ENVIRONMENT AGENCY

The Microbiology of Drinking Water (2002) - Part 1 - Water Quality and Public Health

Methods for the Examination of Waters and Associated Materials

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Methods for the Examination of Waters and Associated Materials

This booklet contains details of the practices and procedures that should be adopted for taking samples for microbiological analysis.

Within this series there are separate booklets dealing with different topics concerning the microbiology of drinking water. Other booklets include

Part 2 - Practices and procedures for sampling
Part 3 - Practices and procedures for laboratories
Part 4 - Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including *E. coli* O157:H7)
Part 5 - Isolation and enumeration of enterococci by membrane filtration
Part 6 - Methods for the isolation and enumeration of sulphite-reducing clostridia and *Clostridium perfringens* by membrane filtration
Part 7 - The enumeration of heterotrophic bacteria by pour and spread plate techniques
Part 8 - Methods for the isolation and enumeration of *Aeromonas* and *Pseudomonas aeruginosa* by membrane filtration
Part 9 - Methods for the isolation and enumeration of *Salmonella* and *Shigella* by selective enrichment, membrane filtration and multiple tube most probable number techniques
Part 10 - Methods for the isolation of *Yersinia*, *Vibrio* and *Campylobacter* by selective enrichment

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About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on recommended methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are

1 General principles of sampling and accuracy of results
2 Microbiological methods
3 Empirical and physical methods
4 Metals and metalloids
5 General non-metallic substances
6 Organic impurities
7 Biological methods
8 Biodegradability and inhibition methods
9 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with this booklet are listed at the back of the booklet.

Publication of new or revised methods will be notified to the technical press. An index of methods is available from the Secretary.

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

Dr D Westwood
Secretary

January 2002

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 1999 (SI 1999/437). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; "Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Safety Precautions, Notes for Guidance" produced by the Public Health Laboratory Service. Another useful publication is "Good Laboratory Practice" produced by the Department of Health.

Preface

The importance of the provision of a wholesome supply of drinking water has been recognised since at least the times of the Romans with major advances being made during the nineteenth century. Knowledge, understanding and good practice has continued to be gained and developed over time with consequential benefits for public health. The provision of safe drinking water is one of the most important steps that can be taken to improve the health of a community by preventing the spread of water-borne disease. The maintenance of a sufficient supply of wholesome drinking water is a complex undertaking in which individuals from many disciplines have a role. This document aims to assist those individuals in their work to maintain the supply of microbiologically wholesome drinking water.

The first report on "The Bacteriological Examination of Water Supplies" was published as Report 71 in a series of Ministry of Health publications on Public Health and Medical Subjects. It was prepared in 1934 by the Ministry of Health under the Chairmanship of Dr Thomas Carnworth with the help of Sir Alexander Houston, and representatives of the Lister Institute of Preventive Medicine, the London School of Hygiene and Tropical Medicine and the Counties' Public Health Laboratories. The document was revised in 1939. In 1956, the Public Health Laboratory Service assumed responsibility for revising the Report. In 1973, the Department of the Environment (DOE) became responsible for all aspects of the water cycle and established the Standing Committee of Analysts (SCA) to review and keep up to date the methods recommended for water examination in the United Kingdom (UK). The fifth edition, issued in 1983, was therefore produced under the auspices of SCA and published jointly by DOE, Department of Health and Social Security and Public Health Laboratory Service.

Following changes in the water industry in the early 1990s, a further revision of the report was undertaken and produced as "The Microbiology of Water 1994 - Part 1 - Drinking Water", the first in a series of publications dealing with all aspects of the microbiology of water. The second document, "The Microbiology of Recreational and Environmental Water" was published in 2000.

The revised European Community Directive[1] relating to the quality of water intended for human consumption adopted at the end of 1998 has required new water quality regulations to be drawn up for the UK. These regulations, together with rapid developments in sampling and analytical techniques, have meant that a further edition of the guidance is now required. A new format has been adopted so that in future new methods and changes to procedures can be incorporated into separate parts without the need for a revision of the whole document.

This revision is being published, as a series of booklets, under the title of "The Microbiology of Drinking Water" and provides general advice and guidance on many microbiological aspects connected with potable water supplies, as well as giving details of methods. The microbiological safety of water supplies in the UK has been assured in no small measure by regular monitoring and observance of the guidance contained in previous editions of this series of booklets. It is hoped that this new edition of The Microbiology of Drinking Water will be even more useful than its predecessor editions, not only in the UK but also internationally.

The maintenance of microbiologically wholesome drinking water requires the commitment of individuals from many different disciplines and organisations, including, amongst others:

- all personnel within water companies responsible for engineering and operational activities associated with drinking water treatment and supply, and for laboratory analyses and quality assessment;

- those responsible for public health such as Consultants in Communicable Disease Control in England and Wales, Consultants in Public Health Medicine in Scotland and local authority environmental health officials;

- hospital and Public Health Laboratory Service microbiologists, and Public Analysts;

- epidemiologists from the Communicable Disease Surveillance Centre and the Scottish Centre for the Investigation of Environmental Health;

- policy makers in the Department of Health in England and Wales and the Scottish Executive; and

- external regulators, such as the Department of the Environment, Food and Rural Affairs, the Drinking Water Inspectorate in England and Wales, the Drinking Water Inspectorate in Northern Ireland, and the Scottish Executive Environment Department.

The Microbiology of Drinking Water is aimed primarily at the water industry, health authorities and local authorities in the UK. It is recognised that different legislation and organisations apply in England and Wales, and in Northern Ireland and Scotland. However, in order to avoid repetition, complication and confusion, unless there are specific differences that require clarification, references to legislation and particular professionals should be interpreted as applying to the particular circumstances in each country within the UK.

Water Quality and Public Health

1 Introduction

1.1 Objectives and scope

This document recognises the importance to public health of the proper maintenance of a wholesome supply of drinking water. Its objectives are to:

- outline the principles on which the microbiological examination of drinking water are based; and

- give advice and guidance on the interpretation of microbiological results and on the need for remedial action following a failure of microbiological standards.

Over 99 % of the population of the UK are served by a piped mains public supply of treated drinking water, and less than 1 % of the population are served by private supplies. This document is primarily concerned with the microbiological examination and monitoring of public water supplies. Mention is also made of risks that might arise from inadequate treatment, especially following sudden pollution of a source, as well as to contamination within the distribution system. In addition, brief reference is made to the microbiological examination of individual supplies, such as those on trains, ships and aircraft; and in hospitals, institutions, large buildings and factories, and in particular those premises where food and drink are manufactured and prepared.

1.2 Microbiological monitoring

The results of a laboratory examination of any single water sample are representative only of the water at the time at that particular point at which the sample is taken. Satisfactory results from single samples do not justify an assumption that the water is safe to drink at all times. Contamination is often intermittent and may not be revealed by the examination of a single sample. The impression of security given by satisfactory results from microbiological testing of waters at infrequent intervals may, therefore, be false. Indeed, the value of microbiological tests is dependent upon their frequent and regular use. It is far more important to examine a supply frequently by a simple test than to examine a supply occasionally by a more complicated test or series of tests.

Information gained over time through monitoring will provide a comprehensive picture of the range of quality of any particular source of water, any deterioration from which should at once arouse suspicion. A microbiological report based on a single sample can only indicate that, at the time of examination, certain bacteria (either indicative of faecal contamination or general water quality) did or did not grow under laboratory conditions from the sample of water submitted. Sampling techniques and sample transportation can influence sample results and good practice is essential. It should be emphasised that, when site inspection reveals obvious signs that a water supply is subject to contamination, remedial action should be taken without waiting for, and irrespective of, the results of microbiological examination. The protection of public health is of paramount importance.

1.3 Legislation and water quality standards for public water supplies

The new European Directive[1] for drinking water prescribes standards for the quality of drinking water, water offered for sale in bottles or containers and water for use in food production undertakings. The Directive specifies two types of parameter values, namely mandatory and non-mandatory. Mandatory standards, covering 28 microbiological and chemical parameters for mains water, are essential for health and the environment, and have to be met by specified dates. Non-mandatory indicator values, covering 20 further microbiological, chemical and physical parameters are prescribed for monitoring purposes. Any contravention of an indicator value must be investigated, but remedial action need be taken only where there is a risk to public health.

The Water Industry Act[2], the Water Supply (Water Quality) Regulations[3] and the Private Water Supplies Regulations[4] transpose the Directive[1] into English law. (Similar legislation applies to Wales, Scotland and Northern Ireland). However, the new water quality standards in these regulations do not apply until 25 December 2003, and until this date the previously published Water Supply (Water Quality) Regulations[5] still apply for many parameters. The Act places a duty on water companies to supply only water that is wholesome at the time of supply. The time of supply is the moment when water passes from the utility's pipe into pipes owned by the owner of premises or property. Companies are not responsible for a deterioration of drinking water quality that occurs within consumers' premises, but the Directive[1] and Regulations[3] do apply to water that is consumed. The Act[2] also creates a criminal offence of supplying water that is unfit for human consumption.

Wholesomeness is defined[2] by reference to the prescribed concentrations or values and other requirements. Prescribed concentrations or values are specified for microbiological, chemical and physical parameters. National legislation includes some standards and requirements in addition to those required by the Directive[1]. All water covered by the regulations must be microbiologically wholesome. Prescribed concentrations or values for microbiological parameters rely on well-proven indicator organisms, such as coliform bacteria, *Escherichia coli*, enterococci, *Clostridium perfringens*, and colony counts. In addition to meeting standards, water must not contain any micro-organism (other than a parameter) or parasite at a concentration which would constitute a potential danger to human health. Regulations for public water supplies in England and Wales[3] also include a treatment standard for *Cryptosporidium*.

1.4 Legislation and water quality standards for bottled waters

In the UK, the quality of water in bottles or containers is regulated by the Department of the Environment, Food and Rural Affairs, under appropriate legislation[6]. These regulations implement the European Directives[7, 8] relating to the exploitation and marketing of natural mineral waters, and also consolidate legislation on other types of bottled water. All water covered by these regulations must be bacteriologically wholesome, again defined by indicator organisms. In addition to meeting standards, water must not contain any property, element, organism or substance at a concentration or value which by itself or in conjunction with any other property, element, organism or substance would be injurious to health.

2 Microbial indicators of water quality

2.1 Introduction

The use of indicator organisms, in particular the coliform group, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health. These are based upon the principle of the detection of selected bacteria that are indicative of either contamination or deterioration of water quality through the use of simple bacteriological tests. This has been the foundation upon which protection of public health from water-borne disease has been developed. The relatively rare occasions where bacterial or viral illnesses have been caused through public drinking water supplies stand testament to the success of the indicator principle and improvements in water treatment.

2.2 Indicator organisms

Indicator organisms are used to assess the microbiological quality of water. For many pathogens, such as viruses and protozoan parasites, reliable indicators are not available. Even if there were, there is no absolute correlation between the number of indicator organisms and (a) the actual presence or numbers of enteric pathogens or (b) the risk of illness occurring.

The use of indicator bacteria, in particular *Escherichia coli* (*E. coli*) and the coliform bacteria, as a means of assessing the potential presence of water-borne pathogens has been paramount to protecting public health. The analysis of large volumes of sample for faecal indicator bacteria using membrane filtration procedures can be very useful in assessing water treatment efficiency at various points in the treatment process[9].

Many pathogens are present only under specific conditions and, when present, occur in low numbers compared with other micro-organisms. Whilst the presence of coliform bacteria does not always indicate a public health threat, their detection is a useful indication that treatment operations should be investigated[10].

2.3 Rationale for the use of indicator organisms

The key criteria for ideal bacterial indicators of faecal pollution are that they should be universally present in large numbers in the faeces of human and other warm-blooded animals. They should also be present in sewage effluent, be readily detectable by simple methods and should not grow in natural waters. Ideally, they should also be of exclusive faecal origin and be present in greater numbers than faecally transmitted pathogens. No single indicator organism fulfils all these criteria, but the member of the coliform group that satisfies most of the criteria for the ideal indicator organism in temperate climates is *E. coli*. The presence of *E. coli* in a sample of drinking water may indicate the presence of intestinal pathogens. However, the absence of *E. coli* cannot be taken as an absolute indication that intestinal pathogens are also absent. *E. coli* bacteria are the only biotype of the family Enterobacteriaceae which can be considered as being exclusively faecal in origin[10, 11] and it can represent up to 95 % of the Enterobacteriaceae found in faeces[12].

For water quality monitoring and assessment, reliance has been placed on relatively simple and more rapid tests for the detection of faecal indicator bacteria and other coliform bacteria.

These bacteria are easier to isolate and characterise, and are, almost always, present in the faeces of humans and warm-blooded animals.

The bacteriological examination of water is particularly important as it remains the most sensitive method for detecting faecal and, therefore, potentially dangerous contamination. Chemical analysis is, nevertheless, an important aid to the hygienic assessment of a water supply. However, the major role of chemical analysis is to provide process control information for water treatment and for monitoring compliance with prescribed standards. Chemical tests that give additional information on whether faecal contamination may be present include turbidity, colour, total organic carbon, nitrate, nitrite and ammonia.

The significance that can be attached to an individual bacterial faecal indicator varies with each organism and with the degree to which that organism can be associated with faecal matter. Some coliform bacteria may originate from non-faecal sources in the environment (for example, soil, decaying vegetation etc) or may even grow in the aquatic environment. Examples of these coliform bacteria are *Serratia fonticola* and *Klebsiella terrigena* and these bacteria can be commonly found in water. However, these bacteria are of no known health significance. *Citrobacter*, *Klebsiella* and *Enterobacter* are found in faeces and also in extra-intestinal environments such as soil and water[10]. Other coliform bacteria may originate from faecal sources and possess the ability to grow inside taps and pipes, even in the presence of high levels of residual disinfectant[13].

Other bacteria, which possess some of the properties of indicator organisms, include the enterococci and spores of sulphite-reducing clostridia, typified by *Clostridium perfringens*. Enterococci do not multiply in the environment and can occur normally in faeces. Numbers of enterococci in humans are greatly outnumbered by *E. coli* bacteria. When coliform bacteria are present in the absence of *E. coli*, but in the presence of enterococci, this can be indicative of the faecal origin of the coliform bacteria.

Clostridium perfringens are present in faeces in much smaller numbers than *E. coli* or enterococci. Spores of *Clostridium perfringens* are capable of surviving for significantly longer periods than vegetative bacteria, such as coliform bacteria or enterococci. These spores are also more resistant to chlorination. At present, there is conflicting evidence regarding the correlation of the presence of spores or vegetative cells of *Clostridium perfringens* with that of pathogens.

Some limited information can be provided on treatment efficiency or past faecal contamination by determining the count of *Clostridium perfringens* in distribution. The main value of carrying out tests for *Clostridium perfringens* more frequently at a point where water leaves the water treatment works (as permitted by regulations[3]) may be to provide information of the efficiency of the treatment process.

Tests for colony count bacteria growing at 37 °C and 22 °C enable a count to be determined of the heterotrophic bacterial population of the water. The bacteria grown in these tests are not indicators of faecal contamination, although historically, the count at 37 °C was taken to give an indication of faecal contamination. In the UK, the rationale for enumerating heterotrophic plate counts has been to assess the general bacterial content of the water and to monitor trends or rapid changes in water quality.

2.4 Coliform bacteria

Coliform bacteria belong to the family Enterobacteriaceae and share similar cultural characteristics. Typical genera encountered in water supplies are *Citrobacter, Enterobacter, Escherichia, Hafnia, Klebsiella, Serratia* and *Yersinia*. Coliform bacteria are defined as Gram-negative, non-spore-forming, rod shaped bacteria which are capable of aerobic and facultative anaerobic growth in the presence of bile-salts or other surface-active agents with similar growth-inhibiting properties. They usually ferment lactose at 37 °C within 48 hours, possess the enzyme β-galactosidase and are oxidase-negative.

Faecal coliform bacteria possess the characteristics of coliform bacteria but are able to carry out lactose fermentation at 44 °C. The term "faecal coliform" is not precise and has been used to describe coliform bacteria thought to be of faecal origin. The term "thermotolerant coliform" has been used to describe presumptive faecal coliform bacteria.

The historic definition of coliform bacteria is one that is not based on taxonomic characteristics, but rather on a set of criteria derived from practical experience. This definition placed restrictions on the methods by which coliform bacteria could be enumerated. In the previous edition of this booklet[14] a revised definition was introduced based upon the possession of the enzyme β-galactosidase. As a consequence of this, it is now possible to detect coliform bacteria using fluorogenic or chromogenic substrates that demonstrate the presence of the enzyme β-galactosidase. Selective media containing these substrates have been developed which allow the presence of coliform bacteria and *E. coli* to be detected in a single step. Details of methods for the detection and enumeration of coliform bacteria are described elsewhere[15] in this series.

Several members of the coliform group are known to be present in soil and other environmental materials, and are capable of growth in nutrient-rich water and biofilms. As a result, coliform bacteria are no longer considered to be specific indicators of faecal contamination. However, some species of coliform bacteria, although common in the environment, can be associated with human infection but rarely with gastro-enteritis. *Hafnia alvei* can be present in the faeces of humans, animals and birds and can occasionally be present in clinical specimens of non-faecal origin. The main human infections caused by species of *Serratia* are associated with hospital environments, with *Serratia marcescens* associated with wound and systemic infections being the most frequently isolated opportunistic pathogen. *Serratia fonticola*, which can be isolated from water, however, has not to date been detected in clinical specimens. *Enterobacter cloacae* can occur within water distribution systems as a result of re-growth but poses no health risk, although some strains can be associated with hospital acquired (nosocomial) infections. In one incident, strains isolated from a distribution system were different from clinical isolates reported from hospitals in the affected area[16]. Some species of *Klebsiella* are known to cause infection in patients undergoing hospital treatment and where their immune system is weak, with the primary route of infection being by person-to-person contact or via food rather than water-borne transmission (for example, *Klebsiella pneumoniae*). *Klebsiella oxytoca* occurs in the intestinal tract of humans and animals as well as being widespread in the environment, whereas *Klebsiella terrigena* and *Klebsiella planticola* are common in natural waters, soils and plant materials.

When coliform bacteria are isolated from drinking water supplies it is often useful to determine which species of coliform bacteria are present, particularly if problems recur, in order to determine the source and significance of the coliform bacteria being recovered. The potential source of coliform bacteria in water supplies result from sub-optimal operation of water treatment processes or ingress of contamination from breaches in the integrity of the distribution system. These include for example, leaking hatches on service reservoirs, contamination via air-valves and stop valves, infiltration into mains and service reservoirs, cross connections and back-flow effects.

Coliform bacteria can be present in domestic plumbing systems with kitchen taps and sinks being recognised sources of these organisms.

2.5 *Escherichia coli*

E. coli is a coliform bacterium and has historically been regarded as the primary indicator of faecal contamination of both treated and untreated water. As a coliform bacterium it is a member of the family Enterobacteriaceae, and is capable of fermenting lactose or mannitol at 44 °C, usually within 24 hours, and produces indole from tryptophan. Most of the *E. coli* strains possess the enzyme β-glucuronidase, which can be detected using specific fluorogenic or chromogenic substrates. Details of methods for the detection and enumeration of *E. coli* are described elsewhere[15] in this series.

E. coli occurs in the faeces of all mammals, often in high numbers (up to 10^9 per gram of faeces). This widespread faecal occurrence, coupled with methods that for the recovery and enumeration of *E. coli* are relatively simple to conduct, has contributed to the detection of this bacterium as the cornerstone of microbiological water quality assessment for over 100 years[10, 11]. The survival characteristics and susceptibility to disinfection of *E. coli* are similar to those of many other bacterial pathogens, particularly *Salmonella* and *Shigella*, and it does not multiply in temperate surface water or in treated waters. There are situations where *E. coli* is not a suitable indicator of microbiological contamination (for example, disinfected surface waters exposed to *Cryptosporidium* contamination), yet it still remains the best biological indicator for drinking water and public health protection[10]. Many tests for *E. coli* rely upon selective isolation at 44 °C. Some of the strains of *E. coli*, however, do not grow well at this temperature, but will be isolated at 37 °C. These isolates, when identified as *E. coli* still have the same sanitary and operational significance with regard to their faecal origin.

2.6 Intestinal enterococci

Intestinal enterococci are defined as Gram-positive cocci that tend to form in pairs and chains. They are non-spore-forming, oxidase-negative, catalase-negative, possess Lancefield's Group D antigen and hydrolyse aesculin. They can grow aerobically and anaerobically in the presence of bile salts, and in sodium azide solutions, concentrations of which are inhibitory to coliform bacteria and most Gram-negative bacteria. *Enterococcus faecalis* and some related species can reduce 2,3,5-triphenyltetrazolium chloride to the insoluble red dye, formazan.

Enterococci include a number of species that occur in the faeces of humans and warm-blooded animals. The main reason for their enumeration is to assess the significance of the presence of coliform bacteria in the absence of *E. coli*, or to provide additional information when assessing the extent of possible faecal contamination. As such, they are regarded as

secondary indicators of faecal pollution. In human faeces, numbers of enterococci rarely exceed 10^6 per gram of faeces, while in animal faeces they are often more numerous than *E. coli*. Enterococci of faecal origin rarely multiply in water and are more resistant to environmental stress and chlorination than *E. coli* and coliform bacteria[11]. They generally persist longer in the environment, with the exception of *Streptococcus bovis* and *Streptococcus equinus*, which die off relatively rapidly once outside the intestinal tract.

Enterococci can be found in foodstuffs, particularly plant-based products, where their presence is often unrelated to direct faecal contamination. A related group of bacteria, *Aerococcus*, which can also be recovered on some of the media used for the enumeration of intestinal enterococci, are often found in water and on vegetation.

It has been suggested[11] that testing for enterococci can be a useful additional indicator of water treatment efficiency. As these bacteria are resistant to drying, they can be of value for routine assessment after new mains have been laid or when repairs in distribution systems have been carried out, or for assessing pollution by surface run-off to ground or surface waters.

The species of enterococci that occur in faeces and, therefore, are more likely to be found in polluted waters can be divided into two main groups. The first includes *Enterococcus faecalis*, *Enterococcus faecium* and *Enterococcus durans*. These organisms are normally present in the faeces of humans and various animals. The second group includes *Streptococcus bovis*, *Streptococcus equinus* and *Enterococcus avium*. These organisms are not normally found in human faeces. The identification of species may, therefore, give an indication of the source of contamination. Details of methods for the detection and enumeration of enterococci are described elsewhere[17] in this series.

2.7 *Clostridium perfringens*

The genus *Clostridium* contains over 100 species of bacteria and some clostridia have been renamed several times (for example, *Clostridium perfringens* was originally named *Bacillus perfringens* and then *Clostridium welchii*). Sulphite-reducing clostridia are Gram-positive, anaerobic spore-forming rods that reduce sulphite to sulphide. *Clostridium perfringens* is a member of the sulphite-reducing clostridia which is non-motile and is capable of fermenting lactose, reducing nitrate and liquefying gelatin. Most clostridia are strictly anaerobic, but a few species are capable of limited growth in the presence of low levels of oxygen. Most species of *Clostridium* are environmental bacteria. Many are saprophytic, normally inhabiting soil, water and decomposing plant and animal material. These bacteria will, therefore, be present in surface derived source waters.

Clostridium perfringens is the key species of the sulphite-reducing clostridia and is commonly found in human and animal faeces. *Clostridium perfringens* produces environmentally resistant spores that survive in water and in the environment for much longer periods than the vegetative cells of *E. coli* and other faecal indicators. Clostridia are removed from water by coagulation and filtration, but the spores of these bacteria can be resistant to chlorine at concentrations normally used in water treatment. As *Clostridium perfringens* is generally present in faeces in much lower numbers than *E. coli* and enterococci, it is less sensitive as an indicator of faecal contamination. Low numbers may occasionally occur in

water supplies, but they do not represent a risk to health. These bacteria will not grow to significant numbers, or produce toxins, in water supplies, as conditions are usually unsuitable.

The genus C*lostridium*, whilst consisting mainly of saprophytes, contains some species which are regarded as opportunistic pathogenic bacteria (for example, some clostridia are commonly associated with wound infections in humans and animals). Growth tends to be restricted to the site of infection but a wide variety of toxins, some of which are extremely potent, are produced dependent on the particular strain (for example, tetanus, wound botulism and gas gangrene). *Clostridium botulinum* and *Clostridium perfringens* have also been associated with food poisoning, and some strains of *Clostridium perfringens* can produce severe but self-limiting diarrhoea in humans and animals if ingested in large numbers. Details of methods for the detection and enumeration of the sulphite-reducing clostridia and *Clostridium perfringens* are described elsewhere[18] in this series.

2.8 Colony count bacteria

Colony counts are enumerations of the general population of heterotrophic bacteria present in water supplies. The enumerations may represent bacteria whose natural habitat is the water environment or those that have originated from soil or vegetation. Historically, these bacteria have been enumerated on bacteriologically nutrient-rich media with incubation at 37 °C and 22 °C. It is well recognised, however, that only a small fraction of the viable bacterial population present in water is enumerated by the procedures normally employed. Despite this, monitoring of water supplies for colony count bacteria can be useful for monitoring trends in water quality or detecting sudden changes in quality.

The requirement to enumerate colony counts at 37 °C is no longer prescribed in the new Directive[1], however, the requirement to enumerate colony counts at 22 °C remains. In UK legislation[3, 5] colony counts at both temperatures are still required. Colony counts at 37 °C, when compared with those at 22 °C, can be a useful quality indicator, in that they can provide an early indication of a significant deterioration in quality. This can often be demonstrated before coliform bacteria or other indicator bacteria are detected (for example, due to ingress into a distribution system). An increase in the counts at 37 °C (compared with those normally recorded for a supply) may be an indication of contamination, particularly if not accompanied by a similar increase in the corresponding counts at 22 °C. Bacteria recovered in the colony counts at 22 °C generally represent those bacteria naturally present in water and are not of sanitary significance, and thus, have limited public health significance. They may, however, be of greater relevance to the food and drink industries and electronics manufacturers, where high numbers may impact on the quality of products. These counts may be useful in assessing the efficiency of water treatment and the cleanliness and integrity of distribution systems.

An important benefit of determining colony counts at both 37 °C and 22 °C, particularly if carried out regularly from the same site and location, is that the data generated can provide an indication of seasonal and longer-term changes in the general bacteriological quality of the water. Many heterotrophic bacteria are able to multiply within the distribution system network by utilising nutrients derived either from fixtures and fittings or from assimilable or particulate organic carbon in the water. Changes in colony numbers may, therefore, be indicative of the use of inappropriate materials or changes in the quality of the source water. Drinking water supplies derived from surface waters tend to support higher numbers of heterotrophic bacteria than those derived from groundwater sources. This is due to the

difference in concentrations of assimilable carbon associated with each type of source. It is, therefore, not the absolute numbers of colony count bacteria enumerated from a supply that are of importance, but whether, over time, there are significant changes or long-term trends in those numbers. Details of methods for the enumeration of colony counts are described elsewhere[19] in this series.

2.9 Other potential indicators of faecal contamination

E. coli and related coliform bacteria, intestinal enterococci and *Clostridium perfringens* are currently recommended for use as indicator organisms of faecal contamination in water. Other micro-organisms have been suggested for this purpose and these include the *Bacteroides fragilis* group, *Bifidobacterium* species or *Rhodococcus coprophilus*. Also bacteriophages that infect the coliform bacteria (coliphages) and the *Bacteroides fragilis* group have been used. Although some of these alternative indicator organisms have been applied with varying degrees of success to environmental waters, they are not considered suitable for the assessment of water treatment efficacy or treated water quality.

The ability to distinguish between human and animal faecal pollution may be of value in tracing the source of faecal contamination and in the assessment of the adequacy of protection of a water supply, especially in rural areas. Two approaches have been suggested for distinguishing between human and animal faecal pollution. Historically, the ratio between numbers of *E. coli* (or 'faecal coliforms') and enterococci (or 'faecal streptococci') has been used. However, because of the uncertainty associated with the interpretation of ratio estimates this approach is not recommended for differentiating between human and animal sources of pollution[20]. Alternatively, the detection and enumeration of specific organisms may be useful.

Streptococcus bovis is the predominant species of enterococci in cattle and most other farm animals, while being relatively uncommon in humans[21]. Its usefulness as a specific indicator of animal faecal pollution is limited, however, by its short survival time outside of the gut. It may be used when found in conjunction with *Rhodococcus coprophilus* (which is also excreted by farm animals but not by humans) to indicate recent faecal pollution from animals. *Rhodococcus coprophilus* is a much hardier organism and its presence in the absence of *Streptococcus bovis* may suggest more remote animal faecal contamination.

Enterococcus faecalis has been used as an indicator of faecal contamination of human origin as it is particularly associated with humans[22], although *Enterococcus faecium* is sometimes considered to be more prevalent in human faeces. Neither bacterium, however, is specific for human faecal contamination.

3 Water-borne pathogens

3.1 Bacteria

3.1.1 *Campylobacter*

Bacteria of the genus *Campylobacter* are members of the family Spirillaceae and are the most common cause of human bacterial gastro-enteritis in the UK. Campylobacteriosis occurs most frequently in the summer months and the most commonly isolated species is *Campylobacter jejuni*. The organism can be carried asymptomatically by cattle, sheep, poultry and other birds,

and is also isolated from natural waters. A number of outbreaks in the UK have been associated with private water supplies[23]. *Campylobacter* species can survive in water for some days but are highly susceptible to chlorination or ultra violet disinfection at the doses typically used in water treatment and should, therefore, not be a risk in treated drinking water, unless it is subject to significant post treatment contamination. Private water supplies without adequate disinfection may represent a greater risk of infection. *E. coli* is not an adequate indicator for the presence of *Campylobacter* in water, but is appropriate for demonstrating adequacy of water treatment. Details of methods for the detection of *Campylobacter* species are described elsewhere[24] in this series.

3.1.2 *E. coli* O157

Some strains of *E. coli* can cause serious diarrhoeal disease. Several classes of diarrhoeagenic *E. coli* are now recognised, which are defined by the possession of distinct virulence factors. The most important of these are the Vero-cytotoxin-producing *E. coli* (VTEC), in particular VTEC of serogroup O157, but other *E. coli* serogroups may contain VTEC members. Typical symptoms of people infected with *E. coli* O157 range from mild diarrhoea, fever and vomiting to severe, bloody diarrhoea and painful abdominal cramps. In 10 - 15 % of cases, a condition known as haemolytic uraemic syndrone which can result in kidney failure. Individuals of all ages can be affected but children up to ten years old and the elderly are most at risk. The infectious dose for *E. coli* O157 is relatively low compared with other bacterial causes of gastro-enteritis, perhaps as low as 10 organisms.

E. coli O157 can be present in untreated water supplies. VTEC are susceptible to chlorination and ultra violet disinfection at the doses normally used in water treatment. Private water supplies may be at greater risk, but outbreaks associated with *E. coli* O157 (and *Campylobacter*) have been reported. Conventional *E coli* tests are adequate indicator tests for the presence and survival of VTEC and other pathogenic *E. coli* in water.

VTEC may not be isolated or may not be recognised by the normal analytical methods for *E. coli*, and specific isolation methods are required. However, if *E. coli* is detected in a water supply it should be assumed that VTEC could also be present. Details of methods for the detection of *E. coli* O157 are described elsewhere[15] in this series.

3.1.3 *Salmonella*

Species of *Salmonella* are members of the family Enterobacteriaceae and are the causative agents of typhoid and paratyphoid fever, and milder forms of gastro-enteritis. The enteric fevers (typhoid, caused by *Salmonella typhi*, and paratyphoid, caused by *Salmonella paratyphi*) remain important contributors to water-borne disease world-wide, although nowadays very rarely in developed countries. Salmonellae can be subdivided into more than 2000 serotypes. S*almonella typhi* and *Salmonella paratyphi* are only associated with humans but the other salmonellae are found commonly in the faeces of animals and agricultural livestock, and have been found in poultry, eggs and meat products. Food-borne contamination is the major route of infection for these bacteria, but transmission can occur by water contaminated with faecal material. Survival in surface water is limited to hours or days, depending on the amount of contamination and the water temperature. Species of *Salmonella* are susceptible to normal methods of disinfection used in the water industry. Untreated private water supplies and uncovered storage tanks may, however, be at risk from avian (for

example, pigeons and seagulls) faecal contamination that may contain *Salmonella*. *E. coli* is an adequate indicator for the presence and survival of *Salmonella* in water. Details of detection methods for *Salmonella* are described elsewhere[25] in this series.

Over the last 50 years, the incidence of water-borne salmonellosis from treated public water supplies has declined and outbreaks are now very rare.

3.1.4 *Shigella*

Species of *Shigella* are members of the family Enterobacteriaceae and cause bacillary dysentery (shigellosis) in humans. The *Shigella* group is divided into four main sub-groups differentiated by biochemical and serological tests. *Shigella dysenteriae*, *Shigella sonnei*, *Shigella flexneri* and *Shigella boydi* are the main organisms of concern. *Shigella sonnei* is the most common species found in the UK and causes the mildest form of the disease. Person-to-person contact, faecally contaminated food, and less frequently, water are the main sources of contamination. Survival in surface water is limited to hours or days, depending on the amount of contamination and the water temperature. Shigellae are susceptible to chlorination and ultra violet disinfection at the doses used in water treatment. *E. coli* is an adequate indicator for the presence and survival of *Shigella* in water. Details of detection methods for *Shigella* are described elsewhere[25] in this series.

3.1.5 *Yersinia*

Species of *Yersinia* are members of the family Enterobacteriaceae, of which some species cause diseases in humans and other mammals. Human plague, caused by *Yersinia pestis*, is not a water-borne disease. Other species, including *Yersinia enterocolitica*, *Yersinia intermedia*, *Yersinia kristensenii*, *Yersinia frederiksenii* and *Yersinia pseudotuberculosis*, may produce symptoms ranging from subclinical and mild diarrhoeal infections to rare severe infection including septicaemia. Some serotypes of *Yersinia enterocolitica* are more frequently associated with human disease than others. *Yersinia* species can be isolated from natural waters and may be associated with farms and meat processing plants. There is evidence that some species of *Yersinia* can grow in water and can be isolated from inadequately treated drinking water. *Yersinia* species are susceptible to chlorination and ultra violet disinfection at doses normally used in water treatment. *E. coli* is an adequate indicator for the presence and survival of *Yersinia* in water. Details of methods for the detection of *Yersinia* species are described elsewhere[24] in this series.

3.1.6 *Vibrio*

Species of *Vibrio* are members of the Vibrionaceae. Some species, most notably, strains of *Vibrio cholerae*, cause gastro-enteritis in humans. *Vibrio* species occur naturally in brackish and saline waters, and some can survive in fresh water systems.

Vibrio cholerae, which causes cholera, can be divided into approximately 140 O-serovars. The strains that usually produce outbreaks of epidemic cholera are toxin-producing strains of the O1 serovar and a more recently reported serovar, O139. Some other serovars of *Vibrio cholerae* can also cause gastroenteritis. The primary route of transmission for cholera is contaminated water and outbreaks have also been reported following consumption of crops irrigated with sewage-contaminated water. *Vibrio parahaemolyticus* also causes diarrhoea,

often through the consumption of raw, contaminated seafood. *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae* and *Vibrio mimicus* are also recognised as causing diarrhoea. Other species of *Vibrio* are associated with wound infections or septicaemia following exposure to environmental waters. *Vibrio* species can grow in environmental waters, particularly when temperatures rise above 10 °C and may be associated with sediments, plankton and cyanobacterial blooms. *Vibrio* are susceptible to chlorination and ultra violet disinfection at doses normally used in water treatment. Details of methods for the detection of *Vibrio* species are described elsewhere[24] in this series.

3.2 Viruses

3.2.1 Norwalk-like viruses

Norwalk-like viruses (NLV) are classified within the *Caliciviridae* family and were formerly known in the UK as small round structured viruses. They are the most common cause of sporadic and epidemic viral gastro-enteritis in adults, but are also common causes of infections in children. Many strains of NLV have been recognised and they are currently divided into two major genogroups (I and II). The strains are designated by the geographical location where they were initially recognised. The main route of transmission is via person-to-person contact, but food-borne transmission may occur, especially involving raw or inadequately cooked shellfish. Water-borne outbreaks have occurred as a result of sewage contamination of drinking water supplies, but none has been confirmed in the UK. Animal strains of NLV are recognised but are not known to infect humans. The limited information currently available suggests that NLV are sensitive to chlorination. As these viruses may survive in the environment longer than bacteria, the absence of *E. coli* may not always equate with the absence of NLV.

3.2.2 Hepatitis A Virus

Hepatitis A virus is a member of the *Picornaviridae* family of viruses, and is the only member of the Hepatovirus genus in which there is only one serotype. No animal strains are known. The virus replicates in the liver and causes acute but self-limiting hepatitis. Transmission is by direct faecal-oral route and is most common in areas of poor hygiene and poor sanitation. The disease occurs sporadically, though at a low prevalence level in the UK. Shellfish and fresh fruits have caused food-borne transmission. Water-borne outbreaks have been recognised after sewage contamination of drinking water, but none have been reported in the UK. Hepatitis A viruses are sensitive to chlorination, but as these viruses may survive in the environment longer than bacteria, the absence of *E. coli* may not always equate with the absence of Hepatitis A viruses.

3.2.3 Other viruses

Enteroviruses (*Picornaviridae* family) are well-established indicators of human enteric viruses in the environment. This is due to the relative ease with which they can be concentrated from sewage-contaminated water and the availability of effective detection methods. Additionally, Enteroviruses replicate in the gastro-intestinal tract and are present in most populations throughout the year. The group includes poliovirus, Coxsackievirus B and echovirus. Enterovirus infections are commonly asymptomatic, but may cause flu-like symptoms, occasionally meningitis and, rarely, paralysis. Enterovirus infection does not result

in gastro-enteritis unless as part of a more generalised illness. Vaccination campaigns utilising live poliovirus are undertaken world-wide resulting in widespread occurrence of the virus in the environment. Infections with other Enterovirus serotypes are common world-wide with different serotypes predominating from year to year. Water-borne transmission has not been confirmed, although as person-to-person transmission is the main route and results in many asymptomatic infections, it would be difficult to identify. Details of detection methods for Enteroviruses are described elsewhere[26] in this series.

Rotaviruses (*Reoviridae* family) comprise six serogroups and are further divided into serotypes and genotypes. Serogroup A is the most common human rotavirus infection, although members of Group B and C can also infect humans. Infection first occurs below the age of one year and rotavirus is the most important pathogen causing gastro-enteritis of this age group. Subsequent infections throughout life are usually asymptomatic. A few water-borne outbreaks world-wide have been reported.

The Adenoviruses (*Adenoviridae* family), which include many different serotypes, replicate in the gastro-intestinal tract and are shed into sewage. Only serotypes 40 and 41 are known to cause gastro-enteritis in humans, mostly in babies. Infection involving drinking water has not been recognised. The Astroviruses (*Astroviridae* family) include at least eight serotypes that infect humans, causing gastro-enteritis, particularly in children. Water-borne infections have been reported.

The above viruses may survive in the environment for longer periods than bacteria, and the absence of *E. coli* may not be an adequate indicator for the environmental presence of these viruses in all circumstances. These viruses are sensitive to chlorination.

Classic calicivirus is the name given to a distinct group of Caliciviruses, and are recognised by clear cup-shaped markings on virions when examined by electron microscopy. This group is also known as Sapporo virus or Sapporo-like viruses. They are part of the family *Caliciviridae*, but are distinct from the NLV. No water-borne infections have been recognised.

3.3 Protozoa

The enteric protozoa that cause human illness are usually transmitted by the consumption of food and drink, although environmental contamination and poor hygiene are also important transmission routes. Many cause particular problems in immuno-compromised patients, particularly in people infected with HIV and individuals with T-cell deficiencies. The protozoa that are of most concern are *Cryptosporidium*, *Giardia* and *Toxoplasma*, although *Cyclospora* has been identified in a number of food-borne outbreaks. Water-borne outbreaks of infection with protozoa have been reported.

3.3.1 *Cryptosporidium*

Cryptosporidium species are the cause of a diarrhoeal disease that can last for up to several days to a few weeks. A chronic life threatening infection with watery diarrhoea can occur in people with compromised immune systems. There have been several outbreaks of gastro-enteritis linked to drinking water, contaminated swimming pool and recreational water use, and drinking water is an important identifiable source of human cryptosporidiosis. Other sources include contamination associated with farm visits and food-borne infection.

Cryptosporidium oocysts have a low infectious dose (from 10 to 1000 organisms) and individual strains have been found to differ in their infectivity. Natural water sources are commonly contaminated with oocysts from animal and human faeces.

Cryptosporidium species includes a number of types that are infectious to humans. *Cryptosporidium parvum* Type 1 is infectious to humans but does not infect most agricultural and laboratory animals. *Cryptosporidium parvum* Type 2 has a wider host range and is infectious to humans, sheep, cattle and laboratory animals. *Cryptosporidium meleagridis* isolated from humans is similar to isolates from birds. *Cryptosporidium felis* is infectious to cattle, cats and humans. The main *Cryptosporidium* strains associated with human disease in the UK are *Cryptosporidium parvum* Types 1 and 2.

The oocysts of *Cryptosporidium* are infectious when excreted in faeces, and pass into rivers and lakes via sewage works and agricultural run-off. The oocysts are resistant to environmental conditions and disinfectants such as chlorine, and can pass into drinking water when there are failures in filtration processes or contamination of source waters. There is increasing evidence that oocysts are susceptible to ultra violet disinfection. Conventional indicator bacteria are not good indicators of *Cryptosporidium* contamination, and water supplies that are at risk of contamination are subject to continuous monitoring under the UK Regulations[3]. Details of detection methods for *Cryptosporidium* oocysts are described elsewhere[27] in this series.

3.3.2 *Giardia*

Giardia species are flagellated protozoans that parasitize the small intestines of mammals, birds, reptiles and amphibians, and giardiasis is a common cause of diarrhoea. The symptoms of giardiasis range from asymptomatic to a transient or persistent acute stage, with steatorrhoea, intermittent diarrhoea, and weight loss, or to a sub-acute or chronic stage that can mimic gallbladder or peptic ulcer disease. Sources of infection, in addition to humans, are thought to include wild and domestic animals. Experimental inoculation indicates that *Giardia* has a low infective dose (10 to 25 cysts).

Outbreaks of infection related to drinking water have been described, although outbreaks have mostly been associated with recreational water use. The cysts of *Giardia duodenalis* are relatively resistant to chlorine, although less resistant than *Cryptosporidium* oocysts. The cysts can remain viable in cold water for several months. Details of detection methods for *Giardia* cysts are described elsewhere[27] in this series.

3.3.3 *Cyclospora*

Cyclospora is a coccidian parasite that causes protracted watery diarrhoea. It occurs world-wide, but in the UK, infection is uncommon, and is normally associated with travel to developing countries. Outbreaks linked to drinking water have been reported. Person-to-person contact is not thought to occur, because the oocysts need to mature (sporulate) under environmental conditions outside the host for one to two weeks before they become infectious. The oocysts have been reported to be relatively resistant to chlorine.

The risk of *Cyclospora* being transmitted via treated mains water in the UK is considered to be low. In developing countries, transmission is likely to be through sewage contaminated

water and the contamination of fruit and vegetables with sewage contaminated water used for irrigation or pesticide application.

3.3.4 Microsporidia

Microsporidia are protozoa with characteristic morphology including a lack of mitochondria and possession of a distinctive coiled polar tube in the spores. Two species, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*, are a common cause of chronic diarrhoea in immuno-compromised individuals, and they may infect a range of agricultural animals. As viable spores are passed by infected patients, person-to-person transmission and contamination of water with human waste are potential routes of transmission. The difficulty of isolating organisms by tissue culture means that reliable information on the sensitivity of spores to chlorine is not available for all species. The relatively recent emergence of species of Microsporidia as human pathogens and the difficulties of diagnosis mean that water-borne associations cannot yet been clearly demonstrated, although spores have been found in non-potable water.

3.3.5 *Toxoplasma gondii*

Toxoplasma gondii is a parasite which forms oocysts in cats, and cysts within a secondary host's (other mammals or birds) tissues. The life cycle is completed when the carnivorous primary host consumes the secondary host. Humans are infected by consuming inadequately cooked meat from infected secondary host species such as agricultural animals, or from oocysts occurring in food or water. The sporulated oocysts of *Toxoplasma* are very resistant to environmental conditions and disinfectants.

Outbreaks of infection have been associated with food, milk, water and environmental contamination with cat faeces. Demonstrating that an outbreak has occurred is difficult, as diagnosis is undertaken using serological techniques, and most infections are not usually serious enough to cause a visit to a physician. Water-borne infections arise through oocysts, from infected wild cats, getting into drinking water.

3.3.6 *Entamoeba histolytica*

Entamoeba histolytica causes amoebic dysentery and abscesses in the liver and other organs. The cysts of *Entamoeba histolytica* are morphologically identical to those of the non-pathogenic *Entamoeba dispar,* and much of the scientific literature may relate to *Entamoeba dispar*. *Entamoeba histolytica* is not endemic in the UK and water-borne infections mostly arise when consuming contaminated food or water in countries where it is endemic. Infection is common world-wide, particularly in poor countries with inadequate sanitation. Outbreaks of infection associated with drinking water are rare.

3.4 Emerging pathogens

An association between the consumption of drinking water and infectious diseases may arise from information on outbreaks and epidemiological studies. Over the last century, the range of pathogenic organisms linked to drinking water has increased. Emerging water-borne pathogens are defined as those whose prevalence has increased within the past two decades or threaten to increase in the near future. Their emergence may be due to the spread of new

agents, recognition of infections that have been present in the population but have gone undetected, realisation that an established disease has an infectious origin, or the reappearance (or "re-emergence") of known infections after a period of decline in incidence.

The occurrence of organisms may increase or decrease as a result of changes in water supply, water use, weather patterns, or technological changes in pathogen detection and typing that allow a better understanding of real incidence. In addition, these changes may be due to changes in public health systems, changes in the prevalence of particular infections in the community and in animals, the recognition of new water problems and a greater understanding of new and old infectious diseases.

The organisms that are of particular interest are those that can pass through conventional water treatment processes, those that are able to grow within distribution systems, and those that are able to colonise domestic plumbing systems. The risks to consumers may be greater with private water supplies, but the number of people that may be infected is greater with public mains water supplies.

Emerging organisms include *Cyclospora cayetanensis*, *Burkholderia pseudomallei*, *E. coli* O157, Norwalk-like viruses, *Toxoplasma gondii*, the newly recognised species of *Cryptosporidium* capable of infecting humans (including *Cryptosporidium meleagridis* and *Cryptosporidium felis*), toxic cyanobacteria, *Acanthamoeba* species, *Entamoeba histolytica* and Hepatitis E virus. In addition, other organisms that can be considered as potential emerging water-borne pathogens include *Helicobacter pylori*, *Mycobacterium avium*, *Mycobacterium avium paratuberculosis*, and the microsporidia (particularly *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*). Many of the diseases caused by these organisms are uncommon within the UK, and the epidemiological evidence for their role in drinking water related infection is variable. It is likely that more drinking water associations with known and novel pathogens may arise in the future and vigilance in this area is desirable. Of particular interest are epidemiological studies that estimate the burden of excess illness in the community due to drinking water, and the organisms responsible for causing infectious intestinal diseases[28].

4 Other organisms

4.1 More common bacteria

Many species of bacteria occur naturally in ground and surface waters. A proportion of these may survive water treatment or may subsequently be introduced into treated water as a result of contamination. Some of these organisms may be able to grow if temperatures are high enough, if sufficient nutrients are available in the water, if inappropriate construction materials are used, or as a result of poor maintenance. In many cases, this may not be a problem; in some cases, if growth is sufficient, the result may lead to deteriorating organoleptic quality of the water or, potentially, a risk to public health.

4.1.1 The *Pseudomonas* group

The family Pseudomonadaceae contains a number of genera that have been split from the genus *Pseudomonas* as the taxonomy has become more clearly understood. The members of the Pseudomonadaceae are Gram-negative rods, and are widespread in nature occurring commonly

in water and soil. Many are capable of growth in relatively low-nutrient environments. When the organisms gain access to treated water they may proliferate in certain circumstances by utilising nutrients either present in the water or derived from unsuitable materials used in the construction of distribution systems or domestic plumbing installations. Similarly, they may grow in water contained in bottles (particularly plastic) and on surfaces such as plastic tubing within drinks vending machines. Some species can be pathogenic for humans and are particularly important as a cause of nosocomial (hospital acquired) infection because of their resistance to many antibiotics and disinfectants and their ability to colonise aquatic low-nutrient environments. Heavy colonisation of water systems with *Pseudomonas* species, particularly the fluorescent species *Pseudomonas fluorescens* and *Pseudomonas putida*, can lead to taste and odour problems without any concomitant risk of disease.

4.1.1.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is ubiquitous in fresh water, sewage and soil and can also be derived from the faeces of animals and humans. The organism can grow in very low nutrient aqueous environments and can survive for many months in water at ambient temperatures. *Pseudomonas aeruginosa* is an important opportunistic pathogen and is particularly significant as a cause of nosocomial infections. *Pseudomonas aeruginosa* causes a wide range of infections, but the vast majority of people exposed to *Pseudomonas aeruginosa* suffer no adverse health effects. Community acquired infections arising from *Pseudomonas aeruginosa* are often localised and are associated with contact with contaminated water. Eye infections are commonly associated with contact lens use and can be contracted as a result of contact lens solutions becoming contaminated or where contaminated tap water has been used in lens care. Although small numbers of *Pseudomonas aeruginosa* may be present in mains drinking water the organisms are not infectious if swallowed, except possibly in profoundly immuno-compromised individuals. The number of *Pseudomonas aeruginosa* present in public mains water is rarely likely to be sufficient to cause infections unless they are allowed to multiply. Taps, however, can become colonised locally with *Pseudomonas aeruginosa* and other organisms, so tap water should never be used for cleaning or rinsing contact lenses or their storage containers. Details of methods for the detection and enumeration of *Pseudomonas aeruginosa* are described elsewhere[29] in this series. The routine examination of mains water for *Pseudomonas aeruginosa* is not recommended, but in view of its importance as an opportunistic pathogen, testing may sometimes be required.

4.1.1.2 Other *Pseudomonas*-like species

A number of other species formerly within the genus *Pseudomonas* can also cause infections, particularly in debilitated hospitalised patients. Of these species, the most important is *Burkholderia cepacia* because of its resistance to antibiotics and its ability to grow in distilled water and dilute disinfectants. *Stenotrophomonas maltophilia* is also a relatively common cause of hospital acquired infection. Other species that have occasionally been associated with water associated hospital infections include *Ralstonia pickettii* and *Sphingomonas paucimobilis.*

4.1.2 *Aeromonas*

Species of *Aeromonas* are members of the Aeromonadaceae. They are natural inhabitants of fresh water environments and, consequently, are common in source waters. Although the understanding of the taxonomy of the genus has improved it remains difficult to readily

identify the species that can be defined by molecular genetic methods. The majority of species are generally motile and are common in natural water where they may sometimes form a large proportion of the total heterotrophic bacterial flora. Aeromonads can be present in high numbers in fresh waters both in the presence and absence of faecal pollution. High numbers are common in sewage effluents but are usually of different species to those found in pristine waters. *Aeromonas* species are, generally, readily killed by chlorine and other commonly used water disinfectants. However, any survivors of the initial treatment or any aeromonads entering the distribution system post-treatment may multiply significantly. Aeromonads are capable of growth in relatively low-nutrient environments. Thus, the presence of *Aeromonas* in drinking water does not indicate faecal pollution but may reflect deteriorating water quality. Control of *Aeromonas* in drinking waters can be achieved with increased disinfectant residuals in distribution, although chlorine residuals in excess of 0.2 mg/l may be needed for protracted periods to achieve control.

Despite frequent isolation of aeromonads from drinking waters there is a lack of epidemiological evidence to demonstrate unequivocal association of their presence with illness in the community. Nonetheless, it would seem advisable to adopt a strategy (such as treatment to maximise organic carbon removal, to shorten residence times in distribution and to provide better control of chlorine residuals [(11)]) that limits re-growth of these organisms in distribution systems. Whilst there is no need to test routinely for *Aeromonas*, examination for aeromonads may prove useful when investigating distribution problems. Details of methods for the detection and enumeration of *Aeromonas* are described elsewhere[(29)] in this series.

4.1.3 *Legionella*

The genus *Legionella* includes over 40 species of bacteria that occur naturally in the aquatic environment. Occasionally, bacteria cause infections in humans and these infections are collectively called legionellosis. The most common infection is Legionnaires' disease which is an acute severe pneumonia. The most common cause of Legionnaires' disease is *Legionella pneumophila* which can be subdivided into 16 serogroups, of which serogroup 1 is the most common type isolated from patients and the environment. In addition, several species of *Legionella* can cause a short-lived, self-limiting influenza-like illness without pneumonia (Pontiac fever or Lochgoilhead fever). At least 18 species of *Legionella* have been associated with disease in humans but *Legionella pneumophila* remains the most common cause in the UK. Infection normally results from the inhalation of an aerosol derived from water containing the bacterium, and has most often been associated with hot and cold water systems in, for example, large buildings, cooling towers and evaporative condensers and spa pools.

Although *Legionella* species occur naturally in water they can only grow with the assistance of other micro-organisms. They prefer warm water at temperatures between 30 - 45 °C. *Legionella* species have been shown to be capable of growth within a variety of protozoa, particularly amoebae, and can grow in association with other bacteria in biofilms. Many protozoa are intrinsically more resistant to biocides (such as chlorine) than *Legionella*, so that growth within the protozoan provides protection. Numbers of legionellae in drinking water are typically too low to cause infection or be readily detected, and conditions for significant growth of *Legionella* are unlikely in well-maintained drinking water distribution systems in the UK. This is because temperatures usually remain below 20 °C and the maintenance of disinfectant residual concentrations limits the growth and survival of legionellae in water. In normal situations, the growth of *Legionella* can be controlled by the application of good

design and maintenance combined with some relatively simple precautions. Consistently keeping water below 20 °C in cold water systems, or close to 60 °C in hot water systems, will normally minimise the growth of *Legionella*. Rigorous attention to cleanliness and adherence to a good biocide or disinfectant regime enables control in cooling systems and spa pools. Guidance on the control of *Legionella* in water systems is given elsewhere[30]. Further information on *Legionella* and details of methods for their detection are described elsewhere[31] in this series.

4.1.4 Mycobacteria

Mycobacteria are a group of bacteria that are characterised by their slow growth. Apart from the obligate pathogen *Mycobacterium tuberculosis* (which causes tuberculosis), the genus *Mycobacterium* includes a number of other species which cause disease in humans. The organisms primarily occur in water and soil. The species of most concern are *Mycobacterium avium* and its close relatives *Mycobacterium intracellulare* and *Mycobacterium scrofulaceum*. These are commonly grouped together and referred to as the *Mycobacterium avium* complex. The source of *Mycobacterium avium* complex appears to be the environment and infection is thought to occur by inhalation or ingestion. There is growing evidence to indicate that untreated and treated water can be a source of infection. Other species that have been associated with outbreaks of disease in which water systems may have been the source include *Mycobacterium kansasii* (lung infections), *Mycobacterium genavense* (disseminated disease), *Mycobacterium xenopi* (lung infections), *Mycobacterium abscessus* (wound infections), and *Mycobacterium fortuitum* (various infections including skin, wound and lung). *Mycobacterium marinum* causes skin infections (swimming pool granuloma) associated with swimming pools. Bottled water has also been shown to contain mycobacteria and could, therefore, be a potential source of infection for immuno-compromised patients. Spa and swimming pools may also become colonised and have been reported to be sources of infections.

Mycobacteria are ubiquitous in the environment being found in soil, house dust, water (including wastewater, surface water, groundwater and drinking water), animals and poultry. *Mycobacterium avium* will grow in water to which no additional nutrients have been added. Water treatment processes, particularly coagulation and sand filtration, appear to reduce numbers of mycobacteria but removal is likely to be incomplete. Re-growth or extended survival and accumulation may occur within distribution systems, either within the mains water distribution network or in water systems of buildings. In common with other aquatic bacteria, mycobacteria grow in biofilms on surfaces within water distribution systems. Mycobacteria are relatively resistant to chlorine and many species can survive free chlorine levels of 1 mg/l. Considering their widespread occurrence in source waters and their survival characteristics, it is not surprising that these saprophytic mycobacteria can colonise domestic hot and cold water systems.

4.2 Micro-organisms affecting taste, odour and appearance

Ideally, drinking water should be clear and acceptable to the palate. In practice, however, the aesthetic properties of a drinking water will depend to a large extent on its source and any subsequent treatment or microbial activity. In most instances, when there is adverse comment regarding the appearance, taste or odour of a drinking water, the causes tend to be physical or chemical in nature rather than microbiological.

Nevertheless, musty, mouldy or earthy tastes and odours may result from the growth of fungi or actinomycetes in water pipes. These tastes and odours are primarily associated with the production of secondary metabolites (notably geosmin and 2-methylisoborneol) or the biomethylation of chlorinated phenols (for example, trichloroanisole from trichlorophenol). Other compounds, which are produced via microbial decomposition, can impart fishy, swampy or septic odours to waters, whilst rotten-egg odours can be generated via the reduction of sulphate and sulphite to hydrogen sulphide by some bacteria (for example, *Desulfovibrio desulfuricans* and some species of *Clostridium*).

Micro-organisms growing in biofilms in pipes can result in the corrosion of iron pipes. A consequence of this is the discoloration of drinking water due to elevated levels of iron in the water, or to the accumulation of (brown) iron or (black) manganese deposits, or iron-stained material being dislodged from pipes or sediments.

Actinomycetes and certain algae can also cause taste problems by growth in the raw water, particularly storage reservoirs. This situation can often be controlled by the inclusion of appropriate use of granular or powdered activated carbon in treatment processes.

Further information on microbially-mediated taste, odour and appearance problems and their investigation is given elsewhere[32] in this series.

4.3 Cyanobacteria and animalcules

4.3.1 Cyanobacteria (blue-green algae)

Cyanobacteria occur naturally in many in-land, standing bodies of water and can often be seen forming a surface scum or bloom. These bacteria thrive in warm, shallow and nutrient-rich lowland waters and examples include *Anabaena*, *Aphanizomenon*, *Microcystis* and *Oscillatoria*. Some species produce toxins that can be found in mucus material which is secreted by cells. There is no evidence, however, that these toxins pose a risk to public health via treated water supplies as they are destroyed by treatment. One of the main problem with Cyanobacteria is that raw water blooms can affect treatment efficiency by blocking filtration systems, and can cause adverse tastes and odours in the treated water, although these may be removed with appropriate treatment.

4.3.2 Animalcules in distribution water

Some distribution systems, especially those carrying treated organic-rich lowland water, can become infested with small aquatic animals. The most commonly occurring of these animals are *Nais* and nematode worms, the “water louse” *Asellus*, the “freshwater shrimp” *Gammarus*, and, occasionally, the larvae of midges and flies. The detection of these animals, or their faeces (frasse), at the tap may cause concern, but the health significance of these animals is likely to be low. It is possible that both the animals and their faeces could harbour pathogenic protozoa, bacteria and viruses. Occurrence should, therefore, be minimised by appropriate water treatment, and flushing and swabbing of distribution systems. Some animals are able to reproduce within distribution systems and, if a population becomes established, controlled permethrin treatment may be required for eradication.

5 Outbreaks of water-borne disease and their prevention

5.1 Introduction

The maintenance of microbiologically wholesome drinking water supplies requires the commitment of individuals from many different disciplines including: professionals in water companies, consultants in communicable disease control, medical officers of environmental health, environmental health officers, public analysts and hospital and public health laboratory service microbiologists.

Good communication and liaison between all individuals concerned with the provision of drinking water and public health is essential to enable appropriate action to be taken whenever water quality problems occur. Communications should be maintained by regular contact between all parties. Each organisation should have procedures in place to ensure that contact details (for example, name, job title, telephone, facsimile, e-mail etc) essential for communication with other organisations are readily available to all relevant staff and are kept up to date. This is particularly important where "duty standby" schedules are used when contact, especially during out of normal working hours, is to be made.

If drinking water supplies become contaminated with microbial pathogens, or there is a risk of microbiological contamination, immediate action should be taken to protect public health. The water company and health authority and local authority will need to consider the issue of advice and guidance (for example, a notification to boil water). This action should be taken if contaminated water gains access to the distribution system or cannot be prevented from entering the distribution system. Other measures include informing consumers not to drink the water and to provide alternative supplies (for example, water in bottles or bowsers).

Any notification to boil water should be issued in such a way that as many people affected as possible receive the information as soon as possible. The procedures chosen to achieve this will depend on particular circumstances but could include individual visits, leaflet drops, the use of loudspeaker vans and media announcements. Arrangements for issuing the advice or providing alternative supplies should be part of all water companies' emergency arrangements and should be reviewed and rehearsed regularly. Careful consideration is needed to ascertain whether issuing advice will prevent illness in the community. Aspects to consider include the nature and time of the contamination, and which organisms are known or suspected of being present[33].

5.2 Outbreaks of water-borne disease

During the last century there have been numerous water-borne or water associated outbreaks of disease in the UK. Recognition of the causative agent, however, is dependent upon the level of knowledge pertaining at the time as to which pathogens might be water-borne and the available capability for their isolation. The decline of recent outbreaks has been attributed to improvements in water treatment and the widespread use of chlorine as a water disinfectant[34]. Since 1980, however, other pathogens capable of being transmitted via water have been recognised and detected. The most important of these are the protozoan pathogens *Cryptosporidium* and *Giardia*, and the bacterium *Campylobacter*.

Improvements in epidemiological investigations and the development of a framework for assessing the strength of association between human illness and water exposure[35] has

allowed a more confident ascription of water-borne outbreaks. The numbers of outbreaks in the UK associated with water for the ten-year period 1991 to 2000 are given in Table 1. Outbreaks prior to this have been reviewed elsewhere[34, 36].

Table 1 Outbreaks of illness associated with public and private drinking water supplies and swimming pools in the UK 1991 – 2000.

Pathogen	Public Supplies	Private Supplies	Swimming Pools
Cryptosporidium	24	4	24
Giardia		1	
Campylobacter	4	16	
E. coli O157	2	4	
Salmonella		1	
Unknown		2	

5.3 Prevention of water-borne outbreaks

Water-borne outbreaks occur following the consumption of drinking water contaminated with pathogenic micro-organisms. This contamination may originate from the source water due to inadequate or ineffective water treatment or it can occur post-treatment within the distribution system.

The main risk areas for contamination of public mains water supplies are:

a) abnormal contamination of the raw water source;
b) water treatment breakdown;
c) water treatment operating above design capacity or under stress;
d) non-availability of electricity, treatment chemicals or essential materials;
e) water mains bursts and repairs;
f) mains renovation and renewal;
g) structural faults in service reservoirs; and
h) vandalism.

In addition, it is recognised that bacteria (except some spores) and viruses are susceptible to the disinfection regimes normally employed in the treatment of public water supplies, and their potential occurrence can be monitored using indicator organisms. However, encysted forms of protozoa such as *Cryptosporidium* and *Giardia* are likely to survive and physical barriers such as effective flocculation and sedimentation, sand filters or membrane filters are required for their control.

It is not the purpose of this document to provide detailed advice on water treatment or on identifying and managing risks. Each water company should have detailed procedures for providing and operating adequate water treatment and distribution systems[37], and contingency plans for resolving problems as soon as they are identified or predicted. Contingency plans should be practical and rehearsed regularly. Particular importance should be placed on the liaison with organisations such as local authorities, health authorities and the emergency services. Lines of communication should be reviewed regularly to ensure that contact details are up to date. When contamination of water supplies is known or suspected,

protection of public health is paramount and contingency plans should include mechanisms for early warning of consumers and, if necessary, the provision of alternative water supplies.

In general, the same comments apply to private water supplies. Local authorities have responsibility for monitoring private water supplies in the UK and advice on treatment and risk assessment is available elsewhere[38].

5.4 Water sources and water treatment

5.4.1 Water sources and their protection

No source of water that is intended for human consumption can be assumed to be free from pollution. All sources have different microbiological qualities and may be subject to natural or manufactured sources of pollution that may result in the deterioration of water quality to the point where treatment is no longer effective in removing all of the contamination. Much can be done to limit pollution by regular inspection and monitoring of water sources, by encouraging the use of good agricultural practice and by controlling effluent discharge. More detailed guidance is given elsewhere[39 - 42].

The microbiological quality of upland reservoirs, which depend for their supply on catchment areas and feeder streams, is usually very good. These catchment areas should be inspected regularly and agricultural and recreational activities associated with reservoirs should be controlled to minimise excessive contamination by spillage, drainage or access of animals and people, particularly with regard to sources of *Cryptosporidium*.

The microbiological quality of rivers, lakes and lowland reservoirs may be much poorer than that of upland reservoir waters. The quality may deteriorate suddenly due to spillage of effluents, or heavy rain causing direct run-off from land or the operation of storm overflows. Where such incidents occur, treatment processes may become stressed and require careful control. Water companies should be aware of potential sources of pollution into rivers, and any pollution incident likely to affect water treatment processes should be notified to them as quickly as possible. The provision of bank-side storage can reduce the impact of pollution events.

Groundwater forms an important part of water resources in the UK and provides up to 35 % of the potable water supplies. It is usually of much better quality than surface water and often only requires chlorination before being made available within distribution systems. Aquifer contamination may be caused directly by the seepage of materials or by the movement of groundwater of poor quality into areas of better water quality. Because aquifer pollution can exist for very long periods of time and may be impossible to remedy, it may be preferable to take preventative measures to minimise pollution rather than provide remedial action or develop alternative sources of water.

Spring water is typically collected and stored in secure underground chambers and covers should be raised clear of the ground and surrounding vegetation. Wells and boreholes should be lined to a depth sufficient to prevent the entry of any surface pollution or polluted sub-soil water. There should be an effective seal between the lining and the ground. The head of a well or borehole should be protected and the installation should be checked at regular intervals to ensure that ingress does not occur. Records for the percolation area showing

sewers, septic tanks, cesspools, waste disposal sites, soak-aways or other sources of potential pollution should be maintained. Water undertakers should be consulted if any new sites or structures are proposed. There may be areas where the establishment of a protection zone might be considered appropriate to prevent contamination of the aquifer. Consideration should be given to the geological nature of the aquifer and the presence of overlying drift that may provide additional protection. The accumulation and spreading of treated bio-solids and agricultural wastes might be prohibited or restricted in protection zones.

5.4.2 Sources of contamination

The quality of many source waters will depend upon geology, soil type, natural vegetation, climate and run-off characteristics. Disruption of natural geology and heavy rainfall can dramatically affect water quality. Wild animals and birds can also be natural sources of zoonotic pathogens.

All types of water sources may be subjected to contamination by agricultural activity. Free-range animals may excrete faeces into water, and animals like cattle have a habit of wading into water and stirring up sediments. Rainfall can result in the run-off of faecal matter from agricultural and other rural lands into rivers, lakes, reservoirs and springs. Much can be done to reduce the risk of water contamination from slurry spillage, or the use of slurry on land followed by surface run-off, by the adoption of appropriate agricultural practices and aquifer protection policies.

Recreational activity may cause pollution through direct contamination of water with faecal material or indirectly by faulty drainage or leakage from sewers and septic tanks provided as part of public access facilities. Proper control of recreational activities or treatment commensurate with the recreational use of water should give adequate protection. Where the public has access to reservoirs, consideration should be given to the provision of toilets and hand-washing facilities.

The discharge of effluents from sewage treatment works, septic tanks and cesspools can dramatically increase the microbial content of surface waters. The installation of septic tanks and cesspools should be in accordance with national standards[43]. The discharge of industrial effluents, particularly from abattoirs and cattle markets, may also contain large numbers of pathogenic micro-organisms which increase the risk of contamination. Slurries and solid waste from sewage treatment and animal waste should be spread on land only with strict control in accordance with the Code of Practice for the Agricultural Use of Sewage Sludge[44] and The Safe Sludge Matrix[45] taking into account any protection or buffer zones.

5.4.3 Monitoring of water sources

Regular monitoring of source waters will provide information about general water quality and establish seasonal variations in water quality and changes in response to weather and other factors. Under circumstances where the microbiological quality of the water deteriorates or where there is suspicion of microbial contamination, investigations should be undertaken and any appropriate remedial action taken. Consideration should be given to the introduction of more detailed microbiological monitoring, including additional tests for bacteria, viruses and intestinal parasites. New water sources for which the microbiological quality is unknown

should be subjected to appropriate microbiological monitoring in order to establish the water quality so that the adequacy of proposed treatment regimes can be assessed.

5.4.4 Water treatment and supply

The objective of water treatment is to produce wholesome water that meets the statutory requirements and is microbiologically and chemically safe for consumption, is not corrosive towards materials in contact with water and is aesthetically acceptable. The range of treatment processes includes clarification and sedimentation, filtration and disinfection. Depending on the source and nature of the water, one or more of these processes can be used. Whilst each of the treatment processes is able to reduce the numbers of particular micro-organisms, no process can ever ensure their complete removal. In the UK, therefore, disinfection (usually by chlorination) is the final safeguard against water-borne microbial contamination.

When chlorine is used, the dose should be selected so that the chemical demand of the water is satisfied and that an adequate contact time is achieved before water is supplied to consumers[11]. An appropriate chlorine residual should be maintained throughout the distribution system and should preferably be present in water at consumers' taps if there is a perceived risk of microbial growth. This may provide an indication of the absence of post-treatment contamination[11]. It is essential, therefore, that chlorine residuals are monitored regularly both at water treatment works and in distribution[3].

Micro-organisms differ in their susceptibility to chlorine (in decreasing order of resistance: protozoan cysts, bacterial spores, enteric viruses and enteric bacteria). However, the combination of chlorine concentration and contact time necessary for inactivation of enteric viruses and pathogenic bacteria can be achieved by a well-managed water treatment works. Nonetheless, certain incidents of water-borne disease have occurred as a result of inadequate chlorination, or because no such facility was installed or used[35, 36].

Chlorination of drinking water can impart chlorinous tastes and odours resulting in complaints from some consumers. Additionally, concerns have been raised regarding the formation of disinfection by-products (most notably, the trihalomethanes). It is, therefore, prudent to manage chlorination and residual chlorine levels but without prejudicing the microbiological quality. It continues to be the case that the microbiological safety of potable water supplies is of paramount importance [11] and the benefits of chlorination have been re-affirmed.

While the proper design, operation and maintenance of treatment works is of the utmost importance, microbiological monitoring at an appropriate frequency is necessary to allow adequate assessment of the hygienic quality and safety of drinking water. The information derived from microbiological tests should, however, be assessed in the light of thorough practical and working knowledge of the conditions applying at the source, throughout all the stages of treatment to which the water is subjected, and in the distribution system. Failure or inadequacy of treatment processes, particularly chlorination, may have serious consequences, but other hazards also cause deterioration in microbiological quality. These include contamination via air-valves and stop valves, infiltration into mains and service reservoirs, cross-connections, back-flow and venturi effects. Sudden changes in the microbiological quality of ground waters can occur through cesspool leakage, from accidental or illicit contamination of the gathering grounds or by polluting material gaining access through faults

or fissures in the strata or through defects in the well or borehole lining. Heavy rain following prolonged drought may enhance the risk of pollution of water sources and service reservoirs if the structures are unsound. Increased pumping from wells, perhaps as a result of prolonged drought, may also lead to the pollution of previously satisfactory sources. Whenever these or other environmental conditions occur, the frequency of microbiological examination should be increased; the location of sampling being carefully chosen so that any changes in quality may be identified quickly and appropriate action taken.

5.4.5 Biofilms in water supply

In low-nutrient aqueous environments, micro-organisms preferentially colonise surfaces rather than grow in the planktonic phase. Nutritional levels are higher at surfaces and bacteria are protected from adverse environmental influences. The organisms colonising surfaces become embedded in a matrix of extra-cellular polymeric substances that are produced by organisms. This layer of growth is termed a biofilm and in water distribution systems is usually quite thin, not exceeding a few hundred micrometres. In both natural environments and water distribution systems, biofilms are usually composed of complex mixtures of micro-organisms including bacteria, fungi and protozoa. The metabolic by-products of one organism can provide nutrients for other organisms. This enables organisms that would otherwise be unable to grow by themselves, such as *Legionella pneumophila*, to proliferate. Biofilm distribution can be patchy and can vary considerably, even over distances of a few millimetres or less. Biofilms can also accumulate organic and inorganic debris from external sources by the adsorption of silt, sediments, inorganic precipitates and corrosion products. These materials may provide additional nutrients for microbial growth.

No material that comes in contact with water is immune to colonisation, but some materials may support or promote more growth than others. To maintain water quality during distribution, construction materials should not promote growth. Non-metallic materials should comply with BS 6920[46], which includes a test for growth promotion.

Most of the growth that takes place in distribution systems probably occurs in biofilms and the majority of the planktonic organisms may be derived from organisms leaving the biofilm or by the biofilm breaking up. Organisms that have survived disinfection, including environmental strains of coliform bacteria, can become attached to biofilms where they may subsequently grow. Biofilms are important because they contribute to many causes of the problems that can occur in water distribution systems. They may promote or cause corrosion of pipes, can be responsible for off-flavours, contribute to discoloured water, harbour pathogens, increase the chlorine demand and provide a site for the re-growth of some strains of coliform bacteria. Biofilms also protect organisms from disinfection. The contact time, by chlorine, required to produce a particular degree of disinfection of the organisms in a biofilm may be hundreds, or even thousands, of times greater than that required to achieve an equivalent degree of disinfection or death for the same organisms suspended in water[47]. Thus, it is possible for biofilms to continue to survive and grow even when the water contains residual chlorine at the concentrations normally used in drinking water. This reduction in disinfection efficiency is caused by the diffusion of the disinfectant being reduced by the biofilm and alterations in the physiology of the organisms growing in the biofilm, and is less for monochloramine than chlorine.

Enumerating the number of bacteria in a body of water in a pipe (planktonic phase) provides a poor estimate of the total microbial activity in a water system. This is because many of the organisms in the planktonic phase do not grow on conventional culture media. Unfortunately, it is difficult to measure the degree of biofilm formation routinely because of problems of collecting representative samples of biofilm. Other physical and chemical determinations such as total and assimilable organic carbon, dissolved oxygen and temperature could be made in areas where biofilms are considered to be a problem. The fact that micro-organisms are not detected in samples of water does not mean that biofilms are absent.

6 Water in food production and other special considerations

Water supplied by a water company is the legal responsibility of that company which is required to supply wholesome water to the point at which it is supplied to the consumer. Once it has left the company's water distribution system, its quality (with some exceptions in respect of the lead and copper parameters) becomes the responsibility of the owner or occupier of the premises supplied. The extent of these responsibilities is described in section 73 of the Water Industry Act[2].

Incorrectly installed water fittings and systems, back-flow from or cross contamination with other water sources and poorly designed or maintained water storage and distribution systems can lead to contamination of water supplies. The Water Supply (Water Fittings) Regulations[48] prescribe the requirements to prevent contamination, waste and misuse of the mains water supply. The water supply industry operates an evaluation and testing scheme for water fittings, which includes tests for their potential to promote microbiological growth. It is advisable to ensure that fittings are Water Regulations Advisory Scheme approved[49].

Wholesome water may be required in circumstances where it is prevented from being supplied directly from a mains water supply. Special care will be required to ensure that the water is wholesome, and remains so throughout the duration of the supply. If there is any doubt about maintaining the wholesomeness of a supply, water outlets should be clearly labelled to show that the water is not to be used for drinking. Boiling such water before use and providing bottled water may be a more viable alternative than trying to maintain a wholesome supply. However, even under such circumstances, care should be taken to ensure that the storage facility is clean, well maintained and disinfected regularly.

6.1 Water in food production

Various Regulations[3, 5] require a water company to supply wholesome water up to the point where it is no longer the responsibility of the water company. Wholesomeness is defined by prescribed concentrations or values specified in the regulations. Wholesome water is not sterile water, and as such, may not be suitable for use, without further treatment, in all manufacturing processes. This matter is for the manufacturing processor to ascertain.

Plumbing systems in large buildings can become very complicated. Incorrectly installed water fittings and systems, poorly maintained storage facilities, localised heating, back-flow, cross contamination and permeation where supply pipes of unsuitable material are laid are all potential sources of possible contamination.

Water supplies within food production premises should be subject to risk and hazard assessment to ensure that appropriate water quality is maintained throughout the production process. Detailed advice is available elsewhere[50, 51].

6.2 Hospitals and other institutions

Some buildings may be supplied by a water company or have their own private water supplies or use a blend of supplies. Most buildings may have one or more storage facilities to balance fluctuating demand and enable a supply to be maintained for limited periods during emergencies. Many hospitals and other large complexes consist of a variety of buildings of different ages with pipe-work constructed of different materials. There are often long and complicated pipe runs, sometimes with "deadlegs". Poorly constructed systems may contain inappropriate materials or allow temperature rises in cold water supplies.

Water samples for microbiological examination should be taken from a rising mains tap (for example, a kitchen tap). Depending on knowledge of the distribution system and water use within the building, consideration should be given to the following additional sampling points:

(a) Inlet of storage tanks where water is used for drinking, food preparation or culinary purposes;

(b) Outlet of storage tanks where water is used for drinking, food preparation or culinary purposes. If a storage tank is large, samples should be taken from possible stagnant areas, and this may involve "dip" sampling[52];

(c) Other representative points from the building's system relative to the pipe-work distribution network (for example, beginning, middle and end of distribution branches). The plumbing arrangements should be checked to ascertain the location of dead end mains;

(d) The supplies to kitchens, ice making machines, highly specialised washers or water purification machines.

Bacteriological analysis depends on local circumstances but as a minimum should consist of the analysis for coliform bacteria, *E. coli* and enterococci. Colony counts may give useful information on water turnover and identify potential problem areas. If *E. coli* or enterococci are detected, investigations into the source of contamination should be instigated without delay and further samples taken for examination. Analysis for *Pseudomonas aeruginosa* or *Aeromonas* may be useful when conducting investigations.

The routine sampling of drinking water systems for *Legionella* is not normally required, provided the water temperature does not exceed 25 °C, and ideally, remains below 20 °C. Sampling programmes, when applied, should follow recommended advice[30]. If required, samples might normally be collected from tanks, the furthest outlet(s) from the tank and any taps in areas of particular concern. Samples from taps may be collected without disinfecting the tap if the intention is to look for colonisation of the tap, or after disinfection, if the intention is to investigate colonisation of the system. For routine monitoring, when the intention is directed to confirming control to the point of use, it might be appropriate to

collect samples without disinfecting the taps. Hot water systems are more likely to require sampling particularly if methods of control, other than the recognised temperature regime are followed. Samples may be collected from various locations including the calorifier outlet or the nearest tap to it; the return supply to the calorifier or the nearest tap to it; and the base of the calorifier where drain valves are fitted. In addition, other locations include the furthest outlet from the calorifier; and outlets in areas of particular concern. In complex systems, samples should be collected so that they are representative of the area sampled. In order to be representative of the hot water system as a whole, samples should be of treated, circulating water and not be taken from temporarily stored water (for example, through thermostatic mixer controlled taps and showers). These may also require sampling depending upon the results of the risk assessment.

6.3 Tankers and bowsers

Tankers and bowsers used to supply wholesome drinking water should preferably be reserved solely for that purpose. If others are used, they should be appropriately cleaned prior to use. They should be constructed of material that does not support microbiological growth, be capable of withstanding 50 mg/l free chlorine, be designed so that they can be completely drained and emptied and be easily accessible for internal examination and disinfection. All hatches should be close fitting and capable of being locked. Tankers and bowsers should be drained when not in use. All water storage facilities should be cleaned and disinfected by chlorination before being filled with wholesome water.

Water is particularly vulnerable to contamination during filling operations. Standpipes and hoses should be protected from contamination and flushed before filling the tanker or bowser. Hose nozzles should be kept immersed in a suitable disinfectant solution prior to use.

6.4 Drinking water tanks in buildings and on ships, trains, planes and coaches

All tanks that are used to store and supply drinking water should be reserved solely for that purpose. They should be constructed of material that does not support microbiological growth, be capable of withstanding 50 mg/l free chlorine, be designed so that they can be completely drained and emptied and be easily accessible for internal examination and disinfection.

All water storage facilities should be cleaned and disinfected by chlorination before being filled with wholesome water. They should be regularly drained, cleaned and disinfected at appropriate intervals.

Water is particularly vulnerable to contamination during filling operations. Standpipes and hoses should be protected from contamination and flushed before filling the tank. Hose nozzles should be kept immersed in a suitable disinfectant solution prior to use. Cross-connections with non-potable water systems and back flow should be avoided.

Ideally, samples should be taken from taps rather than by using “dip” sampling techniques as this should minimise the chances of contaminating the samples.

6.5 Drinks vending machines

Drinks vending machines may be hand-filled or permanently connected to a water supply system. The quality of water supplied to a drinks vending machine is of paramount importance to the quality of the final product dispensed. Although there is the potential for drinks vending machines to increase the microbiological loading of the final product, due to the presence of drink powders (including those that are milk-based) and warm conditions, it is uncommon to find such contamination. Modern machines effectively control these hazards by a combination of design and cleaning requirements. Machines should be cleaned on a regular basis with particular attention being paid to the dispensing point. Parts of the vending machine that come in contact with powdered ingredients should be regularly cleaned either on- or off-site, if necessary by replacement with clean parts. Hot drinks should be supplied from a reservoir within the machine that maintains a suitable temperature.

Sampling and analysis for indicator organisms and colony counts for drinks vending machines should include water entering the machine and the vended water at the tap station. If the machine is hand-filled, samples should also be taken from the storage tank. Further details and advice are given elsewhere[53].

6.6 Domestic filters, point-of-entry and point-of-use devices

There are many commercial devices available that may improve the microbiological or chemical quality of water. These include granular activated carbon filters, ion exchange filters, and reverse osmosis units. These devices may be fitted to the water supply as it enters the premises (point-of-entry-device) or at a single tap (point-of-use-device).

Manufacturer's instructions should always be followed when installing and maintaining such devices. A non-return valve is usually necessary to prevent contamination of the incoming water supply. Filter elements should be changed or regenerated at recommended intervals, otherwise growth of contaminating micro-organisms may occur leading to a deterioration of water quality.

6.7 Ice making machines

Ice machines are normally coupled directly to the incoming water supply. As with vending machines, there is the potential for the final product to become contaminated with micro-organisms if the machine and the dispensing area are not kept clean, or if normal hygiene procedures are not followed. Sampling and analysis for indicator organisms and colony counts should include the water entering the machine and in any storage tank, in addition to ice at the dispensing point.

6.8 Bottled water

In the UK, the quality of bottled water is regulated[6] by the Food Standards Agency. In addition to the statutory sampling and analysis requirement, consideration should be given to sampling for indicator organisms and colony counts at the source, at various stages during the bottling process and at the final product stage. If contamination is detected, production might need to be halted until the wholesomeness of the final product is assured.

7 Private water supplies

7.1 Definition

Private water supplies are defined[2] as any supply of water provided otherwise than by a statutorily appointed water undertaker. For the purposes of the legislation, a private supply is always defined by the source of the water. It does not include public mains water after it enters a private service reservoir or a private distribution system (for example, in a large building). Some private water supplies are not covered by drinking water quality legislation because they are used for industrial, irrigation or animal purposes only.

Although in the UK there are some private supplies in urban areas, particularly those used for industrial purposes, most private supplies are situated in more remote, rural parts of the country. The source of the supply may be a well, borehole, spring, river, stream, lake or pond. The supply may serve only one property, or several properties through a network of pipes.

In England, Scotland and Wales there are about 70,000 private water sources supplying about 450,000 people with water for domestic purposes. Approximately 40,000 of these supplies serve people in a single dwelling. However, more people consume private supplies of water, such as that used for food production purposes and for supplying other premises such as hospitals, hotels or campsites.

7.2 Legislation and guidance

In the UK, legislation continues the long standing duty of local authorities to keep themselves informed of the sufficiency and wholesomeness of both public and private water supplies in their areas. Current Regulations[4] require local authorities to take samples and cause them to be analysed, in order to protect public health. The regulations cover only those supplies used for domestic purposes for drinking, cooking, washing, food preparation and the commercial use of food production.

The water quality standards for private supplies[4] are, essentially, the same as those for public supplies[3]. Sampling requirements for private supplies depend upon whether the supplies are classed as large or small supplies. Local authorities have powers under the Act[2] to require improvements to be made to unwholesome supplies. Legislation is designed to enable local authorities to tailor their actions to the particular circumstances of individual supplies. Detailed guidance on risk assessment and treatment for local authorities and owners and users of private water supplies is available elsewhere[38].

7.3 Public health considerations

It is important that local authorities, owners and users of private water supplies are aware of the risks from untreated water and potential contamination and that considerable emphasis is placed on assessing these risks. Action should be taken to prevent water from becoming contaminated rather than relying on treatment.

Although many private water sources provide a safe supply of water there are risks of contamination that, generally, do not apply to public water supplies. These include:

- farm animals may have unrestricted access to the source catchment, wellhead or spring collecting chamber;

- sources have inadequate protection from contamination from surface runoff and agricultural activity;
- possible proximity of private sewage systems; and
- inadequate or poorly maintained treatment facilities.

Protection can be provided by fencing, to keep farm animals away, and having suitable drainage channels to divert surface run-off and rain. Boreholes and wells should be covered and sealed and collecting-chambers maintained in good condition and protected from animal access and certain agricultural activities.

If water is known or suspected of being microbiologically contaminated it should be boiled before use or alternative supplies provided.

8 Microbiological monitoring

8.1 Actions in the event of a microbiological standard being infringed

Further investigation should be instigated the same day when presumptive evidence of either coliform bacteria, *E. coli*, enterococci, *Clostridium perfringens* or any pathogen is detected in any water sample taken from water leaving treatment works, service reservoirs, water towers or consumers' taps. Investigations following confirmed isolation of *Clostridium perfringens* need not be conducted if is shown that its occurrence is not associated with health risks or operational changes. As a minimum, further investigations should include:

(a) Examination of further samples from the same location as the original sample and from related points. (This examination should include the analysis of the same type of organisms as were examined in the original sample, and may include examination for a wider range of organisms such as enterococci and *Clostridium perfringens* if these organisms were not included in the original analysis).

(b) Appropriate confirmatory tests on the presumptive colonies detected in the original sample.

(c) Where appropriate, immediate checks on:

(i) the operation of the treatment works (for example, the proper functioning of chemical dosing, filtration or disinfection systems);

(ii) contamination of the distribution system through (for example, burst or leaking mains, ingress into a service reservoir, back-siphonage, loss of pressure or cross connections);

(iii) raw water sources to ascertain possible contamination;

(iv) the sampling tap; and

(v) sampling and laboratory procedures.

No further action need be necessary if the investigations at (b) do not confirm the presence of coliform bacteria, *E. coli* or other organisms presumptively detected. The results of additional operational samples and checks, however, should be recorded. If presumptive organisms are detected repeatedly in a distribution system or at a particular site, further investigations should be carried out to determine the identity of the organisms and their source. If these investigations show evidence of actual or potential microbiological contamination, then effective remedial action should be initiated immediately to ensure that satisfactory microbiological conditions are restored. This is particularly important if large numbers of indicator bacteria are detected. This action will depend on local circumstances and the perceived potential effect on the health of consumers but it could include, where appropriate:

(a) consideration of whether to advise consumers to boil water to be used for drinking and food production purposes, or to advise not to use the water, or to provide alternative water supplies;

(b) increasing the disinfection dose at the treatment works or in the distribution system;

(c) correcting the operation of treatment works, including chemical dosing, filtration and disinfection;

(d) repairing, cleaning, flushing or disinfecting mains and service reservoirs;

(e) identifying, and then correcting, any source of water contamination; and

(f) changing or protecting raw water sources.

In some cases, the remedial action may be of a short-term nature pending the completion of longer-term measures.

8.2 Responses to significant microbiological water quality failure

If there is an immediate health risk, immediate emergency action should be taken.

If any evidence is detected of actual or potential serious microbiological contamination, such as high counts of *E. coli*, the presence of specific pathogens or significant failure of the disinfection or filtration processes, then urgent action should be taken. There should be a presumption that water in supply is a potential health hazard, in which case water companies should immediately obtain expert advice. In such cases, water companies should:

(a) Take urgent action to protect consumers. This action may include:

(i) continuing to supply but advising consumers not to use water for drinking and cooking, or to boil water for such purposes;

(ii) switching to temporary alternative supplies or providing suitable alternative supplies to vulnerable groups of consumers such as babies etc;

(iii) shutting off the supply and providing suitable alternative supplies;

(iv) issuing advice to all water users; and

(v) providing information to the press and local radio.

(b) Take all reasonable action to rectify the situation and restore water supplies back to normal as soon as possible.

(c) Notify, without delay, relevant officers of the local authorities and health authorities in accordance with agreed procedures[3] and consult with regard to the appropriate action being taken and to be taken. At all times, close liaison between parties concerned should be maintained.

(d) Carry out increased operational monitoring according to the nature and seriousness of the situation and keep records of all such monitoring and the actions taken.

(e) Notify appropriate water quality regulators as soon as possible in accordance with agreed procedures.

It is important that a water company has documented contingency arrangements to deal with emergencies. These procedures should be kept up to date. All appropriate staff should be familiar with the procedures and they should be rehearsed from time to time.

8.3 Response to water quality complaints

A water company should ensure that its response to complaints from consumers about drinking water quality is appropriate to the type of complaint. Complaints about illness may be the first indication of a serious problem arising and these complaints should, therefore, always be investigated promptly, and water samples taken and analysed.

Microbiological analysis in respect of other types of water quality complaint is usually of limited value. However, the detection of high colony counts, coliform bacteria, *Pseudomonas*, actinomycetes and/or fungi or evidence of the presence of biofilms may give indications to the origin of these causes of complaint[32].

8.4 Sampling for operational evaluation

The microbiological quality of water leaving water treatment works, in service reservoirs and in distribution should be reviewed regularly. Such reviews may detect seasonal or weather-related trends, or the effects of changes in treatment processes, or the effects brought about by re-zoning or blending of water. It is possible that the monitoring required for regulatory purposes is insufficient to provide data for trend reviews. Where this is the case, water companies should consider carrying out additional operational sampling surveys.

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