

National Collaborating Centre for Environmental Health

Centre de collaboration nationale en santé environnementale

Understanding microbial indicators for drinking water assessment: interpretation of test results and public health significance

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Introduction

Boil water advisories are issued when water testing detects higher than accepted amounts of microbial indicators or when there are deficiencies with water treatment. The fear of not issuing a boil water advisory when there is truly a health risk associated with water consumption can lead water operators to call for boil water advisories as a precautionary measure. Yet, issuance of boil water advisories can have unintended consequences. Repetitive boil water advisories affect their effectiveness and can translate into a loss of confidence in drinking water systems.¹ There is also an economic impact at the local level, causing a financial loss for the community.¹

Often practitioners interpret the presence of microbial indicators to mean that there is a public health risk, when this is not always true. This document aims to assist environmental health officers in interpreting drinking water laboratory results by describing the types of microbial indicators used, the ways in which they are reported, and their limitations. This document is meant to help EHOs in their decision-making regarding whether or not to intervene (e.g., calling some type of advisory).

Most common microbial indicators: current use

From a microbial perspective, the main public health concern associated with drinking water is enteric disease.²⁻⁴ Because it is impractical to look for all known enteric pathogens that may contaminate drinking water, microbial safety is assessed through detection of indicators of faecal pollution.⁵ A microbial indicator of faecal pollution is an organism occurring in high numbers in human or animal faeces. If detected, it indicates potential faecal (human or animal) contamination of the water body or distribution system under investigation and the consequent potential presence of enteric pathogens. These microbial indicators are generally not themselves human pathogens.

Four main indicators of microbial safety of drinking water have been used: Heterotrophic plate counts (HPC), total coliform (TC), feacal coliform (FC) and *Escherichia coli* (*E.coli*).

The heterotrophic group of bacteria encompass a broad range of bacteria that uses organic carbon sources to grow. Colony counts of heterotrophic bacteria, referred to as HPC, provide an indication of the general load of aerobic and facultative anaerobic bacteria of a water sample. This indicator is also known as standard plate count (SPC), aerobic plate count (APC) and total plate count (TPC). The total coliform group is a large collection of bacteria that are mostly found in the environment (Figure 1). The feacal coliform group is a subset of the total coliform group

that principally exists in faeces, while *E. coli* belongs to the faecal coliform group and is the only member that is specific to the intestinal tract of warm-blooded animals.

Figure 1. The total coliform, faecal coliform and E.coli groups



Adapted from Washington State Department of Health. "Coliform bacteria in drinking water - Total coliform, faecal coliform and *E.coli* (figure)." Retrieved Oct 31, 2012, from <u>http://www.doh.wa.gov/CommunityandEnvironment/DrinkingWater/Contaminants/Colifor</u> <u>m.aspx</u>.

Heterotrophic Plate Count Bacteria (HPC)

HPC reflects the load of general aerobic bacteria in the water system. Although HPC was used in the late 1800s to assess the "purity" of source water, ⁶ it is no longer used as a health-related indicator.⁷ Today, significant changes in HPC serve as an alert for possible deterioration of water quality, triggering further investigation.⁶ An increase in HPC in finished water can indicate a problem with water treatment or a change in quality of the water source, prior to treatment.⁶ When HPC levels leaving the treatment plant are acceptable, but levels in the distribution system are above baseline levels, regrowth may be occurring in the distribution system.⁶

Total Coliforms (TC)

TC are no longer used as an indicator of faecal contamination because advances in the science of taxonomy show that they are not specific to the intestine of humans or warm-blooded

mammals and can also be found in the environment.^{5,8} The presence of TC in water samples can indicate the presence of a biofilm or can serve as an indicator of treatment efficiency because of their sensitivity to chlorine. However, HPC is a better indicator for that purpose because it covers a wider range of bacteria.⁹

Faecal Coliform (FC) or Thermotolerant Coliform

As mentioned above, the faecal coliform group is a subset of the total coliform group and comprises bacteria such as *E. coli* and *Klebsiella pneumoniae*. Although still in use in some jurisdictions, the lack of specificity of the coliform test to faecal pollution of drinking water has long been documented.^{5,10,11} For example, the presence of high *Klebsellia* spp. counts has been associated with various vegetation and pulp and paper effluent in the absence of faecal contamination.^{5,10-12} This is why a positive result for FC should be interpreted with caution.^{5,13} Boil water advisories solely based on FC-positive samples should be eliminated.¹⁴

E.coli

E.coli is the most reliable indicator of enteric diseases and is therefore the indicator of choice to indicate occurrence of recent faecal contamination in drinking water systems.^{4,5,8,15} One exception is in tropical climates where *E.coli* may be present and multiply in waters not directly subject to faecal pollution.¹⁶ Only some strains of *E.coli* bacteria are capable of causing disease^{8,17,18} and only under certain conditions (e.g., *E.coli* O157:H7). When a water sample is tested for the presence of the indicator *E.coli*, results refer to mainly non-pathogenic strains of *E.coli*.

Limitations of microbial indicators

None of the microbial indicators currently in use for routine monitoring of drinking water quality is perfect because no single indicator represents all potential pathogens that can be present in the water. In order to respond to evolving challenges posed by water microbiology, researchers continue to search for the perfect suite of microbial indicators. In this regard, a few other indicators have been proposed, however most of them are not conducive to routine monitoring or have not been integrated into routine monitoring programs. More information regarding their identities and roles are provided in the review by Payment.⁴

The major drawback associated with microbial indicators is the length of time needed to culture the microorganisms. Methods for water analysis are described in detail in the *Standard Method for The Examination of Water and Wastewater*,¹⁹ a reference book for analysis of water microbiology. Depending on the method used, it takes 24 to 48 hours for laboratory results to come back, which means the water has already been consumed before the operator knows it is safe to drink. Another disadvantage of microbial tests is related to the cost of analysis compared with the cost of physical and chemical tests performed on site. Finally, as suggested by a few researchers²⁰ and described in the section on "Public health significance of laboratory results" of this document, the absence of microbial indicators does not guarantee that water is safe to drink.

Laboratory test results

This section explains the main coding systems found in laboratory reports and examines results through a public health lens.

Deciphering laboratory reports

Three examples of laboratory reports for water sampling results are included in the Appendix. The type of information included in laboratory reports can vary greatly from one laboratory to another. Some reports will only provide the type of test, the results, and whether there is significant evidence for bacterial contamination. Others will be more comprehensive with the name of the test, the results with reference to guidelines for drinking water, and the detection limit of the test. Although, any questions regarding tests results can be addressed to the manager of the laboratory who completed the test, this section explains some of the information that may be found.

• Example of coding systems related to microbial tests: MF, MPN, PA.

There are two types of tests: presence/absence and quantitative tests.

Quantitative tests include the Most Probable Number test (MPN) and Membrane Filtration tests (MF). The MPN technique, also referred to as the Multiple Tube Fermentation Technique, is a technique based on serial dilution of the sample in test tubes containing a selective liquid media. At the end of the incubation, the analyst counts the number of positive test tubes to estimate the number of coliforms or *E.coli* present in the sample. The MF test refers to a technique where 100 ml of the sample is filtered onto a membrane. The membrane is placed on a growth selective media for coliforms/*E.coli*. After incubation, colonies are counted.

MF tests are considered the "gold standard" but both methods, MPN and MF, have advantages and disadvantages that are not discussed in this document. Although quantitative methods are still in use, the tendency is to move away from numerical data for drinking water analysis due to statistical and methodological uncertainties associated with counts.²¹ The US Environmental Protection Agency (USEPA) has already embraced the presence/absence concept for compliance monitoring.^{14,21}

Presence/absence tests (PA) consist of an enzyme-substrate liquid-broth medium usually sold as test kits like collert®, or collagTM. These tests do not provide any information regarding the concentration of the indicator present in the sample.

• Example of coding system to qualify results: EST, TMTC, TNTC, ND, NDOG

For quantitative methods, bacteria can be overgrown, which is defined as "the crowding and/or confluent and/or non-identifiable microbial growth on a plate"²²; in practice terms, "overgrown" means too many bacteria to provide an accurate count. This would appear as NDOG (no data, overgrown plate), ND (no data), TNTC (too numerous to count), TMTC (too many to count) or EST (estimated count).

• Detection limit

The detection limit is the lowest number of microorganisms that can be reliably detected under the stated experimental conditions. When no organisms are detected, the result is reported as < detectable level per volume analysed. Results are usually reported per 100 ml of sample. If 100 ml of water was analysed, the detection limit is < 1 colony forming units (CFU) per 100 ml. If the sample had to be diluted by half, only 50 ml of the sample was analysed; the detection limit becomes < 2 CFU per 100 ml.

Public health significance of laboratory test results

This section explains the limitations associated with the non-detection or the detection of microbial indicators.

No detection

The non-detection of *E.coli* does not guarantee that water is safe to drink because a water sample is only a snapshot^{1,21} of the water being distributed and because some organisms are more resistant to chlorine/disinfectant than the indicator *E.coli*.⁴ A negative *E.coli* result does not necessarily reflect sanitary water conditions because *E.coli* and coliforms, in general, are more sensitive to disinfection than more chlorine-resistant pathogens such as viruses²³ and *Cryptosporidium* oocysts.²⁴

One famous example is the prospective epidemiological study published by Payment et al. (1997).²³ In this study, authors examined self-reported gastrointestinal diseases in persons who drank water that met drinking water standards for faecal coliforms. Results showed that 14–40% of gastrointestinal illnesses were attributable to tap water that met drinking water standards.²³ Due to the short duration of illnesses, authors suggested that viruses may have been the cause. The 1993 Millwaukee outbreak²⁴ is another illustration of the limitations associated with current water quality indicators: water samples were free of total and faecal coliforms throughout the outbreak, but were contaminated by *Cryptosporidium* oocysts.¹⁸ This deficiency is important, knowing that of the 185 reported outbreaks attributed to known pathogens associated with drinking water between 1974 and 2003 in Canada, 41% were caused by protozoa.¹

Positive results or above baseline results

Of the indicators described here (HPC, TC, FC and EC), *E.coli* is the only microbial indicator associated with public health significance. However, because of the limitations associated with the use of indicators, prescribed microbial monitoring only indicates possible water quality issues.¹⁸ A positive *E.coli* is not a confirmation that the water is contaminated by waterborne pathogens; *E.coli* assays detect indicators of faecal pollution but do not target *E.coli* O157:H7 and may or may not capture other pathogens.¹⁴ Therefore, a positive result for *E.coli* is not necessarily a measure of waterborne pathogen occurrence.¹⁴ A positive *E.coli* test can also result from contamination during the process of sampling and may not be representative of the water body; this is ruled out by collecting another sample to confirm the presence/absence of this indicator.

Because of the above limitations, a positive result for *E.coli* should always trigger further investigation. Indicators and possible reasons for their presence in drinking water are found in Table 1. Positive results for microbial indicators (EC, FC) or any number above baseline levels (HPC) should be compared to non-microbial treatment performance parameters such as chlorine residual and turbidity.

Conclusion

Although not perfect, *E.coli* is the best common microbial indicator available to date to inform of public health risks associated with the consumption of drinking water. Due to uncertainties associated with numerical data, the tendency of regulatory agencies is to move towards a

presence/absence concept for compliance monitoring. It is important for practitioners to understand that the presence of indicators (*E.coli* or faecal coliforms) is not a confirmation that the water is contaminated. Microbial test results, whether positive or negative, should be interpreted from a water source to tap perspective.

Acknowledgements

We would like to thank Ray Copes and Lorraine McIntyre for providing us with laboratory reports. We also want to extend our appreciation to Lorraine McIntyre, Mona Shum and Tom Kosatsky for review and comments.

Microbial Indicator	Possible reasons for detection of indicators	MAC* or baseline level ²⁵
E. coli	 Recent faecal contamination, possible presence of pathogenic organisms. 	None detectable per 100 mL.
Faecal coliforms	 Inadequate treatment and disinfection, bacterial re-growth, or infiltration in the distribution system. 	 None detectable per 100 mL in treated water.
Total coliforms	 When present in the water leaving the drinking water treatment plant, indicates a serious treatment failure.²⁵ When present in the water sampled from the distribution system (but not in water leaving the treatment plant), indicates that the distribution system may be vulnerable to contamination or may be experiencing bacterial re-growth.²⁵ May not be related to the occurrence of a faecal contamination. 	 None detectable per 100 mL in water leaving the plant. For the distribution system, it depends on the sampling frequency and therefore the system size.²⁵
Heterotrophic plate count	 A spike in finished water can indicate a problem with water treatment or a change in quality of the water source, prior to treatment. A spike within the distribution system indicates that bacterial re-growth may be occurring in the distribution system. 	Baseline level and range will be specific to each system, depending on site-specific characteristics.

Table 1. Common microbial indicators and possible reasons for their presence in drinking water

*Maximum Acceptable Concentration

Appendix

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