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Bacteriological Quality

Historically, water has played a significant role in the transmission of human disease. Typhoid fever, cholera, infectious hepatitis, bacillary and amoebic dysenteries and many varieties of gastrointestinal disease can all be transmitted by water. The introduction of water treatment with disinfection and the implementation of bacteriological surveillance programs to ensure the delivery of safe water have resulted in a dramatic decrease in the occurrence of water-related illness. The occasional occurrence of waterborne disease outbreaks, however, points out the continuing importance of strict supervision and control over the quality of public and private water supplies.

Contamination by sewage or human excrement presents the greatest danger to public health associated with drinking water, and bacteriological testing continues to provide the most sensitive means for the detection of such pollution. Although modern microbiological techniques have made possible the detection of pathogenic bacteria, viruses and protozoa in sewage and sewage effluents, it is not practical to attempt to isolate them as a routine procedure from samples of drinking water. Pathogens present in water are usually greatly outnumbered by normal intestinal bacteria, which are easier to isolate and identify. The presence of such organisms indicates that pathogens *could be* present; if they are absent, disease-producing organisms *are probably* also absent.

It should be emphasized that no bacteriological analysis of water can take the place of a complete knowledge of the conditions at the sources of supply and throughout a system. Contamination is often intermittent and may not be revealed by the examination of a single sample. The most a bacteriological report can prove is that, at the time of examination, bacteria indicating faecal pollution did or did not grow under laboratory conditions from a sample of water. Therefore, if a sanitary inspection shows that a well is subject to contamination or that water is inadequately treated or subject to contamination during storage or distribution, then the water should be considered unsafe irrespective of the results of bacteriological examination.

Pollution Indicator Organisms

The Coliform Group

Almost from the time of their first isolation from faeces in the late 19th century, the coliform group of bacteria has been used as an indicator of the bacteriological safety of water.[1] The coliform group merits consideration as an indicator of pollution because these bacteria are always present in the intestinal tracts of humans and other warm-blooded animals and are excreted in large numbers in faecal wastes. Although the sanitary significance of some coliform strains is questionable, all members of the group may be of faecal origin, and it should be assumed that they are of faecal origin unless it can be proven otherwise. Finally, water is not a natural medium for coliform organisms, and their presence must at least be regarded as indicative of pollution in its widest sense.

1. Definitions

The coliform group has been defined in the 16th edition of *Standard Methods for the Examination of Water and Wastewater*[2] as follows:

(1) all aerobic and facultative anaerobic, Gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C; or

(2) all organisms that produce a colony with a golden-green metallic sheen within 24 hours on an Endo-type medium containing lactose.

These definitions are not to be regarded as identical but, rather, refer to two groups that are roughly equivalent in sanitary significance. Both groups contain various species of the genera *Escherichia*, *Klebsiella*, *Enterobacter* and *Citrobacter*. Two characteristics—β-galactosidase positive and cytochrome oxidase negative—should be added to provide common traits to link the definitions. The β-galactosidase test would provide a definitive test for lactose fermentation, whereas the cytochrome oxidase test would serve to exclude members of the genus *Aeromonas*, which are frequently responsible for false-positive coliform

reactions. However, participants at a U.S. Environmental Protection Agency (EPA) workshop in 1981 agreed that the presence of *Aeromonas hydrophila* in drinking water presents a potential health hazard. Although it is taxonomically not a coliform, they recommended that it should be included in the total coliform group for the purpose of assessing drinking water quality.[3]

The faecal coliform group includes that portion of the total coliform group that is capable of forming gas within 24 hours in EC medium at 44.5°C *or* that produces a blue colony on m-FC broth within 24 hours at 44.5°C. This group comprises the genera *Escherichia* and, to a lesser extent, *Klebsiella* and *Enterobacter*. The organism most commonly thought of as an indicator of faecal pollution is *Escherichia coli*. Complete identification of *E. coli* in terms of modern taxonomy would require an extensive series of tests that would be impractical for routine water bacteriology. The detection and identification of the faecal coliform group in accordance with the simpler operational definitions given above are currently preferred. A membrane filter method has been developed for the direct enumeration of *E. coli*,[4] but it has not been extensively evaluated with drinking water.

2. Differentiation of Organisms

It was recognized at an early date that some strains included in the total coliform group were not common in faecal material. Organisms of the *Klebsiella*, *Enterobacter* and *Citrobacter* genera (intermediate–aerogenes–cloacae [IAC] subgroups) have been found in soils[5–7] and on vegetation;[8–10] in faeces, however, they are present in much smaller numbers than *E. coli*, which is characteristically the predominant coliform in warm-blooded animal intestines.[11,12] Attempts have therefore been made to differentiate members of the coliform group and to relate their physical and biochemical characteristics to their natural sources and habitats.

MacConkey[13,14] defined the aerogenes group on the basis of fermentative reactions with five sugars and the ability to produce acetylmethylcarbinol in the Voges-Proskauer (VP) reaction. Coliforms can also be differentiated by the ratio of carbon dioxide to hydrogen produced.[15] Coliforms derived from non-faecal sources produced two or more times as much carbon dioxide as hydrogen; in faeces-derived strains, the ratio was 1:1. Low-ratio cultures also produced indole from tryptophan.[16] Clark and Lubs[17] were able to correlate the gas ratio data with the much easier to perform methyl red (MR) test. Low-ratio cultures—faecal coliforms—turned the methyl red indicator a brilliant red. Koser[18,19] found the MR and VP tests inadequate for faecal coliform characterization and suggested a citrate utilization procedure to differentiate coliforms in polluted water ($citrate^-$) from those in unpolluted water ($citrate^+$). Based on an analysis of the literature on coliform differentiation, Parr[20,21] chose the indole, MR, VP and citrate tests as the combination of four procedures that would yield the best classification and introduced the mnemonic IMViC to facilitate the expression of results. IMViC types ++−−, +−−− and −+−−, or the *Escherichia* group, were to be considered of faecal origin.

Because of the high labour costs involved in preparing media and conducting biochemical identification tests, it may be advantageous to use one of the many commercially available pre-packaged multiple test kits. A list of some of the suitable test kits is available.[2]

A second approach to coliform differentiation is the elevated temperature test originally proposed by Eijkman in 1904.[22] It was based on his observation that coliforms derived from the intestines of warm-blooded animals produced gas from glucose at 46°C, whereas non-faecal coliforms failed to grow. Perry and Hajna[23] found the procedure, as described by Eijkman, unsatisfactory; however, by decreasing the glucose and adding phosphate as a buffer, they were able to support his hypothesis. However, the technique was low in sensitivity and specificity. Vaughn and Levine[24] increased the specificity by using boric acid to inhibit the growth of *Aerobacter* and *Citrobacter* organisms. They found that, at 43–44°C, 98% of their *E. coli* strains grew, compared with only 3.3% of their *Aerobacter* strains. Perry and Hajna[25,26] described a buffered tryptose lactose bile salts medium (EC) that, when used at 45.5°C, showed improved sensitivity with a slight loss of specificity.

Geldreich, Clark and their co-workers carried out an extensive evaluation of these procedures[27,28] and reached the following conclusions:

(1) 44.5°C was the best temperature for separation of the faecal coliform group;

(2) false negatives and false positives tend to cancel each other out; and

(3) the EC broth described by Perry and Hajna was the best medium for the test.[25,26]

When these findings were applied to studies on coliforms isolated from faeces,[2] soils[7] and vegetation,[9] a greater positive correlation was found with the elevated temperature procedure (96.6%) than with the IMViC series of biochemical reactions (87.2%).

A new method to differentiate coliforms is based on the selective ability of *E. coli* to metabolize 4-methyl-umbelliferyl-β-D-glucuronide (MUG).[29] When MUG is used both as the sole source of energy and the indicator in a medium, it is hydrolysed by *E. coli* to form 4-methylumbelliferone, which fluoresces under longwave ultraviolet light.

3. Survival and Aftergrowth

Although all the coliform genera (*Escherichia*, *Klebsiella*, *Citrobacter* and *Enterobacter*) are present in fresh faeces and in fresh pollution from faecal sources, they may not all persist in water for the same length of time.[2] *Escherichia coli*, for example, is generally most sensitive to environmental stresses and least likely to grow in the environment. *Klebsiella*, *Citrobacter* and *Enterobacter*, on the other hand, are more likely to persist and to grow on organic-rich materials or in organic-rich waters. They may also form a biofilm within the distribution system that is resistant to chlorination and other eradication measures.[30,31] Regrowth of coliforms in the distribution systems presents a serious problem to water purveyors: the sporadic positive coliform results make it difficult to assess the true hygienic status of the water. Although identification to species of positive coliform tests should be performed, the presence of organisms apparently as a result of "aftergrowth" should not be ignored. Corrective action in such cases is required in order to maintain the usefulness of the coliform indicator system.

4. Applications to Water Studies

The faecal coliform test has been shown to be an indicator of the potential presence of enteric pathogens in water. A relationship between the faecal coliform density and the frequency of *Salmonella* detection has been demonstrated.[32,33] At faecal coliform densities of 1 to 200 CFU (colony-forming units) per 100 mL, *Salmonella* was detected in 28% of the water samples examined; this frequency rose to 98% in waters with a faecal coliform count above 2000 CFU per 100 mL. Studies on survival in river water,[34] well water[35] and septic tank water[36] have shown that faecal coliforms persist longer than *Salmonella* organisms. Because it is relatively specific for faecal contamination, the faecal coliform measurement is preferred for monitoring raw water quality and for indicating the potential presence of pathogens at source. It is also of value in testing untreated drinking water supplies. Any untreated supply that contains faecal coliforms should receive disinfection.

The total coliform test, on the other hand, is less reliable as an indicator of faecal pollution. However, because of its superior survival characteristics, the total coliform group is preferred as an indicator of treatment adequacy in drinking water supply systems. The presence of any type of coliform organism in treated water suggests either inadequate treatment or contamination and therefore should not be tolerated.

Heterotrophic Plate Count

Although attainment of a coliform level of less than 10 CFU per 100 mL in a given sample is considered to satisfy the bacteriological requirements for potable water, there are many micro-organisms commonly present in drinking water whose numbers far exceed those of the coliform group and that can interfere with the development of coliforms. The heterotrophic plate count (HPC, formerly known as standard plate count) provides an index of the level of this general bacterial population. No single medium, temperature or incubation time will ensure the recovery of all organisms present in water. The 16th edition of *Standard Methods for the Examination of Water and Wastewater*[2] does, however, specify requirements that will permit a meaningful standard count of selected members of the bacterial population. These counts can be used for quality control in water treatment plants and as a measure of quality deterioration in wells, distribution lines and reservoirs.[37–39] In some jurisdictions, the background colony counts on total coliform membrane filters are used as a convenient and inexpensive index of the HPC. Elevated background counts not only are an indication of increased concentrations of the general bacteria population but can also suppress the development of any coliform bacteria that may also be present.[37]

The general population of bacteria in potable water may include some genera that could, under special circumstances, constitute a health risk. Some species of *Pseudomonas* can become serious secondary pathogenic invaders in post-operative infections, burn cases and the very young.[40–44] *Flavobacterium* has been reported as a primary pathogen for some surgical patients.[45] The HPC is not a true indicator of potential pathogens of this type, as no constant relationship appears to exist between the HPC and the number of pathogens that might be present. It does seem reasonable to assume, however, that chance occurrences are proportionately greater as the general bacterial population increases.

Muller cited evidence from three typhoid fever outbreaks in Germany that supports the use of the HPC as an indicator.[46] A sudden rise of a colony count that had been low for several years signalled the beginning of the 1926 outbreak in Hanover. Four thousand cases of disease were attributed to these unspecified organisms. The high colony counts were detected before the water in the distribution system yielded *E. coli* or coliforms and two weeks before the first cases of typhoid fever were reported.

In addition, disease outbreaks have been attributed to unchlorinated water supplies in which coliforms were not detectable by conventional methods.[47,48] The value

of monitoring the general bacterial population is therefore greatest in supplies where chlorination is not practised.

Bacterial Pathogens Associated with Waterborne Disease Outbreaks

Salmonella and *Shigella*

The survival characteristics in water and the susceptibility to disinfection of *Salmonella* and *Shigella* have been demonstrated to be similar to those of coliform bacteria.[34,35] Therefore, routine monitoring to ensure the absence of coliforms should be adequate to protect drinking water from *most* contamination situations involving these organisms. However, instances have been reported in which these pathogens were isolated from drinking water in the absence of coliforms.[47,48] Coliform suppression by elevated heterotrophic plate counts and poor recovery of stressed coliforms seem to be the most plausible explanations for these discrepancies. The combined use of coliform and HPC guidelines for treated water should provide an adequate indication of the presence of these pathogens.

Campylobacter and *Yersinia*

Waterborne outbreaks of gastroenteritis involving *Campylobacter jejuni* and *Yersinia enterocolitica* have been recorded with increasing frequencies in the past few years.[49–55] In addition, many reports of their isolation from surface and well waters have also been presented.[53,56–61] Since the realization that water can be a potential route of campylobacteriosis and yersiniosis, isolation and enumeration methods have been developed.[2] Rollins and Colwell[62] recently described the presence of viable but non-culturable states of *C. jejuni* in the aquatic environment. They suggested that this non-culturable type could be one reason why *Campylobacter* is not always isolated from water during a waterborne outbreak of campylobacteriosis. The findings of Wang and co-workers[63] indicated that conventional water treatment and chlorination will probably destroy *C. jejuni* and *Y. enterocolitica* in drinking water. The survival characteristics of *C. jejuni* are similar to those of coliforms, but the frequency of isolation of *Y. enterocolitica* is higher in winter months, indicating that it can survive for extended periods and perhaps even multiply when water temperatures are low.[3] In addition, the presence of *Y. enterocolitica* has been demonstrated to be poorly correlated with levels of coliforms and heterotrophic plate counts.[64] A recent paper by Carter *et al*.[65] sheds some doubt on the usefulness of indicator organisms to predict the presence of *Campylobacter* in surface raw water supplies. *Campylobacter* density was often negatively correlated with densities of total and faecal coliforms, faecal streptococci and heterotrophic plate counts. Thus, coliforms may not be adequate indicators of the presence of both *C. jejuni* and *Y. enterocolitica*.

Legionella pneumophila

Legionella pneumophila, the causative agent of legionellosis and Pontiac fever, has been recovered in low concentrations in the drinking water of a number of Canadian cities.[66,67] However, it is not a major component of the bacterial populations of the relatively cold surface waters in Canada. Although chlorination appears to effectively control *Legionella*, the bacteria can colonize various niches in buildings (e.g., cooling towers, hot water tanks, shower heads, aerators) and contaminate the air and potable water. This situation is particularly troublesome in hospitals, where susceptible human populations can be exposed to aerosols containing hazardous concentrations of *L. pneumophila*.[68] In general, the presence of this organism is not sufficient evidence to warrant remedial action in the absence of disease cases.[66,68]

Methodology

Coliform Organisms

In Canada, three methods are currently in use for the detection of coliform organisms in water: the multiple tube fermentation (MTF) procedure, the membrane filter (MF) technique, and a presence–absence (P–A) procedure. The three methods do not give strictly comparable results. At low coliform levels, the confidence limits of both the MTF and MF methods are large; therefore, separate maximum acceptable concentrations are not recommended for each method. The P–A procedure is a qualitative measure of contamination.

1. Multiple Tube Fermentation Procedure

A detailed description of this procedure has been given in *Standard Methods for the Examination of Water and Wastewater*.[2] Briefly, 10-fold dilutions of the water to be tested are inoculated into tubes containing the appropriate medium (five or 10 tubes per dilution). For drinking water, dilution should be unnecessary because of the expected low counts. The presence of gas or heavy growth after 48 hours of incubation at 35°C constitutes a positive presumptive test for coliforms, and confirmatory procedures are required. For faecal coliforms, the presence of gas after 24 hours at 44.5°C is considered a positive completed test. Results are reported as a most probable number (MPN). The MPN is only a *statistical* estimate of the number of bacteria that, more probable than any other, would give the observed result; it is not an actual count of the bacteria present.

High densities of non-coliform bacteria and the inhibitory nature of some MTF media may have an adverse influence on routine coliform monitoring procedures. Many species in the general bacterial population have been shown to inhibit the detection of *E. coli*.[69–71] Seidler *et al*.[72] showed that the recovery of total coliforms by MTF decreased as the concentration of HPC bacteria increased, with the greatest reduction occurring when the HPC densities exceeded 250 CFU/ mL. Le Chevallier and McFeters[73] hypothesized that competition for limiting organic carbon was responsible for the interference of total coliform recovery by HPC bacteria. The recovery of coliform from gas-negative but turbid presumptive MTF tubes has demonstrated the presence of inhibitory compounds in the MTF media. When lauryl tryptose broth was the primary medium, coliform isolations from turbid gas-negative tubes increased the numbers of positive tubes in an MTF analysis by as much as 28%.[74] Comparative studies using brilliant green lactose bile (BGLB) broth and m-Endo LES agar as confirmatory media also demonstrated that BGLB broth can inhibit the growth of some coliforms. Recently, Evans *et al*.[75] developed a procedure to detect false-negative reactions. Using a modified MTF technique, the incidence of coliform detection was twice that of the standard MTF technique for drinking water. In response to these findings, the current edition of *Standard Methods for the Examination of Water and Wastewater*[2] recommends treating all tubes with turbidity, regardless of gas production, as presumptive coliform-positive tubes.

The MTF procedure lacks precision, and results take longer than with the MF technique; it has therefore been largely replaced by the MF technique for the routine examination of drinking water. It is still of value when conditions render the MF technique unusable—for example, with turbid, coloured or grossly contaminated water—and as a comparative procedure.

Recently, a new defined substrate MTF method, known as Autoanalysis Colilert (AC), has been developed in the United States for the simultaneous enumeration of both total coliforms and *E. coli.*[76] Organisms can be detected and identifed at 1 CFU per 100 mL in 24 hours or less. The Colilert medium provides the specific indicator nutrients, ortho-nitro-phenyl-β-D-galactopyranoside (ONPG) and 4-methylumbelliferyl-β-D-glucuronide (MUG), for the target organisms—total coliforms and *E. coli*. A nation-wide evaluation demonstrated no statistical difference in the number of positive tubes by either the standard MTF test or the AC method. The method was recently approved by the U.S. EPA as an acceptable means for the enumeration of coliforms in drinking water.[77] The AC method should undergo testing in Canada before it can be considered as a suitable method for the enumeration and differentiation of coliforms.

2. Membrane Filter Procedure

The membrane filter (MF) procedure was introduced to bacteriological water analysis in 1951 when it was demonstrated to be capable of producing results equivalent to those obtained by the MTF procedure.[78,79] With this technique, the water sample is passed through a filter that retains bacteria; the filter is placed on an appropriate selective/differential medium and incubated; and, after 24 hours, the coliform or faecal coliform colonies are counted. The advantages of the technique were quickly recognized. It made practical the examination of larger volumes of water, thus increasing sensitivity and reliability while allowing a marked reduction in time, labour, equipment, space and material.

Unfortunately, there are several problems associated with the MF technique. The major concern for this and other methods that use stressful selective media is their inability to enumerate coliform bacteria that have been subjected to sublethal injury (e.g., chlorination) in the treatment plant or distribution system. Stressed organisms are often not able to grow on the selective coliform media but can recover through a resuscitation process. Experiments have shown that as many as 90% of the total coliforms present may be injured.[78] False-negative findings such as these could lead to the acceptance of potentially hazardous water quality. High turbidity can also interfere with the MF method. The retention of particulate matter by the filter can interfere with colony development and the production of surface sheens by coliform bacteria.

One of the most significant improvements in the MF technique has been the development of a new medium (m-T7) for the enhanced recovery of stressed coliforms in drinking water.[80] In a subsequent evaluation,[81] the m-T7 agar yielded eight- to 38-fold more coliforms than m-Endo LES agar. M-T7 agar is also suitable for the enumeration of faecal coliform bacteria. However, the medium will have to be extensively tested in Canada before it can be considered as an acceptable method for the recovery of coliform bacteria.

As in the MTF method, other bacteria can also interfere with the recovery of coliforms. Data from the U.S. National Community Supply Survey[37] showed that the recovery of total coliforms using MF decreased as the concentration of HPC bacteria increased. The greatest reduction occurred when the HPC densities exceeded 500 CFU/mL. It was also shown that most water supplies maintaining a total chlorine residual of 0.2 mg/L had an HPC less than 500 CFU/mL. In a study by Clark,[82] a 24-hour HPC of greater than

1000 CFU/mL produced a marked inhibition of total coliform recoveries, but a similar decline in recovery did not occur with the 24-hour total coliform background count or with the 48-hour HPC. Burlingame *et al*.[83] demonstrated that *Pseudomonas aeruginosa* (30 CFU/mL) and *Aeromonas hydrophila* (2 CFU/mL) caused significant reductions in sheen production by coliforms on m-Endo LES agar. *Flavobacterium* sp. and *Bacillus* sp., in contrast, were not inhibitory, even at concentrations greater than 1000 CFU/mL.

Standridge and Sonzogni evaluated two modifications of the MF technique for total coliforms in drinking water containing high background counts.[84] In both cases, roughly 8% of the plates originally classified as coliform negative but overgrown—i.e., confluent growth or greater than 100 background CFU per 100 mL—yielded coliforms. New methods for the verification of total coliforms recovered by MF have been described.[85–87]

Many workers have shown the superiority of one filter manufacturer's product over another's; however, although each author is sure which is best in his/her own work, no brand emerges as clearly superior when the literature as a whole is considered.[88–97] Variation has been attributed to filter sterilization procedures[88,89] and the source of coliforms.[90] It has been suggested that the most critical factors are the filter retention size and the surface opening diameter size.[91,92] Clearly, there is a need for a set of specifications that covers all parameters affecting the efficiency of recovery of organisms on membrane filters. Until such specifications are available, it will be essential to do periodic comparative membrane filter tests with the kinds of water normally encountered, to ensure acceptable results.

3. Presence–Absence Procedure

The presence–absence (P–A) test was developed as a more sensitive, economical and efficient means of analysing drinking water samples.[98] Essentially, it is a modification of the MTF procedure in which only one analysis bottle per sample is used. It is therefore recommended only for the examination of a water supply for which a sequential or consecutive series of samples has been collected.

In comparative tests, the P–A method was as good as or better than the MF technique for the recovery of coliforms in drinking water samples.[82,99,100] In addition, high concentrations of HPC bacteria did not inhibit the recovery of coliforms by the P–A procedure. The P–A test is also technically simpler than the MF procedure. Initial analysis time is less than one minute per sample, and, because the majority of drinking water samples will be negative, confirmatory procedures are usually not required. The qualitative nature of the P–A test is its only shortcoming; consequently, samples giving a positive result will require an MF or MTF analysis to determine numbers of organisms. Recently, a nation-wide evaluation demonstrated no statistical difference in the number of coliform-positive samples by either the standard MTF method or the AC method (described above) used in a P–A mode.[101]

In 1989, the U.S. EPA approved the use of the presence–absence concept as a suitable means of detecting total coliforms in drinking water.[102]

General Bacterial Population

In the latest edition of *Standard Methods for the Examination of Water and Wastewater*,[2] two additional media have been included for the enumeration of HPC bacteria. R2A agar can be used for spread and MF methods, whereas m-HPC agar is recommended for membrane filtration use only. In comparative studies,[38,39] R2A and m-HPC agar significantly improved the recovery of the general bacterial population compared with standard plate count agar.

Sampling for Bacteriological Examination

Sample Size

A minimum volume of 100 mL should be examined by the MTF procedure in order to obtain a reliable estimate of the mean probable number of coliform organisms at the expected low levels in treated drinking water. A test series consisting of one 50-mL volume and five 10-mL volumes is suggested in the World Health Organization's *International Standards for Drinking Water* for water expected to be of good quality.[103] Smaller volumes, dilutions or other combinations of tubes may be more appropriate for waters of doubtful quality.

With the MF method, if the sample is expected to contain less than 100 coliform organisms per 100 mL, the filtration of 100 mL is necessary. For more polluted samples, the volume should be chosen to give an MF count between 10 and 100. If the volume to be filtered is less than 10 mL, the sample should be diluted with sterile water or buffer so that a minimum of 100 mL is filtered. Although a minimum sample volume of 100 mL is recommended with both procedures, examination of larger volumes, which is practical with the MF method, will increase both the test sensitivity and reliability.

A sample of 200 mL will provide sufficient volume for a coliform determination by one of the three methods and also for a heterotrophic plate count. In addition, enough sample will remain if membrane filtration is required to complement a P–A determination.

Frequency of Sampling

The World Health Organization lists the following factors that should be taken into account when determining sampling frequency:[103,104]

(1) past frequency of unsatisfactory samples;
(2) source water quality;
(3) the number of raw water sources;
(4) the adequacy of treatment and capacity of the treatment plant;
(5) the size and complexity of the distribution system; and
(6) the practice of disinfection.

These variables preclude application of a universal sampling frequency formula. The frequency of sampling should therefore be established by the control agency after due consideration of local conditions. It is recommended, however, that a minimum of four samples per month be examined for water supply systems. For practical and economic reasons, sampling of private wells should be restricted to times when the risk of contamination is greatest.

As a general guide, one sample per 1000 persons served should be examined each month for supplies serving up to 100 000 persons. For supplies serving populations over 100 000, it is considered justifiable to reduce the sampling increment to one per 10 000 persons per month: in systems serving populations of this size, the interval between successive samples will be very short. The samples should be taken at regular intervals throughout the month. It must be emphasized that the above figures are only general guides. In water supplies with a history of high-quality water production, it may be possible to reduce the number of samples taken for bacteriological analysis and to apply the laboratory resources thus liberated to increase the surveillance on supplies with known problems. This practice of basing sampling requirements on the population served recognizes the limited resources available for surveillance of smaller water supply systems; however, small water supplies have more facility deficiencies[105] and are responsible for more disease outbreaks than large ones.[106] Therefore, in addition to population, emphasis should be placed on perceived problems based on sanitary surveys.

Experts at a 1981 U.S. EPA workshop recommended a minimum sampling frequency of five per month.[3] This value was based on the calculation that if at least 60 samples per year are collected and 95% of these do not contain coliforms, then there is a 95% probability that the fraction of water distributed during the year containing coliforms is less than 10%.

If disinfection is practised in water supply systems where the source is or could be contaminated, failure of the disinfection system could result in a serious health hazard. Constant monitoring of the disinfectant residual concentration and bacteriological quality is therefore necessary to ensure that immediate remedial action can be taken if water of doubtful quality enters the distribution system. A check on the disinfection process and bacteriological examination of water entering the distribution system should be made daily.[103,104] Where this is impractical—for example, in the smallest supplies—reliance may have to be placed on residual chlorine determinations. This recommendation does not apply to supplies served by sources of excellent quality in which disinfection is practised to increase the safety margin.

Location of Sampling Points

The location of sampling points in a distribution system must be decided by the surveillance agency. Samples should be taken at the point where the water enters the system and from representative points throughout the network, although not necessarily the same points on each occasion. If the water supply is obtained from more than one source, the location of sampling points in the distribution system should ensure that water from each source is periodically sampled. The majority of samples should be taken in potential problem areas: low-pressure zones, reservoirs, dead ends, areas at the periphery of the system farthest from the treatment plant and areas with a poor previous record. Although this practice is recommended, Pipes and Christian[107] found no significant differences in the frequency of coliform occurrences between peripheral and non-peripheral sampling locations in a distribution system.

Handling of Samples

Proper procedures for collecting samples must be observed to ensure that the sample is representative of the water being examined. Detailed instructions on the collection of samples for bacteriological analysis are given in *Standard Methods for the Examination of Water and Wastewater*.[2] As the way in which samples are collected has an important bearing on the results of their examination, sample collectors should be properly trained for the work.

To avoid unpredictable changes in the bacterial flora of the sample, examination should be started as soon as possible after collection. The sample should be transported to the laboratory in an iced cooler. The interval between collection of the sample and the beginning of its examination should not exceed 24 hours. When greater delays are anticipated, a delayed incubation procedure should be employed. The delayed incubation procedure, described in *Standard Methods for the Examination of Water and Wastewater*,[2] is a modification of the standard MF technique, which permits transport of the membrane, after filtration, to a

distant laboratory for incubation and completion of the test. Alternatively, if transportation time exceeds 24 hours, the sample should be processed and arrangements made to have another sample collected as soon as the first sample is received. Thus, if the late sample contains coliforms, a repeat sample will already have been received or will be in transit. Recent reports[108,109] support the belief that samples should be stored under refrigeration to minimize changes in populations and concentrations. Samples should be identified with the date, location and any special conditions. When examination will be delayed, it is particularly important to record the time and temperature of storage, as this information should be considered in the interpretation of results.

Guidelines and Recommendations

Recommendations for the Treatment of Raw Supplies

As modern water treatment technology can produce high-quality drinking water even from a heavily polluted raw water source, no raw water bacteriological guidelines are recommended. However, close monitoring of all aspects of the raw water quality is required so that treatment processes can be adjusted in accordance with any variation detected. Because it is specific for enteric pollution, the faecal coliform test is preferred for assessing the microbial quality of raw water. The presence of faecal coliform organisms should be regarded as indicative of hazardous contamination. In the absence of faecal coliforms, the presence of total coliform organisms may be due to relatively less recent faecal pollution or to the presence of normal indigenous bacteria.

If past experiences have demonstrated that the raw water could harbour pathogens for which coliforms are not good indicators (e.g., *Giardia lamblia*, *Yersinia enterocolitica*), then the raw water may be periodically monitored for these pathogens.

Raw water quality will vary with both time and location. The frequency of sampling for bacteriological examination of a particular water should therefore be established by the surveillance agency in co-operation with the local control agency.

When water supplies are obtained from polluted sources, effective treatment must be provided to ensure the safety of the consumers. It is suggested that raw water coliform measurements be used to assist in determining treatment requirements as follows:

(1) If more than 10% of the raw water samples in any 30-day period have a faecal coliform density greater than 100 per 100 mL or a total coliform density greater than 1000 per 100 mL, the water should receive complete treatment, consisting of coagulation–flocculation, sedimentation, filtration and disinfection. If the total coliform index exceeds 5000 per 100 mL in more than 10% of the samples, auxiliary treatment—consisting of pre-chlorination or pre-sedimentation, or their equivalents, and post-chlorination—should be used. Other advanced forms of treatment approved by the control agency may be considered equivalent to any of the traditional methods named above.

(2) If more than 10% of the raw water samples in any 30-day period have a faecal coliform density in the range 10 to 100 per 100 mL or a total coliform density between 100 and 1000 per 100 mL, the water should receive a combination of some of the following processes: coagulation–flocculation, sedimentation and filtration (partial treatment), or equivalent advanced forms of treatment approved by the control agency, followed by disinfection.

(3) If any raw water sample contains faecal coliforms or if more than 5% of the samples in any consecutive 30-day period have a total coliform density greater than 10 per 100 mL, disinfection is required.

All supplies derived from surface water sources should receive coagulation, filtration and disinfection as a minimum treatment. Supplies obtained from shallow groundwater sources should also receive disinfection as required by the control agency.

It should not be inferred that these guidelines will guarantee production of adequate-quality drinking water from every raw water source. For example, partial treatment may be necessary to remove turbidity even when the coliform counts are low. In addition, satisfaction of other water quality criteria may dictate the use of unit processes not mentioned in the above scheme.

Potable Water Quality

1. Coliform, Coliform Background and Heterotrophic Plate Counts

Effective treatment including disinfection should yield water free from any coliform organisms no matter how polluted the source water may have been. The presence of any type of coliform organism in treated water therefore suggests either inadequate treatment and disinfection or contamination of the water in the distribution system after treatment.

The routine analysis for coliform bacteria should be supplemented by heterotrophic plate counts or by background colony counts on the total coliform membrane filters. The sudden rise of an HPC or background count that has been traditionally low should give rise to concern even in the absence of a concomitant rise in the coliform count. This is particularly relevant in situations in which elevated concentrations of the general bacteria population hinder the recovery of coliforms and prevent the detection of a threat to public health. Thus, the maximum acceptable concentration (MAC) for total

coliforms is no organisms detectable per 100 mL. Because coliforms are not uniformly distributed in water and are subject to considerable variation in enumeration, drinking water that fulfils the following conditions is considered to be in compliance with the total coliform MAC:

(1) No sample should contain more than 10 total coliform organisms per 100 mL, none of which should be faecal coliforms; and

(2) No consecutive samples from the same site should show the presence of coliform organisms; and

(3) For community drinking water supplies:

(a) not more than 10% of the samples based on a minimum of 10 samples should show the presence of coliform organisms; and

(b) not more than one sample from a set of samples taken from the community on a given day should show the presence of coliform organisms.

If any of the above criteria are exceeded, corrective action should be taken immediately, in consultation with the local control agency. The most common immediate actions include increasing chlorine dosage, flushing water mains, using an alternative source of water and advising consumers to boil drinking water.

If less than 10 total coliform organisms per 100 mL are detected from a single sample, or if the sample contains more than either 200 background colonies on a total coliform membrane filter per 100 mL or 500 HPC colonies/mL, the site should be resampled. If the presence of coliforms is reconfirmed (see condition 2, above), the cause should be determined and corrective action taken as appropriate. If there is a recurrence of unacceptable background or heterotrophic plate counts, an inspection of the system should be undertaken to determine the cause. If remedial action is deemed necessary, special sampling should continue until consecutive samples comply with the guidelines.

2. Pathogenic Micro-organisms

For some potential pathogenic bacteria (e.g., *Salmonella*, *Shigella* and *Campylobacter jejuni*), the absence of coliforms in treated water is a good indication that these pathogens are probably also absent. If, however, past experience has demonstrated that the raw water could harbour pathogens for which coliforms are not good indicators (e.g., *Giardia lamblia* and *Yersinia enterocolitica*), then the treated water should be routinely examined (or treated) for the presence of these pathogens. Properly treated and distributed drinking water should be free of pathogenic micro-organisms.

3. Nuisance Organisms

This category comprises a morphologically and physiologically diverse group of organisms that include planktonic and sessile algae, fungi, crustacea and protozoa, as well as actinomycetes and iron and sulphur bacteria. These organisms may produce objectionable tastes, colour, odour and turbidity and may interfere with treatment processes by clogging strainers and filters. In addition, although not themselves pathogenic, certain planktonic organisms may harbour pathogenic bacteria and viruses, thus protecting them from disinfection by chlorine. It is difficult, however, to specify any quantitative limit on nuisance organisms, because individual species differ widely in their ability to produce undesirable effects. Most of these organisms can be controlled relatively easily by the usual water treatment processes. Furthermore, the problems of taste, odour, colour and turbidity, which may be caused by these organisms, are covered indirectly by the limits on the physical characteristics of water. The frequency of sampling and analysis for nuisance organisms should be determined by the control agency, based on the likelihood of significant kinds and concentrations of such organisms being present.

4. Maintenance of a Chlorine Residual

Where chlorine disinfection is practised, a chlorine residual should be maintained throughout the distribution system. Maintenance and monitoring of a chlorine residual offers two benefits. First, a trace of chlorine will suppress the growth of organisms within the system and may afford some protection against contamination from without; second, the disappearance of the residual provides an immediate indication of the entry of oxidizable matter into the system or of a malfunction of the treatment process. It is therefore recommended that a free chlorine residual be maintained and monitored daily throughout the entire system. It is recognized, however, that excessive levels of free chlorine may result in taste and odour problems. In these cases, the control agency may provide guidance as to the type and concentration of chlorine residual to ensure a microbiologically safe water.

When a residual concentration measured at a sampling point is less than that required by the control agency, another sample should be taken immediately. If this sample is also unsatisfactory, the line should be flushed and sampling continued until a satisfactory concentration is obtained. If the residual does not return to the allowable minimum, the chlorine dosage should be increased. If increasing the chlorine dosage is ineffective, or if excessive chlorination is required, a sanitary survey for potential sources of contamination should be made in co-operation with the responsible control agency, and special samples for bacteriological

analysis should be taken. Should all these measures prove inadequate, the control agency should be consulted for further advice, and action should be taken was appropriate.

5. Sampling and Sample Size

The sampling frequency and location of sampling points should be decided by the control agency after due consideration of local conditions. In general, the number of samples for bacteriological testing should be increased in accordance with the size of the population served. The following table is offered as a guide:

Population served	**Minimum no. of samples per month**
Up to 5000	4
5000 to 100 000	1 per 1000 persons
Over 100 000	90 + 1 per 10 000 persons

The samples should be taken at regular intervals throughout the month. Chlorine residual tests should be made when bacteriological samples are taken. The majority of samples should be taken in potential problem areas.

For private wells, samples should be collected at times of highest risk of contamination—e.g., spring thaw, heavy rains or dry periods. New or renovated wells should also be initially sampled three times, one to three weeks apart.

The sample volume should be sufficient to carry out all the tests required. For treated drinking water, a minimum volume of 100 mL should be examined for the total coliform determination regardless of which method is used. The maximum volume for analysis by the P–A test is usually 100 mL; however, 200 mL of sample should be collected, as a heterotrophic plate count and subsequent examination by the MF method may be required.

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