. Being supported by scientific data.

The AIP further states that “the principals and assumptions underlying these recommendations are that:”

1. Maintaining a maximum contaminant level goal (MCLG) and MCL for *E.col* and Total Coliform monitoring to establish a framework for public water systems to assess for sanitary defects and correct them as appropriate
2. Use of appropriate microbial indicators that a) evaluate effectiveness of treatment, b) determine the integrity of the distribution/storage system, and c) signal the possible presence of fecal contamination.
3. Understand that revisions to the Total Coliform Rule may impact other rules (SWTR, D/DBPRs, etc.)
4. Sanitary defects are related to public health, the RTCR compliments the multi-barrier approach for protecting the distribution system from contamination, and that most public health agencies already have authority to require utilities to correct distribution system defects

5. EPA will continue to consider “decision-relevant data” during the rule-making process.

Other pertinent concepts included in the AIP are:

1. The importance of the integrity of the distribution system to potential health risk, not just treatment alone,
2. The elimination of all “fecal coliform” as a suitable indicator in the RTCR probably since there are simple methods for detecting *E. coli*,
3. The use of the best available analytical methods without sacrificing accuracy, precision, and specificity resulting data on the order of 24 hours to make timely public health decisions. Note: Methods that require “confirmed” and “completed” steps exceed the 24 hour limit,
4. EPA should engage stakeholders in a technical dialogue to review Alternative Test Procedures (ATP) microbial protocol for total coliforms and *E.coli* to review issues such as sensitivity, specificity, matrix interferences, and false positive and false negative results, temperature, and holding times with respect to approved methods for monitoring for total coliforms and *E.coli* in drinking water,
5. The occurrence of total coliforms in a water drinking water sample prompts the water utility to investigate the probable source of the coliforms and to take appropriate action as necessary, but does not signal any health concern if *E. coli* is not present. Such actions include determination if sanitary defects and defects in the distribution system coliform monitoring practices, and
6. Water utilities in consultation with public health agencies should develop a sample siting plan that is representative of the water quality in the distribution system. Note: Because of the costly and intensive resources that are expended when positive coliform samples occurred, utilities should consider installing secure sampling “cabinets” in public buildings where water is always flowing from the sampling spigot. This practice would better ensure that the water sample is not contaminated by the sampling spigot.

The anticipated revisions to the Total Coliform Rule significantly improve the science basis for public health protection. The USEPA will formally elevate the presence of *E.coli* (<1 per 100 mL) as the sole microbial criterion for the probability of unreasonable risk and eliminating the health relevance of coliforms in the absence of *E.coli*. Thus, codifying the health relevance of *E. coli* in drinking water as first suggested in 1885 by Theodore Esherich.

DOES THE JOURNEY END?

While the USEPA is updating the Total Coliform Rule to better reflect the science, the agency has been mandated by a well-intended but ill-advised Congress to examine additional microorganisms and chemicals that are to be considered for future regulation. This law required the development of a “Contaminant Candidate List” (CCL) for microorganisms and chemicals. Once a microorganism or chemical is placed on the

CCL, the USEPA was to then determine as to whether or not there are clinical and epidemiological evidence that warrant regulations for treatment processes that would remove/inactivate this organism or remove the specific chemical.

In the view of some who participated in the development of the initial microbial CCL, there was a lack of rigorous discussion on sufficient clinical and epidemiological evidence that the proposed microorganisms warrant being on the list. Aside from the lack of this critical evidence, the listing of a microorganism implies to the public, water utilities, public health agencies, and the news media that organisms on the CCL when present in drinking water may pose an elevated health risk. It also implies that these microorganism should be monitored for their presence in drinking water even if it is not yet regulated regardless if there may be credible methods (sensitivity, specificity, reproducibility) for detection. The listing of an organism can also set in motion research for detection methods and re-evaluation of treatment processes when in reality there is sufficient evidence that existing treatment practices, i.e. filtration, UV, disinfection, readily remove or inactivate the microorganism. This scenario is also valid for some chemicals on the CCL.

In attempting to improve the credibility of CCL identification process for microorganisms, Edberg et al. (2007) published a review entitled “Issues for Microbial Regulation: *Aeromonas* as a Model,” – this organism was listed on the first CCL. This paper summarized the criteria that should be met before any microorganism can be considered for inclusion on USEPA’s CCL.

The criteria are:

- 1) there is a clinical history of an organism causing disease from the ingestion of drinking water;
- 2) there is epidemiological evidence that drinking water rather than food or other vectors is a major cause of disease;
- 3) there is sufficient evidence that the target organism, if found in water, possesses virulent factors capable of causing disease in humans;
- 4) there is sufficient evidence that the targeted organism is not readily removed or inactivated by multi-barrier conventional treatment processes (e.g., coagulation-filtration-disinfection);
- 5) there is sufficient evidence that the target organism, if surviving conventional treatment, will be viable, virulent, and present in sufficient numbers (infectious dose) to cause the onset of disease;
- 6) there are robust analytical methods for the target organism which have acceptable sensitivity, specificity, and reproducibility to measure accurately the presence of the targeted organism in treated drinking water, and
- 7) the performance criteria of analytical method(s) for the targeted organism have been certified by the appropriate public health agency, and there is intra-laboratory field-test performance data to base this certification.

Of the above criteria, the most daunting challenge is the lack of clinical and epidemiological evidence that is required before USEPA can make an informed decision that a specific microorganism constitutes an unreasonable health risk, and thus placed on the CCL. There is, and will most likely remain, insufficient evidence to continue to list most, if not all, of the CCL microorganisms. The basic problem is that USEPA is mandated to create and update a CCL by law, not necessarily based on existing science. It is inconceivable that there are any microorganisms whether “known” or “emerging” which cannot be removed or inactivated by current water treatment processes.

Based on the above criteria neither *Aeromonas* (Edberg et al, 2004) nor *Helicobacter pylori* (Johnson et al, 1997) should not have been included on the CCL. The same conclusion may be true for other microorganisms on the list. It is recommended that the USEPA apply the above criteria in modifying the current CCL list for microorganisms in the future. If the current CCL mandate is unworkable, i.e. the tremendous cost to obtain credible epidemiological data of disease caused by a CCL microorganisms from the ingestion of treated drinking water, the USEPA should inform Congress so that the law could be revised accordingly.

CHALLENGES OF THE 21ST CENTURY

During the latter part of 20th century significant public health gains were realized as research resulted in discovery and implementation of water treatment processes such as membranes, UV disinfection, ozone, chlorine-based disinfection that together resulted in greater health protection. Research also showed how to optimize conventional filtration, the effectiveness of slow-sand filtration for removal of protozoan and other microorganisms, and disinfection practices to minimize the formation of undesirable disinfection by-products. This late 20th century “renaissance” of research by the global research community also resulted in more effective monitoring practices and devices that help ensure that treatment processes were working correctly, e.g. on-line instruments such as particle counters, turbidimeters, chlorine residuals, and the ability to measure for the presence of *E. coli* in 24 hours or less.

However, many of the 20th century challenges continue to be 21st century challenges, and there are new challenges such as the impact of global warming on sustainable water sources and sufficient food for the citizens of the world.

Water is now viewed as a continuum that it is - from precipitation to drinking water to wastewater to reuse to desalination and to the oceans. With all the better and accessible source waters now essentially utilized for drinking water, the use of impaired quality waters (wastewater effluents, brackish ground water, seawater) are more often being used to satisfy the drinking water needs of an ever growing global population. While there are many processes that can convert lower quality waters into potable water, there are many drawbacks such as high energy consumption, complexity of operation and maintenance, and production and disposal of residuals. These drawbacks often preclude their use especially by smaller public water systems.

Some of the 21st Century challenges include:

- **Minimizing Risk.** The acceptance and realization that drinking water cannot be made absolutely “safe” from a microbiological perspective. The various water treatment technologies reduce the probability of acquiring an infection, with or without a demonstrated disease. The severity of an outcome resulting from consumption of contaminated drinking water depends on a variety of factors related to the microorganism and the infected individual. Some of the factors include: virulence of the microorganism, numbers of viable organisms ingested, previously acquired immunity, reduced immunity (i.e. immunosuppressed or immunocompromised), or a general fragility (i.e. infants and older individuals from other diseases). Immunosuppressed, immunocompromised and older individuals having an increased risk to other underlying diseases are typically under medical supervision and are aware of the need to minimize a risk of infection and the precautions to take. Drinking water is usually a minor factor because pathogens in water are the same as those transmitted regularly through personal contact, food, and animals. These individuals are usually given advice on how to manage risk and most pediatricians recommend the use of boiled water for food preparation. (Allen et al. 2008)

The current array of USEPA regulations when implemented provide a level of protection from disease equivalent or higher than from other everyday sources. The concept of the microbial CCL should be abandoned given that current USEPA regulations that specify level of treatment required based on source water quality ensures the appropriate level of risk reduction for both “frank” pathogens and so-called “emerging pathogens.”

- **Integrity of Distribution System.** While significant gains have been realized in drinking water treatment processes that minimize the microbial risk to consumers, the integrity of distribution and storage network pose a higher and ever increasing risk as the water distribution networks continue to deteriorate. The literature documents a significant percentage of water-borne outbreaks have been attributed to distribution system problems that include water main ruptures, chronic leaking, cross-connections, sudden pressure changes allowing for infiltration of outside water, poorly designed and maintained storage facilities, etc. For these reasons, the USEPA as it revises the Total Coliform Rule is developing distribution system requirements that would minimize risk of microbial contamination post treatment. The challenge for the USEPA is to develop workable regulations and the challenge for the drinking water community will be the capital necessary to repair or replace the aging infrastructure. The magnitude of capital needed will require water rate increases for years and decades to adequately renew the integrity of the distribution system to minimize risk from water-borne disease.
- **Role of Microbial Monitoring.** Historically microbiological monitoring has been the cornerstone for assessing potential health risk in drinking water. The inability

to directly measure for the presence of human pathogens led to the use of indicators, i.e. coliforms, “fecal coliforms,” and more recently *E.coli*. of fecal contamination. Based on the science, *E. coli* is now the recognized organism most often associated with pathogen occurrence and there are now rapid, simple, and low-cost methods for determining its presence. Total coliforms can be used as an indicator of “water quality,” but not health risk although there continues to be a mindset for some that coliforms still can be used for assessing risk.

While the assessment of health risk has based in part on the presence or absence of indicator bacteria, in reality microbial monitoring as now practiced or required is a very imperfect and may be questionable approach to assessing health risk. Given the number of water samples to be analyzed by statute, the very minuscule volumes tested (100mL x number of samples required per month as compared to the total volume of water produced in a month, e.g. 0.0000002%), the different sensitivity and specificity of approved methods, and the complexity of water distribution networks, it is difficult to conclude that samples free of coliforms and *E.coli* denote that drinking water is risk-free.

Data from three large water utilities performing microbiological tests that exceeded their specific regulatory requirements tested 0.0000002 to 0.0000005 percent of their treated drinking water or 99.9999995 to 99.99999998 percent of the delivered drinking water was not tested. These data do not include the extensive and appropriate microbial testing that utilities perform after water main breaks, water main replacement and renewal, or responding to customer complaints. Given the inconsequential percentage of water tested as prescribed by regulations, it would be difficult to conclude that there would be a system-wide elevated health risk when there is a total coliform/*E.coli* positive sample. Follow-up re-sampling is certainly warranted but assuming increased health risk to the population is not.

While microbial monitoring is ONE tool that can be used for assessing water quality and risk, there are other parameters that appear to provide a greater degree of confidence in the safety of drinking water.

These parameters include:

- Appropriate treatment processes as determined by source water quality;
- Continuous on-line monitoring of the treatment processes that ensure the processes are optimized;
- Trained or certified operators;
- Appropriate scheduled operation and maintenance of the treatment processes;
- Appropriate disinfection residuals when the water leaves the plant and the maintenance of residuals throughout the distribution and storage network;

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- An effective cross-connection and leak detection program and management of the distribution system to minimize water main ruptures; and,
- Hazard Analysis Critical Control Point (HACCP) strategies may be appropriate for medium to large systems, although certain elements of HACCP may be used by smaller water utilities.

Microbial monitoring (*E.coli*) is especially important during repair and renovation of water mains, during the repair of water main ruptures, and catastrophic events, e.g., hurricanes, earthquakes.

In recent years there has been growing interest in the potential use and practicality of molecular methods for pathogen detection that are reported to be more rapid than cultural methods.

There are several challenges that need to be addressed and understood when considering molecular methods. Some of these include:

- Of the array of microbial pathogens that may be present in treated drinking water, which one (s) should be selected to best determine the risk from drinking water?
- What are the costs of equipment, supplies, technical expertise, facilities, reagents, etc. required to perform these methods?
- What size water utility (population served) would have adequate resources to routinely use in confidence molecular methods?
- As with other approved microbial methods now used to assess the safety of drinking water, how would these methods be approved (standardized) and by whom?
- Does the presence of microbial nucleic material in drinking water pose a health risk since nucleic material is not infectious; recall the purpose of treatment is to physically remove, e.g., filtration, or inactivate pathogens, i.e. physically destroy the organism that would result in the release of nucleic material?
- What “positive” result or signal would signal an increased health risk and what would be the appropriate response?

While there will be continued research on the development of molecular methods for assessing the safety of drinking water, the above questions need to be addressed at some point, and would the cognizant health agencies permit the use of these methods in lieu of or in addition to the cultural methods, e.g. *E.coli* ?

Given the inherent 18-24 hour delay with indicator (cultural) *E.coli* methods and the small volume tested in comparison to the large volume of water delivered to consumers, it seems more appropriate to use both biological and non-biological parameters (turbidity, disinfectant residuals). Instruments that measure these non-biological parameters can be installed on-line at the treatment plant and throughout the distribution system (particularly disinfectant residuals) to determine changes in water quality. As more reliable and less costly on-line instruments become available, water utilities should consider greater use of these instruments as they provide real-time monitoring of water

quality changes and indirectly potential microbiological changes. The measurement of HPC bacteria could be especially useful in locating areas within the distribution and storage network where bacterial regrowth is occurring resulting in loss of residual and elevated taste and odor. The HPC media R2A has been found to enumerate more bacteria than other media, therefore providing better data as to the HPC populations in the water and water mains.

SMALL WATER SYSTEM ISSUES

There continues to be chronic problems with small public systems that are associated with a high percentage of water-borne outbreaks in the US, Canada, and worldwide. These systems serve small populations (<3300 by EPA definition) often with no filtration or even adequate disinfection. While many small systems use ground water and only use disinfection, often surface and groundwater systems have dilapidated distribution and storage infrastructure, and often no cross-connection requirements.

A chronic problem is the lack of capital to fund treatment and distribution system upgrades, and an unwillingness of residents to increase water rates until there is a water-borne disease outbreak in their community. Also, some consultant firms may not be interested in small system projects because of low profit margins, lack of guaranteed capital, very small capital available, and difficulty in being paid in a timely manner. This situation will not change unless there is a long term commitment of stakeholders (health agencies, consultants, small system consumers, and financial institutions, etc) to work together to help improve the situation. Admittedly there are a significant number of very small systems that realistically will find it difficult to install the appropriate treatment.

One approach that might improve the above situation would be the development of a “primer” that develops a matrix (quality of source water, size and age of system, integrity of the distribution system, current and possible higher water rates, topographical location, power source, etc.). This matrix would produce a “cookie cutter” approach that consultants could use and the health agencies would accept for treatment options OR infrastructure upgrades for specific small systems. It is envisioned that no extensive pilot studies would be necessary and the consultant would be indemnified when recommending treatment options based on the matrix analysis. Financing could be from a state or provincial agency that would loan the funds (not a grant) based on the feasibility of the project (cost and ability for the community to pay). The loan fund, once established, would be renewed by payments from the small systems that previously received a loan.

One technology that seems well suited for small systems is slow sand filtration that requires no chemicals, can often utilize gravity from the source to the consumer, and can be operated/maintained with minimum education and training. Many slow sand filters are operating successfully in North and South America. There are also tablet chlorinators that are inexpensive and easy to operate and maintain. UV disinfection is also a viable treatment technology to consider for medium to small water systems, and membrane treatment is another emerging cost-effective technology.

RECOMMENDATIONS FOR USING THE APPROPRIATE MICROBIAL INDICATOR FOR ASSESSING DRINKING WATER QUALITY AND RISK

- Use the coliform group to monitor treatment efficiencies/water quality changes in distribution system and storage systems.
- Discontinue use of fecal coliform methods to quantify fecal loading in source waters
- Discontinue use of fecal coliform methods for drinking water and source waters
- Use liquid-based methods for best sensitivity (better than MF methods susceptible to clogging and spreading colonies)
- Use MUG-based methods for rapid, specific, and completed test for *E.coli*
- Use *E.coli* methods that don't require confirmation step

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Figure 1: SEM showing bacteria in water main tubercle – New Haven, CT (Allen et al. 1980)

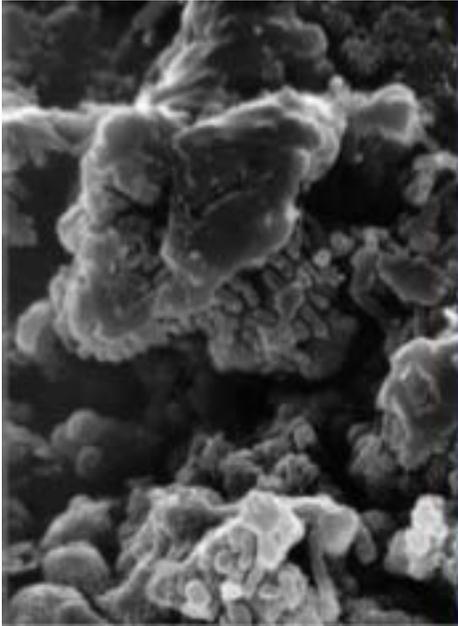


Figure 2 - SEM showing bacteria in water main tubercle – New Haven, CT (Allen et al. 1980)

