The Microbiology of Water 1994
Part 1—Drinking Water

Report on Public Health and Medical Subjects No 71

Methods for the Examination of Waters and Associated Materials

HMSO

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London: HMSO

First published 1994
Second impression 1995

ISBN 0 11 753010 7

Foreword

This volume (the sixth edition) is the revision of the 1983 DoE/DH/PHLS publication 'The Bacteriological Examination of Drinking Water Supplies 1982'.

Whilst the main aim of this edition is to provide users with up to date methods for the microbiological examination of drinking water, additional information on the regulatory background, sampling, quality assurance and statistical considerations, and health and safety requirements are included.

This revision also addresses a change in the definition of coliform organisms. In the past, the definition was based on the behaviour of organisms in lactose fermentation methods traditionally used for coliform isolation. This presented a barrier to the development of new and improved methodologies since it ultimately required the demonstration of the production of gas from lactose during fermentation. When 'Guidance on Safeguarding the Quality of Public Water Supplies' was published in 1989 it contained a definition of coliform organisms based on the possession of genes necessary to break down lactose, namely the gene for ß-galactosidase. This revision confirms that definition and removes the requirement for the demonstration of gas production as a necessary characteristic of coliform organisms. The use of a genetically based definition opens up possibilities for the use of new techniques, provided the performance is demonstrated to be equal to, or better than, the performance of methods described in this volume.

Members of the committee assisting with this booklet

Grateful acknowledgement is made to all members and colleagues who contributed draft manuscripts and to those who made comments and helpful suggestions.

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Grateful acknowledgement is also made to the following organisations who commented on the final draft.

Association of Public Analysts
Department of Health
Public Health Laboratory Service
Scottish Association of Directors of Water and Sewerage Services
Water Companies' Association
WRc plc
Water Services Association

Contents

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, groundwater, river and seawater, waste water and effluents as well as sewage sludges, sediments and biota. In addition, short reviews of the more important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods especially for chemical parameters should be fully evaluated with results from performance tests reported. 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 encompassing at least ten degrees of freedom 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. It is recognised that the performance criteria expected for chemical parameters will not be strictly applicable to microbiological methods. 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 in the series 'Methods for the Examination of Waters and Associated Materials' and their continuous revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is managed by the Drinking Water Inspectorate. At present there are nine working groups, each responsible for one section or aspect of water quality analysis. They are:

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage works control methods
9.0 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 and organisations associated with this booklet are listed at the front.

Publication of new or revised methods will be notified to the technical press. An index of methods and the more important parameters and topics is available from HMSO (ISBN 0 11 752669 X).

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

14 May 1994

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 any regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 1988 SI 1988/1657. 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 and these should be consulted and be readily accessible to all analysts. Amongst such publications are those produced by the Royal Society of Chemistry, namely 'Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory, 5th edition, 1992'; by Member Societies of the Microbiological Consultative Committee, 'Guidelines for Microbiological Safety, 1986, Portland Press, Colchester'; and by the Public Health Laboratory Service 'Safety Precautions, Notes for Guidance'. Another useful publication is produced by the Department of Health entitled 'Good Laboratory Practice'.

Preface

The importance for public health of the provision of a wholesome supply of drinking water has been recognised since the 19th century. Knowledge, understanding and good practice have developed over time with consequent benefits to public health. Today the maintenance of a sufficient supply of wholesome drinking water is a complex undertaking in which individuals from many disciplines take part. This report 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 the first in a series of Ministry of Health publications on Public Health and Medical Subjects. This Report was prepared in 1934 by a small Ministry of Health committee under the Chairmanship of the late Dr Thomas Carnworth with the help of the late 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. In 1956 the Public Health Laboratory Service (PHLS) Water Committee 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. The fifth edition, issued in 1983, was therefore produced under the auspices of the Standing Committee of Analysts and published jointly by DoE, Department of Health and Social Security (DHSS) and PHLS.

A further revision of the Report has become necessary as a result of major changes in the United Kingdom water industry, including the passing of the Water Act 1989 now consolidated into the Water Industry Act 1991 (the Act), the Water Supply (Water Quality) Regulations 1989 (the Regulations), and the Private Water Supplies Regulations 1991 (the Private Supplies Regulations). This revision has been produced by Working Group 2 of the DoE Standing Committee of Analysts.

The new edition is intended to be the first in a series of publications dealing with all aspects of the microbiology of water. As in previous editions, the main section is devoted to reference methods for the isolation, enumeration and identification of micro-organisms found in water supplies that are considered to be of significance, either for sanitary or other reasons. For the first time, tentative methods are included. These are considered to be capable of giving useful results but require further evaluation before achieving fully validated status. Other new sections deal with microbiological aspects of source water quality, water treatment and distribution, and statistical considerations, quality assurance and an expanded section on laboratory safety.

Practical advice on water quality matters is included in "Guidance on Safeguarding the Quality of Public Water Supplies" (the Guidance Document) and its Scottish equivalent. Definitions of relevant organisms are recommended which depart from those traditionally used by water microbiologists. With respect to coliform organisms, the demonstration of the production of gas is now no longer considered relevant. Definitions are more scientifically based and will include organisms of possible sanitary significance that would previously have been excluded by the former 'method-related' definitions. Being no longer 'method-related' they will enable immunological, genetic and electrometric techniques to be used for the detection and confirmation of coliforms provided these methods are shown to be of equivalent or better performance than those described here.

The maintenance of microbiologically wholesome drinking water supplies requires the commitment of individuals from many different disciplines: professionals in water undertakers, consultants in communicable disease control, environmental health officers, public analysts and hospital and PHLS microbiologists. Good communication and liaison between all these individuals is essential to enable appropriate action to be taken whenever water quality problems occur.

In considering the public health aspects of water supplies, local authorities should consult their medical advisors. In most cases this will be the Consultant in Communicable Disease Control (CCDC) (in Scotland, the Consultant in Public Health Medicine) who also acts as Proper Officer for the Local Authority. Where this is not the case, the local authority should consult the Director of Public Health (DPH) for the district health authority (DHA). Revised guidance on the role of the CCDC was issued by the Department of Health (DH) in November 1993 (HSG(93)56). Advice was issued by the Welsh Office to the Welsh authorities in April 1989 (WHC(89)1). It is emphasised that it is the responsibility of the CCDC (or, in the absence of a CCDC, the DPH) to give medical advice if there is any microbiological evidence to suggest that a water supply may be unwholesome. If there is evidence that the water is unsafe to drink, it is the duty of the CCDC to advise immediately on any further action and investigations considered necessary. If water-borne infections are suspected, the CCDC should assume executive responsibility and a co-ordinating role for the control of any outbreak of disease. The CCDC should usually seek advice from the local public health laboratory or its equivalent and, if necessary, the assistance of the regional epidemiologist and the PHLS Communicable Disease Surveillance Centre (CDSC). HSG(93)56 makes it clear that suitable local arrangements at all levels should be made to ensure that there is direct and continuing liaison between all those concerned with communicable disease control; this includes water undertakers, local authorities, DHA and other National Health Service (NHS)authorities and the PHLS. The importance of clear lines of communication, both within and between these organisations, cannot be over-emphasised.

The Regulations apply to all water from public supplies for drinking, cooking, washing or food production. The same standards for wholesomeness are included in the Private Supplies Regulations. Although water suppliers are required to ensure the wholesomeness of their product, it is well recognised that it is difficult to exercise control over changes in water quality to all premises in their areas. Local authorities are required to take samples from private water supplies and have the power to take samples from public water supplies.

As well as giving details of approved methods, this Report gives general advice on many microbiological aspects connected with potable water supplies. The microbiological safety of these supplies in the United Kingdom has been assured in no small measure by regular monitoring and observance of the recommendations contained in previous editions of Report 71. It is hoped that this revised issue will be found to be even more useful than before, not only in the United Kingdom but also internationally.

Chapter 1: Introduction

This Report, recognising the importance to public health of the proper maintenance of a wholesome supply of drinking water, has been prepared with the following objectives:

—to outline the principles on which the microbiological examination of drinking water is based;

—to recommend techniques for sampling and examination to ensure good scientific practice and comparability of results; and

—to give advice on the interpretation of the microbiological results and on the need for consequent action in the event of failure of microbiological standards.

Almost the whole of the population of the United Kingdom is served by a piped public supply of treated drinking water and less than 1% of the population are served by private supplies. This Report is primarily concerned with the microbiological examination and monitoring of public water supplies—not only as the water is distributed, but also as it is abstracted and treated. Mention is also made of risks which might arise from inadequate treatment, especially following a sudden pollution of the source, as well as to contamination within the distribution system. In addition, since the Regulations apply to all water for drinking purposes, brief reference is made in this Report to the microbiological examination of particular supplies, such as those on trains, ships and aircraft, and in hospitals, food establishments and similar premises.

A single laboratory examination of any water does not justify the conclusion that all is well and that the supply will remain suitable for drinking purposes. Contamination is often intermittent and may not be revealed by the examination of a single sample. The impression of security given by microbiological testing of a water at infrequent intervals may, therefore be quite 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 occasionally by a more complicated test or series of tests.** Information gained in the course of time through monitoring will provide a comprehensive picture of the range of quality at any particular source of water, any lapse from which must at once arouse suspicion. The most a microbiological report based on a single sample can prove is that at the time of examination, certain bacteria indicative of faecal contamination did or did not grow under laboratory conditions from the sample of water as received and tested. It must be emphasised that, when site inspection shows a water supply to be obviously subject to contamination, remedial action should be taken without waiting for, and irrespective of, the results of microbiological examination.

The European Community Directive relating to the Quality of Water Intended for Human Consumption, Directive 80/778/EEC (the Directive), lays down standards for the quality of drinking water and water for use in food production undertakings. The Water Act 1989 (now consolidated in the Water Industry Act 1991, which came into force on 1st December 1991), the Water Supply (Water Quality) Regulations 1989 and the Private Water Supplies Regulations 1991 transpose the Directive into the law of England and Wales. Similar legislation has been introduced in Scotland and is in the course of introduction in Northern Ireland.

The Act places a duty on water undertakers to supply only water that is wholesome at the time of supply. The time of supply is the moment when water passes from the company's pipe into pipes owned by the owner of premises or property. Companies are not responsible for a deterioration of drinking water quality which occurs within consumers' premises, although water undertakers are required to take certain steps if there is a risk that the standards for lead, copper or zinc might be breached as a result of corrosion of the consumers' pipes or fittings. Wholesomeness is defined by reference to the standards and other requirements of the Regulations. The Act also creates a criminal offence of supplying water that is unfit for human consumption. Under the Act, the Secretary of S for the Environment and the Secretary of State for Wales are empowered to appo technical assessors. The Drinking Water Inspectorate came into being on 2nd Janu 1990 to fulfil that function. The Inspectorate advises the Secretaries of State as to whet water companies are supplying wholesome water and are monitoring, recording a reporting on the quality of drinking water in accordance with legal requirements.

The Regulations originally applied only to water supplied for domestic purposes (that drinking, washing and cooking). Following amendments made to the Act by the Fo Safety Act 1990 (the Food Act), the Regulations have been amended, and now also app to water supplied for food production purposes. Prescribed concentrations or valu (PCV) are specified for microbiological, chemical and physical parameters. Mandato sampling frequencies for treatment works and water supply zones are specified accordi to the volume of water supplied or the population served. In addition, weekly monitori for microbiological parameters is specified for service reservoirs and water towers in us Regulations under the Food Act prescribe standards for drinking water supplied in seale containers, which includes bottles (see Appendix E).

Private water supplies are defined in the Act as any supplies of water provided otherwis than by a statutorily appointed water undertaker. The definition includes water abstracte from a source on the premises on which it is used or consumed, bulk supplies purchase from a water undertaker and subsequently redistributed, and also sources or supplies use to produce bottled water. The Private Supplies Regulations set standards of wholesome ness and specify the duties of local authorities in respect of sampling and analysis o private water supplies. Where a supply is unwholesome or insufficient the Act allows loca authorities to serve a notice requiring either improvements to the supply or connection o the premises to a public supply. The Department of the Environment has issued Circula 24/91 and the Welsh Office, Circular 68/91, which provides guidance to local authorities on their duties to sample and analyse private water supplies. Similar guidance has been issued by the Scottish Office Environment Department (Circular 20/1992). Advice on treatment has been given in 'Manual on Treatment of Private Water Supplies' (DoE/WO/ SO 1993). The quality of natural mineral water is covered by the Natural Mineral Waters Regulations 1985 (SI 1985/71 as amended by SI 1990/2487) (see Appendix E).

Chapter 2: Public Health Aspects

2.1 Water-borne Infections

The microbiological safety of potable water depends upon the exclusion of pathogenic micro-organisms that cause infections via the gastro-intestinal tract. The recognition in the 19th century that cholera and typhoid fever could be spread by the ingestion of water contaminated with excrement laid the foundations of present day treatment processes and piped distribution, the design and operation of which must always ensure that potable water cannot transmit such infections. Other treatment objectives should never take precedence over this primary requirement.

2.1.1 Water-borne pathogens

Many micro-organisms have now been recognised as capable of being transmitted by water, including bacteria, protozoa and viruses. Their essential properties are that they are present in large numbers in human or animal excreta or both, and are relatively resistant to environmental decay. Many are highly likely to cause infections if ingested in small numbers. Examples of those known to have caused water-borne infections are listed in Table 1.

Table 1 Known Water-borne Pathogens

Bacteria

Campylobacter species
Escherichia coli (certain serotypes)
Salmonella species (including *S.typhi*)
Shigella species
Streptobacillus moniliformis
Vibrio species (including *V.cholerae*)
Yersinia enterocolitica

Protozoa

Balantidium coli
Cryptosporidium species
Entamoeba histolytica
Giardia intestinalis (= *lamblia*)

Viruses

Hepatitis A virus
Hepatitis E virus
Small round structured viruses
(for example Norwalk virus)
Rotaviruses

Other organisms causing enteritis are known to be present in human and animal excreta and therefore have the potential to cause water-borne infections. These include the aeromonas group, *Bacillus cereus*, certain clostridia, *Plesiomonas shigelloides*, *Isospora* species and some enteric viruses. Certain enteroviruses belonging to the poliovirus and coxsackievirus groups have occasionally been isolated in small numbers from drinking water but there is no evidence that illness has resulted; direct person-to-person transmission being the usual route. A more detailed consideration of viruses in the water cycle is given in 'Methods for the isolation and identification of human enteric viruses from water and associated materials' (SCA 1994) (see section 3.7).

Organisms normally present in the environment, such as saprophytic mycobacteria, *Pseudomonas aeruginosa* and some aeromonads, have the potential to cause infections in immuno-compromised people and have at times been suspected of being transmitted by the water route. *Listeria monocytogenes* is widely distributed in the environment and is a possibility that it might be found in water. However, while probable food- listeriosis has been noted there is no suggestion of transmission by drinking w Infections caused by *Legionella pneumophila*, *Naegleria fowleri* and *Acanthamoeb* associated with warm water but require special circumstances to enable infection to o notably direct inhalation of water or aerosols. Thus infection is not directly attributab drinking water supplies.

Most enteric pathogens are found in the intestinal tract of persons suffering from a infections caused by organisms which are present in large numbers in excreta. S infections, such as typhoid fever, may produce a prolonged carrier state after the a phase is over, and the organisms can be excreted in relatively small numbers for m years. Organisms derived from these sources will then be present in sewage.

Animals may also harbour in their gut organisms pathogenic for humans and n therefore be a significant source of pollution. Gulls pose a particular problem catchments, at refuse tips and sewage treatment works. Modern intensive farm methods result in the production of large quantities of slurry which may subsequen contaminate watercourses from which water is abstracted for drinking. This is though have been the cause of an outbreak of water-borne cryptosporidiosis reported in 19 (Richardson et al 1991).

In addition to the drinking of contaminated water, its use in the preparation of food, whi may allow the multiplication of enteric pathogens, presents obvious dangers.

The routes by which enteric pathogens can gain access to drinking water are numerou Contamination of catchment areas will result in the presence of pathogens in surfac waters from which drinking water may be abstracted. Less obviously, ground water ma also be contaminated.

Outbreaks of water-borne infection have usually resulted from defects in one or mor treatment processes (or lack of any treatment) or because there has been ingress o pathogens as a result of activities causing damage to some part of the distribution system Defects in plumbing, cross-connections, back flow and pressure fluctuations can caus contamination of distribution systems, and can result in the transmission of organisms causing infection.

2.1.2 Outbreaks of water-borne infections

The recognition of an outbreak of water-borne infection will, in part, depend upon the continuing surveillance of the prevalence of communicable infections in a particular locality. This will depend initially on the observations by general practitioners or by the local CCDC of a sudden increase in the incidence of infections that could be water-borne. The monitoring by water undertakers of consumer complaints is also important and helpful. Sometimes the CCDC or Environmental Health Officer (EHO) may receive hearsay information suggesting the possibility of a water-borne infection. Other early intimations may arise from an increase in possible water-borne infections (noted by a diagnostic microbiology laboratory) being reported to the CCDC and/or the CDSC, or in Scotland the Communicable Disease and Environmental Health (Scotland) Unit (CD(S)U). Any suspicion of a water-borne outbreak should be notified immediately to the water undertaker, with whom close liaison is essential. If a water-borne outbreak is confirmed, the water undertaker must notify the Drinking Water Inspectorate, whilst the health authority should notify the Department of Health. If a private supply is implicated the primary responsibility for investigation and control lies with the local authority and the DHA in the affected area. The local water undertaker, while not directly involved, should be made aware and can often provide technical help and advice.

The possibility that an outbreak of water-borne infection may result from operational problems such as a failure of disinfection, filter breakdown or a major pollution incident must always be considered by water undertakers, and in these circumstances the local and district health authorities in the area of the supply must be notified as soon as possible.

The National Rivers Authority (NRA) in England and Wales, and in Scotland the River Purification Authorities, have a statutory responsibility for maintaining and upgrading the quality of surface waters and for the protection of groundwater supplies. As all public water supplies are derived from these sources, water undertakers must be informed of any deterioration in quality and pollution incidents which may result in the need for temporary cessation of abstraction or urgent modification of the treatment processes (or other action) at a treatment works.

Close and continuing liaison between water undertakers, district health authorities and local authorities is essential to ensure that appropriate action can be taken to investigate and control outbreaks of water-borne infectious diseases in the local community. The Group of Experts on Cryptosporidium in Water Supplies, (Badenoch Group), (DoE/DH 1990) recommended that district health authorities, local authorities and water companies should together draw up an Outbreak Control Plan (OCP) for the control of water-borne infection in the community, and that this plan should provide for the establishment of an Outbreak Control Team (OCT) to be called into being by the DPH or the CCDC should an outbreak be suspected. The OCT must include representatives from the district health authority, local authority and water undertaker.

Depending on the size of the outbreak, assistance may need to be sought from the regional epidemiologist and from CDSC, who should in any case be kept informed. The assistance of the nearest public health laboratory may be required if there are limited facilities in local NHS or other laboratories.

In the event of an outbreak of water-borne disease, the OCT will be responsible for ensuring that appropriate action is taken without delay. Arrangements will depend on the nature and size of the incident and may require the provision of an alternative supply of drinking water or the issuing of advice to boil the water until the source of the infection has been identified and eliminated. When the need to issue advice to boil water is being assessed the requirements of others, including food, dairy and pharmaceutical industries, dentists and veterinarians and also patients in hospitals or on home dialysis, will need to be considered. It is essential that there is close liaison between district health and local authorities and water undertakers to ensure that clear meaningful advice is given to all persons and organisations likely to be affected. The media can assist with communication of this advice.

The importance of a clear system of emergency communication within the water undertaking between the scientific, managerial and operational staff is self-evident. This will ensure that any communication with consumers and the media, and any remedial action that may be required at the treatment works or in the distribution system can be instituted without delay. General guidance on the measures that may be required is given in 'Guide to the microbiological implications of emergencies in the water services' (WAA 1985) and 'Operational guidelines for the protection of drinking water supplies' (WAA 1988), both originally published by the Water Authorities Association (now the Water Services Association), while the wider public health implications are addressed in detail in 'Drinking Water Supplies: a Microbiological Perspective' (DH 1993a).

Chapter 3: Microbiological Examination of Drinking Water: Rationale

Full examination of a water supply embodies several lines of investigation incluc topographical, chemical, biological and microbiological (of which bacteriology is cially important) and organoleptic characterization. Each line of investigation has its u and indications. This report deals mainly with bacteriogical aspects, but other mic biological aspects are also covered.

Bacteriological examination is particularly important because it remains the m sensitive method of detecting faecal and therefore potentially dangerous pollution.

For several reasons, monitoring for the presence of specific pathogenic bacteria, virus and other agents in water is impracticable and indeed unnecessary for routine contr purposes. Any pathogenic micro-organisms present in polluted water are usually great outnumbered by the normal commensal bacterial flora of the human or animal intestine Although it may be possible to isolate microbial pathogens from contaminated wate especially when it is heavily polluted, large volumes (several litres) of the water may nee to be examined. Selective media and special equipment may also be required for th isolation, and subsequent identification of organisms may involve biochemical, serologi cal and/or other tests. Reliance is therefore placed on relatively simple and more rapid tests for the detection of certain commensal intestinal bacteria, especially *Escherichia coli* and other coliform organisms, because they are easier to isolate and characterise and because they are almost always present in the faeces of humans and warm-blooded animals, and hence in sewage, in large numbers. The presence of such faecal indicator organisms in a sample of drinking water thus denotes that intestinal pathogens could be present, and that the supply is therefore potentially dangerous to health (Waite 1991). The absence of these faecal indicator organisms, however, cannot be taken as an absolute indication that intestinal pathogens are also absent.

Chemical analysis, though lacking the sensitivity of bacteriology in this respect, is nevertheless an important aid to the hygienic assessment of a water supply; however, its major roles are to provide process control information for water treatment and to monitor for compliance with the prescribed standards. Biological examination is used to detect the presence of algae and animal life which may impede performance of filtration and gain access to supplies through suboptimal water treatment or defects in the distribution network. Topographical examination of catchment areas and water supply networks may reveal potential risks which would pass unnoticed by any other method.

3.1 Organisms Indicative of Faecal Pollution

The search for organisms indicative of faecal pollution instead of for pathogens themselves is universally accepted for monitoring the microbial pollution of water supplies. Ideally, the detection of these indicator organisms should denote the potential presence of intestinal pathogens. Indicator organisms should be abundant in faeces and sewage, and be absent or at least occur in very small numbers in all other sources. They should be capable of easy isolation, identification and numerical estimation and unable to grow in the aquatic environment. In practice there is no organism which consistently meets all these criteria, but in temperate climates most of them are fulfilled by the bacterium *Escherichia coli*. This organism is reaffirmed as the essential indicator of pollution by faecal material of human or animal origin. Other organisms which possess some of these properties can also be used to provide supplementary information in certain circumstances. They include other coliform organisms, faecal streptococci, *Clostridium perfringens* and possibly other intestinal commensals. The particular test or combination of tests to be used in water examination must be left to the discretion of the microbiologist, as they will depend on the nature of the sample, the actual circumstances and the information required.