

Microbiological Aspects

Session Objectives

- To highlight the number and range of pathogens that may be found in water.
- To describe some of the key preventative and monitoring actions which maintain and improve microbiological water quality.
- To introduce the concept and use of indicator bacteria in water quality monitoring.
- To describe the principal indicator bacteria used and their key characteristics which make them suitable for use as indicators.
- To emphasise the value of *E.coli* and thermotolerant faecal coliforms as routine indicators.

Microbiological Aspects

Summary

The wide variety of waterborne diseases is the most important concern about water quality, and their public health impact has far-reaching implications. The pathogens concerned include many types of viruses, bacteria, protozoa and helminths, which differ widely in size, structure and composition. This implies that their survival in the environment and resistance to water treatment processes differs significantly. However, the waterborne transmission of infectious diseases can be controlled effectively by practical and economic methods. The approach must be based on protection of the source, selection of appropriate treatment methods, fail-safe application of the treatment methods, well protected distribution networks and appropriate quality monitoring. Relatively simple and inexpensive indicator methods are available for routine monitoring of the microbiological safety of water and the efficiency of treatment processes. Most reliable results are obtained by high frequency testing for indicator organisms selected for particular purposes. For instance, routine monitoring programmes for drinking-water may be based on tests for faecal streptococci, thermotolerant coliform organisms or *Escherichia coli*. Under certain circumstances, tests for the heterotrophic plate count and coliphages may be included. These tests are simple, inexpensive and yield results in a relatively short time. More complicated and expensive tests such as those for human viruses and protozoan parasites are required only for particular purposes, including research and assessment of the efficiency of treatment processes.

1 Introduction

Waterborne diseases are the most important concern about the quality of water. Developing countries and rural communities are particularly vulnerable. In developed countries the mortality due to waterborne diseases is low, but the socio-economic impact is phenomenal (Avendano *et al*, 1993; Payment, 1993).

Waterborne diseases are typically caused by enteric pathogens which belong to the group of organisms basically transmitted by the faecal-oral route. In other words, they are mainly excreted in faeces by infected individuals, and ingested by others in the form of faecally contaminated water or food. Some of the pathogens may be of animal origin. Some may also be transmitted by personal contact, droplet transfer, or inhalation of contaminated aerosols. Water may also play a role in the transmission of pathogens which are not faecally excreted. These include opportunistic pathogens which are members of the normal flora of the external human body. Some of these pathogens are natural inhabitants of certain water environments. Most waterborne pathogens are distributed world-wide, but outbreaks of some, for instance cholera and hepatitis E, tend to be regional. Dracunculiasis is geographically limited to rural areas in India, Pakistan, and sixteen countries in sub-Saharan Africa.

1.1 Enteric pathogens typically transmitted by the faecal-oral route

Bacteria: *Salmonella* spp, *Shigella* spp, pathogenic *Escherichia coli*, *Campylobacter* spp, *Vibrio cholerae* and *Yersinia enterocolitica*.

Viruses: Hepatitis A and E, enteroviruses, adenoviruses, small round structured viruses including Norwalk virus, astro and rota viruses.

Protozoa: *Entamoeba histolytica*, *Giardia intestinalis*, *Cryptosporidium parvum*.

1.2 Helminths

Infections contracted by exposure to, or ingestion of, infectious larval stages of human parasites released by specific snails or cyclops:

Schistosoma spp (schistosomiasis, bilharziasis) and *Dracunculus medinensis* (dracunculiasis guinea worm). The latter is not faecally excreted but typically transmitted by water and of major public health importance in some countries.

1.3 Opportunistic pathogens

Infections of the skin and mucous membranes of the eye, ear, nose and throat:

Pseudomonas aeruginosa, *Aeromonas*, and species of *Mycobacterium*.

Infections contracted by the inhalation of contaminated aerosols:

Legionella spp (legionellosis), *Naegleria fowleri* (primary amoebic meningoencephalitis) and *Acanthamoeba* spp (amoebic meningitis, pulmonary infections).

1.4 Toxins from cyanobacteria

Toxins released by blooms of cyanobacteria (blue-green algae) such as *Microcystis aeruginosa* may adversely affect the health of animals and possibly also humans.

1.5 Nuisance organisms

A variety of non-pathogenic micro-organisms, and small plants and animals, may under undesirable conditions thrive in water supplies and cause turbidity, taste and odour, or visible animal life, which are aesthetically objectionable.

Bacterial contamination of drinking-water has resulted in numerous cases of infectious disease. The massive cholera epidemic in Latin America, which spread from Peru to several other countries, and the recent one in Rwanda, are reminders of the speed with which certain waterborne diseases can spread.

Viruses feature prominently among the wide variety of waterborne pathogens. Examples include the 1991 outbreak with 70,000 cases of hepatitis E caused by polluted drinking-water in Kanpur (Grabow *et al*, 1994a). Reasons for the high incidence of waterborne viral infections include excretion in exceptionally high numbers by infected individuals, relatively high resistance to unfavourable environmental conditions including water treatment and disinfection processes, and a minimal infectious dose which may be as low as a single viable viral particle (Payment, 1993). The impact of viral infections is aggravated by secondary and even tertiary transmission by routes other than the water which caused the original infection (Morens *et al*, 1979). Epidemiological studies on waterborne viral infections are complicated by the absence

of clinical symptoms in many individuals, particularly children, while all infected individuals excrete viruses at similar rates.

Recent years have seen a substantial increase in the number of waterborne *Giardia* and *Cryptosporidium* outbreaks. The cysts and oocysts of these protozoan parasites are extremely resistant to water treatment and disinfection processes, and their minimal infectious dose is low (Casemore, 1991; Craun, 1991).

Despite modern technology and know-how, waterborne diseases continue to have a major public health and socio-economic impact, and at least in parts of the world their incidence may even increase (Craun, 1991). Challenges to control waterborne diseases are complicated by continuous changes in the composition and priority of waterborne pathogens. Factors which affect the occurrence of pathogens include changes in population densities, socio-economic situations, standard of living, education, vaccination, climate, geography, urbanisation, migration and travelling, and public health policies.

The role of microbiological analysis is very important in a strategy for the control of waterborne diseases based on appropriate treatment systems, appropriate operation of the treatment systems, and appropriate quality monitoring.

2 Water Treatment and Disinfection Technology

A wide variety of treatment systems and disinfection processes are available to ensure the safety of water supplies. At the low technology and inexpensive end of the range there are methods such as simple sand filtration of water, the addition of household bleach to a bucket of drinking-water, storage of water, the exposure of water to sunlight, or boiling of drinking-water. At the other end of the range there are multiple-barrier treatment trains capable of the direct reclamation of drinking-water from waste water. All of these systems are capable of producing safe water. Consideration of the quality of available raw water sources is an integral part of the selection of appropriate treatment methods. The challenge is to select the appropriate system for each particular situation. Each situation has to be evaluated in its own merit, based on considerations such as the raw water quality, intended use of the water, financial resources, and technological capabilities.

3 Operation of Water Treatment Systems

The wide variety of treatment systems capable of producing safe water mentioned above, are without exception subject to potential breakdown and human failure in operation, supervision and quality surveillance. There is not even an indication that in the foreseeable future we can hope for a practical fail-safe water treatment system. Successful operation and supervision of treatment systems, improvement of technical capabilities, and training programmes aimed at meeting water quality requirements, are very important. The production of safe water is not possible without fail-safe operation and supervision of treatment systems (Bellamy, 1993).

4 Microbiological Water Quality Monitoring

Transmission of diseases by treated water supplies can be ascribed to inappropriate treatment methods, failure in operation and supervision, or shortcomings in quality monitoring. In fact, it can theoretically be argued that all waterborne diseases can be prevented by appropriate monitoring and corrective measures taken in good time. Since there is no indication that we can expect to see practical fail-safe treatment systems, or an elimination of human failure or error in the operation and supervision of treatment systems, appropriate microbiological quality monitoring will remain an indispensable component of strategies for the control of waterborne diseases.

Regular inspection of sanitary and hygienic aspects of raw water sources, treatment facilities and distribution networks is an important component of quality monitoring programmes, and is particularly important with regard to pathogens such as viruses and protozoan parasites which are not readily detectable in water.

4.1 Indicator organisms

Since it would be practically impossible to test water for each of the wide variety of pathogens that may be present, microbiological water quality monitoring is primarily based on tests for indicator organisms. There is no single indicator organism that can universally be used for all purposes of water quality surveillance. Each of the wide variety of indicators available for this purpose has its own advantages and disadvantages, and the challenge is to select the appropriate indicator, or combination of indicators, for each particular purpose of water quality assessment.

Indicators most commonly used are of faecal or sewage origin, and the following are some of the most important requirements of such indicators:

- a) Present whenever pathogens are present.
- b) Present in the same or higher numbers than pathogens.
- c) Specific for faecal or sewage pollution.
- d) At least as resistant as pathogens to conditions in natural water environments, and water purification and disinfection processes.
- e) Non-pathogenic.
- f) Detectable by simple, rapid and inexpensive methods.

Ideally, various other properties are desirable, such as counts which are directly related to those of pathogens. However, the fundamental and most important requirement is that pathogens should be absent or inactivated whenever indicators are absent or inactivated.

Many indicators have been studied and recommended for water quality assessment (ISO, 1990; Standard Methods 1992). Evaluation of the reliability of indicators is carried out by comparison of their incidence and survival in water and treatment processes with that of selected pathogens, by epidemiological studies on the consumers of water supplies, by calculations based on the minimal infectious dose of pathogens, and by experiments with human volunteers (Regli *et al.*, 1991). The following is a summary of the most important features of commonly used indicators:

4.1.1 *Escherichia coli*

This species is a member of the group of faecal coliform bacteria. *Escherichia coli* has the important feature of being highly specific for the faeces of man and warm-blooded animals. For all practical purposes these bacteria cannot multiply in any natural water environment and they are, therefore, used as specific indicators for faecal pollution. They are generally distinguished from other thermotolerant coliforms by the ability to yield a positive indole test within 24 hours at 44.5°C. More recently, *E. coli* is also identified by possession of the enzyme β -glucuronidase, which hydrolyses the fluorogenic substrate 4-methyl-umbelliferyl- β -D-glucuronide (MUG) with release of the fluorogen which can be observed in liquid media under ultraviolet light. Media based on hydrolysis of MUG are commercially available under names such as "Colilert". Such complex sets of tests for the final confirmation of *E. coli* are not recommended as a routine.

4.1.2 Thermotolerant coliform bacteria

This term refers to certain members of the group of total coliform bacteria which are more closely related to faecal or sewage pollution, and which generally do not readily replicate in water environments. This group of bacteria is also known as faecal coliforms, presumptive *E. coli*, faecal *E. coli*, faecal coli, etc. Thermotolerant coliforms are primarily used for the assessment of faecal pollution in waste water and raw water sources. They are detectable by simple and inexpensive tests, and are widely used in routine water quality monitoring. The test methods used are the multiple tube and membrane filtration using mFC medium and incubation for 24 hours at 44.5°C. In the membrane filtration individual colonies can be identified, and the presence of *Escherichia coli* provides strong evidence of faecal pollution.

4.1.3 Coliform bacteria (total coliforms)

The term "coliform bacteria" refers to a vaguely defined group of Gram-negative bacteria which have a long history in water quality assessment. In outdated literature these bacteria go by all sorts of names, including coliforms, colis, etc. Some of the bacteria included in this group are almost conclusively of faecal origin, while other members may also replicate in suitable water environments. These bacteria, which can be determined by simple and inexpensive tests, are primarily used for assessment of the general sanitary quality of finally treated and disinfected drinking-water. Methods used are multiple tube or membrane filtration using LES Endo agar and incubation for 24 hours at 35-37°C. More recently coliform bacteria are also identified by their possession of the enzyme β -D-galactosidase, which hydrolyses chromogenic substrates such as ortho-nitrophenyl- β -D-galactopyranoside (ONPG), resulting in release of the chromogen and a colour change in liquid media.

The primary purpose of coliform tests is not to detect faecal pollution but to screen the general sanitary quality of treated drinking-water supplies.

4.1.4 Enterococci

Enterococci, sometimes referred to as faecal streptococci, is a group of bacteria more closely related to faecal pollution than total coliforms because most members of this group do not replicate as readily in water environments. These Gram-positive bacteria tend to be more resistant than faecal coliforms (Gram-negative), and are detectable by practical techniques, such as membrane filtration using m-enterococcus agar and incubation at 44.5° or 37°C for 48 hours. Presently the group is considered to primarily include only *Enterococcus faecalis*, *E. faecium*, *E. durans* and *E. hirae*. More recently enterococci are identified by the ability to hydrolyse 4-methyl-umbelliferyl- β -D-glucoside (MUD) in the presence of thallium acetate, nalidixic acid and 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) resulting in release of the fluorogen which in liquid media is readily detectable under ultraviolet light.

4.1.5 Sulphite-reducing clostridia

An important advantage of these Gram-positive anaerobic bacteria is that their spores are more resistant to conditions in water environments, as well as treatment and disinfection processes, than most pathogens, including viruses. Clostridia are sometimes considered as too resistant, and their inclusion in water quality guidelines as too stringent. One of the members of the group, *Clostridium perfringens*, is like *E. coli* highly specific for faecal pollution. Clostridia generally occur in lower numbers in waste water than coliform bacteria. Detection methods are relatively expensive and time-consuming.

4.1.6 Heterotrophic plate count

This test is also known as the total or standard plate count. The test detects a wide variety of organisms, primarily bacteria, which give an indication of the general microbiological quality of water. The test is simple and inexpensive, yields results in a relatively short time, and has proved one of the most reliable and sensitive indicators of treatment or disinfection failure. The generally used test method is pour plates using a rich growth medium such as yeast extract agar and incubation for 48 hours at 37°C.

4.1.7 Other indicators

A variety of other indicators has been used in water quality assessment, including cytopathogenic human viruses, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, acid-fast bacteria, *Legionella* species, *Candida albicans*, and endotoxins. All of these have advantages for certain purposes.

4.2 Protozoan parasites

The cysts and oocysts of intestinal parasites such as *Giardia* and *Cryptosporidium* species are exceptionally resistant, and they generally occur in low numbers in raw and treated water supplies (Casemore, 1991; Bellamy *et al*, 1993). In addition, they are not readily detectable, and their behaviour in water treatment and disinfection processes differs extensively from that of commonly used indicators. Quality control is, therefore, generally based on specifications for raw water quality and the efficiency of treatment processes rather than indicators or testing for cysts and oocysts (Regli *et al*, 1991).

4.3 Human viruses

The incidence and behaviour of human viruses in water environments and treatment processes may differ extensively from that of faecal indicators for reasons such as:

- a) Viruses are excreted only by infected individuals, and coliform bacteria by almost all people and warm-blooded animals. Numbers of viruses in water environments are, therefore, generally lower than those of indicators such as faecal coliforms by several orders of magnitude.
- b) Viruses are excreted for relatively short periods in numbers of up to 10^{12} /g of faeces, while coliform bacteria are excreted fairly consistently in numbers of about 10^9 /g of faeces.
- c) The structure, composition, morphology and size of viruses differs fundamentally from that of bacteria, which implies that behaviour and survival in water differ.

In view of the above differences it is not surprising that bacterial indicators such as coliform bacteria have shortcomings as indicators for viruses. These shortcomings have been confirmed in epidemiological studies and research on the incidence of indicators and viruses in water supplies. Ideally water quality surveillance should, therefore, include tests for viruses. Unfortunately, however, tests for viruses are relatively expensive, complicated and time consuming, and require sophisticated facilities and know-how. In addition, the great majority of viruses concerned are not detectable by conventional virological cell culture techniques. Control of the virological safety of water is, therefore, as in the case of protozoan parasites, often based on raw water quality and specifications for purification and disinfection processes rather than testing of the treated water (Regli *et al*, 1991).

4.4 New developments in microbiological water quality monitoring

4.4.1 Bacteriophages

Bacteriophages (phages) are viruses which infect bacteria. In terms of size, structure, morphology and composition they closely resemble human viruses. The behaviour of phages in water and related environments, and their resistance to unfavourable conditions, treatment systems and disinfection processes do, therefore, more closely resemble those of human viruses than bacterial indicators of faecal pollution.

Phages can replicate only in specific host bacteria, which implies that the phages of *E. coli* (coliphages) are, like their hosts, related to faecal pollution. Phages commonly used in water quality assessment include the groups of phages known as somatic and male-specific coliphages, which each have their own indicator advantages and disadvantages. Phages which infect *Bacteroides fragilis* strain HSP40 are highly specific for human faeces, and can be used to distinguish between faecal pollution of human and animal origin (Grabow *et al*, 1994b).

Evidence supporting the indicator value of phages is accumulating, and their inclusion in quality monitoring protocols is gaining ground rapidly.

4.4.2 Virological analysis of water

Although desirable, virological analysis is not included in many routine surveillance protocols because of cost, complexity and time. In addition, the great majority of viruses concerned are not detectable by conventional techniques. However, progress is being made in the development of more practical and meaningful techniques, and virological monitoring for certain purposes is becoming more feasible. Challenges include the recovery of small numbers of viruses from large volumes of water, the detection of a wider variety of viruses, and reduction in the cost of testing (Standard Methods, 1992).

4.5 Indicator strategies in water quality surveillance

Since no single indicator can fulfil all the needs of water quality surveillance, best results are obtained by using appropriate combinations of indicators for various purposes. Each of these indicators offers certain information, which in combination yields a reliable picture of the quality of the water under investigation. For instance, indicators selected for monitoring the quality of treated drinking-water supplies may primarily be based on tests for *Escherichia coli* to detect faecal pollution. However, under certain circumstances indicators such as the heterotrophic plate count, total coliforms and somatic coliphages may yield valuable additional information.

Breakdown in treatment plants, and human error in operation and supervision, generally take place without warning, in fact, like a thief at night they tend to strike when least expected. This implies that quality surveillance programmes should make provision for microbiological monitoring at the highest possible frequency, in order to detect problems at the earliest possible stage. Since monitoring programmes are subject to many variables and considerations, including the raw water source and treatment system concerned, as well as available financial resources, facilities and manpower, it is not possible to formulate universal sampling protocols. Each case has to be evaluated in its own merit. With regards to sampling frequencies, it is important to keep in mind that it is better to run simple and inexpensive tests at high frequency than complicated and expensive tests at low frequency.

Important principles in sampling procedures include aseptic collection in sterile containers, and delivery at the laboratory for testing preferably within two hours of collection. The inclusion of samples collected at the consumer's tap is advisable, and so is the collection of samples at different times of the day and different days of the week.

5. Microbiological Water Quality Guidelines

Water quality guidelines and standards recommended by various authorities are similar in that they intend to ensure the minimum risk of infection. However, they differ in detail because of considerations such as economic and technical capabilities, and perceptions of acceptable risks of infection.

The *Guidelines* state that drinking-water must not contain waterborne pathogens. More specifically, *E. coli* or thermotolerant coliforms should not be present in 100 ml samples of drinking-water at any time, for any type of water supply, treated or untreated, piped or unpiped. In the case of large supplies, where sufficient numbers of samples are examined, total coliforms

are acceptable in the distribution system in a maximum of 5% of samples taken throughout any 12 month period (Annex 1).

If guideline values are exceeded, immediate investigative action must be taken, including repeat testing, and thorough inspection of the treatment plant and its operation, the raw water source, and general hygiene of the water distribution system.

It is recognised that, in the great majority of rural water supplies in developing countries, faecal contamination is widespread and achieving the guideline values for *E. coli* or thermotolerant coliforms is often not possible. Under these conditions, the national surveillance agency should set medium-term targets for the progressive improvement of water supplies, as recommended in Volume 3 of *Guidelines for drinking-water quality* (Surveillance and control of community supplies).

Because routine monitoring techniques are not available for viruses, protozoa and helminths of health significance, the *Guidelines* recommend protection of the source and treatment techniques to ensure their absence. The degree of treatment required is a function of the nature (ground or surface water) and level of faecal contamination of the source.

To ensure the absence of viruses, the *Guidelines* recommend that the following conditions of disinfection with chlorine be met:

Residual free chlorine	≥	0.5 mg/litre
Contact time	≥	30 minutes
pH	<	8.0
Median turbidity	≤	1 Nephelometric Turbidity Unit (NTU)
Maximum turbidity	=	5 NTU

The control of pathogenic protozoa and guinea-worm requires efficient filtration since these organisms are rather resistant to disinfection.

6. Conclusions

- Waterborne diseases have a major public health and socio-economic impact, and are the most important concern of water quality.
- Strategies for the control of waterborne diseases must be based on the selection of appropriate water sources and treatment systems, fail-safe operation of these treatment systems, and reliable bacteriological quality monitoring.
- A wide variety of treatment and disinfection systems is available for reliable production of microbiologically safe drinking-water.
- Reliable guidelines for the microbiological safety of drinking-water are available.

References

Avendano P, Matson D O, Long J, Whitney S, Matson C C and Pickering L K (1993). Costs associated with office visits for diarrhea in infants and toddlers. *Pediatric Infectious Diseases Journal* 12, 897-902.

Bellamy W D, Cleasby J L, Logsdon G S and Allen M J (1993). Assessing treatment plant performance. *Journal of the American Water Works Association* 85, 34-38.

Casemore D P (1991). The epidemiology of human cryptosporidiosis and the water route of infection. *Water Science and Technology* 24, 157-164.

Craun G F (1991). Causes of waterborne outbreaks in the United States. *Water Science and Technology* 24, 17-20.

Grabow W O K, Favorov M O, Khudyakova N S, Taylor M B, and Fields H A (1994a). Hepatitis E seroprevalence in selected individuals in South Africa. *Journal of Medical Virology* (in press).

Grabow W O K, Neubrech T E, Holtzhausen C S and Jofre J (1994b). *Bacteroides fragilis* and *Escherichia coli* bacteriophages: excretion by humans and animals. *Water Science and Technology*. (Submitted for publication).

ISO (1990). Detection and enumeration of coliform organisms, thermotolerant coliform organisms, and presumptive *Escherichia coli*.

Part 1: Membrane filtration method (9308-1:1990).

Detection and enumeration of coliform organisms, thermotolerant coliform organisms, and presumptive *Escherichia coli*.

Part 2: Multiple tube (most probable number) method (9308-2:1990).

Morens D M, Zweighaft R M, Vernon T M, Gary G W, Eslie J J, Wood B T, Holman R C and Dolin R (1979). A waterborne outbreak of gastroenteritis with secondary person-to-person spread: Association with a viral agent. *The Lancet* i, 964-966.

Payment P (1993). Prevalence of disease, levels and sources. In: *Safety of Water Disinfection: Balancing Chemical and Microbial Risks*. International Life Sciences Institute, Washington DC.

Regli S, Rose J B, Haas C N and Gerba C P (1991). Modelling the risk from giardia and viruses in drinking-water. *Journal of the American Water Works Association* 92, 76-84.

Standard Methods (1992). *Standard Methods for the Examination of Water and Wastewater*, 18th Edition. Eds.: A E, Clesceri L S, Eaton A D. American Public Health Association, Washington DC. pp 9-1 - 9-147.

Annex 1
BACTERIOLOGICAL QUALITY OF DRINKING-WATER

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

Microbiological Aspects

Presentation Plan

Section	Key points	OHP
Introduction	<ul style="list-style-type: none"> • very many different types of microorganisms in water, some of which are harmful (pathogens) and some of which are not • pathogens may be bacteria, viruses or parasites 	Table 1 & 2
Pathogens in water	<ul style="list-style-type: none"> • <i>Guidelines</i> considered many types of pathogens including bacteria, viruses, protozoa and helminths • Pathogens have distinguishing properties that make them very different from chemical pollutants and influence GV setting • GV not set for pathogens as there is no lower tolerable limit, any ingestion of pathogens represents some risk to health 	1
Monitoring and preventative actions	<ul style="list-style-type: none"> • water quality is prone to rapid variability and failures often discrete events • water quality failure may be caused by poorly protected sources, inadequate or failures in treatment or leaking distribution systems • to ensure water quality need to use four key approaches: water quality analysis, sanitary inspection, source protection & minimum treatment requirements • water quality analysis only identifies contamination once it has occurred, sanitary inspection identifies potential risks and source protection and minimum treatment limit risks 	2
The need for indicators	<ul style="list-style-type: none"> • analysis of pathogens difficult, expensive and is essentially a reactive process - fails to provide a warning about potential problems • therefore need a system to identify water supplies which represent a health risk before disease outbreaks occur • risks associated with faecal contamination, so use indicator bacteria which indicate faecal contamination 	3
Properties of indicators	<ul style="list-style-type: none"> • faecal indicators should be present in water where there is faecal contamination • indicators should be present in greater numbers than pathogens • they should have the same resistance to disinfectants and environmental stress as the most resistant pathogens • they should not multiply under environmental conditions • should be easy and cheap to carry out analysis of indicators 	4, 5

Section	Key points	OHP
Examples of indicators	<ul style="list-style-type: none"> • number of indicators may be used • these have different characteristics and may be used for different purposes or under different conditions • examples of indicator bacteria include: total coliforms, thermotolerant coliforms, <i>E.coli</i>, faecal streptococci, <i>Clostridium</i> and <i>Pseudomonas</i> • these all derive purely or in part from human faeces and are present in large numbers • indicator bacteria are not particularly effective in indicating presence of viruses which are more resistant and persistent than bacteria • coliphages may also be used as indicators, although this is under debate at present 	6
Principal indicator bacteria	<ul style="list-style-type: none"> • the most commonly used indicators are total, thermotolerant coliforms and <i>E.coli</i> • total coliforms grow at 37°C, but do not come from a purely faecal origin • presence of total coliforms in water supplies indicates a leakage or biofilm problem and thus a potential risk from ingress of surface water • <i>E.coli</i> is the most commonly used faecal indicator bacteria for which thermotolerant (faecal) coliforms are an accepted substitute • - there should be zero thermotolerant coliforms in 100 ml of drinking water 	7
The use of other indicators	<ul style="list-style-type: none"> • other indicators may be used for other reasons such as indication of operational problems or because they are more resistant to disinfection than coliforms • faecal streptococci are more persistent than <i>E.coli</i> and rarely multiply in polluted water, but may come from animal faeces • however, they tend to be present in lower numbers than <i>E.coli</i> 	

Problems in Setting Guideline Values for Individual Pathogens

- Pathogens are discrete and not in solution
- Pathogens often in clumps or adhere to suspended solids
- Cannot predict likelihood of infectious dose from average concentration
- Infection and disease development dependent on invasiveness, virulence and immunity
- Dose-response not cumulative



Ensuring Microbiological Quality

- Water quality analysis
- Sanitary inspection
- Source protection
- Minimum treatment requirements



Need for Faecal Indicator Organisms

- Pathogen analysis:
 - » expensive
 - » impracticable
 - » techniques may be time consuming
 - » reactive
- Reliance is therefore placed on relatively simple and more rapid tests for the detection of certain intestinal bacteria which indicate that faecal contamination could be present.



Characteristics of the Ideal Faecal Indicator

- 1 Should be present in wastewater and contaminated water when there are pathogens
- 2 Should be present when there is a risk of contamination by pathogens
- 3 Should be present in greater numbers than the pathogens
- 4 Should not multiply in environmental conditions under which pathogens cannot multiply
- 5 The indicator population should correlate with the degree of faecal contamination.



Characteristics of the Ideal Faecal Indicator (continued)

- 6 The survival time in unfavourable environmental conditions should exceed that of pathogens
- 7 Should be more resistant to disinfectants and other stresses than the pathogens
- 8 Should present no health risk
- 9 Should be easy to enumerate and identify by simple methods
- 10 Should have stable characteristics and give consistent reactions in these analyses



Examples of Indicator Organisms

	Percentage in mammal faeces	LOG ₁₀ /g
Total coliforms	Viable, but many environmental sources	
Thermotolerant coliforms	100 (environmental source in tropical waters?)	7-9
<i>E.coli</i>	100	7-9
Faecal streptococci	100	5-6
<i>Clostridium perfringens</i>	13-35	6-7
<i>Pseudomonas aeruginosa</i>	3-15	3-5
<i>Bacteriodes fragilis</i>	100	7-10
Coliphages		
• Somatic	60	1-8
• F-Specific	6	1-2



Principal Indicator Bacteria

Principal indicator bacteria are:

- *Escherichia coli*
- Faecal coliforms (95% are *E.coli* , $\pm 44^{\circ}\text{C}$)
- Faecal streptococci



Microbiological Aspects

Table 1 : Examples of Pathogens Considered in the Guidelines

Pathogen	Health significance	Persistence in water supplies	Resistance to chlorine	Relative infectious dose	Important animal reservoir
Bacteria					
<i>Campylobacter jejuni, C.coli</i>	High	Moderate	Low	Moderate	Yes
Pathogenic <i>E.coli</i>	High	Moderate	Low	High	Yes
<i>Salmonella typhi</i>	High	Moderate	Low	High	No
<i>Shigella</i> spp.	High	Short	Low	High	No
<i>Vibrio cholera</i>	High	Short	Low	High	No
<i>Yersina enterocolitica</i>	High	Long	Low	High (?)	Yes
<i>Pseudomonas aeruginosa</i>	Moderate	May multiply	Moderate	High (?)	No
<i>Aeromonas</i> spp.	Moderate	May multiply	Low	High (?)	No

Microbiological Aspects

Table 2 : Examples of Pathogens Considered in the Guidelines (continued)

Pathogen	Health significance	Persistence in water supplies	Resistance to chlorine	Relative infectious dose	Important animal reservoir
Viruses					
Adenoviruses	High	?	Moderate	Low	No
Enterovirus	High	Long	Moderate	Low	No
Hepatitis A	High	?	Moderate	Low	No
Norwalk virus	High	?	?	Low	No
Rotavirus	High	?	?	Moderate	No (?)
Small round virus	Moderate	?	?	Low (?)	No
Protozoa					
<i>Entamoeba histolytica</i>	High	Moderate	High	Low	No
<i>Giardia intestinalis</i>	High	Moderate	High	Low	Yes
<i>Cryptosporidium parvum</i>	High	Long	High	Low	Yes
Helminths					
<i>Dracunculus medinensis</i>	High	Moderate	Moderate	Low	Yes