2. Microbiological aspects

2.1 Agents of significance

2.1.1 Waterborne infections

Infectious diseases caused by pathogenic bacteria, viruses, and protozoa or by parasites are the most common and widespread health risk associated with drinking-water.

Infectious diseases are transmitted primarily through human and animal excreta, particularly faeces. If there are active cases or carriers in the community, then faecal contamination of water sources will result in the causative organisms being present in the water. The use of such water for drinking or for preparing food, contact during washing or bathing, and even inhalation of water vapour or aerosols may then result in infection.

2.1.2 Orally transmitted infections of high priority

The human pathogens that can be transmitted orally by drinking-water are listed in Table 1 (p. 10), together with a summary of their health significance and main properties. Those that present a serious risk of disease whenever present in drinking-water include *Salmonella* spp., *Shigella* spp., pathogenic *Escherichia coli, Vibrio cholerae*, *Yersinia enterocolitica*, *Campylobacter jejuni*, and *Campylobacter coli*, the viruses listed in Table 1, and the parasites *Giardia* spp., *Cryptosporidium* spp., *Entamoeba histolytica*, and *Dracunculus medinensis*. Most of these pathogens are distributed worldwide. However, outbreaks of cholera and infection by the guinea worm *D. medinensis* are regional. The elimination of all these agents from water intended for drinking has high priority. Eradication of *D. medinensis* is a recognized target of the World Health Assembly (World Health Assembly resolution WHA44.5, 1991).

2.1.3 Opportunistic and other water-associated pathogens

Other pathogens are accorded moderate priority in Table 1 or are not listed, either because they are of low pathogenicity, causing disease opportunistically in subjects with low or impaired immunity, or because, even though they cause serious diseases, the primary route of infection is by contact or inhalation, rather than by ingestion.

Opportunistic pathogens are naturally present in the environment and are not formally regarded as pathogens. They are able to cause disease in people with impaired local or general defence mechanisms, such as the elderly or the very young, patients with burns or extensive wounds, those undergoing immunosuppressive therapy, or those with acquired immunodeficiency syndrome (AIDS). Water used by such patients for drinking or bathing, if it contains large numbers of these organisms, can produce various infections of the skin and the mucous membranes of the eye, ear, nose, and throat. Examples of such agents are *Pseudomonas aeruginosa* and species of *Flavobacterium*, *Acinetobacter*, *Klebsiella*, *Serratia*, *Aeromonas*, and certain “slow-growing” mycobacteria.

Certain serious illnesses result from inhalation of water in which the causative organisms have multiplied because of warm temperatures and the presence of nutrients. These include Legionnaires’ disease (*Legionella* spp.) and those caused by the amoebae *Naegleria fowleri* (primary amoebic meningoencephalitis) and *Acanthamoeba* spp. (amoebic meningitis, pulmonary infections).

Schistosomiasis (bilharziasis) is a major parasitic disease of tropical and sub-tropical regions, and is primarily spread by contact with water during bathing or washing. The larval stage (cercariae) released by infected aquatic snails penetrates the skin. If pure drinking-water is readily available, it will be used for washing, and this will have the benefit of reducing the need to use contaminated

surface water.

It is conceivable that unsafe drinking-water contaminated with soil or faeces could act as a carrier of other parasitic infections, such as balantidiasis (*Balantidium coli*), and certain helminths (species of *Fasciola, Fasciolopsis, Echinococcus, Spirometra, Ascaris, Trichuris, Toxocara, Necator, Ancylostoma, Strongyloides* and *Taenia solium*). However, in most of these, the normal mode of transmission is ingestion of the eggs in food contaminated with faeces or faecally contaminated soil (in the case of *Taenia solium*, ingestion of the larval cysticercus stage in uncooked pork) rather than ingestion of contaminated drinking-water.

2.1.4 Toxins from Cyanobacteria

Blooms of *Cyanobacteria* (commonly called blue-green algae) occur in lakes and reservoirs used for potable supply. Three types of toxin can be produced, depending upon species:

- hepatotoxins, produced by species of *Microcystis*, *Oscillatoria*, *Anabaena*, and *Nodularia*, typified by microcystin LR:R, which induce death by circulatory shock and massive liver haemorrhage within 24 hours of ingestion;

- neurotoxins, produced by species of *Anabaena*, *Oscillatoria*, *Nostoc*, *Cylindrospermum*, and *Aphanizomenon*;

- lipopolysaccharides.

Table 1. Orally transmitted waterborne pathogens and their significance in water supplies

Pathogen	Health significance	Persistence in water supplies[a]	Resistance to chlorine[b]	Relative infective dose[c]	In r
Bacteria					
Campylobacter jejuni, C. coli	High	Moderate	Low	Moderate	
Pathogenic					
Escherichia coli	High	Moderate	Low	High	
Salmonella typhi	High	Moderate	Low	High[d]	
Other *salmonellae*	High	Long	Low	High	
Shigella spp.	High	Short	Low	Moderate	
Vibrio cholerae	High	Short	Low	High	
Yersinia enterocolitica	High	Long	Low	High(?)	
Pseudomonas aeruginosa[e]	Moderate	May multiply	Moderate	High(?)	
Aeromonas spp.	Moderate	May multiply	Low	High(?)	
Viruses					
Adenoviruses	High	?	Moderate	Low	
Enteroviruses	High	Long	Moderate	Low	
Hepatitis A	High	?	Moderate	Low	
Enterically transmitted non-A, non-B hepatitis viruses, hepatitis E	High	?	?	Low	
Norwalk virus	High	?	?	Low	
Rotavirus	High	?	?	Moderate	
Small round viruses	Moderate	?	?	Low(?)	
Protozoa					
Entamoeba histolytica	High	Moderate	High	Low	
Giardia intestinalis	High	Moderate	High	Low	
Cryptosporidium parvum	High	Long	High	Low	
Helminths					
Dracunculus medinensis	High	Moderate	Moderate	Low	

? - not known or uncertain

[a] Detection period for infective stage in water at 20°C: short, up to 1 week; moderate, 1 week to 1 month; long, over 1 month.

[b] When the infective stage is freely suspended in water treated at conventional doses and contact times. Resistance moderate, agent may not be completely destroyed.

[c] Dose required to cause infection in 50% of health adult volunteers; may be as little as one infective unit for some viruses.

[d] From experiments with human volunteers (see section 2.1.7)

[e] Main route of infections is by skin contact, but can infect immunosuppressed or cancer patients orally

There are a number of unconfirmed reports of adverse health effects caused by algal toxins in drinking-water, including an epidemiological study of mild, reversible liver damage in hospital patients receiving drinking-water from a reservoir with a very large toxic bloom of Microcystis aeruginosa. Only activated carbon and ozonation appear to remove or reduce toxicity; however, knowledge is impeded by the lack of suitable analytical methods. There are insufficient data to allow guidelines to be recommended, but the need to protect impounded surface water sources from discharges of nutrient-rich effluents is emphasized.

2.1.5 Nuisance organisms

There are a number of diverse organisms that have no public health significance but which are undesirable because they produce turbidity, taste and odour, or because they appear as visible animal life in water. As well as being aesthetically objectionable, they indicate that water treatment and the state of maintenance and repair of the system are defective. Examples include:

- seasonal blooms of cyanobacteria and other algae in reservoirs and in river waters, impeding coagulation and filtration and causing coloration and turbidity of water after filtration;

- in waters containing ferrous and manganous salts, oxidation by iron bacteria, causing rust-coloured deposits on the walls of tanks, pipes and channels, and carry-over of deposits in the water;

- microbial corrosion of iron and steel pipes by iron and sulfur bacteria;

- production of objectionable tastes and odours, with a low threshold, e.g., geosmin and 2-methylisoborneol by actinomycetes and cyanobacteria;

- colonization of unsuitable non-metallic fittings, pipes, jointing com-pounds and lining materials by microorganisms able to utilize leached organic compounds;

- microbial growth in distribution systems encouraged by the presence of biodegradable and assimilable organic carbon in water, often released by oxidative disinfectants (chlorine, ozone); this growth may include Aeromonas spp., which can produce false positive reactions in the coliform test;

- infestation of water mains by animal life, feeding on microbial growth in the water or on slimes, for example crustacea (*Gammarus pullex*, *Crangonyx pseudogracilis*, *Cyclops* spp., and *Chydorus sphaericus*), *Asellus aquaticus*, snails, mussels (*Dreissena polymorpha*), bryozoa (*Plumatella*), *Nais* worms, nematodes, and larvae of chironomids (*Chironomus* spp.)

and mosquitos (*Culex* spp.); in warm weather, slow sand filters can sometimes discharge chironomid larvae by draw-down into the filtered water.

The only positively identified health hazard from animal life in drinking-water arises with the intermediate stage of the guinea worm, *Dracunculus medinensis*, which parasitizes the water flea, *Cyclops*.

2.1.6 Persistence in water

After leaving the body of their host, pathogens and parasites gradually lose viability and the ability to infect. The rate of decay is usually exponential, and a pathogen will become undetectable after a certain period. Pathogens with low persistence must rapidly find a new host and are more likely to be spread by person-to-person contact or faulty personal or food hygiene than by drinking-water. Because faecal contamination is usually dispersed rapidly in surface waters, the most common waterborne pathogens and parasites are those that have high infectivity or possess high resistance to decay outside the body. Persistence in water and resistance to chlorination are summarized in Table 1.

Persistence is affected by several factors, of which temperature is the most important. Decay is usually accelerated by increasing temperature of water and may be mediated by the lethal effects of ultraviolet radiation in sunlight acting near the water surface. Viruses and the resting stages of parasites (cysts, oocysts, ova) are unable to multiply in water. Conversely, relatively high amounts of biodegradable organic carbon, together with warm temperatures and low residual concentrations of chlorine, can permit growth of *Legionella, Naegleria fowleri, Acanthamoeba*, the opportunistic pathogens *Pseudomonas aeruginosa* and *Aeromonas*, and nuisance organisms during water distribution.

2.1.7 Infective dose

Waterborne transmission of the pathogens listed in Table 1 has been confirmed by epidemiological studies and case histories. Part of the demonstration of pathogenicity involves reproducing the disease in suitable hosts. Experimental studies of infectivity provide relative information, as shown in Table 1, but it is doubtful whether the infective doses obtained are relevant to natural infections. For example, many epidemics of typhoid fever can be explained only by assuming that the infective dose was very low. Individuals vary widely in immunity, whether acquired by contact with a pathogen or influenced by such factors as age, sex, state of health, and living conditions. Pathogens are likely to be widely dispersed and diluted in drinking-water, and a large number of people will be exposed to relatively small numbers. Hence, the minimal infective doses and the attack rates are likely to be lower than in experimental studies. If food is contaminated by water containing pathogens that multiply subsequently, or if a susceptible person becomes infected by water, subsequently infecting others by person-to-person contact, the initial involvement of water may be unsuspected. Hence, improvements in water supply, sanitation, and hygiene are closely linked in control of disease in a community.

The multifactorial natures of infection and immunity mean that experimental data from infectivity studies and epidemiology cannot by used to predict infective doses or risk precisely. However, probabilistic modelling has been used to predict the effects of water treatment in reducing attack rates from very low doses of viruses and *Giardia* and thereby to confirm water treatment criteria

2.1.8 Guideline values

Pathogenic agents have several properties that distinguish them from chemical pollutants:

- Pathogens are discrete and not in solution.

- Pathogens are often clumped or adherent to suspended solids in water, so that the likelihood

of acquiring an infective dose cannot be predicted from their average concentration in water.

• 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-negarive 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	10000
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)	Maximu loadin (per 100
Screening	NA[c]	NA	NA	NA	NA	NA
Plain sedimentation	50	60	600	50	1000	10000
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 water-borne 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). It must be stressed that any regular occurrence of coliform organisms is a matter of concern and should be investigated.

Virological quality

Drinking-water must essentially be free of human enteroviruses to ensure negligible risk of transmitting viral infection. Any drinking-water supply subject to faecal contamination presents a risk of viral disease to consumers. Two approaches can be used to ensure that the risk of viral infection is kept to a minimum: providing drinking-water from a source verified free of faecal contamination, or adequately treating faecally contaminated water to reduce enteroviruses to a negligible level.

Virological studies have shown that drinking-water treatment can considerably reduce the levels of viruses but may not eliminate them completely from very large volumes of water. Virological, epidemiological, and risk analyses are providing important information, although it is still insufficient for deriving quantitative and direct virological criteria. Such criteria cannot be recommended for routine use because of the cost, complexity, and lengthy nature of virological analyses, and the fact that they cannot detect the most relevant viruses.

The guideline criteria shown in Table 5 are based upon the likely viral content of source waters and the degree of treatment necessary to ensure that even very large volumes of drinking-water have a negligible risk of containing viruses.

Ground water obtained from a protected source and documented to be free from faecal contamination from its zone of influence, the well, pumps, and delivery system can be assumed to be virus-free. However, when such water is distributed, it is desirable that it is disinfected, and that a residual level of disinfectant is maintained in the distribution system to guard against contamination.

The water must meet guideline criteria for turbidity and pH (see Table 5), bacteriological quality (see Table A2.1), and parasitological quality (see below).

Parasitological quality

It is not possible to set guideline values for pathogenic protozoa, helminths, and free-living organisms, other than that these agents should not be present in drinking-water, because one or very few organisms can produce infection in humans. The analytical methods for protozoan pathogens are expensive and time-consuming and cannot be recommended for routine use. Methods for concentrating the transmission stages of *Giardia* and *Cryptosporidium* from large volumes of water are being standardized (see Volume 2). When facilities are available for studying the incidence of these parasites in surface water, these methods could be used to measure the efficiency of water treatments in removing them and the incidence of carriage of these parasites by animal vectors in the watershed. This will enable the epidemiology and zoonotic relationships of these parasites to be better understood. The control of pathogenic parasites and of other invertebrate animal life in water mains is best accomplished by proper operation and control of water treatment processes and distribution practices. In particular, the attainment of the bacteriological criteria (see Table A2.1) and the application of treatments for virological reduction (see Table 5) should, except in extraordinary cases of extreme contamination by parasites, ensure that the water has a negligible risk of transmitting parasitic diseases.

Table 5. Recommended treatments for different water sources to produce water with negligible virus risk[a]

Type of source	Recommended treatment
Ground water	
Protected, deep wells; essentially free of faecal contamination	Disinfection[b]
Unprotected, shallow wells; faecally contaminated	Filtration and disinfection
Surface water	
Protected, impounded upland water; essentially free of faecal contamination	Disinfection
Unprotected impounded water or upland river; faecal contamination	Filtration and disinfection
Unprotected lowland rivers; faecal contamination	Pre-disinfection or storage, filtration, disinfection
Unprotected watershed; heavy faecal contamination	Pre-disinfection or storage, filtration, additional treatment and disinfection
Unprotected watershed; gross faecal contamination	Not recommended for drinking-water supply

[a] For all sources, the median value of turbidity before terminal disinfection must not exceed 1 nephelometric turbidity unit (NTU) and must not exceed 5 NTU in single samples.

Terminal disinfection must produce a residual concentration of free chlorine of $\geq$0.5 mg/litre after at least 30 minutes of contact in water at pH<8.0, or must be shown to be an equivalent disinfection process in terms of the degree of enterovirus inactivation (>99.99%).

Filtration must be either slow sand filtration or rapid filtration (sand, dual, or mixed media) preceded by adequate coagulation-flocculation (with sedimentation or flotation). Diatomaceous earth filtration or a filtration process demonstrated to be equivalent for virus reduction can also be used. The degree of virus reduction must be >90%.

Additional treatment may consist of slow sand filtration, ozonation with granular activated carbon adsorption, or any other process demonstrated to achieve >99% enterovirus reduction.

[b] Disinfection should be used if monitoring has shown the presence of *E. coli* or thermotolerant coliform bacteria.

2.4 Monitoring

2.4.1 Approaches and strategies

The monitoring of drinking-water quality ideally consists of two components:

- continual control of quality on a routine basis to as certain that treatment and distribution comply with the given objectives and regulations;

- periodic microbiological and public health surveillance of the entire water supply system from source to consumer.

The continual control function is an integral part of the responsibilities of the water supply agency, through which the waterworks management ensures the satisfactory performance of the treatment processes, the quality of the product water, and the absence of secondary contamination within the distribution network. An independent body should verify that the waterworks correctly fulfils its duties. This surveillance function usually rests with the health authorities at the local, regional, and national levels.

2.4.2 Sampling frequencies

The frequency of sampling will be determined by the resources available. The more frequently the water is examined, the more likely it is that chance contamination will be detected. There are two main points to be noted. Firstly, the chance of detecting pollution that occurs periodically, rather than randomly, is increased if samples are taken at different times of day and on different days of the week. Secondly, frequent examination by a simple method is more valuable than less frequent examination by a complex test or series of tests. Sampling frequencies for raw water sources will depend upon their overall quality, their size, the likelihood of contamination, and the season of the year. They should be established by local control agencies and are often specified in national regulations and guidelines. The results and information from sanitary inspection of the gathering grounds will often indicate whether increased vigilance is needed.

Sampling frequencies for treated water leaving the waterworks depend on the quality of the water source and the type of treatment. Minimum frequencies are: one sample every 2 weeks for waterworks with a ground water source; and one sample every week for waterworks with a surface water source.

The frequency of sampling must be greater where the number of people supplied is large, because of the higher number of people at risk. Advice on the design of sampling programmes and on the frequency of sampling is given in ISO standards (Table 6) and in national regulations. The minimum frequencies shown in Table 7 are recommended for water in the distribution system.

Samples should be spaced randomly within each month and from month to month, and should be taken both from fixed points, such as pumping stations and tanks, and from random locations throughout the distribution system, including points near its extremities and taps connected directly to the mains in houses and large multi-occupancy buildings, where there is a greater risk of contamination through cross-connections and back-siphonage. Frequency of sampling should be increased at times of epidemics, flooding, emergency operations, or following interruptions of supply or repair work. With systems serving small communities, periodic sanitary surveys are likely to yield more information than infrequent sampling.

Table 6. A list of International Organization for Standardization (ISO) standards for water quality giving guidance on sampling

ISO standard no.	Title (water quality)
5667-1:1980	Sampling - Part 1: Guidance on the design of sampling programmes
5667-2:1982	Sampling - Part 2: Guidance on sampling techniques
5667-3:1985	Sampling - Part 3: Guidance on the preservation and handling of samples
5667-4:1987	Sampling - Part 4: Guidance on sampling from lakes, natural and man-made
5667-5:1991	Sampling - Part 5: Guidance on sampling of drinking-water and water used for food and beverage processing
5667-6:1990	Sampling - Part 6: Guidance on sampling of rivers and streams

Table 7. Minimum sampling frequencies for drinking-water in the distribution system

Population served	Samples to be taken monthly
Less than 5000	1 sample
5000-100 000	1 sample per 5000 population
More than 100 000	1 sample per 10 000 population, plus 10 additional samples

No general recommendation can be made for unpiped supplies and untreated water, because the quality and likelihood of contamination will vary seasonally and with local conditions. The frequency should be established by the local control agency and reflect local conditions, including the results of sanitary surveys.

2.4.3 Sampling procedures

Detailed advice on the procedures to be used for sampling different sources of water or treatment plants and distribution systems and at the tap are given in Volume 3 of *Guidelines for drinking-water quality* and in standard methods (Table 6) and other references, which should be consulted. However, the following general points should be noted.

Care must be taken to ensure that samples are representative of the water to be examined and that no accidental contamination occurs during sampling. Sample collectors should, therefore, be trained and made aware of the responsible nature of their work. Samples should be clearly labelled with the site, date, time, nature of the work, and other relevant information and sent to the laboratory for analysis without delay.

If the water to be examined is likely to contain chlorine, chloramine, chlorine dioxide, or ozone, then sodium thiosulfate solution should be added to neutralize any residual disinfectant. A properly controlled concentration of thiosulfate has no significant effect on the coliform organisms, including *E. coli,* either in chlorinated or in unchlorinated water samples during storage. If heavy metals, particularly copper, are present, then chelating agents (e.g., edetic acid (EDTA)), should also be added.

When samples of disinfected water are taken, the concentration of residual disinfectant at the sampling point and the pH should be determined at the time of collection.

When a number of samples are to be taken for various purposes from the same location, the sample for bacteriological examination should be collected first to avoid the danger of contamination of the sampling point.

Samples must be taken from different parts of the distribution system to ensure that all parts of

the system are tested. When streams, lakes, or cisterns are being sampled, the water must be taken from below the surface, away from banks, sides of tanks, and stagnant zones, and without stirring up sediments. Taps, sampling ports, and the orifices of pumps should, if possible, be disinfected and a quantity of water run to waste to flush out the standing water in the pipe, before the sample is taken. Sampling ports in treatment processes and on water mains must be carefully sited, to ensure that samples are representative. The length of pipework to the tap should be as short as possible.

The changes that may occur in the bacterial content of water on storage can be reduced to a minimum by ensuring that samples are not exposed to light and are kept cool, preferably between 4 °C and 10 °C, but not frozen. Examination should begin as soon as possible after sampling and certainly within 24 hours. If samples cannot be cooled, they must be examined within 2 hours of sampling. If neither condition can be met, the sample should not be analysed. The box used to carry samples should be cleaned and disinfected after each use to avoid contaminating the surfaces of bottles and the sampler's hands.

2.4.4 Surveillance programme requirements

Surveillance is the continuous and vigilant public health assessment and overview of the safety and acceptability of drinking-water supplies. Each component part of the drinking-water system - the source, treatment, storage, and distribution - must function without risk of failure. A failure in one part will jeopardize and nullify the effects of other parts that function perfectly, as well as the care that has been taken to ensure that they do so. Water is liable to contamination at all stages in the process of supply, hence the need for constant vigilance. At the same time, careful and intelligent assessment of likely sources of risk and breakdown are needed before a supply is planned and installed and, indeed, continuously thereafter, because of changing conditions and potential sources of contamination. Contingency plans must be made to deal with any emergencies that may arise through natural or man-made disasters, such as accidents, hostilities and civil commotions, or cessation in supplies of essential chemicals used in treatment.

An essential part of surveillance is the establishment of a proper network for regulation and command. At the highest level, this means the establishment and enforcement of national standards, the promulgation of national guidelines for achieving compliance with the laws and standards and, at the level of the water supply agency, the promotion of local codes of good waterworks practice, together with formal instruction and training. A regulatory inspectorate, with national authority, should be established to ensure that the legal requirements are met and compliance with standards is achieved. This body should be separate from that representing the interests of the water provider.

Both the water provider and the regulatory inspectorate should have properly equipped laboratory facilities with trained and properly qualified personnel, adequate facilities for sustaining the level of monitoring required on a regular basis, and sufficient capacity to carry out additional examinations as required to meet special needs. Operational staff at the waterworks should also be appropriately trained and qualified.

Lines of communication and command must be established at the outset and must be properly understood by all staff, to the highest levels. This is to ensure effective functioning of day-to-day operations. It is also to ensure that immediate remedial action is taken when emergencies and contamination are discovered; bacteriological failures must be acted on as soon as discovered, which means that the findings of the microbiologist must carry authority with the engineer and operational staff. The lines of communication needed in an emergency will be complex, involving not only different public bodies but also geographical boundaries of responsibility. Appropriate instructions must be drawn up and understood at each site.

The scope of surveillance, with examples covering the points made in this section, has been considered in a separate WHO publication, which should be consulted (see Bibliography). The

importance of surveillance is highlighted repeatedly by official reports of serious outbreaks of waterborne disease, which usually reveal deficiencies in more than one area. Surveillance procedures are described further in Volume 3 of the *Guidelines for drinking-water quality.*

The levels of surveillance of drinking-water quality differ widely in developing countries, just as economic development and provision of community water supplies vary. Surveillance should be developed and expanded progressively, by adapting the level to the local situation and economic resources, with gradual implementation, consolidation, and development of the programme to the level ultimately desired.