CHAPTER

20

Indicator Microorganisms

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20.1 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 such examinations by looking first for certain indicator microorganisms whose presence indicates that pathogenic microorganisms may also be present. Developed at the turn of the last century for assessing 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 easily be isolated and quantified by simple bacteriological methods. Detection of these bacteria in water means 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 20.1. Unfortunately, no one indicator meets all 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 20.2 and 20.3.

20.2 TOTAL COLIFORMS

The coliform group, which includes *Escherichia, Citrobacter, Enterobacter,* and *Klebsiella* species, is relatively easy to detect. Specifically, this group includes all aerobic and facultatively anaerobic, gram-negative, non–spore-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 most of this 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.

TABLE 20.1 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 20.2 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
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}

However, it has been learned that a number of deficiencies in the use of this indicator exist (Table 20.4).

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., sewage sludge amended) after periods of heavy rainfall. Of greatest concern is the growth or recovery of injured coliform bacteria in a distribution system because this may give a false indication of fecal contamination (Fig. 20.1). Coliforms may colonize and grow in the biofilm found on the 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 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/ml. Finally, the longer survival and greater resistance to disinfectants of pathogenic

TABLE 20.3 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	3,980
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	1,580

Modified from Geldreich (1978).

TABLE 20.4 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).

enteric viruses and protozoan parasites limit the use of coliform bacteria as an indicator for these organisms. Still, the coliform group of bacteria has proved its merit in assessing 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.**

20.2.1 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 Chapter 10.1.3). This test consists of three steps: a presumptive test, a confirmed test, and a completed test. In the **presumptive test** (Fig. 20.2a), 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, then 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** (Fig. 20.2b), 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 Fig. 20.2) 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.

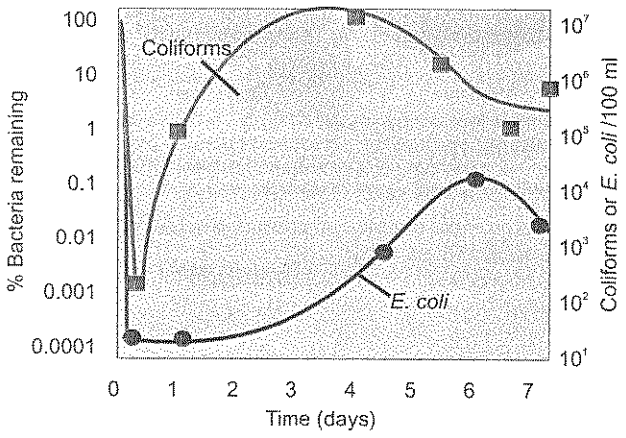

FIGURE 20.1 Regrowth of coliforms and *E. coli* in sewage effluent after inactivation with 5 mg/l chlorine. (From Shuval *et al.*, 1973.)

20.2.2 The Membrane Filter (MF) Test

The 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 (Fig. 20.3) (see also Chapter 10.2.1.3). 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 designed to permit growth and differentiation of the organisms being sought. For example, if total coliform organisms are sought, a modified Endo medium 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 Fig. 20.3). To determine the number of coliform bacteria in a water sample, the colonies having a green sheen are enumerated.

20.2.3 The Presence–Absence (P–A) Test

Presence–absence tests (P–A tests) are not quantitative tests; instead, they answer the simple 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 P–A test or an MPN assay. The Colilert system (Fig. 20.4) 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-

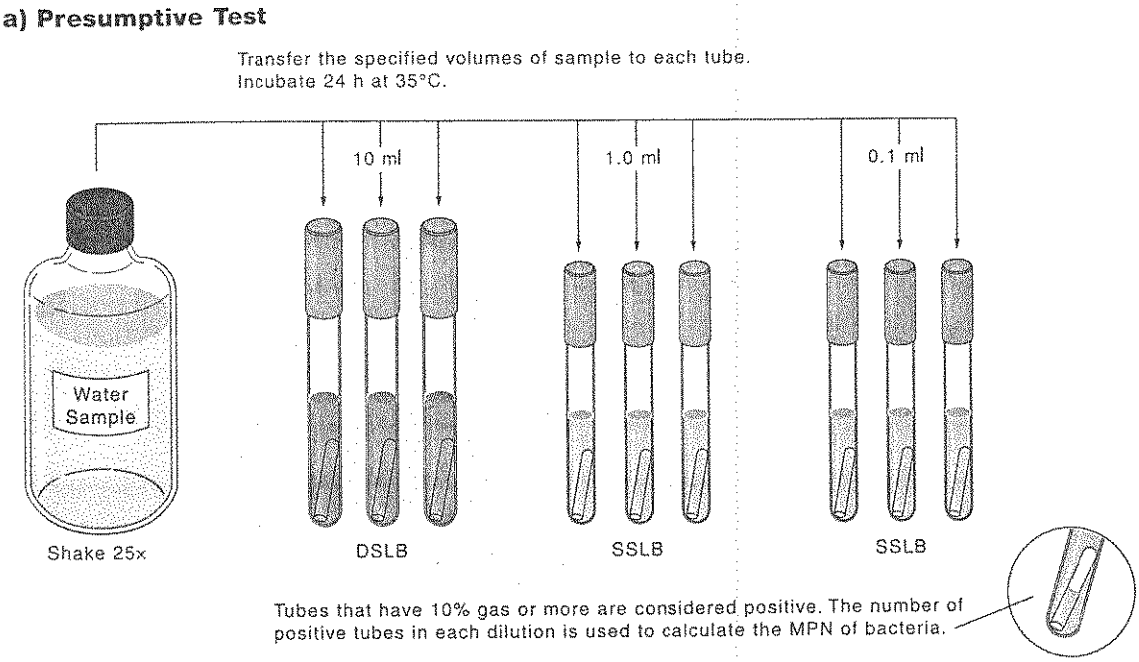

FIGURE 20.2 Procedure for performing an MPN test for coliforms on water samples: (a) presumptive test and (b) confirmed test. (From *Pollution Science* © 1996, Academic Press, San Diego, CA.)

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) (Fig. 20.5), 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

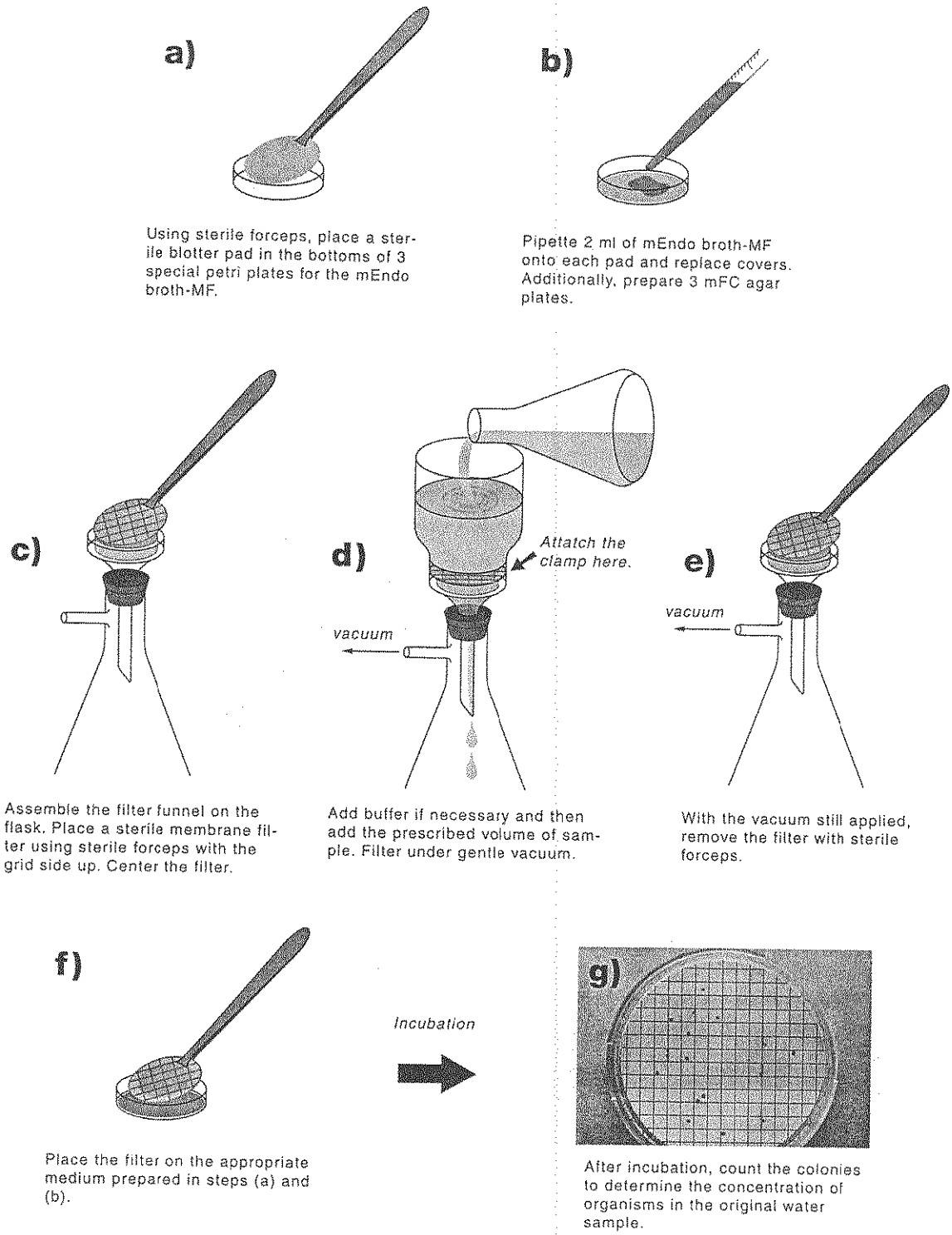

FIGURE 20.3 Membrane filtration for determining the coliform count in a water sample using vacuum filtration. (From *Pollution Science* © 1996, Academic Press, San Diego, CA.)

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 (Fig. 20.4a). After 24 hours of incubation, samples positive for total coliforms turn yellow (Fig. 20.4b), whereas *E. coli*–positive samples fluoresce under long-wave UV illumination in the dark (Fig. 20.4c).

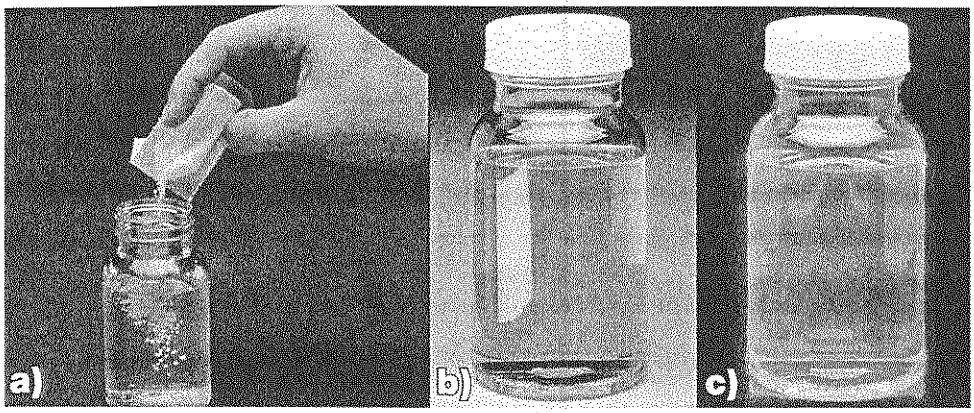

FIGURE 20.4 Detection of indicator bacteria with Colilert. (a) Addition of salts and enzyme substrates to water sample; (b) yellow color indicating the presence of coliform bacteria; (c) fluorescence under long-wave ultraviolet light indicating the presence of *E. coli*. Photographs courtesy of IDEXX, Westbrook, ME.

20.3 FECAL COLIFORMS

Although the total coliform group has served as the main indicator of water pollution for many years, many 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.** 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, then, 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 outside the intestine in these waters, 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). 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.

20.4 FECAL STREPTOCOCCI

The fecal streptococci are a group of gram-positive Lancefield group D streptococci (Fig. 20.6). The fecal streptococci belong to the genera *Enterococcus* and *Streptococcus* (Gleeson and Gray, 1997). The genus *Enterococcus* includes all streptococci that share certain biochemical properties and have a wide range of tolerance of adverse growth conditions. 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. faccalis,* and *Ent. gallinarium.* In the water industry the genus is often given as *Streptococcus* for this group. Of the genus

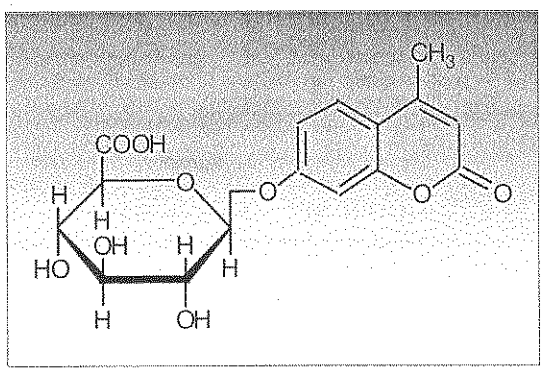

FIGURE 20.5 The structure of 4-methylumbelliferyl-β-d-glucuronide (MUG).

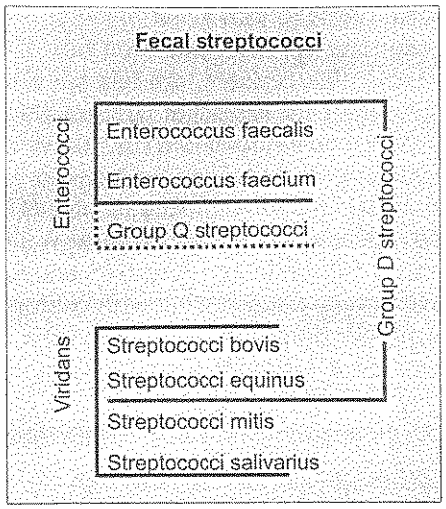

FIGURE 20.6 Definition of the terms "enterococci," "group D streptococci," and "fecal streptococci" based on *Streptococcus* species belong to each group.

Streptococcus, only *S. bovis* and *S. equinus* are considered to be true fecal streptococci. These two species of *Streptococcus* are predominately 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 20.5). 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 aesulin 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,

TABLE 20.5 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

and *Ent. faecium* are considered to be more specific to the human gut. Fecal streptococci are considered to have certain advantages over the coliform and fecal coliform bacteria as indicators.

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

The enterococci have been suggested as useful indicators of risk of gastroenteritis for recreational bathers and standards have been recommended (Cabelli, 1989). They have been suggested as useful indicators of the presence of enteric viruses in the environment.

20.5 *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. 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).

Other anaerobic bacteria such as *Bifidobacterium* and *Bacteroides* have been suggested as potential indicators. Because some of the *Bifidobacterium* are primarily associated with humans, they could potentially help distinguish between human and animal contamination. However, better and more standard methods are needed for detection of all of the anaerobic bacteria in the environment before they can be adequately monitored in a routine fashion.

20.6 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 chlo-

rinated distribution water are shown in Table 20.6 (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 drinking water. In drinking water, the number of HPC bacteria may vary from less than 1 to more than 10^4 CFU/ml, and they 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 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 by

TABLE 20.6 Identification of HPC Bacteria in Untreated Drinking Water and in the Chlorinated Distribution System

	Distribution water	Untreated drinking water
Organism	% of the total number of organisms identified	% of the total number of organisms identified
Acinomycetes	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 cloacae	0	0.6
Klebsiella pneumoniae	0	0
Serratia liquefaciens	0	0.6
Unidentified	18.7	17.8

Modified from LeChevallier *et al.* (1980).

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 seen widespread use and is recommended for 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.

20.7 BACTERIOPHAGE

Because of their constant presence in sewage and polluted waters, the use of bacteriophage (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 bacteriophage closely resemble those of enteric viruses. For these reasons, they have also been used extensively to evaluate virus resistance to disinfectants, to evaluate virus fate during water and wastewater treatment, and as surface and groundwater tracers. The use of bacteriophage 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 phage in particular have been studied: the **somatic coliphage**, which infect *E. coli* host strains through cell wall receptors, and the **F-specific RNA coliphage**, which infect strains of *E. coli* and related bacteria through the F^+or sex pili. A significant advantage of using coliphage 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 coliphage (Fig. 20.7) in volumes ranging from 1 to 100 ml. The F-specific coliphage (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, ϕ174, MS-2, and PRD-1 are the ones most commonly used as tracers and for evaluation of disinfectants. Because F-specific phage 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 contribute to their consideration as an index of waste-

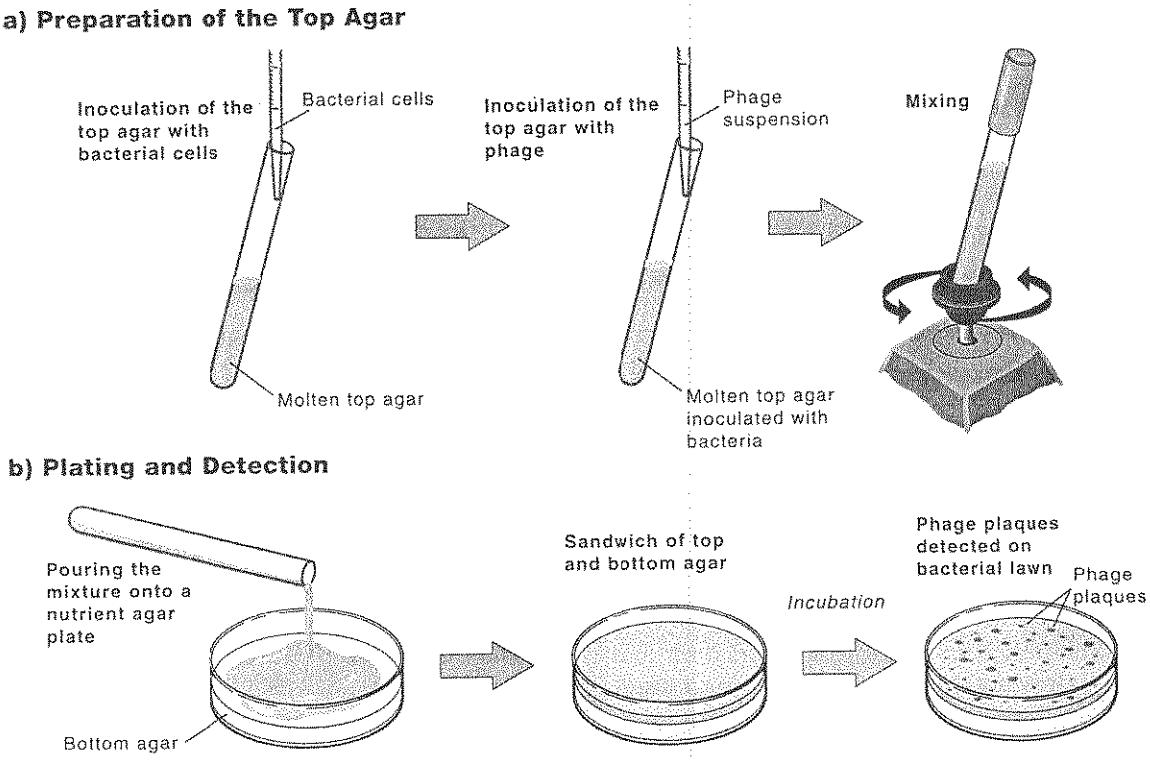

FIGURE 20.7 Technique for performing a bacteriophage assay. (From Pepper *et al.*, 1995.)

water contamination and as potential indicators of enteric viruses.

Bacteriophage 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 bacteriophage active against these organisms have the potential to be suitable indicators of viral contamination.

Bacteriophage 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 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.

20.8 OTHER 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, and 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 gram-negative rods, facultatively anaerobic, that are included in the family Vibrionaceae. Only *Aeromonas hydrophila* has received attention as an organism of potential sanitary significance. Aeromonas occur 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.

Table 20.7 summarizes potential applications of indicator organisms in the assessment of water quality.

20.9 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 16.1). In contrast, standards for recreational waters and wastewater reuse are determined by the individual states. Micro-

TABLE 20.7 Water Quality Indicators, Their Significant Sources, and Potential Uses

Indicator	Significant source[a]	Potential use[b]
Coliforms	F S I R A	S
Fecal coliforms	F S I R A	F S
Enterococcus	F S	F S A D
Clostridium perfringens	F S	F S D
Candida albicans	F S	P F S
Bifidobacteria	F S	F S A D
Coliphage	S	S
Pseudomonas aeruginosa	S I R A	P S N
Aeromonas hydrophila	S I R A	P S N

Modified from Cabelli (1978).

[a] Relative to other sources: F, feces of warm-blooded animals; S, sewage; I, industrial wastes; R, runoff from uncontaminated soils; A, fresh and marine waters.

[b] Potential use: P, pathogen; F, fecal indicators; S, sewage indicator; A, separation of human from lower animal sources; D, proximity to fecal source; N, indicator of nutrient pollution.

bial standards set by various government bodies in the United States are shown in Table 20.8. Standards used by the European Union are given in Table 20.9.

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 exists. 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 (Fig. 20.8) and fecal coliforms. No 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 re-

TABLE 20.8 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 oval/4 g
California	
Wastewater reclamation for irrigation	≤2.2 MPN coliforms
Arizona	
Wastewater reclamation for irrigation of golf courses	25 fecal coliforms/100 ml 125 enteric virus/40 liters No detectable *Giardia*/40 liters

TABLE 20.9 Drinking Water Criteria of the European Union

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

From European Union (1995).

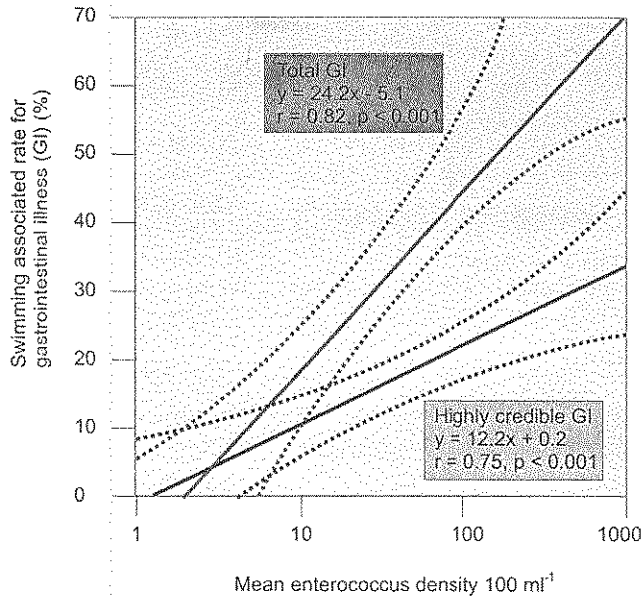

FIGURE 20.8 Dose–response relationships produced by the work of Cabelli *et al.* (1982).

lationships to illness and that other bacterial indicators were more predictive of illness rates (Kay and Wyer, 1992). These differences probably arise because of 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, length of observation, etc. Various guidelines for acceptable numbers of indicator organisms have been in use (Table 20.10), 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 will do 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 random fashion so that the entire system is characterized. For drinking water, no detectable coliforms are allowed in the United States (Table 20.8). However, in other countries some 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 setting because of the often skewed distribution of bacterial numbers. This prevents one or two high values from giving overestimates of high levels of contamination, which would appear to be the case with **arithmetric averages** (see Table 20.11).

Geometric averages are determined as follows.

$$\log \bar{x} = \frac{\Sigma (\log x)}{N} \qquad \text{(Eq. 20.1)}$$

$$\bar{x} = \text{antilog} (\log \bar{x}) \qquad \text{(Eq. 20.2)}$$

where N is the number of samples $\bar{x}$ is and 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 20.10 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/ml Fresh water[b] 33 enterococci/100 ml 126 fecal coliforms/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.

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 arithmetric 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 3000.

TABLE 20.11 Arithmetric 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 = arithmetric average	1.46 = log $\bar{x}$ antilog $\bar{x}$ = 29 29 = geometric average

[a] MPN, most probable number.

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?

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