INTERNATIONAL STANDARDS FOR DRINKING-WATER

Third Edition



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PREFACE

International Standards for Drinking-Water was first published by WHO in 1958 as an aid to the improvement of water quality and treatment. The standards have been adopted in whole or in part by a number of countries as a basis for the formulation of national standards, and were cited in the International Sanitary Regulations as applicable in deciding what constitutes a pure and acceptable water supply at ports and airports.

In 1963 a second, revised, edition of the International Standards was published. Increasing knowledge of the nature and effect of various contaminants, and improved techniques for identifying and determining their concentrations. have led to a demand for further revision of the recommendations. Accordingly, WHO convened an Expert Committee in Geneva in March 1971. and this third edition is the outcome of the Committee's deliberations.

The present volume is considerably shorter and more manageable than the second edition, more than two-thirds of which was devoted to a detailed description of approved methods of water examination. As these appear in other readily available publications the present edition simply refers the reader to descriptions published elsewhere. Certain other material has been omitted, such as the list of suggested subjects for research, and less space is devoted to the evidence considered by the Committee when recommending limits for the concentrations of individual substances. Research workers interested in such matters are referred to the Committee's report, in which its reasoning is more fully discussed.

In the preparation of the material for this publication, use was made of many sources, including earlier editions of the International Standards, the 1970 edition of the European Standards for Drinking-Water.² The Bacteriological Examination of Water Supplies (26), the 1962 edition of Public Health Service Drinking Water Standards (86), the 12th edition of Standard Methods for the Examination of Water and Wastewater (3). Water Treatment and Examination (42), and the water standards of the Ministry of Health of the USSR.3

In publishing this revised edition of International Standards for Drinking-Water, WHO hopes to stimulate further investigations of such problems as the provision of safe and potable water to all communities, the function of

Who Expert Committee on Health Criteria for Water Supplies, Report (unpublished document).
 European standards for drinking-water, 1970, Geneva, World Health Organization, 2nd ed.
 The numbers in brackets refer to the list of references on page 64.

water quality in maintaining public health and reducing disease, and the improvement of treatment processes to ensure the maintenance of high standards in water supplied to consumers. It is recognized that the criteria embodied in these standards cannot be considered final and that future developments may make further revision necessary. Constructive criticism and suggestions based upon experience will be welcomed by WHO.

1. Introduction

Water intended for human consumption must be free from organisms and from concentrations of chemical substances that may be a hazard to health. In addition, supplies of drinking-water should be as pleasant to drink as circumstances permit. Coolness, absence of turbidity, and absence of colour and of any disagreeable taste or smell are of the utmost importance in public supplies of drinking-water. The situation, construction, operation, and supervision of a water supply, its reservoirs, and its distribution system must be such as to exclude any possible pollution of the water.

Some countries have established national standards of quality and have achieved a certain degree of uniformity in methods of analysis and in the expression of the results of such analyses. Others, however, still lack official standards of quality or have no recognized methods for assessing quality. At regional and international conferences sponsored by the World Health Organization, the problems of establishing standards of quality for a safe and acceptable water supply and of devising suitable methods for the examination of water have been discussed by groups of experienced hygienists and engineers. Great progress could be achieved throughout the world if the various methods of examination could be made easily comparable by the adoption of uniform methods of expressing the results; furthermore, outbreaks of water-borne disease could be avoided through stricter control by the responsible health authorities of the quality of the water distributed for drinking purposes. The World Health Organization has therefore studied the situation, in collaboration with member governments and with the assistance of a number of experts, in an effort to offer technical guidance to the health administrations of countries wishing to revise their regulations on waterquality control and to bring them up to date.

1.1 Purpose

It is hoped that this publication will be of value to operators of water supply systems and others concerned with the treatment and distribution of water, and that it will be of assistance to countries wishing to establish their own national standards or to revise existing standards. It is also hoped that it will be of particular value to health authorities in ensuring that the supplies of water that reach the public are safe and pleasant to use. Some guidance is given on the principles to be adopted in choosing a source of water to be used as a public supply.

1.2 Scope

This publication is concerned with the minimum requirements as to chemical and bacterial quality that supplies of water for domestic use can reasonably be expected to satisfy. Though it is desirable that the quality of the water supplied to individuals and small communities should not be inferior to that of water supplied to the public in large communities, it is not considered that all such water could reasonably be expected to conform to the standards suggested for supplies distributed through a piped distribution system. It is, however, important that local health authorities should exercise some control over at least the bacterial quality of water supplied to individuals and small communities.

Some countries are fortunate in having an abundant supply of water from deep wells and underground springs, while others have to make extensive use of rivers, lakes, and other sources of surface water. In yet other areas, the provision of an adequate volume of water is the most pressing problem. It is felt, however, that the recommendations as to chemical and bacterial quality made in the main body of the publication should apply, whatever the original source of the water may have been.

The standards of chemical and bacterial quality and the various methods recommended here are not, and cannot be, the last word on the subject. New methods are constantly being introduced and developed, and it is expected that the methods suggested, and even the standards, will be revised from time to time.

Sections on virological examination, pesticides and polynuclear aromatic hydrocarbons have been added to this edition. Much more information is required on these topics and also on the danger to health of the toxic or potentially toxic substances that may be found in water—for some of these, tentative limits have been proposed in a later section. Mention is also made in a later section of the new chemicals that are from time to time introduced for the treatment of water, and it is essential to ensure that no danger of toxic hazards arises from their use.

1.3 Arrangement of Material

This publication is concerned primarily with methods of ensuring that supplies of drinking-water do not constitute a danger to the health of the consumers. It has been divided into sections on bacteriological, virological, biological, radiological, physical and chemical examination and sampling. Section 2 on bacteriological examination is concerned with: (1) the choice of organisms to be used as indicators of pollution; (2) the methods that it is suggested should be used for the detection of these organisms; and (3) the standards of bacterial quality that might reasonably be set for supplies of drinking-water. This is followed by Sections 3 and 4 on virological examination

and biological examination respectively. Although neither can be regarded as part of the routine examination of drinking-water, it may be necessary to carry out such examinations on water from time to time, and more is known about them now than when previous editions were prepared. Section 5 on the radiological examination of drinking-water follows.

Section 6 on physical and chemical examination is concerned primarily with the tentative limits of concentration that should be set for certain toxic substances that may constitute a danger to health; methods have been recommended for detecting and estimating these substances. Consideration has also been given in Section 6 to the approximate critical concentrations above which other chemical substances may affect the health of the consumer. The highest desirable and maximum permissible concentrations of chemical substances that affect the acceptability of water for domestic use have also been listed. The methods that may be used in the estimation of these chemical substances are indicated and references given to publications in which full technical details can be found. In the part of Section 6 dealing with chemical examination, it is suggested that certain methods should be used in the general examination of supplies for their aesthetic, physical, and chemical characteristics, in order to make the results obtained in different laboratories more easily comparable.

In Section 7 on sampling, methods of sampling for purposes of bacteriological, virological, biological, radiological, and physical and chemical examinations are outlined, and advice is given on when and how frequently samples should be collected for each purpose. Some advice on the storage and transport of samples is also given in this section.

Examples of forms for reporting the results of bacteriological and chemical examination are given in Annex 1; tables of Most Probable Numbers—with confidence limits—for use in bacteriological examination, are given in Annex 2.

1.4 Expression of Results

In view of the importance of uniformity in the methods of expressing the results of physical, chemical, and bacteriological examination of water, the terms in which it is recommended that these results should be expressed are first described.

Although the expression of the results of chemical analysis in terms of milliequivalents per litre (mEq/1) is necessary in striking a balance between anions and cations, it is considered that the results of chemical analysis in general should be expressed in milligrammes per litre, since this method of expression is well-known and widely used. Milliequivalents per litre should be used for the expression of total hardness and total alkalinity, for which milligrammes per litre are not appropriate.

Wherever possible, chemical components should be expressed in terms of ions; volumes should be expressed in millilitres (ml), and temperature should

be measured in degrees Celsius (°C). In bacteriological examinations, the total number of micro-organisms developing on solid media should be expressed as colonies counted per 1 ml of water, the medium, the duration, and the temperature of incubation being stated. Estimates of the numbers of coliform organisms, *Escherichia coli (E. coli)*, and other organisms indicative of pollution should be given in terms of most probable numbers (MPN) per 100 ml, when counted by a multiple-tube method, or as colonies per 100 ml, when counted on a membrane filter. In radiological examinations, radioactivity should be expressed in picocuries per litre (pCi/1). In physical examinations, electrical conductivity should be expressed in microsiemens per centimetre (μ S/cm). For the expression of results of examinations for turbidity, colour, odour, and taste, see Table 3 (p. 38).

1.5 Surveillance

The importance of a sanitary survey of sources of water cannot be overemphasized.

No bacteriological or chemical examination, however careful, can take the place of a complete knowledge of the conditions at the sources of supply and throughout the distribution system. Every supply should be regularly inspected from source to outlet by experts, and sampling—particularly for purposes of bacteriological examination—should be repeated under varying climatic conditions, especially after heavy rainfall and after major repair or construction work. It should be emphasized that, when sanitary inspection shows that a water, as distributed, is liable to pollution, it should be condemned irrespective of the results of chemical or bacteriological examination. Contamination is often intermittent and may not be revealed by the chemical or bacteriological examination of a single sample, which can provide information only on the conditions prevailing at the moment of sampling; a satisfactory result cannot guarantee that the conditions found will persist in the future.

With a new supply, the sanitary survey should be carried out in conjunction with the collection of initial engineering data on the suitability of a particular source and its capacity to meet existing and future demands. The sanitary survey should include the detection of all potential sources of pollution of the supply and an assessment of their present and future importance. In the case of an existing supply, a sanitary survey should be carried out as often as required for the control of pollution hazards and the maintenance of the quality of the water.

It is considered that the responsibility of the surveillance authority goes beyond that of merely pronouncing that water as delivered satisfies, or fails to satisfy, a certain quality standard. Surveillance should include the giving of advice on how defects can be removed and quality improved; this, in turn, implies a knowledge of the water supply system, including the treatment processes, and close liaison with the laboratory workers and water supply operators concerned.

1.5.1 Choice of a raw water source

When a choice has to be made between alternative sources, the quality of the raw water (and hence the extent of the treatment required) as well as to the adequacy and reliability of the sources, from a quantitative point of view, together with the potentialities for expansion in the future, must be considered. The choice of a source requiring a minimum amount of treatment must always be regarded as preferable to the installation of sophisticated purification plant. Removal of pollutants from an industrial effluent before it is discharged into a body of water is often simpler and more reliable than an attempt to remove them from water intended for domestic use taken from some other point in the same body of water.

Nothing in these standards should be regarded as implying approval of the degradation in any respect of an existing water source of a quality superior to that recommended. Existing and potential sources of water should, as far as possible, be protected against pollution, even though there may be no immediate intention of developing them.

1.5.2 Adequacy of treatment

The treatment that a water may require before it is distributed as a public supply does not come within the scope of this publication; treatment should, however, be adequate to deal with changes in the quality of the raw water and produce a finished product of consistently high quality however great the demand on the supply may be.

Chlorination, or other form of disinfection, is not always sufficient, in itself, as a method of obtaining a supply of adequate quality from every raw water. Other forms of treatment—such as coagulation and filtration—are required, before disinfection, to make certain raw waters fit for distribution as public supplies. It should, moreover, be emphasized that the quality of drinking-water is dependent on the quality of the raw water, particularly with regard to those mineral constituents that are not normally removed in water treatment.

In addition to its action as a disinfectant in the final stage of treatment, chlorine has two beneficial effects when added in quantities sufficient to maintain a residual concentration. The first is that the residual may afford some protection against subsequent contamination of the treated water within the distribution system; normal concentrations, of the order of 0.2 mg/l, are too low to have much of an effect of this kind, but larger doses, such as those given in an emergency, can provide some protection, though not against a massive intrusion of pollution. The second is the possibility

of supplementing bacteriological testing with the much simpler colorimetric test for free and combined residual chlorine. Disappearance of the residual chlorine is an immediate indicator of the entry of oxidizable matter, or of a malfunctioning of the treatment process that should have removed it before chlorination. As chlorine residual tests can be carried out in minutes (compared with the hours required for bacteriological examinations) and by unskilled staff without laboratory facilities, it is recommended that maximum use should be made of the potentialities of such tests as a supplement to, though not as a substitute for, the bacteriological testing programme described later in this publication.

When a disinfectant residual is being maintained in the distribution system, the following two points should be noted:

- (1) high concentrations of an oxidizing disinfectant may cause aftergrowth in the system since oxidized organic matter able to maintain bacterial growth is then available when the residual has been absorbed;
- (2) seasonal variations for example in temperature, may well prevent the residual from reaching the periphery of the distribution system, thus causing the difficulties described under (1).

1.5.3 Distribution system maintenance

The inside surfaces of the mains and service pipes comprising the underground distribution system of any water supply are frequently coated with a biological layer that may include slimes, algae, sponges, *Dreissena* and other molluscs, iron bacteria, and various organisms that may harbour nematodes, *Daphniae*, and similar forms of animal life. There is no evidence that any of these constitute a direct health hazard, although it has been suggested that viruses may be concentrated, for instance, within the gut of *Asellus*.

The acceptability of the water may be affected when any of these organisms emerge from consumers' taps, and there is always the risk that the biological layer may be loosened by an increase in water velocity (e.g., when water is used for fire-fighting purposes), by an alteration in the chlorine dosage (e.g., when the concentration is increased during an epidemic, or after a mains repair), or by the mixing of waters of different quality characteristics (e.g., as a consequence of desalination or water re-use). In such cases, discoloration, odours, or turbidity may render the water undrinkable.

Regular flushing or foam swabbing⁴⁹ of mains is recommended to prevent the formation of this biological layer, coupled with a dosage of pyrethrins or other harmless pesticide for the control of animal life, if any organisms have become too firmly established for removal by flushing. Chlorination of mains and reservoirs after cleaning or repair is an additional precaution, and prevention of cross-connexions or back-syphonage is essential if contamination of the system is to be avoided.

2. Bacteriological Examination

This publication is concerned mainly with the routine surveillance of water supplies. When a new source of water is being considered it is important that a full bacteriological examination should be carried out. Such an examination should include colony counts of micro-organisms on non-selective media, and an examination for faecal streptococci and possibly also for Clostridium perfringens (Cl. welchii), as well as for coliform organisms and Escherichia coli. Examinations of this nature should also be carried out at other times when the chief of the laboratory of the responsible authority considers them to be necessary. Special circumstances may require further examinations to be carried out, for example for pathogenic organisms, or for "nuisance" bacteria.

An example of a form for reporting the results of a bacteriological examination is given in Annex 1.

2.1 Organisms as Indicators of Pollution

2.1.1 Organisms indicative of faecal pollution

The greatest danger associated with drinking-water is that it may recently have been contaminated by sewage or by human excrement; even the dangers of animal pollution must not be overlooked. If such contamination has occurred sufficiently recently, and if it has been caused partly by cases or carriers of such infectious diseases as enteric fever or dysentery, the water may contain the living pathogens of these diseases. The drinking of such water may result in fresh cases of the diseases concerned. Although modern bacteriological methods have made it possible to detect these pathogenic bacteria in sewage and sewage effluents, it is not practicable to attempt to isolate them as a routine procedure from samples of drinking-water. When pathogenic organisms are present in faeces or sewage they are almost always greatly outnumbered by the normal excremental organisms, and these normal organisms are easier to detect in water. If they are not found in the water it can, in general, be inferred that disease-producing organisms are also absent, and the use of normal excremental organisms as an indicator of faecal pollution in itself introduces a margin of safety.

The organisms most commonly used as indicators of pollution are E. coli and the coliform group as a whole. E. coli is of undoubted faecal origin, but the precise significance of the presence in water of other members of the coliform group has been much debated. 10, 11, 74, 89 All the members of the coliform group may be of faecal origin, and the worst possible interpretation should therefore be attached to their presence in water; thus, from a practical point of view, it should be assumed that they are all of faecal origin unless a non-faecal origin can be proved. Quite apart from the question of their use as indicators of faecal pollution, organisms of the coliform group as a whole are foreign to water and their presence must at least be regarded as indicative of pollution in its widest sense.

The search for faecal streptococci, of which the most characteristic type is *Streptococcus faecalis*, may well be of value in confirming the faecal nature of pollution in doubtful cases.

Faecal streptococci regularly occur in faeces in numbers that vary but are usually considerably smaller than those of *E. coli*. In water they probably die and disappear at approximately the same rate as *E. coli*, and usually more rapidly than other members of the coliform group. When, therefore, organisms of the coliform group, but not *E. coli*, are found in a water sample, the finding of faecal streptococci is important confirmatory evidence of the faecal nature of the pollution.

Anaerobic spore-forming organisms, of which the most characteristic is Cl. perfringens (Cl. welchii), are also regularly present in faeces, though generally in much smaller numbers than E. coli. The spores can survive in water longer than organisms of the coliform group and usually resist chlorination at the doses normally used in waterworks practice. The presence of spores of Cl. perfringens in a natural water suggests that faecal contamination has occurred and, in the absence of organisms of the coliform group, that such contamination took place some considerable time ago.

Examination for faecal streptococci and anaerobic spore-forming organisms may also be of value when water samples are examined at infrequent intervals, and when a new source of supply is being considered, so that as much information as possible is obtained about the quality of the water.

2.1.2 Total content of micro-organisms

Colony counts on nutrient agar at 37°C and at 20°C are not infrequently used in the bacteriological examination of water. The colony count alone is of little value in detecting the presence of faecal pollution, since organisms of all types capable of growing at these temperatures will be counted. A series of colony counts from a source such as a deep well or a spring may be of considerable value—a sudden increase in the colony count from such a source may give the earliest indication of contamination. Colony counts frequently repeated for a series of points in a treatment plant are of considerable value in the control of waterworks treatment; they are also of

value when a new source of supply is being considered and as much information as possible about the quality of the water is being collected.

An isolated colony count is thus rarely of value; even a series of colony counts is of little value in the case of raw surface waters, because of the wide variations that occur—due, for example, to changes in climatic conditions.

Plates of nutrient agar and of gelatin are useful in examining water for certain "nuisance" bacteria, such as green-fluorescent pseudomonads and organisms that liquefy gelatin rapidly; these, although they are not important from the point of view of safety, may give rise to difficulties in dairies and in food processing.

2.1.3 Recommendations

Water circulating in the distribution system, whether treated or not, should not contain any organism that may be of faecal origin. The absence of organisms of the coliform group, as defined below, should be considered as a fairly reliable indication that pollution is absent. Their presence should be assumed to be due to faecal pollution unless a non-faecal origin can be proved. Should coliform organisms be found, further investigation is required to determine their source.

The coliform group includes all Gram-negative, non-spore-forming rods capable of fermenting lactose with the production of acid and gas at 37°C in less than 48 hours.

E. coli is definitely of faecal origin and its presence should be considered as a sure indication of faecal pollution calling for immediate action. For the purpose of the hygienic analysis of water, E. coli is regarded as a Gramnegative, non-spore-forming rod capable of fermenting lactose with the production of acid and gas at both 37°C and 44°C^a in less than 48 hours; it produces indole in peptone water containing tryptophane and is incapable of utilizing sodium citrate as its sole source of carbon.

Frequent bacteriological examinations are essential for hygienic control. Frequent tests by simple methods are much to be preferred to a series of more complex tests at longer intervals. The volume of the sample should be sufficient for carrying out the tests required and should preferably not be less than 100 ml.

When repairs or extensions to water supply installations or distribution systems have been carried out, it is essential that a bacteriological examination of the water should be performed after the part of the system concerned has been disinfected and before it is put into service.

[•] In at least one country mannitol has been used successfully, in place of lactose, for the 44°C fermentation test. Its use avoids difficulties with strains of E. coll that are deficient in permease.

2.2 Recommended Methods for the Detection and Estimation of Organisms Indicative of Pollution

2.2.1 Detection of coliform organisms and E. coli

The two basic methods used for the detection and enumeration of coliform organisms in water are the multiple tube method, 3. 26 in which measured volumes of water are added to volumes of a suitable liquid medium, and the membrane filtration method, 3. 18. 14. 26. 81. 92. 93 in which measured volumes of water are filtered through a membrane filter. The two methods do not give strictly comparable results, one reason for this being that counts on membrane filters give no indication of gas production from lactose.

(a) Multiple tube method

The examination in liquid media starts with the presumptive coliform test, in which the water sample is inoculated into bottles or tubes containing a suitable liquid medium; these are then incubated and, after the appropriate period of time, examined for the reaction given by coliform organisms. The test is called presumptive because the reaction observed may occasionally be due to the presence of some other organism or combination of organisms, and the presumption that the reaction is due to coliform organisms has to be confirmed. The proportion of false positive reactions obtained depends both on the bacterial flora of the water under examination and on the medium used.

By the inoculation of suitable volumes of water into a number of tubes, an estimate of the number of coliform organisms present in a given volume of water can be obtained from statistical tables. Tables showing the most probable number of coliform organisms in the original sample for various combinations of positive and negative results are given in Annex 2.

In the past a variety of different media have been used in different countries for the presumptive coliform test. Much work on chemically defined media has been carried out in the past 15 years, 19, 32, 39, 40, 48, 75, 77, 90, 91 and it is now possible to recommend that MacConkey broth 15, 26, 89 with bromcresol purple as an indicator 89 and a standardized concentration of bile salts 12 or a glutamate medium—incubated at 37°C for up to 48 hours—should be used for these tests. Several glutamate media are in use, 19, 75, 77, 91 but recent comparisons 75, 77 indicate that the improved formate lactose glutamate medium, originally described by Gray, 40 but with the minerals modified, 26, 77 is the most generally satisfactory.

Confirmatory tests. The presumptive test should be followed by at least a rapid confirmatory test for coliform organisms and E. coli, the most suitable being the subculture of each presumptive positive tube into two tubes of brilliant green bile broth, 3, 15, 63 lactose-ricinoleate broth, 76 or

MacConkey broth,^a one of which should be incubated at 37° C for up to 48 hours for confirmation of the presence of coliform organisms, and the other incubated at 44° C and inspected after 6 and then after 24 hours⁸⁸ to decide whether or not *E. coli* is present.

Further confirmation of the presence of *E. coli*, if desired, can be obtained by testing for indole production at 44°C. Where complete confirmation is necessary, presumptive positive tubes can be plated on to a solid medium, such as lactose agar, Endo medium, eosin methylene-blue agar or Mac-Conkey agar, and individual colonies picked off for identification by the indole, and citrate utilization tests,^{3, 15, 26, 72} and by testing for fermentation of lactose at 37°C and 44°C.

Volume of water to be examined. At least 100 ml of water are required for bacteriological examination. The volumes to be used in tests in liquid media will depend on the quality of the water to be examined, and the series to be used in a particular instance will depend on the bacteriologist's experience with that water. With waters expected to be of good quality, one 50-ml volume and five 10-ml volumes would be suitable, whereas with waters of doubtful quality one 50-ml, five 10-ml and five 1-ml quantities could be used. With heavily polluted waters, the original water may have to be diluted by a factor of 100, 1000, or even more, in order to obtain some negative reactions in the series put up, and thus obtain a finite figure for the MPN. Whatever the series used, the volumes of water in the individual tubes and the number of tubes containing each volume of water should be such that an estimate of the MPN of coliform organisms present in 100-ml of the original water can be obtained from statistical tables.

(b) Membrane filtration method

The alternative method of counting coliform organisms in water is by filtering a measured volume of the sample through a membrane composed of cellulose esters or certain other substances. All the bacteria present are retained on the surface of the membrane and, by incubating the membrane face upwards on suitable media and at the appropriate temperatures and then counting the colonies that develop on the surface of the membrane, it is possible to obtain within a total incubation time of 18 hours direct presumptive coliform counts and direct *E. coli* counts that do not depend on the use of probability tables. Counts on membranes are, however, subject to statistical variations and replicate counts of the same water sample will not, in general, show the same number of organisms (for confidence limits see *The Bacteriological Examination of Water Supplies*²⁶).

^{*} If MacConkey broth is used for confirmatory tests, it is recommended that presumptive positive tubes derived from chlorinated waters should be plated on to a solid medium to confirm the presence of coliform organisms, since false reactions in MacConkey broth, both at 37°C and at 44°C, may be caused by spore-bearing anaerobic organisms.⁵⁹

Neither spore-bearing anaerobes, which may be a cause of false presumptive reactions in MacConkey broth, nor mixtures of organisms, which may cause false presumptive reactions in any liquid medium, cause false results on membranes. It is, however, not possible to detect gas production on a membrane.

Filtration apparatus and outline of technique. Essentially the filtration apparatus consists of a porous carbon or sintered glass disc supported in silicone rubber gaskets fitted in a base to which can be clamped a cylindrical funnel graduated at 50 ml and 100 ml. The membrane filter is supported on the porous disc. For filtration, the filter-holding assembly is mounted on a filter-flask with a side arm that can be connected to an electric vacuum pump, a filter pump operating on water pressure. or a simple hand-operated aspiration pump. After a measured volume of water has been filtered through the membrane under suction, this is removed and placed. face upwards, on a suitable solid medium in a Petri dish or on a pad soaked in liquid medium in a Petri dish. Descriptions and illustrations of the apparatus and its method of use are given in Standard Methods for the Examination of Water and Wastewater³ and in The Bacteriological Examination of Water Supplies. 26 Details of the sterilization of the apparatus and of the membranes, the media that can be used, and the incubation procedure are also given in these two publications. Separate membranes and different incubation procedures are required for examinations for total coliform organisms and for E. coli.

After incubation the membranes should be examined with a hand lens under good lighting. The appearance of the colonies will depend on the medium used, but all colonies of the appropriate appearance should be counted irrespective of size. If necessary, individual colonies can be transferred from the membranes into liquid media for confirmation, or on to a solid medium from which colonies can be taken for full confirmatory tests.

Volume of water to be examined. The coliform count and the E. colicount are made for separate volumes of water. If the samples are expected to contain less than 100 coliform organisms in 100 ml, the filtration of 100 ml for each test is necessary. The volumes of polluted samples should be so chosen that the number of colonies to be counted on the membranes lies between 10 and 100. When the volume to be filtered is less than 10 ml, the sample should be diluted with sterile dilution water so that a minimum of 10 ml is filtered.

Advantages and disadvantages of membrane filtration method. The outstanding advantage of the membrane filtration method is the speed with which results can be obtained, including an E. coli count. This enables rapid corrective action to be taken when required; it also enables the waterworks plant to be put back into service more quickly when a negative result is obtained. In the laboratory, there is a saving in technical labour and in the

amount of media and glassware required. It is also possible, where it is not practicable for a sample to be taken immediately to a fully equipped laboratory, for a sample to be filtered through a membrane at the site of collection or in a local laboratory with limited facilities and sent on a transport medium to a fully-equipped laboratory for examination. Reference will be made to such procedures in Section 7, which deals with sampling.

Membranes are unsuitable for waters of high turbidity in association with low counts of coliform organisms since, in such instances, the membrane will become blocked before sufficient water can be filtered. Membranes are also unsuitable for water containing few coliform organisms in the presence of many non-coliform organisms capable of growing on the media used, since the non-coliform organisms are then liable to cover the whole membrane and interfere with the growth of the coliform organisms. If non-gas-producing lactose-fermenting organisms predominate in the water, membranes will be unsuitable because of the high proportion of false positive results obtained.

Some of the original membrane techniques require a change of medium after the first few hours of incubation. In some of the newer techniques this has been replaced by a change of temperature. This can be effected either by transferring containers of membranes from one incubator to another or by using special apparatus to provide an automatic change of temperature at the appropriate time.

Since the results given by the membrane filtration method are not necessarily the same as those obtained by the multiple tube method, it is essential that, before adopting membrane filtration as a routine procedure in any laboratory or for any particular water supply, an adequate series of parallel tests by the two methods should be carried out in order to establish that the membrane filtration method gives results corresponding to those given by the multiple tube method; the latter should be regarded as the method of reference.

2.2.2 Detection of faecal streptococci and anaerobic spore-forming organisms

On those occasions on which it is considered desirable to supplement the examination for coliform organisms and *E. coli* by examination for faecal streptococci or anaerobic spore-forming organisms, the following methods can be recommended.

Faecal streptococci. Methods commonly used for the detection and estimation of the number of faecal streptococci are:

(1) The inoculation of multiple portions of water into tubes of glucose azide broth.^{26, 41} The inoculated tubes are then incubated at 37°C for 72 hours. As soon as acidity is observed, a heavy inoculum is subcultured into further tubes of glucose azide broth, and incubated at 45°C for 48 hours; all tubes showing acidity at this temperature contain faecal streptococci.²⁶

- (2) The inoculation of multiple portions of water into tubes of buffered azide-glucose-glycerol broth (BAGG medium). The inoculated tubes are then incubated at 45°C for up to 48 hours. Growth with the production of acid is almost definite evidence of the presence of faecal streptococci, but this can be confirmed by the microscopical examination of Gram-stained films from tubes showing acid production.³⁵
- (3) A membrane filtration technique.^{26, 83} The technique is essentially the same as that described in Section 2.2.1 for the membrane filtration method of counting coliform organisms, except that a different medium and a different incubation procedure are used. After filtration, the membrane is placed on a well-dried plate of glucose-azide agar.⁸³ This is then incubated at 37°C for four hours and then at 44°C or 45°C for 44 hours.²⁶ All red or maroon colonies are counted as faecal streptococci.⁹³

Anaerobic spore-forming organisms. Methods commonly used for the detection and estimation of the number of spores of Cl. perfringens in water are:

- (1) The inoculation of multiple portions of water—previously heated at 75°C for 10 minutes to destroy non-spore-forming organisms—into differential reinforced clostridial medium (DRCM)³⁸ in screw-capped bottles. The bottles should be filled up if necessary so as to leave only a small air space. They should then be incubated at 37°C for 48 hours. A positive reaction will be shown by blackening of the medium due to reduction of the sulfite and precipitation of ferrous sulfide. Any clostridium may produce this reaction. A loopful from each positive bottle should be subcultured into a tube of litmus milk ²⁶ that has been freshly steamed and cooled. The tubes should then be incubated at 37°C for 48 hours. Those containing Cl. perfringens will produce a "stormy-clot" in which the milk is acidified and coagulated and the clot disrupted by gas.
- (2) A sulfite-reduction method using a solid medium.^{15, 27} Volumes of water are mixed with the melted medium in tubes or in Petri dishes. When the medium has set, it is incubated at 37°C or 44°C for 24 to 48 hours. The black colonies in the depth of the medium are counted. The presence of black colonies of over 3 mm in diameter indicates contamination with *Cl. perfringens.*⁶¹

Some workers prefer to heat the water to 75-80°C for 10 minutes before adding it to the medium in order to destroy non-sporing organisms.

2.3 Standards of Bacterial Quality Applicable to Supplies of Drinking-Water

Some supplies of drinking-water are chlorinated or otherwise disinfected before being distributed; others are not. There does not, however, appear

to be any logical reason for setting different bacteriological standards for supplies that are disinfected and for those that are not so treated. Efficient chlorination yields a water that is virtually free from coliform organisms, and, if supplies that are distributed without chlorination or other form of disinfection cannot be kept up to the bacteriological standard that can reasonably be expected of disinfected water, steps should be taken to chlorinate this water or disinfect it in some other way.

It would seem to be reasonable, however, to make a distinction between water from supplies distributed by means of a piped distribution network and water from supplies not so distributed, since it may not be practicable to keep the latter up to the standards proposed for supplies distributed through a piped network.

In the consideration of bacterial standards for supplies of drinking-water distributed through a piped network, it must be remembered that the quality of the water in the distribution system itself may not be the same as that of the water entering the system, since a water that is perfectly satisfactory when it enters the system may undergo some deterioration before it reaches the consumer's tap. Two points should be stressed: (1) the necessity of maintaining a sufficiently high pressure throughout the whole distribution system to prevent contamination from entering the system along the length of the mains by back-syphonage; and (2) the necessity for every distribution system to have available a means of chlorination to deal with accidental pollution, which is always a possibility.

2.3.1 Piped supplies

2.3.1.1. Water entering the distribution system

- (a) Chlorinated or otherwise disinfected supplies. Efficient treatment, culminating in chlorination or some other form of disinfection, should yield a water free from any coliform organisms, however polluted the original raw water may have been. In practice this means that it should not be possible to demonstrate the presence of coliform organisms in any sample of 100 ml. A sample of the water entering the distribution system that does not conform to this standard calls for an immediate investigation into both the efficacy of the purification process and the method of sampling. It is important, however, in testing chlorinated waters, that presumptive positive tubes should always be subjected to appropriate confirmatory tests.
- (b) Non-disinfected supplies. Where supplies of this sort exist, no water entering the distribution system should be considered satisfactory if it yields E. coli in 100 ml. If E. coli is absent, the presence of not more than 3 coliform organisms per 100 ml may be tolerated in occasional samples from established non-disinfected piped supplies, provided that they have been regularly and frequently tested and that the catchment area and

storage conditions are found to be satisfactory. If repeated samples show the presence of coliform organisms, steps should then be taken to discover and, if possible, remove the source of the pollution. If the number of coliform organisms increases to more than 3 per 100 ml, the supply should be considered unsuitable for use without disinfection.

2.3.1.2 Water in the distribution system

Water that is of excellent quality when it enters the distribution system may undergo some deterioration before it reaches the consumer's tap. Just as much deterioration may occur in the distribution system of a chlorinated supply in which there is little or no residual chlorine in the water reaching the consumer as in that of a non-disinfected supply, so that in this respect the two are on the same footing. Coliform organisms may gain access to the water in the distribution system from booster pumps, from the packing used in the jointing of mains, or from washers on service taps. In addition, the water in the distribution system may become contaminated from outside. for example, through cross-connexions, back-syphonage, defective service reservoirs and water tanks, damaged or defective hydrants or washouts, or through inexpert repairs to domestic plumbing systems. Although coliform organisms derived from tap washers or the jointing material of mains may be of little or no sanitary significance, the entry of contamination into the water in the distribution system from outside is at least as potentially dangerous as the distribution of originally polluted and insufficiently treated water.

Ideally, all samples taken from the distribution system, including consumers' premises, should be free from coliform organisms. In practice, this standard is not always attainable, and the following standard for water collected in the distribution system is therefore recommended:

- (1) Throughout any year, 95% of samples should not contain any coliform organisms in 100 ml.
 - (2) No sample should contain E. coli in 100 ml.
- (3) No sample should contain more than 10 coliform organisms per 100 ml.
- (4) Coliform organisms should not be detectable in 100 ml of any two consecutive samples.

If any coliform organisms are found the minimum action required is immediate re-sampling. The repeated finding of 1 to 10 coliform organisms in 100 ml, or the appearance of higher numbers in individual samples suggests that undesirable material is gaining access to the water and measures should at once be taken to discover and remove the source of the pollution.

The presence of any coliform organisms in a piped supply should always

give rise to concern, but the measures—apart from the taking of further samples—that may be considered advisable in order to safeguard the purity of the water supplied to consumers will depend on local conditions.

The degree of contamination may be so great that action should be taken without delay, even before the result of the examination of a repeat sample is known. This is a matter for decision by those who know the local circumstances and who are responsible for safeguarding the health of the community.

2.3.2 Individual or small community supplies

Where it is economically impracticable to supply water to the consumers through a piped distribution network and where reliance has to be placed on individual wells, bores, and springs, the standard outlined above may not be attainable. Such a standard should, however, be aimed at and everything possible should be done to prevent pollution of the water. By relatively simple measures, such as the removal of obvious sources of contamination from the catchment area and by attention to the coping, lining, and covering, it should be possible to reduce the coliform count of water from even a shallow well to less than 10 per 100 ml. Persistent failure to achieve this, particularly if *E. coli* is repeatedly found, should, as a general rule, lead to condemnation of the supply.

3. Virological Examination

It is theoretically possible that virus disease can be transmitted by water free from coliform organisms, but conclusive evidence that this has occurred is lacking.

None of the generally accepted sewage treatment methods yields virusfree effluent. Although a number of investigators have found activated sludge treatment to be superior to trickling filters 73 from this point of view, it seems possible that chemical precipitation methods will prove to be the most effective. With increasing use of reclaimed water as raw water for drinking purposes, the importance of removing organic substances by precipitation as a direct means of removing viruses as well as a means of increasing the efficiency of disinfection must be stressed.

Viruses can be isolated from raw water and from springs. Enteroviruses, reoviruses, and adenoviruses have been found in water, the first named being the most resistant to chlorination. If enteroviruses are absent from chlorinated water, it can be assumed that the water is safe to drink. Some uncertainty still remains about the virus of infectious hepatitis, since it has not so far been isolated, but in view of the morphology and resistance of enteroviruses it is likely that, if they have been inactivated, hepatitis virus will have been inactivated also.

An exponential relationship exists between the rate of virus inactivation and the redox potential. A redox potential of 650 mV (measured between platinum and calomel electrodes) 58 will cause almost instantaneous inactivation of even high concentrations of virus. Such a potential can be obtained with even a low concentration of *free* chlorine, but only with an extremely high concentration of combined chlorine. 59, 60 This oxidative inactivation may be achieved with a number of other oxidants also, e.g., iodine, ozone, and potassium permanganate, but the effect of the oxidants will always be counteracted if reducing components, which are mainly organic, are present. As a consequence, the sensitivity of viruses towards disinfectants will depend on the milieu just as much as on the particular disinfectant used.

Thus, in a water in which *free* chlorine is present, active viruses will generally be absent if coliform organisms are absent. In contrast, because the difference between the resistance of coliform organisms and of viruses to disinfection by oxidants increases with increasing concentration of reducing components, e.g., organic matter, it cannot be assumed that the absence of viable coliform organisms implies freedom from active viruses under circumstances where a free chlorine residual cannot be maintained. For this reason

and because sedimentation and slow filtration in themselves may contribute to the removal of viruses from water, the importance of such treatments must be stressed.

In practice, 0.5 mg/l of *free* chlorine for one hour is sufficient to inactivate virus, even in water that was originally polluted; 0.4 mg/l of free ozone for 4 minutes has been found to inactivate virus, 17, 18 but somewhat more rigorous treatment would perhaps be desirable because the resistance of hepatitis virus to ozone is unknown.

If not even one plaque-forming unit (PFU) of virus can be found in 1 litre of water, it can reasonably be assumed that the water is safe to drink. It would, however, be necessary to examine a sample of the order of 10 litres to obtain a proper estimation of the PFU's at this level. Such examinations cannot be made in ordinary control laboratories, but there should be at least one laboratory in each country or region capable of carrying out virus examinations and also of pursuing further research on this subject.

4. Biological Examination

Biological examination is of value in determining the causes of objectionable tastes and odours in water and controlling remedial treatments, in helping to interpret the results of various chemical analyses, and in explaining the causes of clogging in distribution pipes and filters. In some instances, it may be of use in demonstrating that water from one source has been mixed with that from another.

The biological qualities of a water are of greater importance when the supply has not undergone the conventional flocculation and filtration processes, since increased growth of methane-utilizing bacteria on biological slimes in pipes may then be expected, and the development of bryozoal growths such as *Plumatella* may cause operational difficulties.

Some of the animalcules found in water mains may be free-living in the water, but others such as *Dreissena* and *Asellus* are more or less firmly attached to the inside of the mains. Although these animalcules are not themselves pathogenic, they may harbour pathogenic organisms or viruses in their intestines, thus protecting these pathogens from destruction by chlorine.

Chlorination, at the dosages normally employed in waterworks, is ineffective against certain parasites, including amoebic cysts; they can be excluded only by effective filtration or by higher chlorine doses than can be tolerated without subsequent dechlorination. Amoebiasis can be conveyed by water completely free from enteric bacteria; microscopic examination after concentration is therefore the only safe method of identification. Strict precautions against back-syphonage and cross-connexions are required if amoebic cysts are found in a distribution system containing treated water.

The cercariae of schistosomiasis can be detected by similar microscopic examination, but there is, in any case, no evidence to suggest that this disease is normally spread through piped water supplies.

The cyclops vector of the embryos of *Dracunculus medinensis*—which causes dracontiasis or Guinea-worm disease—can be found in open wells in a number of tropical countries. They are identifiable by microscopic examination. Such well supplies are frequently used untreated, but the parasite can be relatively easily excluded by simple physical improvements in the form of curbs, drainage, and apron surrounds. For further information on biological examination and on parasites spread by water, see *Water Treatment and Examination*.⁴²

5. Radiological Examination

5.1 Levels of Radioactivity in Drinking-Water

The identification of the critical path by which released radioactivity may reach the group of people likely to receive the highest dose is at present of great concern. Nevertheless, operational standards for the population at large, in the form of concentration levels, should be used for routine surveillance procedures.

The radioactivity levels given below are based on the recommendations of the International Commission on Radiological Protection (ICRP). They have been derived from the maximum permissible concentrations in water (MPC_w) for occupational exposure to the respective nuclides ⁴⁵. ⁴⁶. ⁴⁷ by multiplying the figures for the gonadal or whole body exposure by a factor of 1/100, so as to make them applicable to consumers of drinkingwater belonging to the "total population".

The following levels are proposed:

Gross alpha activity 3 pCi/l Gross beta activity 30 pCi/l

These levels are applicable to the mean of all the activity measurements obtained during a 3-month period. However, when significant radioactive contamination of the water supply is suspected, individual water samples should be radioanalysed. Furthermore, single samples with unexpectedly high values should be investigated without delay.

The methods for the analysis of gross alpha and gross beta activities should be selected in the light of local conditions and in collaboration with the appropriate authorities. Procedures for the measurement of activity levels of specific radionuclides have been published.⁹⁶

Radioactivity in drinking-water should be kept to a minimum, and it is therefore recommended that radioactive wastes should not be discharged indiscriminately into sources that are to be used for supplies of drinking-water. However, the values given include naturally occurring radioactivity as well as any radioactivity that may have reached the water as a result of radioactive fallout or the use of nuclear energy. They represent a level below which water can be considered potable without undergoing more complex radiological examination, but the following comments should be borne in mind:

Alpha activity. Before the analysis is started, the activity of ²²²Rn and ²²⁰Rn should be eliminated by proper aeration of the water sample. The

contribution of the short-lived daughter products of these isotopes can be excluded by allowing them to decay and then measuring the activity.

Alpha activity of 3 pCi/l or less is acceptable, and no further examination is necessary, even if all of it is due to ²²⁶Ra. However, if the activity exceeds 3 pCi/l, radioanalysis is required in accordance with the following procedure:

Gross alpha activity in pCi/l*	
3 to 10	

More than 10

More than 1000

Examination procedure

- 1. The contribution of the short-lived daughter products of ²²²Rn and 220 Rn should be excluded. If the residual activity still exceeds 3 pCi/1, then:
- 2. Radioanalysis for ²²⁶Ra should be performed. If ²²⁶Ra activity is below 3 pCi/1, no further examination is necessary, but if it exceeds 3 pCi/1 the results should be referred to the appropriate health authorities for further investigations.

Comprehensive radioanalysis is necessary. The results obtained should be referred to the appropriate health authority for further investigations.

Detailed radiological examination (radiochemical determination of ⁹⁰Sr and gammaspectroscopy) is necessary. The results should be

Beta activity. Beta activity of 30 pCi/l or less is acceptable and no further examination is necessary, even if all of it is due to ⁹⁰Sr. However, if the activity exceeds 30 pCi/l, radioanalysis is required in accordance with the following procedure:

Gross beta activity in pCi/la	Examination procedure			
30 to 100	1. The ⁴⁰ K contribution should be excluded. If the residual activity still exceeds 30 pCi/1, then:			
	2. Radioanalysis for 90Sr should be performed. If 90Sr activity is			
	below 30 pCi/1, no further examination is necessary, but if it exceeds			
	30 pCi/1, the results should be referred to the appropriate health			
	authority for further investigations.			
100 to 1000	1. The ⁴⁰ K contribution should be excluded.			
	2. Radioanalysis for ⁹⁰ Sr and ¹²⁹ I should be performed. If ⁹⁰ Sr activity is below 30 pCi/1 and ¹²⁹ I activity is below 100 pCi/1, no further examination is necessary. If these values are exceeded, the results should be referred to the appropriate health authority for further investigations.			
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referred to the appropriate health authority for further investigations.

Where it is suspected that ³H may have reached the water from atmospheric fallout or in effluent from nuclear power stations, a special examination for this radionuclide should be carried out. It cannot be measured by the techniques used in gross beta determinations, and special instruments such as liquid scintillation spectrometers are required. If ³H is detected at levels

of 1000 pCi/l or more, the appropriate health authorities should be consulted.

^a Mean of all analyses during a 3-month period.

6. Physical and Chemical Examination

6.1 Purpose

Chemical analysis has a wide range of uses in the investigation of water supplies. This publication, however, is concerned primarily with the protection of users of water supplies from dangers to health. With this in mind, attention is therefore mainly directed to the detection and estimation of toxic chemical substances, to pesticides—including insecticides, herbicides, and fungicides—to specific chemical substances that may affect health, and to characteristics affecting the acceptability of water for domestic use.

Whereas frequent bacteriological examination is required for the hygienic control of drinking-water supplies, chemical examination is required much less frequently; the collection of samples and the frequency of sampling are discussed more fully in Section 7.

With the object of encouraging greater uniformity in the methods of carrying out the more general examination of water for physical, chemical, and aesthetic characteristics, a list is given of the tests that are commonly performed, and of a number of recommended methods of carrying them out.

6.2 Toxic Chemical Substances

A number of chemical substances, if present in certain concentrations in supplies of drinking-water, may constitute a danger to health. The limits for these substances should be related to the daily intake of drinking-water, and values are given in Table 1 based on an assumed average daily intake of 2.5 litres by a man weighing 70 kg. Tentative limits for some toxic substances have been worked out on the basis of the available toxicological data and the body burden from other sources—food and air for example. The intake of these substances from the environment cannot be avoided, but it may be possible to control that fraction of it associated with drinking-water. If the levels in drinking-water are high, their possible effect has to be considered in relation to the body burden resulting from other sources in the particular locality.

The tentative limits given in Table 1 should be considered in conjunction with the explanatory notes that follow; they will be subject to review from time to time as more information becomes available on the toxicity in drinking-water of the substances concerned.

TABLE 1. TENTATIVE LIMITS FOR TOXIC SUBSTANCES IN DRINKING-WATER

Substance	Upper limit of concentration	Methods of estimation
Arsenic (as As)	0.05 mg/l	(a) Polarographic estimation. ⁶¹ (b) Atomic absorption spectrophotometric method. ²⁶ , ⁶⁶ , ⁷⁹
		(c) Use of Gutzeit generator. ³ , ¹⁶ , ⁴³ , ⁸⁰
Cadmium (as Cd)	0.01 mg/l	Dithizone method. ³
Cyanide (as CN)	0.05 mg/l	Can be estimated by means of a number of methods of which the following are generally in use and are equally satisfactory:
		(a) Titration with silver nitrate in dilute ammoniscal solution using diphenyl carbazide as an adsorption indicator. ⁶⁷
!		(b) Colorimetric method: conversion of cyanide to either cyanogen chloride or cyanogen bromide, and coupling with a suitable aromatic amino compound, such as dimedone,54 pyrazolone3 or sulfanilic acid.62
!		(c) Colorimetric method: yellow ammonium sulfide converts cyanide to thiocyanate in slightly alkaline solution; the thiocyanate reacts quantitatively with ferric iron to form coloured ferric thiocyanate. ³³ , ⁹⁹
Leed	0.1 mg/l	(a) Polarographic estimation. ⁵¹
(as Pb)		(b) Atomic absorption spectrophotometric method. 25, 79
	1	(c) Colorimetric methods. ³ , ¹⁶ , ³⁷ , ⁴³ , ⁸⁰
Mercury (total (as Hg)	0.001 mg/l	Neutron activation analysis. ⁴⁴ Atomic absorption. ³⁴
Selenium (as Se)	0.01 mg/l	Colorimetric method using gum arabic solution, hydroxylamine hydrochloride, sulfur dioxide and concentrated hydrobromic acid. ³ , ¹⁶

Explanatory Notes to Table 1

Arsenic. Figures higher than that quoted are found in a number of Latin American countries and levels up to 0.2 mg/l are not known to have caused difficulties. Some epidemiological studies have suggested that arsenic is carcinogenic but no real proof of the carcinogenicity to man of arsenic in drinking-water has been forthcoming. It would seem wise to keep the level of arsenic in drinking-water as low as possible.

Cadmium. The results of animal studies suggest that very small amounts of cadmium can produce nephrotoxic and cardiovascular effects. The repro-

ductive organs of animals are specifically affected after parenteral administration of very small amounts of cadmium salts. The toxicity of cadmium may depend on the presence of other trace elements, for example, zinc and selenium. Cadmium may be derived from natural or industrial sources, or from cadmium compounds used in the production of plastic water pipes. The level proposed is the lowest concentration that can be conveniently measured.

Cyanide. In the consideration of limits for cyanide in drinking-water, it was noted that the acceptable daily intake (ADI) for man of hydrogen cyanide residues in some fumigated foods has been established at 0.05 mg per kg of body-weight. The amount of cyanide found in water is small and the level given in Table 1 is such as to ensure that the source of water is not too highly contaminated by industrial effluents and also that the treatment of the water has been adequate, since cyanide is readily destroyed by conventional treatment processes.

Lead. The tentative level for this substance has been increased from 0.05 mg/l, as given in the second edition of the International Standards, to 0.10 mg/l because this level has been accepted in many countries and the water has been consumed for many years without apparent ill effects. It is difficult to reach a lower level in countries where lead pipes are used. The danger of plumbosolvency can be reduced by appropriate treatment of soft waters. Lead compounds are used as stabilizers in some plastic pipes and may leach out. The total body-load of lead should be reduced to a level at which equilibrium between absorption and elimination can be maintained. There is no direct evidence that tissue accumulations at existing levels of intake are harmful or potentially harmful to man. The maximum acceptable load of lead from food and beverages has been tentatively placed at 0.005 mg per kg per day. a

Mercury. Toxicological data indicate that mercury is a cumulative poison. No acceptable daily intake for man can be estimated on the basis of the information at present available. The figure given is related to levels found in natural water.

Selenium. Selenium intoxication in man produces rather ill-defined symptoms but can cause well-defined illness in animals; selenium is known, however, to be an essential trace element for some species. Selenium compounds have been shown to protect the rat against the toxic effects of cadmium and mercuric cations.⁷¹ Because of the geological association of selenium with sulfur, a limit has been set for it in Table 1.

In addition to the substances listed, others such as barium, beryllium, cobalt, molybdenum, nitrilotriacetate, thiocyanate, tin, uranium, and vanadium should be controlled in drinking-water. Insufficient information is at present available to enable tentative limits to be given for these substances.

See Wld Hith Org. techn. Rep. Ser., 1967, No. 373, 15.

When chemicals—particularly new chemicals—are used in water treatment, care should be taken to ensure that their use does not give rise to a toxicity hazard. If polymeric coagulant aids, such as polyacrylamide, are used in the treatment of water intended for drinking, it is necessary to ensure that any toxic components, e.g., monomer, are reduced to safe levels by insisting on an adequate specification of the chemicals used. Lists of approved coagulant aids with agreed specifications have been published in the USA by the Food and Drug Administration and in the United Kingdom by the Committee on Approval of New Substances in Treatment of Potable Water.

6.3 Pesticides

The term pesticides also includes insecticides, herbicides, and fungicides. Pesticides are kept under constant review by joint FAO/WHO meetings of experts. The concept of acceptable daily intake (ADI) serves as a guideline for the toxicological evaluation of pesticide residues, 97, 98 and on this basis a number of such residues were evaluated and re-evaluated in 1965–1970 and the findings published in FAO/WHO monographs. The ADI is based entirely on toxicological evidence. The acceptable daily intake of a chemical is defined as "the daily intake which, during an entire lifetime, appears to be without appreciable risk on the basis of all the known facts at the time". a

Although the ADI concept applies mainly to the evaluation of residues in food, the intake from other possible contaminated sources should also be taken into account. Experience up to now supports the general opinion that the residues of pesticides that may occur in community water supplies, mostly of the order of a few micrograms per litre, make only a minimal contribution to the total daily intake of pesticides for the population served.

Contamination of ground or surface water with pesticides may be the result of direct intentional application (e.g., in the control of aquatic weeds or insects), the discharge of industrial effluents or spray liquid residues, the accidental contamination of a surface source, or percolation or leaching out by rain from treated agricultural land. Such contamination should be prevented as far as possible because of the influence of pesticides on the water biocoenosis and the danger of accumulation in the food chain. For this reason, extensive protective measures for catchment areas, water supply streams, and underground water sources are recommended.

Knowledge of actual instances of contamination of drinking-water with pesticide residues and of the circumstances in which they have occurred is desirable. If it is necessary to use pesticides for intentional treatment of drinking-water sources, complete information must be available so that the risk to the quality of the water from residues can be evaluated and the

[•] FAO Agricultural Series, 1970, No. 84, p. 39; Wid Hith Org. techn. Rep. Ser., 1970, No. 458, p. 39.

influence of such residues on the water biocoenosis can be estimated. The conditions under which residues disappear from water sources and the efficiency of water purification methods must be known before permissible limits for pesticide residues in drinking-water can be proposed.

Very low concentrations of some pesticides cause organoleptic changes in the water, so that it is not acceptable to the consumer, irrespective of any known toxicity hazard. Conventional methods of water treatment do not remove all pesticide residues, but special treatment processes can remove some of them.

There should be at least one centre in each country or region capable of carrying out investigations into pesticide residues in drinking-water.

6.4 Specific Chemical Substances that may Affect Health

6.4.1 Fluorides

Fluorides occur naturally in many public water supplies and, if present in excessive amounts, may give rise to dental fluorosis in some children. When present in much higher concentrations, they may eventually cause endemic cumulative fluorosis with resulting skeletal damage in both children and adults.

In the assessment of the safety of a water supply with respect to the fluoride concentration, the total daily fluoride intake by the individual must be considered. Apart from variations in climatic conditions, it is well known that, in certain areas, fluoride-containing foods form an important part of the diet. These facts should be borne in mind in deciding the concentration of fluoride to be permitted in drinking-water.

Fluorides are also regarded as an essential constituent of drinking-water, particularly with regard to the prevention of dental caries in children. If the fluoride concentration in the drinking-water of a community is less than 0.5 mg/l, the incidence of dental caries is likely to be high. To prevent the development of dental caries in children, a number of communal water supplies are fluoridated to bring the fluoride concentration within the range shown in Table 2.

The figures in Table 2, which are adapted from those given in the 1962 edition of *Public Health Service Drinking Water Standards*,⁸⁶ give the recommended control limits for the concentration of fluorides (expressed as F) in drinking-water for various ranges of the annual average of maximum daily air temperatures. The temperatures used with Table 2 should be based on data obtained over a minimum of five years.

Annual average of maximum daily air temperature in *C	Recommended control limits for fluorides (as F) in mg/!		
	Lower	Upper	
10 –12	0.9	1.7	
12.1-14.6	0.8	1.5	
14.7-17.6	0.8	1.3	
17.7–21.4	0.7	1.2	
21.5-26.2	0.7	1.0	
26.3-32.6	0.6	0.8	

TABLE 2. RECOMMENDED CONTROL LIMITS FOR FLUORIDES IN DRINKING-WATER

Methods recommended for the estimation of fluorides in water are: (a) the colorimetric method using zirconium-alizarin reagent (colour, turbidity, chlorine and phosphates must be removed or the sample must be distilled before examination);^{3. 43} (b) an electrochemical method using the Orion electrode;²¹ and (c) the SPADNS colorimetric method.³

6.4.2 Nitrates

In some circumstances, nitrates have been shown to present a health hazard to infants, and possibly older children, if they are present in drinking-water at concentrations greater than 45 mg/l (expressed as NO₃) because, after reduction to nitrite, they may give rise to methaemoglobinaemia. In view of the small quantity of water consumed by infants directly as drinking-water, or indirectly as prepared food, it should not be difficult to find an alternative source of water of low nitrate content for this vulnerable section of the population. Concern has recently been expressed over the possibility of nitrosamine formation in vivo. Nitrosamines may arise as the products of the reaction between ingested nitrites, some of which may also be formed by the action of gut bacteria on ingested nitrates from various sources including water, and on secondary or tertiary amines present in food. Because of their carcinogenic potential, nitrosamines are a possible hazard to the health of man. It may eventually become necessary to reduce the level of nitrates in water if it is found that this source makes a significant contribution to the hazard to human health arising from nitrosamines. From the toxicological point of view, the considerations applicable to nitrates also apply to any nitrites present in drinking-water.

Methods recommended for the estimation of nitrates in water are: (a) the phenoldisulfonic acid method; $^{3, 16, 22}$ (b) the brucine method; $^{3, 22, 89, 99}$ (c) reduction with a zinc-copper couple followed by Nesslerization, either directly or after distillation; 48 and (d) the salicylic acid method. 37

6.4.3 Polynuclear aromatic hydrocarbons

Some polynuclear aromatic hydrocarbons (PAH) are known to be carcinogenic and their presence in water supplies and the consequent potential hazard to man have been noted. The concentration of six representative PAH compounds (fluoranthene, 3,4-benzfluoranthene, 11,12-benzfluoranthene, 3,4-benzpyrene, 1,12-benzperylene, and indeno [1,2,3-cd] pyrene) should therefore not, in general, exceed 0.2 μ g/l. This concentration can be measured by means of a modified liquid-liquid extraction method. Higher concentrations indicate remaining pollution and insufficient treatment.

While routine examination of ground water for PAH is not necessary, treated surface water should be examined periodically. Consequently, at least one centre capable of carrying out investigations on PAH in drinking-water is desirable in each country or region. Further research into their presence and significance in drinking-water is required.

6.5 Substances and Characteristics Affecting the Acceptability of Water

Certain substances present in water and certain characteristics of the water, although not constituting a hazard to the health of the people using it, may affect its acceptability as a domestic supply. A list of such substances and characteristics is given in Table 3, together with an indication of the undesirable effects they may produce, figures—where appropriate—for the highest desirable and maximum permissible levels recommended, and a number of recommended methods for use in detection and estimation. All the methods given in the twelfth edition of Standard Methods for the Examination of Water and Wastewater³ can be regarded as satisfactory.

6.6 General Examination for Physical, Chemical and Aesthetic Characteristics of Water

Although this publication is concerned primarily with the hygienic control of water supplies, it has been thought wise to include a list of the tests commonly carried out to determine the physical, chemical and aesthetic characteristics of water, and to indicate a number of recommended methods for conducting them.

Many of the tests that are about to be considered are not directly concerned with the safety of the water for supply to the public, but with its pleasantness for use, its suitability as a piped supply, and the waterworks control of any treatment that is applied to the water. Considerable variations in the amount of organic matter, albuminoid nitrogen, nitrite and phosphate—as well as in the amount of ammonia and nitrate—should, however, be taken as in indication of the possibility of pollution. In some circumstances, the examination of a sample from the distribution system for a single

TABLE 3. SUBSTANCES AND CHARACTERISTICS AFFECTING THE ACCEPTIBILITY OF WATER FOR DOMESTIC USE

Substance or characteristic	Undesirable effect that may be produced	Highest desirable level	Maximum permissible level	Methods of estimation
Substances causing discoloration	Discoloration	5 units*	50 units a	(a) Comparison with platinum-cobalt standards.
			ļ	(b) Comparison with standardized glass discs. 3, 43
Substances causing odours	Odours	Unobjectionable	Unobjectionable	Test cold and when heated.
1			ı	Test at several dilutions. ³
Substances causing tastes	Tastes	Unobjectionable	Unobjectionable	Test at temperature not lower than 16°C; test at several dilutions. ⁵⁹
Suspended matter	Turbidity Possibly gastrointestinal irri- tation	5 units ⁵	25 units®	(a) Turbidimetric methods, either visual or photo- electric. ³ , ³⁷ , ⁴³
				(b) Comparison with standards in bottles.3
Total solids	Taste Gastrointestinal irritation	500 mg/l	1500 mg/l	Gravimetric, after evaporation and drying. ³
pH range	Taste Corrosion	7.0 to 8.5	6.5 to 9.2	(a) By means of an electric pH meter with glass electrodes. ³ , ³⁷ , ⁴³
				(b) Use of indicator solutions in a comparator; useful for preliminary estimations in the field. ³⁷ , ⁴³
Anionic detergents	Taste and foaming	0.2 mg/l	1.0 mg/l	Methylene-blue extraction method.3,67
Mineral oil	Taste and odour after chlori- nation	0.01 mg/l	0.30 mg/l	Gas chromatography.6

[•] On the platinum-cobalt scale.

^b Turbidity units.

^{*} Different reference substances are used in different countries.

TABLE 3 (continued)

Substance or characteristic	Undesirable effect that may be produced	Highest desirable level	Maximum permissible level	Methods of estimation
Phenolic compounds (as phenol)	Taste, particularly in chlori- nated water	0.001 mg/l	0.002 mg/l	Colorimetric methods, preferably after distillation: (a) Using diszotized sulfanilic acid. ⁴³ (b) Indophenol method. ⁴³ (c) 4-Aminoantipyrine method. ³ (d) Using p-nitroaniline. ³⁷
Total hardness	Excessive scale formation	2mEq/a,b (100 mg/l CaCOs)	10mEq/l (500 mg/l CaCO ₃)	(a) Versenate (EDTA) method using Eriochrome Black T as an indicator. 3, 37, 43, 85 (see also reference 82) (b) By calculation from calcium and magnesium and other hardness-producing cations if present in significant amounts.3
Calcium (as Ca)	Excessive scale formation	75 mg/l	200 mg/i	(a) Versenate (EDTA) method using murexide as an indicator. ³ , ¹⁶ , ³⁷ , ⁴³ , ⁶⁵ (b) Volumetric method. Precipitate calcium as calcium oxalate, dissolve in sulfuric acid, and titrate with standard potassium permanganate solution. ³ , ¹⁶ , ³⁷ , ⁴³ (c) Gravimetric method. Precipitate calcium with ammonium oxalate. Ignite and weigh as calcium oxide. ³ , ¹⁶ , ³⁷ (d) Atomic absorption spectrophotometric method. ²⁶ , ⁷⁹
Chloride (as Ci)	Taste; corrosion in hot-water systems	200 mg/l	600 mg/l	(a) Titration using standard silver nitrate solution and potassium chromate indicator. ³ , ¹⁶ , ³⁷ , ⁴³ (b) Colorimetric method. ⁷⁸ (c) Titration with mercuric nitrate at approximately pH 3.1. Diphenylcarbazone and bromphenol blue used as indicators. ¹⁶

[•] If the hardness is much less than this, other undesirable effects may be caused, for example, heavy metals may be dissolved out of pipes.
• ImEq/I of hardness-producing ion = 50 mg CaCOs/I = 5.0 French degrees of hardness = 2.8 (approx) German degrees of hardness = 3.5 (approx) English degrees of hardness.

TABLE 3 (continued)

Substance or characteristic	Undesirable effect that may be produced	Highest desirable level	Maximum permissible level	Methods of estimation
Copper (as Cu)	Astringent taste; discoloration and corrosion of pipes, fittings and utensils	0.05 mg/l	1.5 mg/l	(a) Atomic absorption spectrophotometric method. ²⁵ , ⁷⁹ (b) Colorimetric method using diethyl-dithiocarbamate. 16, 37, 43 (c) Cuprethol method. ³ (d) Bathocuproine method. ³
Iron (total as Fe)	Taste; discoloration; deposits and growth of iron bacteria; turbidity	0.1 mg/l	1.0 mg/l	Colorimetric methods: (a) Phenanthroline method. ³ , ¹⁶ , ⁸⁰ (b) Thiocyanate method. ⁴³ , ⁸⁰ (c) Bipyridyl method. ⁸⁵ , ⁸⁰ (d) Reduction of ferric salts and formation of an iron-dimethylglyoxime complex. ⁵⁶ , ⁹⁹ (e) Thioglycolic acid method. ⁸⁹
Magnesium (as Mg)	Hardness: taste: gastrointes- tinal irritation in the presence of sulfate	Not more than 30 mg/l if there are 250 mg/l of sulfate; if there is less sulfate, magne- sium up to 150 mg/l may be allowed	150 mg/l	(a) Versenate (EDTA) method. Precipitate calcium as oxalate, and estimate magnesium in supernatant liquid, using Eriochrome Black T as an indicator. ⁷ , 16, 65, 62 (Another versenate method is also available ⁷ , 16, 37). (b) Spectrophotometrically, using titan yellow. ¹⁶ , 80 (c) Atomic absorption spectrophotometric method. ²⁸ , 79
Manganese (as Mn)	Taste; discoloration; deposits in pipes; turbidity	0.05 mg/l	0.5 mg/l	Colorimetric methods: (a) Persulfate method. ³ , ³⁷ , ⁴³ (b) Periodate method. ³ , ¹⁶ (c) Atomic absorption spectrophotometric method. ²⁵ , ⁷⁹
Suifate (as So ₄)	Gastrointestinal irritation when magnesium or sodium are present	200 mg/l	400 mg/l	(a) Versenate (EDTA) method. ⁶² , ⁸⁹ (b) Gravimetric method, weighing as berium sulfate. 3, ¹⁶ , ³⁷ , ⁴³
Zinc (as Zn)	Astringent taste; opelescence and sand-like deposits	5.0 mg/l	15 mg/l	(a) Colorimetric method using dithizone reagent. 3, 15, 37 (b) Microtitration with potassium ferrocyanide. 60 (c) Atomic absorption spectrophotometric method. 25, 79

chemical component—such as chloride or sulfate—may be of great value in demonstrating that the water in the distribution system has been mixed with water from outside, for example, through a cross-connexion. Such an examination may give conclusive information within a few minutes.

Not all the tests given need be carried out on every occasion that a water supply is examined chemically, but it is suggested that the following examinations—some of which are given in Table 3 and some in Table 4—should be carried out as part of the short routine chemical examination of water supplies: appearance, colour, odour, taste, temperature, methyl orange alkalinity, oxidizability, ammonia, nitrite, nitrate (see Section 6.4.2), chloride (and, if the water has been chlorinated, a test to determine the residual chlorine content, both free and total), and possibly also tests for albuminoid nitrogen and iron. The other tests listed in Tables 3 and 4 will probably need to be carried out much less frequently, but this will depend to some extent on local conditions. In any event, all the tests will be required when a new source of supply is being considered. The estimation of total solids is useful in the initial analysis of a water when its suitability as a source for a supply of drinking-water is under consideration, but it is not regarded as valuable in the routine chemical examination of water.

It is hoped that the methods recommended in Table 4 and the suggested methods of expressing the results will be of some value in ensuring the comparability of the results obtained.

Examples of forms for reporting the results of a routine short chemical examination and of a complete chemical examination are given in Annex 1.

TABLE 4. METHODS OF EXAMINATION FOR PHYSICAL, CHEMICAL AND AESTHETIC CHARACTERISTICS OF WATER*

Substance or test	Methods of estimation	Expression of results
Organic matter (oxidizability)	An acid method using potassium permanganate at 100°C on a waterbath for 30 minutes is recommended. ²² In some countries an acid method, at 100°C for 10 minutes or for 20 minutes, or an alkaline method is used. An acid method at 27°C for 4 hours is also used. ⁴³	mg/l oxygen con- sumed. Time and temperature at which test is performed should be stated.
Albuminoid nitrogen	By addition of alkaline permanganate solution to water left in the distilling flask after the distillation of free ammonia (see below). Collect portions of distillate. Nesslerize and compare with standards. ³ , ⁴³	mg/l N
Nitrite	(a) Colorimetric method using sulfanilic acid and naphthylamine hydrochloride ³ or α - naphthylamine. ³⁷ (b) Method using 1-naphthylamine-7-sulfonic acid. ²⁰	mg/l NO2
Ammonia	(a) Nesslerization after distillation. ³ , ⁴³ (b) Direct Nesslerization. ³⁷ , ⁸⁹ (c) Nesslerization after treatment with zinc sulfate and sodium hydroxide. ³	mg/l NH4
Phosphate b and orthophosphate	 (a) Colorimetrically, using ammonium molybdate and stannous chloride³, ³⁷, ⁴³ or tin foil. (b) Colorimetrically, using ammonium molybdate and aminonaphtholsulfonic acid.³ (c) Vanadium phosphomolybdate method.² (d) Method of Murphy and Riley.⁶⁷ (e) Method of Edwards, Molof and Schneeman.²⁴ 	mg/l PO₄
Orthophosphate and polyphosphate	Vanadium phosphomolybdate method.1	mg/l PO4
Total phosphate, or- thophosphate and polyphosphate	Boil with concentrated acid, neutralize and proceed as in (a) or (b) above. ³	mg/l PO4

of the considerable variations are found in the amount of organic matter, albuminoid nitrogen, nitrite, ammonia, and phosphate (listed in the first section of the table together with the estimation of residual chlorine), and also in the amount of nitrate (see Section 6.4.2) and chloride (Table 3), the possibility of pollution should be considered.

Strengths of solutions are also of importance. Identical techniques should be used if results are to be comparable.

In the estimation of phosphates the addition of polyphosphates to the water for softening purposes must be borne in mind. The harmlessness of these substances is not universally accepted, and they may be capable of removing the protective coat from lead pipes. The discharge of large quantities of phosphates into lakes and rivers may result in an over-abundant growth of algae.

TABLE 4 (continued)

Substance or test	Methods of estimation	Expression of results
Residual chlorine	Both free and total residual chlorine should be estimated. ⁵⁸ Methods:	mg/l Cl₂
	(a) Orthotolidine/arsenite amethod. By this method free residual chlorine, combined residual chlorine, and colour due to interfering substances can be estimated.3	
	(b) Acid/orthotolidine method. Free residual chlorine and total residual chlorine can be estimated by this method. ³	
	(c) Diethyl-p-phenylenediamine (DPD) method. 69	
	(d) Methyl orange decolorization method for free residual chlorine. ⁶⁴ , ⁸⁵	
	(e) Amperometric titration method for free residual and combined residual chlorine. ³	
	(f) lodometric titration for total residual chlorine. 3, 43	,
Temperature (measured at time of collecting sample) 3	-	Record to nearest 0.1 °C
Electrical conductivity (or resistivity)	By use of conductivity bridge at 20°C.37, 43	Record in μ S/cm (or MΩ/cm).4
Total alkalinity*	Titration with standardized sulfuric or hydrochloric acid and phenolphthalein and methyl orange as indicators. ^{3, 37}	mEq/l (i.e., ml N acid/l) or mg/l Ca- CO3
Bicarbonate	(a) From alkalinity by calculation.3, 37	mg/I HCOs
	(b) From pH and total carbon dioxide by calcula- tion.4	
	(c) From temperature, pH and total solids by means of nomographs. ³	
Carbonate	(a) From alkalinity by calculation.3, 37	mg/l COs
	(b) By titration with standardized hydrochloric acid with or without the addition of barium chloride solution. ⁵²	
	(c) From pH and total carbon dioxide by calculation.4	
	(d) From temperature, pH and total solids by means of nomographs.3	

In some countries, the manufacture and use of orthotolidine have been prohibited.

 $^{^4}$ The unit of electrical conductivity of water, $\mu S/cm$, is the reciprocal of the unit of electrical resistivity in water, $M\Omega/cm$.

 $^{^{\}circ}$ It is useful to record the phenolphthalein alkalinity and the methyl orange alkalinity separately in terms of mI of standard acid.

TABLE 4 (continued)

Substance or test	Methods of estimation	Expression of results
Hydroxyl ion	(a) From alkalinity by calculation. ³ , ³⁷ (b) By titration with standardized sulfuric or hydrochloric acid using strontium chloride, and phenolphthalein as an indicator ⁵ (see also Dickinson ²²). (c) From temperature, pH and total solids by means of a nomograph. ³	mg/l OH
Free carbon dioxide	(a) Titration with sodium carbonate using phenol-phthalein as an indicator. ³⁹ (b) For aggressive carbon dioxide in hard waters: the marble test using powdered calcium carbonate. ²²	mg/l CO2
Dissolved oxygen	(a) Electrometric method. ²³ (b) Either the Winkler method or one of its modifications. ³ , ¹⁹ , ³⁷ , ⁴³	mg/l O ₂
Aluminium	(a) Colorimetrically, using "aluminon" (the ammonium salt of aurin tricarboxylic acid). ³ , ¹⁶ , ⁸⁰ (b) Colorimetrically, using haematoxylin solution. ⁴³	mg/l Al
Chromium (hexavalent)	(a) Atomic absorption spectrophotometric method, which measures total chromium, ²⁵ , ⁷⁹ (b) Colorimetric methods. ³ , ¹⁶ , ⁴³ , ⁹⁰	mg/l Cr (hexavalent)
Silver	(a) Spectrographic method. ³ (b) Colorimetric method using dithizone. ³	mg/i Ag
Sodium	Flame spectrophotometry using standards. ³	mg/l Na
Potassium	(a) Flame spectrophotometry using standards. ³ (b) Colorimetrically, using sodium cobalt nitrite, sulfuric acid and potassium dichromate. ³	mg/l K
Total silica	(a) Colorimetric or spectrophotometric method, based on yellow colour obtained on formation of ammonium silicomolybdate. ³ , ¹⁶ , ³⁷ , ⁴³ (b) Gravimetric method, using hydrochloric acid or hydrochloric acid and perchloric acid. ³ , ¹⁶ , ³⁷	mg/l SiO2
Hydrogen sulfide	Colorimetric method using p-aminodimethylaniline and ferric chloride. ³	mg/l H ₂ S

7. Sampling

7.1 Sampling for Bacteriological Examination

7.1.1 Frequency of sampling

The frequency of bacteriological examination for hygienic control of the supply, and the location of the sampling points at pumping stations, treatment plants, reservoirs, booster pumping stations, and in the distribution system should be such as to enable the bacterial quality of the water supply to be properly controlled. The frequency of examination should depend on the quality of the water source, the risks of contamination, the complexity of the system, the number of water sources, the length of the distribution system, and the dangers of epidemics arising—for example, at international ports or pilgrim centres—as well as the population served by the supply. 36, 86

Topographical inspection of the whole supply from source to consumers' premises is of the utmost importance, and the authority responsible for health matters should have the services of an expert adviser in deciding on the points from which samples should be taken and the frequency with which samples from each point should be examined. Bacteriological examinations should be carried out by authorized laboratories.

The frequency of examination of routine samples of water from the distribution system, and of routine samples of naturally pure water entering the distribution system, should be based on the size of the population served; these examinations should be spaced out over a period of time, depending on the danger of pollution, the geographical situation, and the protection of the source of supply. When, however, water requires chlorination or some other form of disinfection before passing into the distribution system, a constant check on the bacterial quality of the water entering the system is probably necessary, and the corresponding bacteriological examination should, in principle, be carried out at least once a day.

These recommendations and those given below are intended as a guide only, and the actual number of samples examined and their spacing are matters for decision by the responsible authority in the light of local conditions.

An example of a form for reporting the results of a routine bacteriological examination is given in Annex 1.

7.1.1.1 Recommendations

Where water requires chlorination or some other form of disinfection before entering the distribution system, constant checks both on the residual concentration of the chlorine or other chemical disinfectant and on the bacterial quality are needed. The importance of checking this concentration cannot be too strongly emphasized since it ensures that immediate remedial action can be taken should any inadequately treated, and therefore possibly contaminated, water enter the distribution system as a result, for example, of a failure in chlorination. In principle, the bacteriological examination of chlorinated or otherwise disinfected water as it enters the distribution system from each treatment point should be carried out at least once a day, and with the larger supplies this will no doubt be done. With small supplies, serving a population of 10 000 or less, daily sampling may be impracticable and reliance will have to be placed on proper control of disinfectant dosage with checks on the bacterial quality of the water at, say, weekly intervals. In the smallest supplies, this interval may have to be even longer.

Some supplies that do not require disinfection are none the less chlorinated as an additional precautionary measure. Daily bacteriological examination of such water as it enters the distribution system is probably unnecessary. The frequency of bacteriological examination of non-disinfected water entering the distribution system (see below) could be adopted for this type of water also.

In all supplies that are disinfected, a check on the concentration of the chemical disinfectant should be carried out several times a day, not only at each treatment point but preferably also at several points throughout the distribution system. The efficiency of chlorination and of some other forms of disinfection can be checked most effectively by the use of residual recorders, preferably with automatic control. These, however, require technical supervision and, for the small supply, regular manual testing may be all that is practicable.

The results of all these examinations should be recorded for permanent reference and should be supplemented at least twice a year by an inspection on the spot by engineering and hygiene experts acting on behalf of the responsible authority. A plan of the water supply system should be kept up to date and placed at the disposal of the experts.

TABLE 5. MAXIMUM INTERVALS BETWEEN SUCCESSIVE SAMPLES OF NON-DISINFECTED WATER ENTERING THE DISTRIBUTION SYSTEM

Population served	Maximum interval between successive samples
Less than 20 000	1 month
20 000 to 50 000	2 weeks
50 001 to 100 000	4 days
More than 100 000	1 day

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For samples of non-disinfected water entering the distribution system, the proposed maximum intervals between successive routine examinations are shown in Table 5.

On each occasion, samples should be taken from all the points at which water enters the distribution system.

With regard to samples to be collected from the distribution system—whether the water has been subjected to disinfection or not—the proposed maximum intervals between successive samples and the minimum numbers of samples to be examined in each month are given in Table 6.

Population served	Maximum interval between successive samples	Minimum number of samples to be taken from whole distribution system each month	
Less than 20 000	1 month)		
20 000 to 50 000	2 weeks	1 sample per 5 000 population per month	
50 001 to 100 000	4 days		
More than 100 000	1 day	1 sample per 10 000 population per month	

TABLE 6. MAXIMUM INTERVALS BETWEEN SUCCESSIVE SAMPLES AND MINIMUM NUMBER OF SAMPLES TO BE TAKEN

Both of the above criteria should be satisfied in every distribution system.

The minimum number of samples may be reduced to 1 per 10 000 population per month when the population served exceeds 100 000, since in systems serving populations of this size some samples are examined each day.

The samples should not necessarily be taken from the same points on each occasion, but the expert advisers referred to above should determine the points in the distribution system from which samples should be collected.

It should be emphasized that, in routine control, it is far more important to examine numerous samples by means of a simple test than occasional samples by a more complicated test or series of tests.

The frequencies recommended are the minimum necessary for routine bacteriological examination, and, in unfavourable circumstances or in the event of an epidemic or immediate danger of pollution, or when more stringent control is necessary, as for example with water supplies to dairies or food-processing plants, much more frequent bacteriological examination will be required.

Samples should also be collected more frequently from premises in which there is a danger of contamination—particularly through cross-connexions—and also after repairs to mains have been carried out.

7.1.2 Collection, transport and storage of samples for bacteriological examination

Scrupulous care in the collection of samples for bacteriological examination is necessary to ensure that the sample is representative of the water it is desired to examine, and to avoid accidental contamination of the sample during collection. The way in which samples are collected has an important bearing on the results of their examination and it is important, therefore, that sample collectors should be properly trained for the work.

Where several samples are being collected on the same occasion from the same source, the sample for bacteriological examination should be collected first, in order to avoid the danger of contamination of the sampling point during the collection of the other samples.

Sterilized glass bottles provided with a ground-glass stopper or a metal screw-cap should be used; the stopper and neck of the bottle at least should be protected by a paper or parchment cover, or by thin aluminium foil.

If the water to be sampled contains, or is likely to contain, traces of chlorine, chloramine, or ozone, it is necessary to add to the sampling bottles, before sterilization, a sufficient quantity of sodium thiosulfate (Na₂S₂O_{3.5}H₂O) to neutralize these substances. It has been shown that 0.1 ml of a 3% solution of crystalline sodium thiosulfate in a 170-ml bottle has no significant effect on the coliform or *E. coli* content of unchlorinated water during 6 hours' storage.³¹ This amount of sodium thiosulfate is sufficient to neutralize up to at least 5 mg/l of residual chlorine, and it is therefore recommended that it should be added to all bottles used for the collection of samples for bacteriological examination. If samples of chlorinated water are taken, it is desirable to determine the content of chlorine at the sampling point.

The sampling bottle should be kept unopened until it is required for filling. During sampling, the stopper and neck of the bottle should not be allowed to touch anything. The bottle should be held near its bottom. The bottle should be filled, without rinsing, and the stopper should be replaced immediately.

If a sample of mains water is to be taken from a tap, the tap chosen should supply water from a service pipe directly connected with the main, and not, for instance, one served from a roof cistern. The tap should be cleaned and then flamed to sterilize it. The water should be allowed to run to waste from the tap for at least two minutes before the sample is collected.

When samples are collected directly from a river, stream, lake, reservoir, spring, or shallow well, the aim must be to obtain a sample that is representative of the water that will be taken for purposes of supply to consumers. It is therefore undesirable to take samples too near the bank or too far from the point of draw-off; if this is effected by means of a floating arm, the

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sample should not be taken from too great a depth. In a stream, areas of stagnation should be avoided.

Samples from a river, stream, lake or reservoir can often be taken by holding the bottle near its bottom and plunging it, neck downwards, below the surface. The bottle should then be turned until the neck points slightly upwards, with the mouth facing the direction of the current. If no current exists (as in a reservoir), one should be artificially created by pushing the bottle horizontally forward. If it is not possible to collect samples in this way, a weighted foot may be attached to the bottle, which can then be lowered into the water. Damage to the bank must be avoided, otherwise fouling of the water may occur. Special apparatus is required to collect samples from the depths of a lake or reservoir.

If the sample is to be taken from a well fitted with a hand-pump, water should be pumped to waste for about 5 minutes, the pump outlet should be sterilized, and more water should be pumped to waste before the sample is collected. If the well is fitted with a mechanical pump, the sample should be collected from a previously sterilized tap on the rising main. If there is no pumping machinery, a sample can be collected directly from the well by means of a sterilized bottle fitted with a weighted foot, but in this case care should be taken to avoid contamination of the sample by the surface scum.

When the sample has been collected, it should be clearly labelled and sent to the laboratory without delay, accompanied by a note of all the relevant particulars.

Changes occur in the coliform and *E. coli* content of water samples on storage ^{28, 30} and it is important, therefore, that samples should be examined as soon as possible after collection. Examination should preferably be started within one hour of the collection of the sample, but the interval between collection of the sample and the beginning of its examination should never be allowed to exceed 24 hours.

Where there is likely to be delay in getting samples to the laboratory, vans fitted as laboratories can be used or the sample can be filtered through a membrane at the site of collection or in a local laboratory. The membrane can be placed, after filtration, on an absorbent pad saturated with a transport medium ^{26, 70} in a Petri dish. Transport medium is a very dilute medium on which the organisms survive but do not develop visible colonies in three days at room temperature. Polystyrene Petri dishes are preferable for use for despatch to a central laboratory. Delays of three days have been found to make little difference to counts of coliform organisms and *E. coli*.

Apparatus is available that enables filtration of samples and incubation of the membranes to be carried out in the field.

7.2 Sampling for Virological Examination

7.2.1 Frequency of sampling

It is not practicable for examination for viruses to be carried out as frequently as bacteriological examination. In large communities using surface water or ground water that requires treatment, the frequency of examination for viruses depends on local circumstances.

7.2.2 Collection, transport and storage of samples for virological examination

In principle, the same sampling procedure should be used as for bacteriological sampling, but since clean—but not necessarily sterile—bottles may be used it is possible to use bottles made of a plastic material. The size of the samples should be at least 2 litres. Samples should be sent to the laboratory without delay.

7.3 Sampling for Biological Examination

7.3.1 Collection, transport and storage of samples for biological examination

The following methods of collecting samples can be used for piped water supplies:

- (1) For collecting samples from taps: a large volume of water can be filtered through a membrane filter; after drying, the membrane can be made transparent by treating it with immersion oil and a direct microscopic examination can be carried out; alternatively, a special filter device can be attached to a tap and a large volume of water allowed to pass through it; the deposit can then be examined both microscopically and macroscopically.89
- (2) For collecting samples from mains: a special nylon net or cotton bag can be attached to the outlet of a hydrant and a section of the main washed through, using a high water flow rate. Alternatively, a section of the main can be "swabbed" with a specially designed cylinder made of plastic, 49 or a special three-branched standpipe can be used. 53 The debris found in the bag may then be examined macroscopically and microscopically.

Results can be expressed either in terms of organisms per unit volume of water or per unit area of pipe surface, whichever is appropriate.

In sampling from other than piped supplies, the procedure should be the same as for bacteriological examination. In general, clean bottles of at least 2-litre capacity should be used; previous sterilization of the bottle is not essential. The temperature of the sample should be kept as close as possible to its original value, and the sample should be transported to the laboratory without delay.

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7.4 Sampling for Radiological Examination

7.4.1 Collection of samples for radiological examination

The determination of sampling frequencies and the choice of methods of collection and analysis should take into account the fluctuation of observed activity levels of radionuclides in the water, the vicinity of nuclear installations and other major sources of radiopollution, and the risk of contamination.

Many radionuclides are readily adsorbed on to surfaces and solid particles. It is important, therefore, to choose sampling points in the distribution system and at the sources of supply with care so that the sample will be representative of the water that it is desired to examine. Water samples for radiological examination should be collected in polythene bottles to reduce adsorption on to the walls of the containers to a minimum. The volume of the sample should be at least one litre and it should be examined as soon as possible after the collection of the sample in case radionuclides with a short half-life are present.

It is recommended that each country should have at least one centre where simple radiological examinations can be undertaken.

7.5 Sampling for Physical and Chemical Examination

7.5.1 Frequency of sampling

Whereas frequent bacteriological examination is required for hygienic control of water supplies, chemical examination is required much less frequently.

It is recommended that examination for toxic substances (see Table 1) should be carried out at least once a year; the frequency of this examination should be increased when toxic substances are known to be present at sub-tolerance levels in the source of supply, or in certain special circumstances as, for example, when new industries that may be discharging toxic wastes are established in the area.

Complete chemical examination of all supplies used by the public should be carried out once a year. Short routine chemical examination (see p. 41) should be carried out once a month with supplies serving more than 50 000 inhabitants, or twice a year with supplies serving smaller populations. More frequent chemical examinations may be required for the control of waterworks treatment processes.

Frequent chemical examinations of new sources of supply, both for toxic chemical substances and for general chemical examination, will be required, depending on local circumstances.

7.5.2 Collection, transport and storage of samples for physical and chemical examination

For general chemical examination, a sample of at least 2 litres is required. It should be collected in a chemically clean bottle, made of good quality (neutral) glass that is practically colourless; the bottle should be fitted with a ground-glass stopper, or a polythene-lined plastic stopper, and should be rinsed out at least three times with the water that is to be sampled before it is filled. Polythene bottles may be substituted for glass bottles in certain circumstances—for example, when sending samples by air.

In the collection of samples for chemical analysis, the general recommendations given above for the collection of samples for bacteriological examination should be followed, except that it is not necessary to sterilize taps or pump-outlets unless they are being used for collecting samples for bacteriological examination at the same time. Wherever possible, samples of water for chemical examination should be collected by an experienced sample-collector. The way in which samples are collected has an important bearing on the results of their examination.

Samples should be transported to the laboratory with as little delay as possible and should be kept cool during transport. Chemical analysis should be started as soon as is practicable after the collection of the sample and in any event within 72 hours.

Certain examinations should be carried out at the time the sample is collected: these include temperature, pH and residual chlorine, for example. The estimation of free carbon dioxide should also be carried out at the time the sample is collected, but if this is not practicable a special sample should be collected for this purpose; the bottle should be filled completely and the sample kept cool with ice until it is examined.

A special sample is also required for the dissolved oxygen test. The sample should be collected in a narrow-necked bottle of 200-300-ml capacity having an accurately fitting glass stopper. If the sample is collected from a tap, the water should be passed down a glass tube to the bottom of the bottle and allowed to overflow for 2-3 minutes before the stopper is inserted. When samples are taken from a stream or reservoir, a suitable apparatus to ensure that the water in the sampling bottle is displaced several times should be used. The dissolved oxygen in the sample should be "fixed" on the spot as soon as the sample has been collected.^{3, 22, 37} The water temperature at the time of sampling should be recorded in degrees Celsius.

It is advisable that a special sample should be collected for examination for iron, nitrate, nitrite, and organic matter (oxidizability). The sample should be "fixed" at the time of collection by adding 1 ml of concentrated sulfuric acid for each litre of water.

Examination for hydrogen sulfide should be carried out as soon after the collection of the sample as is practicable.

Annex 1

EXAMPLES OF FORMS FOR REPORTING THE RESULTS OF BACTERIOLOGICAL AND CHEMICAL EXAMINATION OF WATER

The forms that follow are intended for use in reporting the results of the chemical or bacteriological examination of a single sample of water. Other forms may be required for summarizing the results of repeated examinations of samples from a single source, or for comparing the results before and after treatment.

It is assumed that the methods of examination used will be those recommended in the body of the publication. With the exception of temperature, no indication of those chemical or physical examinations that should be carried out at the time of collection of the sample, or of those examinations for which the sample should be "fixed" at the time of collection, is given in the forms. This information is given in the body of the publication.

In chemical examinations, the use of such phrases as "absent", "trace", or "present", should be limited to qualitative tests only.

When a quantitative chemical analysis has been attempted and the result is less than the limit of sensitivity of the method, the result should be expressed as "less than (limit of sensitivity)".

SPECIMEN FORM FOR REPORTING THE RESULTS OF A BACTERIOLOGICAL EXAMINATION OF WATER

•	Name and address of laboratory) on bacteriological examination of water
Name and address of sender:	Sender's reference number :
Nature of sample:	Date and time of collection :
Where collected:	Date and time of commencing examination:
Colony counts*:	of medium in each instance)
MPN* of coliform organisms :	/100 ml
MPN of E. coli :	
MPN*, of faecal streptococci:	/100 ml
MPN* of CI. perfringens :	/100 ml
Date of report :	Remarks:
Date results telephoned:	Signed :

Although bacteriological examination would ordinarily be carried out only for coliform organisms and E. coli, spaces for recording the results of other examinations have been included in the specimen form for use when necessary.

b Where membrane filtration methods are used for these examinations, the words "MPN of" should be replaced by "Number of colonies of" in the report form.

ANNEX 1

SPECIMEN FORM FOR REPORTING THE RESULTS OF A SHORT CHEMICAL EXAMINATION OF WATER

Appearance:	Sender's reference number : Laboratory reference number: : ::::::::::::::::::::::::::::::::	
Date and time of arrival at	story :	
Appearance:	xamination:	
Colour :		
Ammonia (NH4+): mg/l Chlo Iron (Fe++) mg/l Nitri Nitra Nethyl orange alkalinity: mg/l (as Ca Co ₂) Orga	°c	
Iron (Fe ⁺⁺) :mg/l Nitri Nitri Methyl orange alkalinity:mg/l (as Ca Co ₂) Orga	Anions	
Methyl orange alkalinity: mg/l (as Ca Co ₂) Orga	CI-) :mg/i	
Methyl orange alkalinity: mg/l (as Ca Co ₂) Orga	D2 ⁻) :mg/i	
	Da=):mg/l	
or ml N acid/I (mEq/I) (oxid	stter :mg/l	
Residual chlorine (as Cl2)		
Free chlorine :mg/l	in minutes/hours	
Total chlorine:mg/l Albu		

SPECIMEN FORM FOR REPORTING THE RESULTS OF COMPLETE CHEMICAL EXAMINATION OF WATER

	ss of send		Sender's reference number Laboratory reference num				}	mEq/I		m
Nature of sample Date and time of Date and time of Date and time of Where collected	:ination:	:		Alkalinity Phenolphti alkalinity Methyl ora alkalinity	Phenolphthalein alkalinity Methyl orange alkalinity		Free carbon dioxide Aggressive carbon dioxide Dissolved oxygen Residual chlorine (as Cl ₂) Free chlorine Total chlorine			
Appearance:			units			Organic matter (oxidi	zability: c	quantity o	f oxygen consumed *C)	m
Colour :				Albuminoid nitrogen (as N) Total silica (as SiO2) Phenolic compounds (as phenol) Anionic detergents (as reference substance) Hydrogen sulfide (as H2S) Polynuclear aromatic hydrocarbons (PAH) Toxic substances mg/l mEq/l Radiosctivity			pCi			
Cations	mg/l	cd/	Anions OH- CI-	mg/l	mEq/1	Arsenic Cadmium	mg/l	Janea/19	Radioactivity Gross alpha activity Rn activity*	рсі
H+ NH4+ Na+ K+ Ag+ Ca++ Mg++ Fe++ Mn++ Cu++ Cu++ Cu++ Cr (hexavalent)			NO2- NO3- F- HCO3- CO3- SO4 PO4 Metaphosphate and polyphosphates			Cyanide Lead Mercury (total) Selenium			226 Ra activity Gross beta activity 40K activity 90Sr activity 129I activity 3H activity * Rn activity stands for the activity of the short-lived daughter products of 222Rn and 220Rn.	

a State whether measured at time of collection or on arrival at laboratory.

Annex 2

TABLES FOR DETERMINING THE MOST PROBABLE NUMBER (MPN) OF PARTICULAR ORGANISMS PRESENT IN 100 ML OF WATER

These tables indicate the estimated number of organisms of the type for which examination is being made in 100 ml of water, corresponding to various combinations of positive and negative results in the portions used for the test.⁸⁴

TABLE 1. MPN AND 95% CONFIDENCE LIMITS WITHIN WHICH IT CAN LIE, FOR VARIOUS COMBINATIONS OF POSITIVE AND REGATIVE RESULTS WHEN FIVE 10-ml PORTIONS ARE USED

Number of tubes giving positive reaction	MPN	95 % confidence limits		
out of 5 of 10 ml each		Lower limit	Upper limit	
0	0	0	6.0	
1	2.2	0.1	12.6	
2	5.1	0.5	19.2	
3	9.2	1.6	29.4	
4	16.0	3.3	52.9	
6	Infinite	8.0	Infinite	

TABLE 2. MPN AND 95% CONFIDENCE LIMITS WITHIN WHICH IT CAN LIE, FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 10-mi PORTIONS, FIVE 1-mi PORTIONS AND FIVE 0.1-mi PORTIONS ARE USED

Number of tubes giving positive reaction out of		MPN	95 % confidence limits		
5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower limit	Upper limit
0	0	1	2	< 0.5	7
0	1	0	2	< 0.5	7
0	2	0	4	< 0.5	11
1	0	0	· 2	< 0.5	7
1	0	1 1	4	< 0.5	11
1	1	0	4	< 0.5	11
1	1	1	6	< 0.5	15
1	2	0	6	< 0.5	15
2	0	0	5	< 0.5	13
2	0	1 1	7	1 1	17
2	1	0	7	1 1	17

TABLE 2 (continued)

Number of tubes giving positive reaction out of		MPN	95 % confidence limits		
5 of 10 ml each	5 of 1 ml each	5 of 0.1 ml each		Lower limit	Upper limit
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1 1	19
3	0	1	11	2	25
3	1	0] 11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2] 0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	96
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	114
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	154
5	2	0	49	17	126
5	2	1	70	23	168
5	2	2	94	28	219
5	3	0	79	25	187
5	3	1	109	31	253
5	3	2	141	37	343
5	3	3	175	44	503
5	4	0	130	35	302
5	4	1	172	43	486
5	4	2	221	57	698
5	4	3	278	90	849
5	4	4	345	117	999
5	5	0	240	68	754
5	5] 1	348	118	1 005
5	6	2	542	180	1 405
5	5	3	918	303	3 222
5	5	4	1 609	635	5 805
		I .	I	1	

ANNEX 2 59

TABLE 3. MPN AND 95% CONFIDENCE LIMITS WITHIN WHICH IT CAN LIE, FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN ONE 50-ml PORTION AND FIVE 10-ml PORTIONS ARE USED

Number of tubes giving positive reaction out of		MPN	95 % confidence limits		
1 of 50 ml	5 of 10 ml each		Lower limit	Upper limit	
0	1 [1	< 0.5	4	
0	2	2	< 0.5	6	
0	з (4	< 0.5	11	
0	4	5	1	13	
1	0	2	< 0.5	6	
1	1 1	3	< 0.5	9	
1	2	6	1	15	
1	3	9	2	21	
1	4	16	4	40	

TABLE 4. MPN AND 95 % CONFIDENCE LIMITS WITHIN WHICH IT CAN LIE, FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN ONE 50-mi PORTION, FIVE 10-mi PORTIONS AND FIVE 1-mi PORTIONS ARE USED

Number of tubes giving positive reaction out of			MPN	95 % confidence limits	
1 of 50 ml	5 of 10 ml each	5 of 1 ml each	IMF 14	Lower limit	Upper limit
0	0	1	1	< 0.5	4
0	0	2	2	< 0.5	6
0	1	0	1	< 0.5	4
0	1	1 1	2	< 0.5	6
0	1	2	3	< 0.5	8
0	2	0	2	< 0.5	6
0	2	1	3	< 0.5	8
0	2	2	4	< 0.5	11
0	3	0	3	< 0.5	8
0	3	1	5	< 0.5	13
0	4	0	5	< 0.5	13
1	0	0	1	< 0.5	4
1	0	1	3	< 0.5	8
1	0	2	4	< 0.5	11
1	0	3	6	< 0.5	15
1	1	0	3	< 0.5	8
1] 1	1	5	< 0.5	13
1	1 1	2	7	1 1	17
1	1	3	9	2	21
1	2	0	5	< 0.5	13

TABLE 4 (continued)

Number of tubes giving positive reaction out of			MPN	95 % confidence limits	
1 of 50 ml each	5 of 10 ml each	5 of 1 ml each		Lower limit	Upper limit
1	2	1	7	1	17
1	2	2	10	3	23
1	2	3	12	3	28
1	3	0	8	2	19
1	3	1	11	3	26
1	3	2	14	4	34
1	3	3	18	5	53
1	3	4	21	6	66
1	4	0	13	4	31
1	4	1	17	5	47
1	4	2	22	7	69
1	4 1	3	28	9	85
1	4	4	35	12	101
1	4	5	43	15	117
1	5	0	24	8	75
1	5	1	35	12	101
1	5	2	54	18	138
1	5	3	92	27	217
1	5	4	161	39	> 450

TABLE 5. MPN AND 95 % CONFIDENCE LIMITS WITHIN WHICH IT CAN LIE, FOR VARIOUS COMBINATIONS OF POSITIVE AND NEGATIVE RESULTS WHEN FIVE 50-mI PORTIONS, FIVE 10-mI PORTIONS AND FIVE 1-mI PORTIONS ARE USED

Number of tubes giving positive reaction out of			MPN	95 % confidence limits	
5 of 50 ml each	5 of 10 ml each	5 of 1 ml each		Lower limit	Upper limit
0	0	1	1	< 0.5	2
0	1 1	0	1	< 0.5	2
0	1	1	1	< 0.5	2
0	2	0	1	< 0.5	2
0	3	0	1	< 0.5	2
1	0	0	1	< 0.5	2
1	0	1 1	1	< 0.5	2
1	1	0	1	< 0.5	2
1	1	1	1	< 0.5	2
1	2	0	1	< 0.5	2
1	2	1	2	< 0.5	4
1	3	0	2	< 0.5	4
2	0	0	1	< 0.5	2
2	0	1	1	< 0.5	2
2	1	0	1	< 0.5	2

ANNEX 2

TABLE 5 (continued)

Number of tubes giving positive reaction out of		MPN	95 % confidence limits		
5 of 50 ml each	5 of 10 ml each	5 of 1 ml each		Lower limit	Upper limit
2	1	1	2	< 0.5	4
2	2	0	2	< 0.5	4
2	2	1	2	< 0.5	4
2	3	0	2	< 0.5	4
2	3	1	3	1	7
2	4	0	3	1	7
3	0	0	2	< 0.5	4
3	i o	1	2	< 0.5	4
3	1 1	0	2	< 0.5	4
3	1	1	2	< 0.5	4
3	1 1	2	3	1	7
3	2	0	3	1	7
3	2	1	3	1	7
3	2	2	4	1	9
3	3	0	3	1	7
3	3	1	4	1 1	9
3	4	Ó	4	1	9
3	4	1	4	1	9
4	Ò	Ó	2	< 0.5	4
4	o	1	3	1	7
4	o	2] 3	1	7
4	1	ō	3	1 1	7
4	i	i	4	1	9
4	i	2	4	1	9
4	2	Ō	4	1	9
4	2	1	4	1 1	9
4	2	2	5	2	12
4	3	ō	5	2	12
4	3	1	5	2	12
4	3	2	6	2	14
4	4	ō	6	2	14
4	4	1	1 7	3	17
4	5	ò	7	3	17
4	5	ì	8	3	19
5	Ö	Ö	4	1	9
5	Ö	1	4	i	9
5	l ŏ l	2	6	2	14
5	1	ō	5	2	12
5	i	ĭ	6	2	14
5	;	2	7	3	17
5	2	ō	6	2	14
5	2	ĭ	l š	3	19
5	2	2	10	4	23
5	2	3	12	4	28
5	3	ő	9	3	21
5	3	1	11	4	26

TABLE 5 (continued)

Number of tubes giving positive reaction out of			MPN	95 % confidence limits	
5 of 50 ml each	5 of 10 ml each	5 of 1 ml each	MEN	Lower limit	Upper limit
5	3	2	14	5	34
5	3	3	18	6	53
5	4	0	13	5	31
5	4	1	17	6	47
5	4	2	22	7	70
5	4	3	28	9	85
5	4	4	35	11	101
5	5	0	24	8	75
5	5	1	35	11	101
5	5	2	54	18	140
5	5	3	92	27	218
5	5	4	161	39	424

TABLE 6. MPN AND 95% CONFIDENCE LIMITS FOR VARIOUS COMBINATIONS OF POSITIVE RESULTS WHEN THREE 10-ml PORTIONS, THREE 1-ml PORTIONS AND THREE 0.1-ml PORTIONS ARE USED

Number of tubes giving positive reaction out of			MPN	95 % confidence limits	
3 of 10 ml each	3 of 1 ml each	3 of 0.1 ml each		Lower limit	Upper limit
0	0	1	3	< 0.5	9
0	1	0	3	< 0.5	13
1	0	0	4	< 0.5	20
1	0	1	7] 1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1 1	0	15	3	44
2	1 1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	149
3	۱ ۰	0	23	4	120
3	٥ ا	1 1	39	7	130
3	٥	2	64	15	379
3	1 1	0	43	7	210
3	1 1	1 1	75	14	230
3	1 1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1 300
3	3	1 1	460	71	2 400
3	3	2	1 100	150	4 800

Annex 3

MEMBERSHIP OF THE WHO EXPERT COMMITTEE ON HEALTH CRITERIA FOR WATER SUPPLIES

Geneva, 30 March - 5 April 1971

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