Chapter 23

Indicator Microorganisms

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23.1 THE CONCEPT OF INDICATOR ORGANISMS

The routine examination of environmental samples for the presence of intestinal pathogens is often a tedious, difficult and time-consuming task. Thus, it has been customary to tackle this issue by looking for indicator microorganisms, whose presence indicates that pathogenic microorganisms may also be present. Developed for the assessment of fecal contamination, the indicator concept depends on the fact that certain nonpathogenic bacteria occur in the feces of all warm-blooded animals. These bacteria can often be isolated and quantified by simple bacteriological methods more easily than pathogenic microbes. Detection of these bacteria in water can mean that fecal contamination has occurred, and suggests that enteric pathogens may also be present.

For example, coliform bacteria, which normally occur in the intestines of all warm-blooded animals, are excreted in great numbers in feces. In polluted water, coliform bacteria are found in densities roughly proportional to the degree of fecal pollution. Because coliform bacteria are generally hardier than disease-causing bacteria, their absence from water is an indication that the water is bacteriologically safe for human consumption. Conversely, the presence of the coliform group of bacteria is indicative that other kinds of microorganisms capable of causing disease may also be present, and that the water is potentially unsafe to drink.

In 1914, the U.S. Public Health Service adopted the coliform group as an indicator of fecal contamination of drinking water. Many countries have adopted coliforms and other groups of bacteria as official standards for drinking water, recreational bathing waters, wastewater discharges and various foods. Indicator microorganisms have also been used to assess the efficacy of food processing and water and wastewater treatment processes. As an ideal assessor of fecal contamination, it has been suggested that they meet the criteria listed in Table 23.1. Unfortunately, no single indicator meets all of these criteria. Thus, various groups of microorganisms have been suggested and used as indicator organisms. Concentrations of indicator bacteria found in wastewater and feces are shown in Tables 23.2 and 23.3.

Indicators have traditionally been used to suggest the presence of enteric pathogens; however, today we recognize that there is rarely a direct correlation between bacterial indicators and human pathogens (Ashbolt *et al.*, 2001). As such, the use of indicators is better defined by their intended purpose (Table 23.4). Thus, process indicators are used to assess the efficacy of a treatment process

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TABLE 23.1 Criteria for an Ideal Indicator Organism

- The organism should be useful for all types of water.
- The organism should be present whenever enteric pathogens are present.
- The organism should have a reasonably longer survival time than the hardiest enteric pathogen.
- The organism should not grow in water.
- The testing method should be easy to perform.
- The density of the indicator organism should have some direct relationship to the degree of fecal pollution.
- The organism should be a member of the intestinal microflora of warm-blooded animals.

TABLE 23.2 Estimated Levels of Indicator Organisms in Raw Sewage

Organism	CFU* per 100 ml
Coliforms	10^7–10^9
Fecal coliforms	10^6–10^7
Fecal streptococci	10^5–10^6
Enterococci	10^4–10^5
Escherichia coli	10^6–10^7
Clostridium perfringens	10^4
Staphylococcus (coagulase positive)	10^3
Pseudomonas aeruginosa	10^5
Acid-fast bacteria	10^2
Coliphages	10^2–10^3
Bacteroides	10^7–10^{10}

**CFU = colony forming units*

TABLE 23.3 Microbial Flora of Animal Feces

Animal Group	Average Density Per Gram		
	Fecal Coliforms	Fecal Streptococci	*Clostridium perfringens*
Farm animals			
Cow	230,000	1,300,000	200
Pig	3,300,000	84,000,000	3980
Sheep	16,000,000	38,000,000	199,000
Horse	12,600	6,300,000	<1
Duck	33,000,000	54,000,000	–
Chicken	1,300,000	3,400,000	250
Turkey	290,000	2,800,000	–
Animal pets			
Cat	7,900,000	27,000,000	25,100,000
Dog	23,000,000	–	–
Wild animals			
Mouse	330,000	7,700,000	<1
Rabbit	20	47,000	<1
Chipmunk	148,000	6,000,000	–
Human	13,000,000	3,000,000	1580

Modified from Geldreich (1978).

TABLE 23.4 Definitions and Examples of Indicator Microorganisms

Group	Definition and Examples
Process indicator	A group of organisms that demonstrate the efficacy of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection
Fecal indicator	A group of organisms that indicate the presence of fecal contamination, such as the fecal coliforms or *Escherichia coli*
Index and model organisms	A group or species indicative of pathogen presence and behavior, respectively, such as *E. coli* as index for *Salmonella* and male-specific coliphages as models for human enteric viruses

Modified from Ashbolt et al. (2001).

(e.g., drinking water treatment), while fecal indicators indicate the presence of fecal contamination. An index (or model) organism represents the presence and behavior of a pathogen in a given environment.

23.2 TOTAL COLIFORMS

The coliform group, which includes *Escherichia*, *Citrobacter*, *Enterobacter* and *Klebsiella* species, is relatively easy to detect (Figure 23.1). Specifically, this group includes all aerobic and facultatively anaerobic, Gram-negative, nonspore-forming, rod-shaped bacteria that produce gas upon lactose fermentation in prescribed culture media within 48 hours at 35°C.

The coliform group has been used as the standard for assessing fecal contamination of recreational and drinking waters for almost a century. Through experience, it has been learned that absence of this organism in 100 ml of drinking water ensures the prevention of bacterial waterborne disease outbreaks. However, it has also been

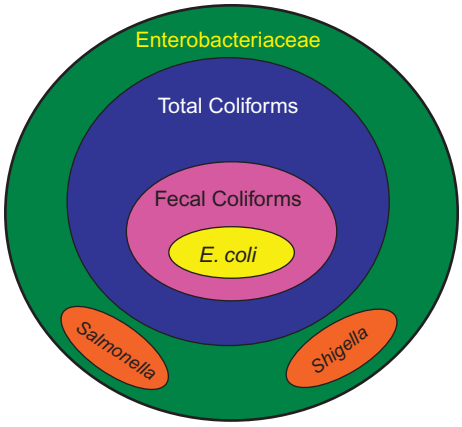

FIGURE 23.1 Relationships between indicators in three Enterobacteriacea.

TABLE 23.5 Deficiencies with the Use of Coliform Bacteria as Indicators of Water Quality

- Regrowth in aquatic environments
- Regrowth in distribution systems
- Suppression by high background bacterial growth
- Not indicative of a health threat
- No relationship between enteric protozoan and viral concentration

Modified from Gleeson and Gray (1997).

learned that a number of deficiencies in the use of this indicator exist (Table 23.5).

All members of the coliform group have been observed to regrow in natural surface and drinking water distribution systems (Gleeson and Gray, 1997). The die-off rate of coliform bacteria depends on the amount and type of organic matter in the water, and its temperature. If the water contains significant concentrations of organic matter and is at an elevated temperature, the bacteria may increase in numbers. This phenomenon has been observed in eutrophic tropical waters, waters receiving pulp and paper mill effluents, wastewater, aquatic sediments and organically enriched soil (i.e., soil amended with biosolids) after periods of heavy rainfall. Of greatest concern is the growth of recovery of injured coliform bacteria in a drinking water distribution system, because this may give a false indication of fecal contamination. Coliforms may colonize and grow in biofilms found on distribution system pipes, even in the presence of free chlorine. *Escherichia coli* is 2400 times more resistant to free chlorine when attached to a surface than when it is suspended as free cells in water (LeChevallier *et al.*, 1988).

Because large numbers of heterotrophic bacteria in the water may mask the growth of coliform bacteria on selective media used for their isolation, true numbers of coliforms may be underestimated. This often becomes a problem when aerobic heterotrophic bacterial numbers exceed 500 per ml. Finally, the longer survival time and greater resistance to disinfectants of pathogenic enteric viruses and protozoan parasites limits the use of coliform bacteria as an indicator for these organisms. However, the coliform group of bacteria has proved its merit in the assessment of the bacterial quality of water. Three methods are commonly used to identify coliforms in water. These are the most probable number (MPN), the membrane filter (MF), and the presence–absence (P–A) tests.

23.2.1 The Most Probable Number (MPN) Test

The MPN test allows detection of the presence of coliforms in a sample and estimation of their numbers (see also Section 10.3.2). This test consists of three steps: a presumptive test, a confirmed test and a completed test. In the presumptive test (Figure 23.2A), lauryl sulfate–tryptose–lactose broth is placed in a set of test tubes with different dilutions of the water to be tested. Usually, three to five test tubes are prepared per dilution. These test tubes are incubated at 35°C for 24 to 48 hours, and subsequently examined for the presence of coliforms, which is indicated by gas and acid production. Once the positive tubes have been identified and recorded, it is possible to estimate the total number of coliforms in the original sample by using an MPN table that gives numbers of coliforms per 100 ml. In the confirming test (Figure 23.2B), the presence of coliforms is verified by inoculating selective bacteriological agars such as Levine's eosin–methylene blue (EMB) agar or Endo agar with a small amount of culture from the positive tubes. Lactose-fermenting bacteria are indicated on the medium by the production of colonies with a green sheen or colonies with a dark center. In some cases, a completed test (not shown in Figure 23.2) is performed in which colonies from the agar are inoculated back into lauryl sulfate–tryptose–lactose broth to demonstrate the production of acid and gas.

23.2.2 The Membrane Filter Test

The membrane filter (MF) test also allows scientists to determine the number of coliforms in a sample, but it is easier to perform than the MPN test because it requires fewer test tubes and less labor (Figure 23.3) (see also Chapter 8). In this technique, a measured amount of water (usually 100 ml for drinking water) is passed through a membrane filter (pore size 0.45 μm) that traps bacteria on its surface. This membrane is then placed on a thin absorbent pad that has been saturated with a specific medium

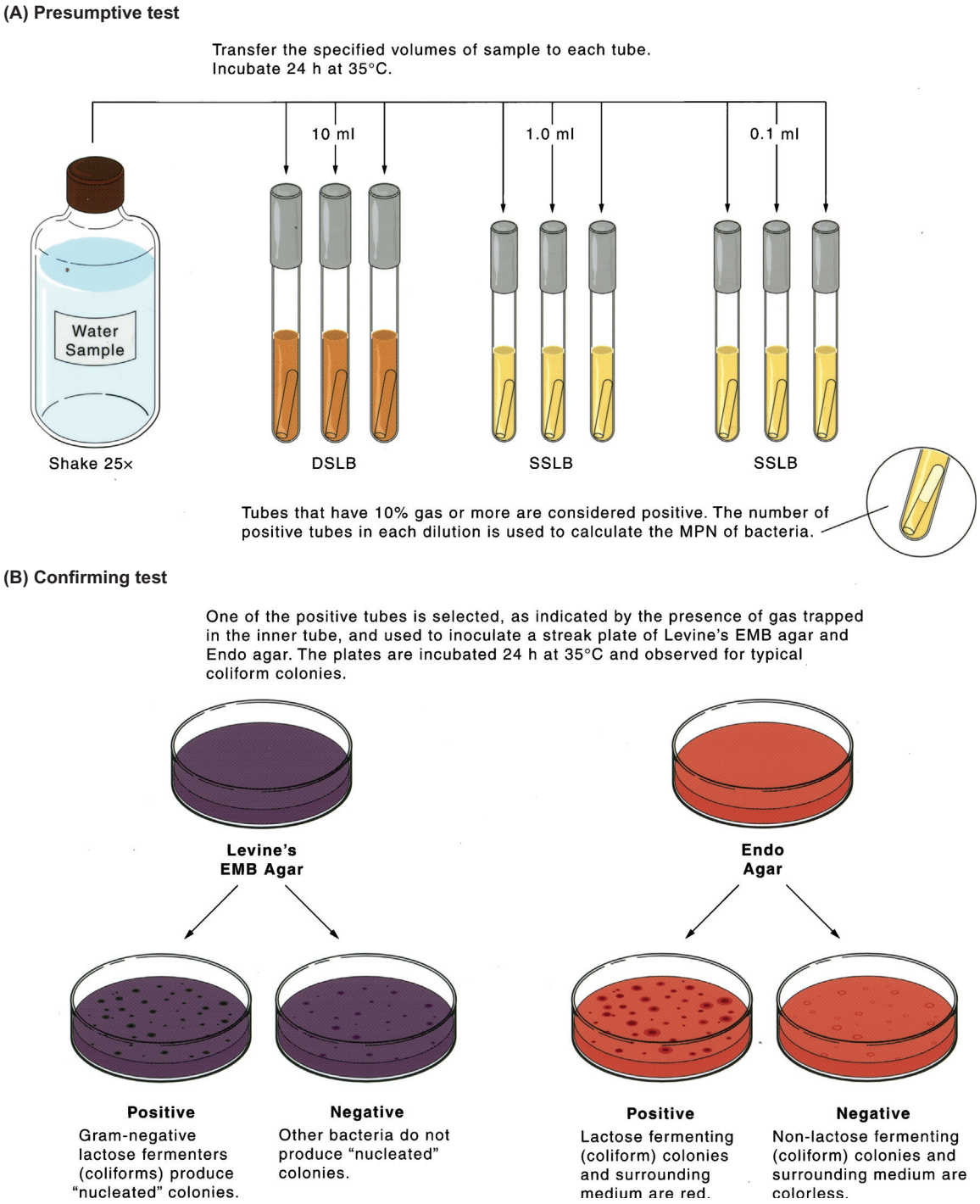

FIGURE 23.2 Procedure for performing an MPN test for coliforms on water samples: (A) presumptive test and (B) confirming test. DSLB = double strength lauryl sulfate broth, SSLB = single strength lauryl sulfate broth.

designed to permit growth and differentiation of the organisms being sought. For example, if total coliform organisms are sought, a modified Endo medium (m-Endo) is used.

For coliform bacteria, the filter is incubated at 35°C for 18–24 hours. The success of the method depends on using effective differential or selective media that can facilitate identification of the bacterial colonies growing on the membrane filter surface (see Figure 23.3). To determine the number of coliform bacteria in a water sample, the colonies having a green sheen with m-Endo media are enumerated.

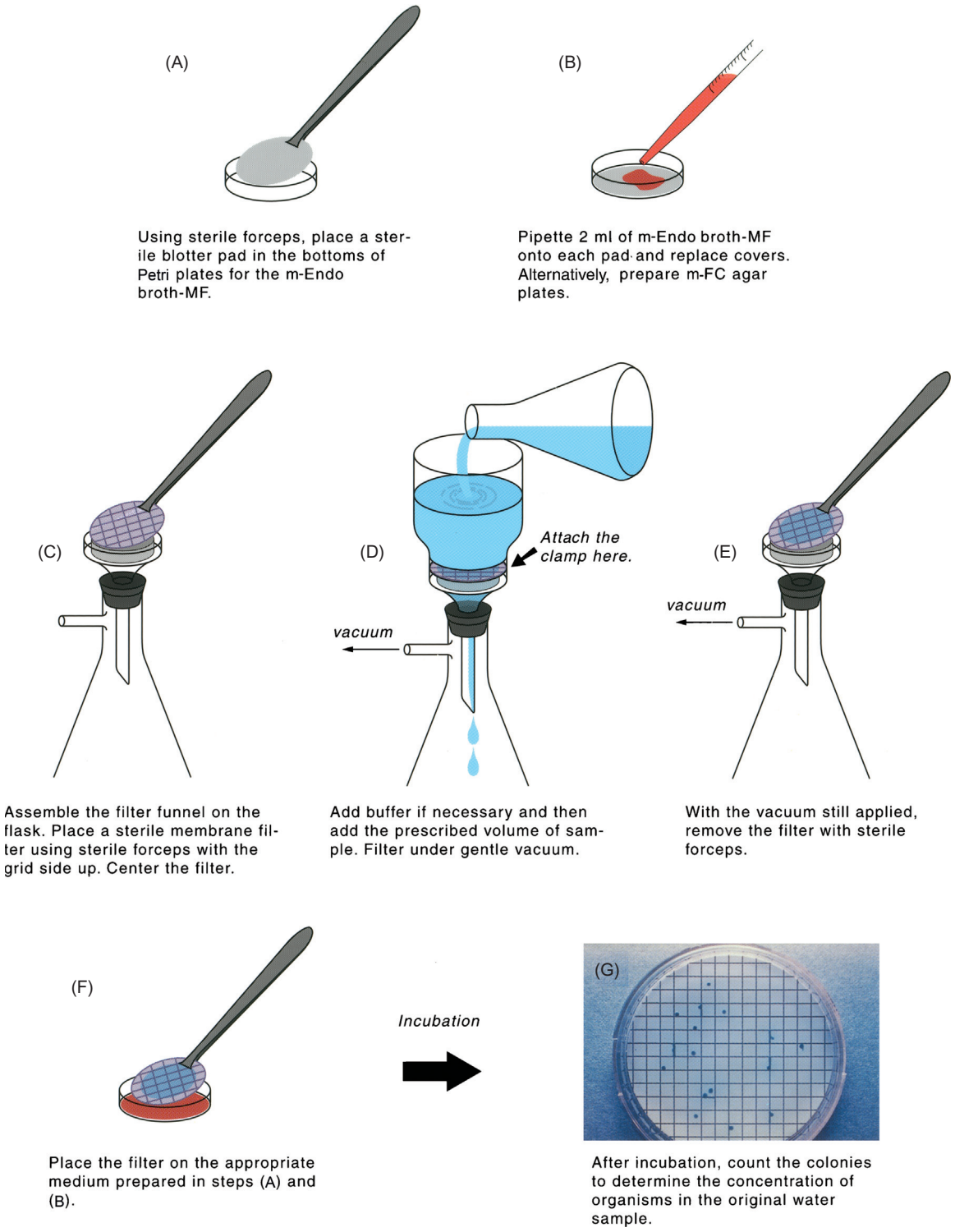

FIGURE 23.3 Membrane filtration for determining the coliform count in a water sample using vacuum filtration.

23.2.3 The Presence–Absence (P–A) Test

Presence–absence tests (P–A tests) are not quantitative tests; instead, they answer the question of whether the target organism is present in a sample or not. A single tube of lauryl sulfate–tryptose–lactose broth as used in the MPN test, but without dilutions, would be used in a P–A test. In recent years, enzymatic assays have been developed that allow the simultaneous detection of total coliform bacteria and *E. coli* in drinking water. The assay can be a simple

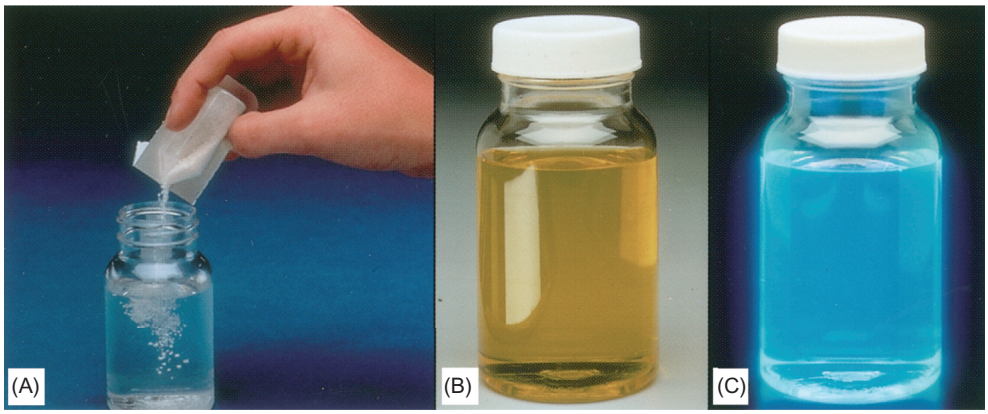

FIGURE 23.4 Detection of indicator bacteria with Colilert. (A) Addition of salts and enzyme substrates to water sample; (B) yellow color indicating the presence of coliform bacteria; (C) fluorescence under long-wave ultraviolet light indicating the presence of *E. coli*.

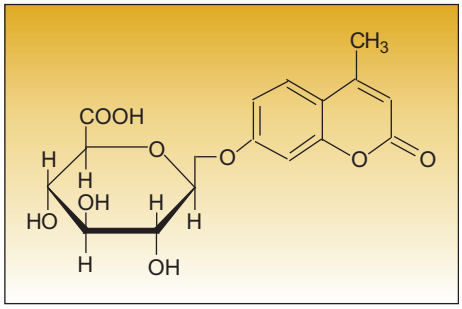

FIGURE 23.5 The structure of 4-methylumbelliferyl-β-*D*-glucuronide (MUG).

P−A test or an MPN assay. The Colilert system (Figure 23.4) is one such assay: it is based on the fact that total coliform bacteria produce the enzyme β-galactosidase, which hydrolyzes the substrate *o*-nitrophenyl-β-*D*-galactopyranoside (ONPG) to yellow nitrophenol. *E. coli* can be detected at the same time by incorporation of a fluorogenic substrate, 4-methylumbelliferone glucuronide (MUG) (Figure 23.5), which produces a fluorescent end product after interaction with the enzyme β-glucuronidase found in *E. coli*, but not in other coliforms. The end product is detected with a long-wave ultraviolet (UV) lamp. The Colilert test is performed by adding the sample to a single bottle (P−A test) or MPN tubes that contain powdered ingredients consisting of salts or specific enzyme substrates that serve as the only carbon source for the organisms (Figure 23.4A). After 24 hours of incubation, samples positive for total coliforms turn yellow (Figure 23.4B), whereas *E. coli*-positive samples fluoresce (blue color) under long-wave UV illumination in the dark (Figure 23.4C).

23.3 FECAL COLIFORMS AND *ESCHERICHIA COLI*

Although the total coliform group has served as the main indicator of water pollution for many years, several of the organisms in this group are not limited to fecal sources. Thus, methods have been developed to restrict the enumeration to coliforms that are more clearly of fecal origin—that is, the fecal coliforms (Figure 23.1). These organisms, which include the genera *Escherichia* and *Klebsiella*, are differentiated in the laboratory by their ability to ferment lactose with the production of acid and gas at 44.5°C within 24 hours. In general, this test indicates fecal coliforms; it does not, however, distinguish between human and animal contamination. The frequent occurrence of coliform and fecal coliform bacteria in unpolluted tropical waters, and their ability to survive for considerable periods of time in these waters outside the intestine, have suggested that these organisms occur naturally in tropical waters (Toranzos, 1991), and that new indicators for these waters need to be developed.

Some have suggested the use of *E. coli* as an indicator, because it can easily be distinguished from other members of the fecal coliform group (e.g., absence of urease and presence of β-glucuronidase), and is more likely to indicate fecal pollution. Fecal coliforms also have some of the same limitations in use as the coliform bacteria, i.e., regrowth and less resistant to water treatment than viruses and protozoa.

Fecal coliforms may be detected by methods similar to those used for coliform bacteria. For the MPN method, EC broth is used, and for the membrane filter method, m-FC agar is used for water analysis. A medium known as m-T7 agar has been proposed for use in the recovery of injured fecal coliforms from water (LeChevallier *et al.*, 1983), and results in greater recovery from water. The Colilert test has the advantage of detecting coliforms and *E. coli*, the principal fecal coliform, simultaneously within 24 hours.

23.4 FECAL STREPTOCOCCI (*ENTEROCOCCI*)

The fecal streptococci are a group of Gram-positive Lancefield group D streptococci (Figure 23.6). The fecal

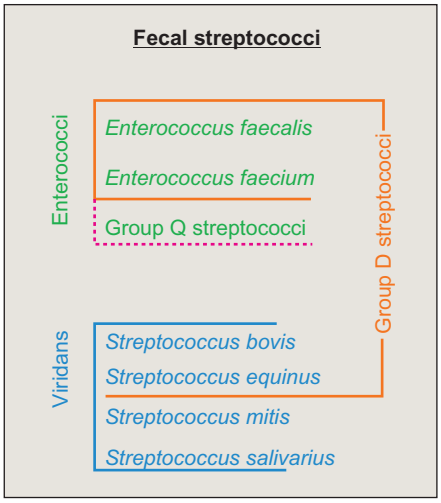

FIGURE 23.6 Definition of the terms "enterococci," "group D streptococci" and "fecal streptococci" based on *Streptococcus* species belonging to each group.

streptococci belong to the genera *Enterococcus* and *Streptococcus* (Sadowsky and Whitman, 2011). The genus *Enterococcus* includes all streptococci that share certain biochemical properties, and have a wide range of tolerance of adverse growth conditions. The enterococci can be found in soil, water, dairy products, food and plants. They are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, pH 9.6 and 45°C, and include *Ent. avium*, *Ent. faecium*, *Ent. durans*, *Ent. faecalis* and *Ent. gallinarium.* In the water industry, the genus is often given as *Streptococcus* for this group. Of the genus *Streptococcus*, only *S. bovis* and *S. equinus* are considered to be true fecal streptococci. These two species of *Streptococcus* are predominantly found in animals; *Ent. faecalis* and *Ent. faecium* are more specific to the human gut. It has been suggested that a fecal coliform/fecal streptococci (FC/FS) ratio of 4 or more indicates a contamination of human origin, whereas a ratio below 0.7 is indicative of animal pollution (Geldreich and Kenner, 1969) (Table 23.6). However, the validity of the FC/FS ratio has been questioned. Further, this ratio is valid only for recent (24 hours) fecal pollution.

Both the membrane filtration method and MPN method may also be used for the isolation of fecal streptococci. The membrane filter method uses fecal *Streptococcus* agar with incubation at 37°C for 24 hours. All red, maroon and pink colonies (due to reduction of 2,4,5-triphenyltetrazolium chloride to formazan, a red dye) are counted as presumptive fecal streptococci. Confirmation of fecal streptococci is by subculture on bile aesculin agar and incubation for 18 hours at 44°C. Fecal streptococci form discrete colonies surrounded by a brown or black halo due to aesculin hydrolysis, and *Ent. faecalis* organisms are considered to be more specific to

TABLE 23.6 The FC/FS Ratio

FC/FS Ratio	Source of Pollution
>4.0	Strong evidence that pollution is of human origin
2.0–4.0	Good evidence of the predominance of human wastes in mixed pollution
0.7–2.0	Good evidence of the predominance of domestic animal wastes in mixed pollution
<0.7	Strong evidence that pollution is of animal origin

the human gut. Enterococci are considered to have certain advantages over the coliform and fecal coliform bacteria as indicators:

- They rarely grow in water.
- They are more resistant to environmental stress and chlorination than are coliforms.
- They generally persist longer in the environment (Gleeson and Gray, 1997).

The concentration of enterococci in surfaces waters has been shown to be related to the risk of gastroenteritis among recreational bathers, and standards have been developed for acceptable levels of enterococci (Cabelli, 1989).

23.5 *CLOSTRIDIUM PERFRINGENS*

Clostridium perfringens is a sulfite-reducing anaerobic spore former; it is Gram positive, rod shaped and exclusively of fecal origin. The spores are very heat resistant (75°C for 15 minutes), persist for long periods in the environment and are very resistant to disinfectants. The hardy spores of this organism limit its usefulness as an indicator because it is often found in soils and sediments. However, it has been suggested that it could be an indicator of past pollution, a tracer of less hardy indicators and an indicator of removal of protozoan parasites or viruses during drinking water and wastewater treatment (Payment and Franco, 1993). It is used as an indicator for drinking water in Europe (Bitton, 2011).

23.6 *BACTEROIDES* AND *BIFIDOBACTERIUM*

Bifidobacterium and *Bacteroides* are anaerobic bacteria that have also been suggested as potential indicators. *Bacteriodes* are Gram-positive rods found in the gut of humans and animals. Because they are strict anaerobes

they do not survive long in the environment, and have been suggested as indicators of recent fecal pollution. They are more common in the human gut than *E. coli* and represent 30% of the total number of fecal isolates (Sadowsky and Whitman, 2011). Because some of the *Bifidobacterium* are primarily associated with humans, it has been suggested that they can be used to help distinguish between human and animal contamination. Until recently the isolation of this strict anaerobe has been difficult; however, use of polymerase chain reaction has made it more feasible.

23.7 HETEROTROPHIC PLATE COUNT

An assessment of the numbers of aerobic and facultatively anaerobic bacteria in water that derive their carbon and energy from organic compounds is conducted via the heterotrophic plate count or HPC. This group includes Gram-negative bacteria belonging to the following genera: *Pseudomonas*, *Aeromonas*, *Klebsiella*, *Flavobacterium*, *Enterobacter*, *Citrobacter*, *Serratia*, *Acinetobacter*, *Proteus*, *Alcaligenes*, *Enterobacter* and *Moraxella.* The heterotrophic plate counts of microorganisms found in untreated drinking water and chlorinated distribution water are shown in Table 23.7 (LeChevallier *et al*., 1980). These bacteria are commonly isolated from surface waters and groundwater, and are widespread in soil and vegetation (including many vegetables eaten raw). Some members of this group are opportunistic pathogens (e.g., *Aeromonas*, *Pseudomonas*), but no conclusive evidence is available to demonstrate their transmission by ingestion of drinking water. In drinking water, the number of HPC bacteria may vary from less than 1 to more than 10^4 CFU per ml, and members are influenced mainly by temperature, presence of residual chlorine and level of assimilable organic matter. In reality, these counts themselves have no or little health significance. However, there has been concern because the HPC can grow to large numbers in bottled water and charcoal filters on household taps. In response to this concern, studies have been performed to evaluate the impact of HPC on illness. These studies have not demonstrated a conclusive impact on illness in persons who consume water with high HPC. Although the HPC is not a direct indicator of fecal contamination, it does indicate variation in water quality, and the potential for pathogen survival and regrowth. These bacteria may also interfere with coliform and fecal coliform detection when present in high numbers. It has been recommended that the HPC should not exceed 500 per ml in tap water (LeChevallier *et al*., 1980).

Heterotrophic plate counts are normally done via the spread plate method using yeast extract agar incubated at 35°C for 48 hours. A low-nutrient medium, R_2A (Reasoner and Geldreich, 1985), has been widely used for

TABLE 23.7 Identification of HPC Bacteria in Untreated Drinking Water and in a Chlorinated Distribution System

Organism	Distribution Water	Untreated Drinking Water
	% of the total number of organisms identified	% of the total number of organisms identified
Actinomycetes	10.7	0
Arthrobacter spp.	2.3	1.3
Bacillus spp.	4.9	0.6
Corynebacterium spp.	8.9	1.9
Micrococcus luteus	3.5	3.2
Staphylococcus aureus	0.6	0
S. epidermidis	5.2	5.1
Acinetobacter spp.	5.5	10.8
Alcaligenes spp.	3.7	0.6
Flavobacterium meningosepticum	2.0	0
Moraxella spp.	0.3	0.6
Pseudomonas alcaligenes	6.9	2.5
P. cepacia	1.2	0
P. fluorescens	0.6	0
P. mallei	1.4	0
P. maltophilia	1.2	5.7
Pseudomonas spp.	2.9	0
Aeromonas spp.	9.5	15.9
Citrobacter freundii	1.7	5.1
Enterobacter agglomerans	1.2	11.5
Escherichia coli	0.3	0
Yersinia enterocolitica	0.9	6.4
Hafnia alvei	0	5.7
Enterobacter aerogenes	0	0.6
Enterobacter clonane	0	0.6
Klebsiella pneumoniae	0	0
Serratia liquefaciens	0	0.6
Unidentified	18.7	17.8

Modified from LeChevallier et al. (1980).

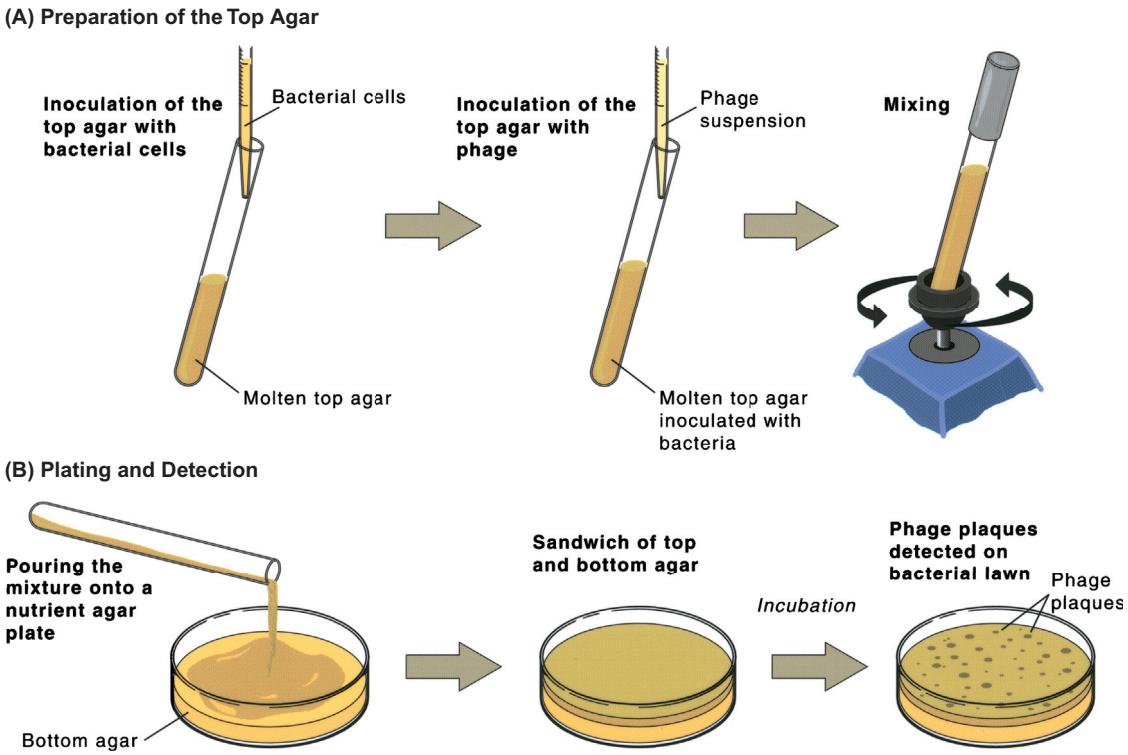

FIGURE 23.7 Technique for performing a bacteriophage assay.

disinfectant-damaged bacteria. This medium is recommended for use with an incubation period of 5–7 days at 28°C. HPC numbers can vary greatly depending on the incubation temperature, growth medium and length of incubation.

23.8 BACTERIOPHAGES

Because of their constant presence in sewage and polluted waters, the use of bacteriophages (or bacterial viruses) as appropriate indicators of fecal pollution has been proposed. These organisms have also been suggested as indicators of viral pollution. This is because the structure, morphology and size, as well as the behavior in the aquatic environment of many bacteriophages, closely resemble those of enteric viruses. For these reasons, they have also been used extensively to evaluate virus resistance to disinfectants, the fate of viruses during water and wastewater treatment, and as indicators for viruses in surface and groundwater. The use of bacteriophages as indicators of fecal pollution is based on the assumption that their presence in water samples denotes the presence of bacteria capable of supporting the replication of the phage. Two groups of phages in particular have been studied: the somatic coliphages, which infect *E. coli* host strains through cell wall receptors, and the F-specific RNA coliphages, which infect strains of *E. coli* and related bacteria through the F^+ or sex pili. A significant

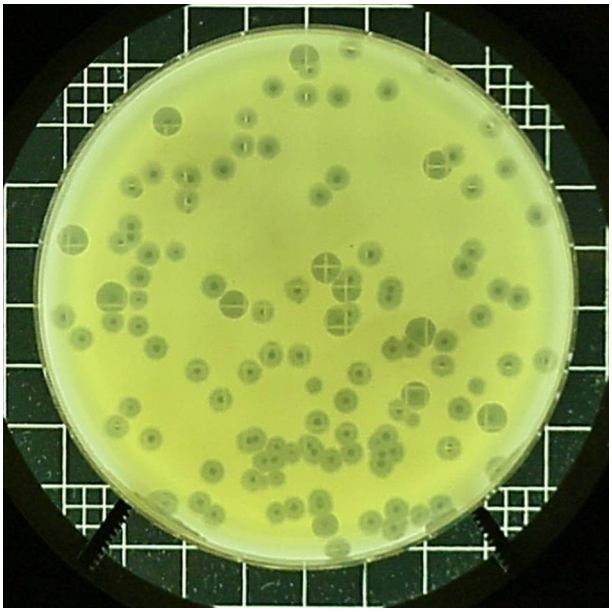

FIGURE 23.8 Bacteriophage plaques on a bacterial lawn.

advantage of using coliphages is that they can be detected by simple and inexpensive techniques that yield results in 8–18 hours. Both a plating method (the agar overlay method) and the MPN method can be used to detect coliphages (Figures 23.7 and 23.8) in volumes ranging from 1 to 100 ml. The F-specific coliphages (male-specific phage) have received the greatest amount of attention

because they are similar in size and shape to many of the pathogenic human enteric viruses. Coliphage f2, φX174, MS-2, Qβ and PRD-1 are the phages that have most commonly been used as tracers and for evaluation of disinfectants. Because F-specific phages are infrequently detected in human fecal matter and show no direct relationship to the fecal pollution level, they cannot be considered indicators of fecal pollution (Havelaar *et al*., 1990). However, their presence in high numbers in wastewaters, and their relatively high resistance to chlorination, contributes to their consideration as an index of wastewater contamination and as potential indicators of enteric viruses.

Bacteriophages of *Bacteroides fragilis* have also been suggested as potential indicators of human viruses in the environment (Tartera and Jofre, 1987). *Bacteroides* spp. are strict anaerobes and are a major component of human feces, so bacteriophages active against these organisms have the potential to be suitable indicators of viral contamination.

Bacteriophages that infect *B. fragilis* appear to be exclusively human in origin (Tartera and Jofre, 1987), and appear to be present only in environmental samples contaminated with human fecal pollution. This may help to differentiate human from animal contamination. They are absent from natural habitats, which is a considerable advantage over coliphages, which are found in habitats other than the human gut. They are unable to multiply in the environment (Tartera *et al*., 1989), and their decay rate in the environment appears to be similar to that of human enteric viruses. However, their host is an anaerobic bacterium that involves a complicated and tedious methodology, which limits their suitability as a routine indicator organism.

23.9 OTHER POTENTIAL INDICATOR ORGANISMS

A number of other organisms have also been considered to have potential as alternative indicator organisms or for use in certain applications (e.g., recreational waters). These include *Pseudomonas* spp., yeasts, acid-fast mycobacteria (*Mycobacterium fortuitum* and *M. phlei*), *Aeromonas* and *Staphylococcus.*

Within the genus *Pseudomonas*, the species of significant public health concern is *P. aeruginosa*, a Gram-negative, nonsporulating, rod-shaped bacterium. The most common diseases associated with this organism are eye, ear, nose and throat infections. It is also the most common opportunistic pathogen causing life-threatening infections in burn patients and immunocompromised individuals. A characteristic of the pseudomonad is that it can produce the blue−green pigment pyocyanin, or the fluorescent pigment fluorescein, or both. Numerous cases of folliculitis, dermatitis, ear (swimmer's ear) and urinary tract infections are due to *P. aeruginosa* associated with swimming in contaminated water, or poorly maintained swimming pools and hot tubs. Because of this association and its consistent presence in high numbers in sewage, *P. aeruginosa* has been suggested as a potential indicator for water in swimming pools, hot tubs and other recreational waters (Cabelli, 1978). However, as this organism is known to be ubiquitous in nature and can multiply under natural conditions (it can even grow in distilled water), it is believed to be of little value for fecal contamination studies.

Coliforms have been used for many years to assess the safety of swimming pool water, yet contamination is often not of fecal origin with infections associated primarily with the respiratory tract, skin and eyes. For this reason, *Staphylococcus aureus* and *Candida albicans*, a Gram-positive bacterium and a yeast, respectively, have been proposed as better indicators of this type of infection associated with swimming. Recreational waters may serve as a vehicle for skin infections caused by *S. aureus*, and some observers have recommended that this organism be used as an additional indicator of the sanitary quality of recreational waters, because its presence is associated with human activity in recreational waters (Charoenca and Fujioka, 1993).

The genus *Aeromonas* includes straight facultatively anaerobic gram-negative rods that are included in the family Vibrionaceae. Only *Aeromonas hydrophila* has received attention as an organism of potential sanitary significance. *Aeromonas* occurs in uncontaminated waters as well as in sewage and sewage-contaminated waters. The organism can be pathogenic for humans, other warm-blooded animals and cold-blooded animals including fish. Foodborne outbreaks associated with *A. hydrophila* have been documented, and it is considered an opportunistic pathogen in humans. Because of its association with nutrient-rich conditions, it has been suggested as an indicator of the nutrient status of natural waters.

23.10 STANDARDS AND CRITERIA FOR INDICATORS

Bacterial indicators such as coliforms have been used for the development of water quality standards. For example, the U.S. Environmental Protection Agency (U.S. EPA) has set a standard of no detectable coliforms per 100 ml of drinking water. A drinking water standard is legally enforceable in the United States. If these standards are violated by water suppliers, they are required to take corrective action or they may be fined by the state or federal government. Authority for setting drinking water standards was given to the U.S. EPA in 1974 when Congress passed the Safe Drinking Water Act. Similarly, authority for setting standards for domestic wastewater discharges is given under the Clean Water Act (see Table 17.1). In contrast, standards for recreational waters and wastewater

reuse are determined by the individual states. Microbial standards set by various government bodies in the United States are shown in Table 23.8. Standards used by the European Union are given in Table 23.9.

Criteria and guidelines are terms used to describe recommendations for acceptable levels of indicator microorganisms. They are not legally enforceable, but serve as guidance indicating that a potential water quality problem may exist. Ideally, all standards would indicate that an unacceptable public health threat exists, or that some relationship exists between the amount of illness and the level of indicator organisms. Such information is difficult to acquire because of the involvement of costly epidemiological studies that are often difficult to interpret because of confounding factors (see Chapter 24). An area where epidemiology has been used to develop criteria is that of recreational swimming. Epidemiological studies in the United States have demonstrated a relationship between swimming-associated gastroenteritis and the densities of enterococci (Figure 23.9) and *E. coli*. No

TABLE 23.8 U.S. Federal and State Standards for Microorganisms

Authority	Standards
U.S. EPA	
Safe Drinking Water Act	0 coliforms/100 ml
Clean Water Act	
Wastewater discharges	200 fecal coliforms/100 ml
Sewage sludge	<1000 fecal coliforms/4 g
	<3 *Salmonella*/4 g
	<1 enteric virus/4 g
	<1 helminth ovum/4 g
California	
Wastewater reclamation for irrigation	≤2.2 MPN/100 ml coliforms
Food and Drug Administration	
Shellfish growing areas[a]	14 MPN/100 ml fecal coliforms

[a]*FDA (2005)*.

TABLE 23.9 Drinking Water Criteria of the European Union

Tap water	
Escherichia coli	0/100 ml
Fecal streptococci	0/100 ml
Sulfite-reducing clostridia	0/20 ml
Bottled Water	
Escherichia coli	0/250 ml
Fecal streptococci	0/250 ml
Sulfite-reducing clostridia	0/50 ml
Pseudomonas aeruginosa	0/250 ml

From European Union (1995).

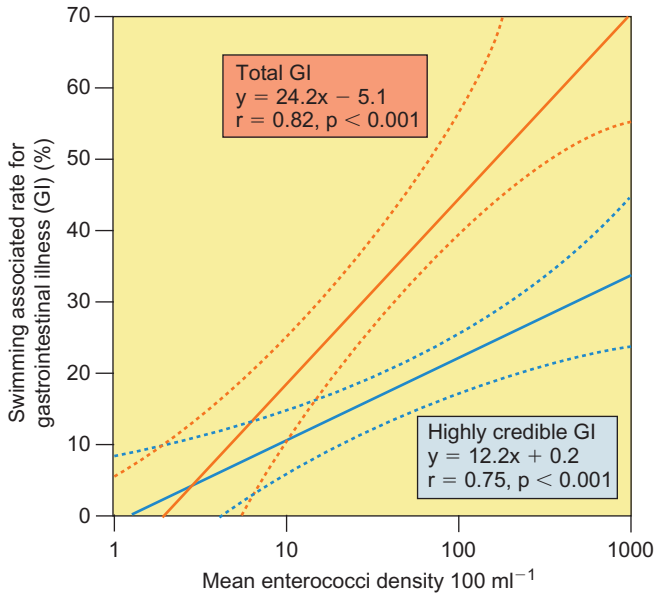

FIGURE 23.9 Dose–response relationships produced by the work of Cabelli *et al.* (1982).

relationship was found for coliform bacteria (Cabelli, 1989). It was suggested that a standard geometric average of 35 enterococci per 100 ml be used for marine bathing waters. This would mean accepting a risk of 1.9% of the bathers developing gastroenteritis (Kay and Wyer, 1992). Numerous other epidemiological studies of bathing-acquired illness have been conducted. These studies have shown slightly different relationships to illness and that other bacterial indicators were more predictive of illness rates (Kay and Wyer, 1992). These differences probably arise for a variety of reasons including: the different sources of contamination (raw versus disinfected wastewater); types of recreational water (marine versus fresh); types of illness (gastroenteritis, eye infections, skin complaints); immune status of the population; and the length of observation. Various guidelines for acceptable numbers of indicator organisms have been in use (Table 23.10), but there is no general agreement on standards.

The use of microbial standards also requires the development of standard methods and quality assurance or quality control plans for the laboratories that conduct the monitoring. Knowledge of how to sample and how often to sample is also important. All of this information is usually defined in the regulations when a standard is set. For example, frequency of sampling may be determined by the size (number of customers) of the utility providing the water. Sampling must proceed in some systematic fashion so that the entire system is characterized. For drinking water, no detectable coliforms are allowed in the United States (Table 23.8). However, in other countries a certain level of coliform bacteria is allowed. Because of the wide variability in numbers of indicators in water, some positive samples may be allowed or tolerance levels or averages may be allowed. Usually, geometric averages are used in standard settings because the distribution of bacterial numbers is often skewed. This prevents one or two high values from overestimating of high levels of contamination, which would appear to be the case of arithmetic averages (see Table 23.11).

Geometric averages are determined as follows:

$$\log \bar{x} = \frac{\sum(\log x)}{N} \qquad \text{(Eq. 23.1)}$$

$$\bar{x} = \text{anti log}(\log\ \bar{x}) \qquad \text{(Eq. 23.2)}$$

where:

N is the number of samples,
$\bar{x}$ is the geometric average, and
x is the number of organisms per sample volume.

As can be seen, standard setting and the development of criteria is a difficult process and there is no ideal standard. A great deal of judgment by scientists, public health officials and the regulating agency is required.

TABLE 23.10 Guidelines for Recreational Water Quality Standards

Country or Agency	Regime (samples/time)	Criteria or Standard[a]
U.S. EPA	5/30 days	200 fecal coliforms/100 ml <10% to exceed 400 per ml
		Fresh water[b] 33 enterococci/100 ml 126 *E. coli*/100 ml
		Marine waters[b] 35 enterococci/100 ml
European Economic Community	2/30 days[c]	500 coliforms/100 ml
		100 fecal coliforms/100 ml
		100 fecal streptococci/100 ml
		0 *Salmonella*/liter
		0 Enteroviruses/10 liters
Ontario, Canada	10/30 days	≤1000 coliforms/100 ml
		≤100 fecal coliforms/100 ml

From Saliba (1993); U.S. EPA (1986).
[a]*All bacterial numbers in geometric means.*
[b]*Proposed, 1986.*
[c]*Coliforms and fecal coliforms only.*

TABLE 23.11 A Comparison of Arithmetic and Geometric Averages of Bacterial Numbers in Water

MPN[a]	Log
2	0.30
110	2.04
4	0.60
150	2.18
1100	3.04
10	1.00
12	1.08
198 = arithmetic average	1.46 = log $\bar{x}$ antilog $\bar{x}$ = 29
	29 = geometric average

[a]*MPN, most probable number.*

QUESTIONS AND PROBLEMS

1. What are some of the criteria for indicator bacteria?
2. What is the difference between standards and criteria?
3. Why are geometric means used to report average concentrations of indicator organisms?
4. Calculate the arithmetic and geometric averages for the following data set: fecal coliforms/100 ml on different days on a bathing beach were reported as 2, 3, 1000, 15, 150 and 4000.
5. Define coliform and fecal coliform bacteria. Why are they not ideal indicators?
6. Why have coliphage been suggested as indicator organisms?
7. What are two methods that can be used to detect indicator bacteria in water?
8. Calculate the most probable number (MPN) for the following dataset:

Volume added to each tube	Number of positive tubes
10 ml	3
1.0 ml	1
0.1 ml	0

9. What is the difference between a fecal indicator organism and a process indicator? Give an example of each.
10. How are bacteriophages used as indicators? What is a coliphage?
11. How many bathers would you expect to develop gastroenteritis: (1) if 35 enterococci/100 ml were detected in the water; or (2) if 100 enterococci/100 ml were present? (See Figure 23.8.)
12. Which indicator would be best for indication of long-term sewage pollution? Which one would be best as a short-term indicator of recent sewage pollution?

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