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Literature Cover Sheet

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Topic: WHO Acceptance of APHA *Standard Methods*

Title: WHO *Guidelines For Drinking Water Quality 2nd Edition*

Author(s): World Health Organization (WHO)

Date: 1996

Source: Volume 1, cover, pp. 14-22, p.173

Highlights:
- While the WHO does not approve any methods, it does recommend that "established standard methods should be used for routine examinations." It specifically mentions the methods of the American Public Health (APHA) which include IDEXX's Colilert®, Colilert®-18, and Colisure™ methods. p.19
- *E. coli* is "recommended as the indicator of choice for drinking-water."p.20
- 100ml presence/absence testing is recommended
- Allowance can be made for coliform organisms in up to 5% of samples taken over any 12-month period, provided *E. coli* is not present. p.22

Guidelines for drinking-water quality

SECOND EDITION

Volume 1
Recommendations

World Health Organization
Geneva

- The likelihood of a successful challenge by a pathogen, resulting in infection, depends upon the invasiveness and virulence of the pathogen, as well as upon the immunity of the individual.
- If infection is established, pathogens multiply in their host. Certain pathogenic bacteria are also able to multiply in food or beverages, thereby perpetuating or even increasing the chances of infection.
- Unlike many chemical agents, the dose response of pathogens is not cumulative.

Because of these properties there is **no tolerable lower limit** for pathogens, and water intended for consumption, for preparing food and drink, or for personal hygiene should thus contain no agents pathogenic for humans. Pathogen-free water is attainable by selection of high-quality uncontaminated sources of water, by efficient treatment and disinfection of water known to be contaminated with human or animal faeces, and by ensuring that such water remains free from contamination during distribution to the user. Such a policy creates multiple barriers to the transmission of infection (see Chapter 6 for a more detailed discussion of the multiple-barrier concept).

As indicated in section 1.3, although many pathogens can be detected by suitable methods, it is easier to test for bacteria that specifically indicate the presence of faecal pollution or the efficiency of water treatment and disinfection (see section 2.2). It follows that water intended for human consumption should contain none of these bacteria. In the great majority of cases, monitoring for indicator bacteria provides a great factor of safety because of their large numbers in polluted waters; this has been reinforced over many years of experience.

2.2 Microbial indicators of water quality

2.2.1 Introduction

Frequent examinations for faecal indicator organisms remain the most sensitive and specific way of assessing the hygienic quality of water. Faecal indicator bacteria should fulfil certain criteria to give meaningful results. They should be universally present in high numbers in the faeces of humans and warm-blooded animals, and readily detectable by simple methods, and they should not grow in natural water. Furthermore, it is essential that their persistence in water and their degree of removal in treatment of water are similar to those of waterborne pathogens. The major indicator organisms of faecal pollution – *Escherichia coli*, the thermotolerant and other coliform bacteria, the faecal streptococci, and spores of sulfite-reducing clostridia – are described briefly below. Details of additional microbial indicators of water quality, such as heterotrophic plate-count bacteria, bacteriophages, and opportunistic and overt pathogens, are given in Volume 2 of *Guidelines for drinking-water quality.*

2.2.2 General principles

While the criteria described above for an ideal faecal indicator are not all met by any one organism, many of them are fulfilled by *E. coli* and, to a lesser extent, by the thermotolerant coliform bacteria. The faecal streptococci satisfy some of the criteria, although not to the same extent as *E. coli,* and they can be used as supplementary indicators of faecal pollution or treatment efficiency in certain circumstances. It is recommended that *E. coli* is the indicator of first choice when resources for microbiological examination are limited. Because enteroviruses and the resting stages of *Cryptosporidium, Giardia,* amoebae, and other parasites are known to be more resistant to disinfection than *E. coli* and faecal streptococci, the absence of the latter organisms will not necessarily indicate freedom from the former. Spores of sulfite-reducing clostridia can be used as an additional parameter in this respect.

2.2.3 *Escherichia coli* and the coliform bacteria

Escherichia coli

Escherichia coli is a member of the family Enterobacteriaceae, and is characterized by possession of the enzymes β-galactosidase and β-glucuronidase. It grows at 44–45 °C on complex media, ferments lactose and mannitol with the production of acid and gas, and produces indole from tryptophan. Some strains can grow at 37 °C, but not at 44–45 °C, and some do not produce gas. *E. coli* does not produce oxidase or hydrolyse urea. Complete identification of *E. coli* is too complicated for routine use, hence certain tests have been evolved for identifying the organism rapidly with a high degree of certainty. Some of these methods have been standardized at international and national levels and accepted for routine use, whereas others are still in the developmental or evaluative stage.

E. coli is abundant in human and animal faeces, where it may attain concentrations in fresh faeces of 10^9 per gram. It is found in sewage, treated effluents, and all natural waters and soils that are subject to recent faecal contamination, whether from humans, agriculture, or wild animals and birds. Recently, it has been suggested that *E. coli* may be found or even multiply in tropical waters that are not subject to human faecal pollution. However, even in the remotest regions, faecal contamination by wild animals, including birds, can never be excluded. As animals can transmit pathogens infective for humans, the presence of *E. coli* or thermotolerant coliform bacteria can never be ignored, because the presumptions remain that the water has been faecally contaminated and that treatment has been ineffective.

Thermotolerant coliform bacteria

These are defined as the group of coliform organisms that are able to ferment lactose at 44–45 °C; they comprise the genus *Escherichia* and, to a lesser extent, species of *Klebsiella*, *Enterobacter*, and *Citrobacter*. Thermotolerant coliforms other than *E. coli* may also originate from organically enriched water such as industrial effluents or from decaying plant materials and soils. For this reason, the often-used term "faecal" coliforms is not correct, and its use should be discontinued.

Regrowth of thermotolerant coliform organisms in the distribution system is unlikely unless sufficient bacterial nutrients are present or unsuitable materials are in contact with the treated water, water temperature is above 13 °C, and there is no free residual chlorine.

The concentrations of thermotolerant coliforms are, under most circumstances, directly related to that of *E. coli*. Hence, their use in assessing water quality is considered acceptable for routine purposes. The limitations with regard to specificity should always be borne in mind when the data are interpreted. Specific detection of *E. coli* by additional confirmatory tests or by direct methods, as described in the research literature, should be carried out if high counts of thermotolerant coliforms are found in the absence of detectable sanitary hazards. National reference laboratories are advised to examine the specificity of the thermotolerant coliform test for *E. coli* under local circumstances when developing national standard methods.

Because thermotolerant coliform organisms are readily detected, they have an important secondary role as indicators of the efficiency of water treatment processes in removing faecal bacteria. They may therefore be used in assessing the degree of treatment necessary for waters of different quality and for defining targets of performance for bacterial removal (see section 2.3).

Coliform organisms (total coliforms)

Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water. The term "coliform organisms" refers to Gram-negative, rod-shaped bacteria capable of growth in the presence of bile salts or other surface-active agents with similar growth-inhibiting properties and able to ferment lactose at 35–37 °C with the production of acid, gas, and aldehyde within 24–48 hours. They are also oxidase-negative and non-spore-forming. By definition, coliform bacteria display β-galactosidase activity.

Traditionally, coliform bacteria were regarded as belonging to the genera *Escherichia*, *Citrobacter*, *Enterobacter*, and *Klebsiella*. However, as defined by modern taxonomical methods, the group is heterogeneous. It includes lactose-fermenting bacteria, such as *Enterobacter cloacae* and *Citrobacter freundii*, that can be found both in faeces and the environment (nutrient-rich waters, soil, decaying plant material), and also in drinking-water with relatively high concentrations of nutrients, as well as species that are rarely, if ever, found in faeces and may multiply in relatively good quality drinking-waters, for example, *Serratia fonticola, Rahnella aquatilis,* and *Buttiauxella agrestis.*

The existence both of non-faecal bacteria that fit the definitions of coliform bacteria and of lactose-negative coliform bacteria limits the applicability of this group as an indicator of faecal pollution. Coliform bacteria should not be detectable in treated water supplies and, if found, suggest inadequate treatment, post-treatment contamination, or excessive nutrients. The coliform test can therefore be used as an indicator of treatment efficiency and of the integrity of the distribution system. Although coliform organisms may not always be directly related to the presence of faecal contamination or pathogens in drinking-water, the coliform test is still useful for monitoring the microbial quality of treated piped water supplies. If there is any doubt, especially when coliform organisms are found in the absence of thermotolerant coliform organisms and *E. coli,* identification to the species level or analyses for other indicator organisms may be undertaken to investigate the nature of the contamination. Sanitary inspections will also be needed.

2.2.4 Faecal streptococci

The term "faecal streptococci" refers to those streptococci generally present in the faeces of humans and animals. All possess the Lancefield group D antigen. Taxonomically, they belong to the genera *Enterococcus* and *Streptococcus.* The taxonomy of enterococci has recently undergone important changes, and detailed knowledge of the ecology of many of the new species is lacking. The genus *Enterococcus* now includes all streptococci that share certain biochemical properties and have a wide tolerance of adverse growth conditions. It includes the species *E. avium, E. casseliflavus, E. cecorum, E. durans, E. faecalis, E. faecium, E. gallinarum, E. hirae, E. malodoratus, E. mundtii,* and *E. solitarius.* Most of these species are of faecal origin and can generally be regarded as specific indicators of human faecal pollution under many practical circumstances. They may, however, be isolated from the faeces of animals, and certain species and subspecies, such as *E. casseliflavus, E. faecalis* var. *liquefaciens, E. malodoratus,* and *E. solitarius,* occur primarily on plant material.

In the genus *Streptococcus,* only *S. bovis* and *S. equinus* possess the group D antigen and are members of the faecal streptococcus group. Their sources are mainly animal faeces. Faecal streptococci rarely multiply in polluted water, and they are more persistent than *E. coli* and coliform bacteria. Their primary value in water quality examination is therefore as additional indicators of treatment efficiency. Furthermore, streptococci are highly resistant to drying and may be valuable for routine control after laying new mains or repairs in distribution systems, or for detecting pollution by surface run-off to ground or surface waters.

2.2.5 Sulfite-reducing clostridia

These are anaerobic, spore-forming organisms, of which the most characteristic, *Clostridium perfringens* (*C. welchii*), is normally present in faeces, although in much smaller numbers than *E. coli*. However, they are not exclusively of faecal origin and can be derived from other environmental sources. Clostridial spores can survive in water much longer than organisms of the coliform group and will resist disinfection. Their presence in disinfected waters may thus indicate deficiencies in treatment and that disinfection-resistant pathogens could have survived treatment. In particular, the presence of *C. perfringens* in filtered supplies may indicate deficiencies in filtration practice. Because of their longevity, they are best regarded as indicating intermittent or remote contamination. They thus have a special value but are not recommended for routine monitoring of distribution systems. Because they tend to survive and accumulate, they may be detected long after and far from the pollution and thus give rise to false alarms.

2.2.6 Coliphages and other alternative indicators

The bacteriophages have been proposed as indicators of water quality because of their similarity to human enteroviruses and their easy detection in water. Two groups have been studied extensively: the somatic coliphages, which infect *E. coli* host strains through cell-wall receptors; and the F-specific RNA-bacteriophages, which infect strains of *E. coli* and related bacteria through the F- or sex-pili. Neither occurs in high numbers in fresh human or animal faeces, but they are abundant in sewage. Their significance is as indicators of sewage contamination and, because of their greater persistence compared with bacterial indicators, as additional indicators of treatment efficiency or groundwater protection.

The bifidobacteria and the *Bacteroides fragilis* group are very numerous in faeces but have not been considered as suitable indicators of faecal pollution (see Volume 2) because they decay more rapidly in water than coliform bacteria and because the methods of examination are not very reliable and have not been standardized.

2.2.7 Methods of detection

Microbiological examination provides the most sensitive, although not the most rapid, indication of pollution of drinking-water supplies. Unlike chemical or physical analysis, however, it is a search for very small numbers of viable organisms and not for a defined chemical entity or physical property. Because the growth medium and the conditions of incubation, as well as the nature and age of the water sample, can influence the species isolated and the count, microbiological examinations may have variable accuracy. This means that the standardization of methods and of laboratory procedures is of great importance if criteria for microbiological quality of water are to be uniform in different laboratories and internationally. International standard methods should be evaluated under local circumstances before being adopted in national surveillance programmes. Established standard methods are available, such as those of the International Organization for Standardization (ISO) (Table 2), of the American Public Health Association (APHA), and of the United Kingdom Department of Health and Social Security. It is desirable that established standard methods should be used for routine examinations. Whatever method is chosen for detection of *E. coli* and the coliform group, some step for "resuscitating" or recovering environmentally- or disinfectant-damaged strains must be used, such as pre-incubation for a short period at a lower temperature.

Table 2. International Organization for Standardization (ISO) standards for detection and enumeration of faecal indicator bacteria in water

ISO standard no.	Title (water quality)
6461-1:1986	Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) - Part 1: Method by enrichment in a liquid medium
6461-2:1986	Detection and enumeration of the spores of sulfite-reducing anaerobes (clostridia) - Part 2: Method by membrane filtration
7704:1985	Evaluation of membrane filters used for microbiological analyses
7899-1:1984	Detection and enumeration of faecal streptococci - Part 1: Method by enrichment in a liquid medium
7899-2:1984	Detection and enumeration of faecal streptococci - Part 2: Method by membrane filtration
9308-1:1990	Detection and enumeration of coliform organisms, thermotolerant coliform organisms, and presumptive *Escherichia coli* - Part 1: Membrane filtration method
9308-2:1990	Detection and enumeration of coliform organisms, thermotolerant coliform organisms, and presumptive *Escherichia coli* - Part 2: Multiple tube (most probable number) method.

2.3 Recommendations

2.3.1 General principles

The provision of a safe supply of drinking-water depends upon use of either a protected high-quality ground water or a properly selected and operated series of treatments capable of reducing pathogens and other contaminants to negligible levels, not injurious to health. Treatment systems should provide multiple barriers to the transmission of infection. The processes preceding terminal disinfection should be capable of producing water of high microbiological quality, so that terminal disinfection becomes a final safeguard. Disinfection is also most efficient when the water has already been treated to remove turbidity and when substances exerting a disinfectant demand, or capable of protecting pathogens from disinfection, have been removed as far as possible.

The search for microbial indicators of faecal pollution is a "fail-safe" concept; in other words, if faecal indicators are shown to be present, then it must be assumed that pathogens could also be present. For this reason, faecal indicator bacteria must never be present in treated water delivered to the consumer, and any detection should prompt immediate action to discover the cause and to take remedial action.

The most specific of the readily detectable faecal indicator bacteria and the one present in greatest numbers in faeces is *Escherichia coli* and it is therefore recommended as the indicator of choice for drinking-water. The thermotolerant coliform test can be used as an alternative to the test for *E. coli.* Thermotolerant coliform bacteria are also recommended as indicators of the efficiency of water treatment processes in removing enteric pathogens and faecal bacteria, and for grading the quality of source waters in order to select the intensity of treatment needed. Total coliform bacteria should not be present in treated water supplies and, if found, suggest inadequate treatment, post-treatment contamination, or excessive nutrients.

2.3.2 Selection of treatment processes

The selection of treatment processes to meet microbiological and chemical requirements can be made only after a careful detailed survey of the source and watershed, as outlined in section 6.2, including assessment of likely sources of pollution. Extensive bacteriological surveys, to include different seasons and weather conditions, can be used to assist in the selection. Regular bacteriological examination of source water after commissioning the treatment plant will establish long-term trends in quality and indicate whether there is a need to revise the treatment given.

2.3.3 Treatment objectives

The multiple-barrier concept of water treatment (see Chapter 6) requires that the removal of pathogens and of pollutants and biodegradable compounds should be as nearly complete as possible before terminal disinfection. Table 3 gives an example of performance objectives for typical urban water treatment processes, based upon loadings and removal of turbidity and thermotolerant coliform bacteria. These levels of performance are capable of being met and exceeded comfortably in normal operation. It is emphasized that the sequence of processes given in Table 3 is only one example from the many possible combinations of processes that are used in normal practice.

Table 3. An example to illustrate the level of performance that can be achieved in removal of turbidity and thermotolerant coliform bacteria in conventional urban water treatment

Stage and process	Turbidity			Thermotolerant coliform bacteria		
	Removal[a] (%)	Average loading (NTU)[b]	Maximum loading (NTU)[b]	Removal[a] (%)	Average loading (per 100 ml)	Maximum loading (per 100 ml)
Micro-straining	NA[c]	NA	NA	NA	NA	NA
Pretreatment[d]	NA	NA	NA	> 99.9	1000	10 000
Coagulation/settling[e]	90	50	300	NA	NA	NA
Rapid filtration[e]	> 80	5	30	80	1	10
Terminal chlorination	NA	1	5	>99.9	<1	2
Mains distribution	NA	<1	<5	NA	<1	<1

[a] Required performance.
[b] NTU, nephelometric turbidity units.
[c] NA, not applicable. Process not designed to remove turbidity and/or bacteria. Micro-straining removes micro-algae and zooplankton.
[d] Pretreatments that can result in significant reductions in thermotolerant coliform bacteria are storage in reservoirs for 3–4 weeks, and pre-disinfection.
[e] Taken together, coagulation, settling, and rapid filtration should be expected to remove 99.9% of thermotolerant coliform bacteria.

The multiple-barrier concept can also be applied to water treatment in rural and remote regions. Table 4 gives an example of treatment objectives for such plants.

2.3.4 Guideline values

It is most important that the reasons for adopting the following guideline values for drinking-water are properly understood and that the guideline values are used only in conjunction with the information given below and in Volume 2.

Table 4. An example of performance objectives for removal of turbidity and thermotolerant coliform bacteria in small-scale water treatment

Stage and process	Turbidity			Thermotolerant coliform bacteria		
	Removal[a] (%)	Average loading (NTU)[b]	Maximum loading (NTU)[b]	Removal[a] (%)	Average loading (per 100 ml)	Maximum loading (per 100 ml)
Screening	NA[c]	NA	NA	NA	NA	NA
Plain sedimentation	50	60	600	50	1000	10 000
Gravel pre-filters (3-stage)	80	30	300	90	500	5000
Slow sand filter	>90	6	60	95	50	500
Disinfection	NA	<1	<5	>99.9	<3	25
Distributed water	NA	<1	<5	NA	<1	<1

[a] Required performance.
[b] NTU, nephelometric turbidity units.
[c] NA, not applicable. Process not designed to remove turbidity and/or bacteria.

Bacteriological quality

Water intended for drinking and household purposes must not contain waterborne pathogens. Because the most numerous and the most specific bacterial indicator of faecal pollution from humans and animals is *E. coli,* it follows that *E. coli* or thermotolerant coliform organisms must not be present in 100-ml samples of any water intended for drinking (see Annex 2, Table A2.1).

This criterion is readily achievable by water treatment (see section 6.3). In nearly all epidemics of waterborne disease, it has been shown that the bacteriological quality of the water was unsatisfactory and that there was evidence of failure in terminal disinfection.

During distribution, the bacteriological quality of water may deteriorate. Coliform bacteria other than *E. coli* can occur in inadequately treated supplies, or those contaminated after leaving the treatment plant, as a result of growth in sediments and on unsuitable materials in contact with the water (washers, packing, lubricants, plastics and plasticizers, for example). They may also gain entrance from soil or natural water through leaky valves and glands, repaired mains, or back-siphonage. This type of contamination is most likely to be found when the water is untreated or undisinfected, or where there is limited or no residual disinfectant. Allowance can be made for the occasional occurrence in the distribution system of coliform organisms in up to 5% of samples taken over any 12-month period, provided *E. coli* is not present (Table A2.1, p. 173). It must be stressed that any regular occurrence of coliform organisms is a matter of concern and should be investigated.

***Table A2.1. Bacteriological quality of drinking-water*[a]**

Organisms	Guideline value
All water intended for drinking	
E. coli or thermotolerant coliform bacteria[b,c]	Must not be detectable in any 100-ml sample
Treated water entering the distribution system	
E. coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100-ml sample
Total coliform bacteria	Must not be detectable in any 100-ml sample
Treated water in the distribution system	
E. coli or thermotolerant coliform bacteria[b]	Must not be detectable in any 100-ml sample
Total coliform bacteria	Must not be detectable in any 100-ml sample. In the case of large supplies, where sufficient samples are examined, must not be present in 95% of samples taken throughout any 12-month period

[a] Immediate investigative action must be taken if either *E. coli* or total coliform bacteria are detected. The minimum action in the case of total coliform bacteria is repeat sampling; if these bacteria are detected in the repeat sample, the cause must be determined by immediate further investigation.

[b] Although *E. coli* is the more precise indicator of faecal pollution, the count of thermotolerant coliform bacteria is an acceptable alternative. If necessary, proper confirmatory tests must be carried out. Total coliform bacteria are not acceptable indicators of the sanitary quality of rural water supplies, particularly in tropical areas where many bacteria of no sanitary significance occur in almost all untreated supplies.

[c] It is recognized that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread. Under these conditions, the national surveillance agency should set medium-term targets for the progressive improvement of water supplies, as recommended in Volume 3 of *Guidelines for drinking-water quality.*