

## 2.0 INDICATOR ORGANISMS AND WATER QUALITY CRITERIA

### 2.1 INTRODUCTION OF INDICATOR BACTERIA

The goal of public health regulation is to limit human exposure to pathogens in water. It would be the best if pathogens can be monitored directly. Ideally, monitoring programs to support public health protection specifically for activities such as shellfish consumption and contact recreation, would measure pathogens directly. Instead, indicator organisms have been used for regulatory purposes, especially for recreational and shellfish growing waters.

Cabelli (1977) noted that the best indicator organism should be the one whose densities correlate best with health hazards associated with one or several given types of pollution sources. He also listed the requirements for an indicator as follows:

- A. The indicator should be consistently and exclusively associated with the source of the pathogens.
- B. It must be present in sufficient numbers to provide an accurate density estimate whenever the level of each of the pathogens is such that the risk of illness is unacceptable.
- C. It should approach the resistance to disinfectants and environmental stress, including toxic materials deposited therein, of the most resistant pathogen potentially present at significant levels in the sources.
- D. It should be quantifiable in recreational waters by reasonably facile and inexpensive methods and with considerable accuracy, precision, and specificity.

These requirements provide a basis to compare available indicators for water quality monitoring.

The objective of this session is to give a brief description of the indicator organisms and their relations with pathogens. The organisms discussed include total and FC, E. coli, fecal streptococcus, and enterococcus, which have been used as indicators in either EPA guidance or state water quality standards. In addition, the EPA 1986 water quality criteria for bacteria, the Texas water quality criteria for contact and noncontact recreational waters, and the water quality criteria for shellfish growing waters by the National Shellfish Sanitation Program (NSSP) are also discussed in this section.

### 2.1.1 Total and Fecal Coliform

By definition (Standard Methods, 1989), the total coliform (TC) group comprises all aerobic and facultative anaerobic, gram-negative, nonspore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48 hours at 35°C. Fecal coliform (FC) are defined as those coliforms which respond at an elevated temperature of 44.5°C. Thus a more accurate name for organisms which show positive on the FC test would be heat tolerant coliforms.

Among the coliform group, there are four genera in the Enterobacteriaceae family, Escherichia, Klebsiella, Citrobacter, and Enterobacter (Metcalf and Eddy, 1991). Some of these genera are common in the intestinal tract of mammals (e.g. Escherichia coli and others are common in soil and on the surface of plants e.g. Klebsiella).

In addition to other kinds of bacteria, each person produces from 100 to 400 billion coliform organisms per day (Metcalf and Eddy, 1991). Since historically the primary public health concern has been diseases transmitted through human wastes, the absence of coliform organisms is taken as an indication that a sample is free of disease-producing organisms. After being isolated and associated with the fecal wastes of warm-blooded animals in late 1800s and early 1900s (Cabelli, 1977), coliforms have been used as indicators for indexing health hazards in drinking and recreational waters.

Coliforms have also shown a measure of correlation with pathogens. For example, Geldreich (1978) found that for FC concentrations less than 200/dL, (100 mL = 1 dL) Salmonella occurrences ranged from 6.5 to 31%. However, at FC concentrations greater than about 1000/dL, the frequency of Salmonella occurrence doubled. For recreational lakes and streams with FC levels from 1 to 200/dL, Salmonella occurred in 28% of the water samples. When FC were about 1000/dL, Salmonella occurrence was 96%.

On the other hand, there are many bacterial species of the four main genera which are common in soil and on the surface of plants which respond positively to the TC or FC test (Dufour 1977; Cabelli, et al., 1982). FC positive results have been found in numerous food processing industry wastes (EPA, 1986) and fish growing ponds (De La Cruz, 1992) all with no mammalian waste sources. Elevated levels are also found in runoff from agricultural fields with very limited mammalian and avian population and have been documented to grow in higher organic strength waters (Jensen, Ritter, and Tyrawski, 1977).

A common theme of these results would appear to be elevated concentrations of organic materials from a wide range of sources can support bacterial populations, a portion of which are capable of responding positively to the TC and FC tests. This would indicate

that the TC/FC test does not meet Cabelli's first requirement for a good indicator organism -- consistent and exclusive association with a pathogen source.

### 2.1.2 Escherichia Coli

Escherichia coli is a member of the coliform bacteria population that may be used to indicate fecal sources. It is a normal and dominant inhabitant of the mammalian digestive tract. However, disease-causing strains of E. coli specie have been isolated from tap water, drinking water sources, and mountain streams (Standard Methods, 1989). Examination of pathogenic E. coli is not easy due to the uncertainty in determining the pathogenic nature of isolated E. coli strains. There is no biochemical marker that can separate pathogenic from non-pathogenic strains and the relationship between serotype and pathogenicity is questionable (Standard Methods, 1989).

The use of E. coli as an indicator organism is somewhat restricted by the fact that (Tchobanoglous and Schroeder, 1985) (1) E. coli is not a single species, (2) certain genera of the coliform group such as Proteus and Aerobacter are normally found outside the human intestinal tract in soil, (3) other organisms found in water that do not represent fecal pollution possess some of the characteristics attributed to E. coli, and (4) E. coli identical to that found in humans is also found in the intestinal tract of other warm-blooded animals. However, primarily because studies had shown that E. coli was a much better indicator of disease risk than was FC, EPA (1986) has recommended that E. coli be used as a criteria for classifying waters for fresh water contact recreation.

### 2.1.3 Fecal Streptococcus

The fecal streptococcus (FS) group consists of a number of species of the genus Streptococcus. They are characterized as gram-positive, cocci bacteria which are capable of growth in brain-heart infusion broth. In the laboratory they are defined as all the organisms which produce red or pink colonies within 48 hours at  $35 \pm 1.0^{\circ}\text{C}$  on KF-streptococcus medium (Standard Methods, 1989). The normal habitat of FS is the gastrointestinal tract of warm-blooded animals so that the presence of them is an indication of contamination of fecal wastes.

FS have been used together with FC to differentiate human fecal contamination from that of other warm-blooded animals. A ratio of FC to FS greater than four was considered indicative of human fecal contamination, while a ratio of less than 0.7 was suggestive of contamination by nonhuman sources (Standard Methods, 1989). This differentiation has been questioned (Dutka and Kwan, 1980) because of variable survival rates of fecal streptococcus group species. Also, disinfection of wastewaters have a significant effect on the ratio of these indicators, which may result in misleading conclusions regarding the source of contaminants. The ratio is also affected by the methods for enumerating FS.

The KF membrane filter procedure has a false-positive rate ranging from 10 to 90% in marine and fresh waters (Standard Methods, 1989). Due to all these reasons, the FC versus FS ratio is of questionable utility in differentiating human and nonhuman sources of positive coliform test results.

#### 2.1.4 Enterococcus

The enterococcus group includes two strains of the FS that is most human specific. These are *S. faecalis* and *S. faecium* (Metcalf and Eddy, 1991). They can be differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at both 10°C and 45°C. Studies at marine and fresh water bathing beaches indicated that swimming-associated gastroenteritis was related directly to the quality of the bathing water and that enterococci were the most efficient bacterial indicator of water quality (Standard Methods, 1989; EPA, 1986). In fact, *S. faecalis* has the advantage over *E. coli* in that it survives better in the aquatic environment (Slanetz and Bartley, 1965). This may be the reason that enterococcus is the only indicator for marine waters selected by EPA in its 1986 water quality criteria.

#### 2.1.5 Discussion on Indicator Organisms

For a number of historical reasons described above, the coliform group has been employed as an indicator of the possible presence of disease producing organisms. Initially, the TC test was most widely used. Since the late 1970's, the FC test has generally supplanted the TC test as being somewhat more specific to mammalian wastes.

While the FC test is undoubtedly an improvement over the TC test, it is by no means the ideal indicator organism. Among the problems with the FC test is that it is subject to false positive results from organisms which are not of enteric origin. EPA studies involving contact recreation found that the FC test results were not highly related to the presence of pathogen concentrations measured. Based on these studies, EPA (1986) recommended that the FC test be replaced by either *E. coli* or enterococci for classification of waters for contact recreation as described in Section 2.2.3. Texas has not acted on that EPA recommendation.

Another weakness of the FC test, and perhaps any indicator organism test geared to human waste, is that there are some bacterial pathogens which are unrelated to human wastes. To the degree that naturally occurring microbial pathogens become a significant public health concern, completely new test procedures may have to be developed.

While the FC test has its limitations and problems, it also has many attributes. Perhaps the most significant attribute is that as a regulatory tool, it has worked long and well. In the case of shellfish quality regulation, coliform testing has been used successfully for well

over fifty years. For the foreseeable future, the FC test will continue to be the basis for much of the regulatory decision making regarding both shellfish harvesting and contact recreation.

## 2.2 DISCUSSION OF FEDERAL AND STATE WATER QUALITY CRITERIA

The objective of this section is to review the current EPA and Texas criteria for bacteria in fresh and marine waters that are applicable to the GBNEP project area. The focus is on coliform and other indicator bacteria used for water quality criteria.

### 2.2.1 General Texas Water Quality Criteria for Bacteria

According to Section 307.4.(i) of the Texas Surface Water Quality Standards (TWC, 1991), the general water quality criterion for bacteria is that a FC concentration of not more than 200 colonies per 100 mL (1 dL) shall apply to all water bodies not specifically listed in Appendix A of Section 307.10. As described in the following sections, this criterion is the same as the 1976 EPA water quality criteria.

### 2.2.2 Texas Water Quality Criteria for Designated Segments

Based on site-specific uses, the TWC established water quality standards for bacteria. For recreation waters, it is divided into two categories - contact and noncontact recreation. Recreational activities involving a significant risk of ingestion of water, including wading by children, swimming, water skiing, diving, and surfing, are defined as contact recreation. Activities involving no significant ingestion risk such as boating are defined as noncontact recreation. The Texas State Water Quality criteria for both contact and noncontact recreation waters are:

#### 1. Contact Recreation

- a. FC content shall not exceed 200 colonies per dL as a geometric mean based on a representative sampling of not less than five samples collected over not more than 30 days.
- b. FC content shall not equal or exceed 400 colonies per dL in more than 10% of all samples, but based on at least five samples, taken during any 30-day period. If 10 or fewer samples are analyzed, no more than one sample shall exceed 400 colonies per dL.

## 2. Noncontact Recreation

- a. FC content shall not exceed 2,000 colonies per dL as a geometric mean based on a representative sampling of not less than five samples collected over not more than 30 days.
- b. FC content shall not equal or exceed 4,000 colonies per dL in more than 10% of all samples, but based on at least five samples, taken during any 30-day period. If 10 or fewer samples are analyzed, no more than one sample shall exceed 4,000 colonies per dL.

In addition, criteria for specific segments are established based on designated uses.

Most of the GBNEP segments fall under the 200 FC/dL general criterion. The exceptions are:

<u>River Basin</u>	<u>Segment Number</u>	<u>Water Quality Criterion (FC/dL)</u>
San Jacinto	1006, 1007	2,000
Galveston Bay	2421, 2422, 2423, 2424, 2432, 2433, 2434, 2435, 2439	14

The criterion of 2,000 FC/dL is for the Houston Ship Channel which is not designated for contact recreation. The criterion of 14 is for the bay areas where shellfish growth is a designated use.

### 2.2.3 EPA Water Quality Criteria for Bacteria

#### A. EPA Criteria for Bathing (Full Body Contact) Recreational Waters

Before 1986, the EPA water quality criteria (EPA, 1976) for bacteria were the same as, and the basis for, the TWC criteria described previously. In 1986, based on the results of a large prospective epidemiological study conducted for EPA by Cabelli, et al. (1982), the current federal bacteriological water quality criteria were derived. The following is a summary of the 1986 EPA criteria.

## B. EPA Criteria for Fresh Waters

EPA (1986) recommends that, based on a statistically sufficient number of samples (generally not less than 5 samples equally spaced over a 30-day period), the geometric mean of the indicated bacterial densities should not exceed one or the other of the following:

<u>E. coli</u>	126 per dL; or
enterococci	33 per dL;

no sample should exceed a one sided confidence limit (C. L.) calculated using the following as guidance:

designated bathing beach	75% C. L.
moderate use for bathing	82% C. L.
light use for bathing	90% C. L.
infrequent use for bathing	95% C. L.

based on a site-specific log standard deviation, or if site data are insufficient to establish a log standard deviation, then using 0.4 as the log standard deviation for both indicators.

## C. EPA Criteria for Marine Waters

Based on a statistically sufficient number of samples (generally not less than 5 samples equally spaced over a 30-day period), the geometric mean of the enterococci densities should not exceed 35 per dL; no sample should exceed a one sided confidence limit using the following as guidance:

designated bathing beach	75% C. L.
moderate use for bathing	82% C. L.
light use for bathing	90% C. L.
infrequent use for bathing	95% C. L.

based on a site-specific log standard deviation, or if site data are insufficient to establish a log standard deviation, then using 0.7 as the log standard deviation for both indicators.

### 2.2.4 Discussion of EPA 1986 Criteria

According to EPA (1986), the major limitations of the new criteria are that the observed relationships between indicator and pathogens may not be valid if the size of the population contributing the fecal wastes becomes too small or if epidemic conditions are present in a community. In both cases the pathogen to indicator ratio, which is approximately

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constant in a large population, becomes unpredictable and therefore, the criteria may not be reliable under these circumstances.

The presence of indicator bacteria, in rural areas, shows the presence of warm blooded animal fecal pollution. Therefore, EPA recommends the application of the above criteria unless sanitary and epidemiological studies show the sources of the indicator bacteria to be non-human and that the "indicator densities are not indicative of a health risk to those swimming in such waters" (EPA, 1986).

The 1976 EPA criteria for swimming waters were based on FC because they were more fecal specific and less subject to variation than TC which were more heavily influenced by storm water runoff. However, based on the observed strength of the relationship between the rate of gastroenteritis and the indicator density, the 1986 EPA criteria adopted E. coli or enterococci as new indicator organisms. EPA also found that no general correlation between E. coli and FC densities could be obtained across different beaches. This finding caused EPA to believe that E. coli or enterococci were superior to the FC test. Thus, EPA strongly recommended states to begin the transition process to the new indicators.

The maximum allowable geometric mean enterococci density of 33/dL for fresh waters and 35/dL for marine waters were obtained by assuming an acceptable swimming-associated rate of gastroenteritis of 8/1000 swimmers for fresh waters and 19/1000 swimmers for marine waters. These acceptable rates are equal to the estimated rate of illness at 200 FC organisms/dL. In other words, EPA based its recommended criteria levels on its best estimate of the risk level that currently exists and society accepts.

While these new EPA criteria would seem to be a technical improvement, there has been some criticism. For example, Fleisher (1991) cited flaws in the marine recreation criteria such as averaging waters of different salinities and lack of sufficient controls on local sources of variation. Whatever the outcome on these very technical points, they would not seem to alter the fundamental conclusions regarding the relatively poor performance of the FC test and the availability of better procedures.

### 2.3 HISTORICAL WATER QUALITY CRITERIA FOR SHELLFISH GROWING WATERS

According to Hunt (1977), following the shellfish borne outbreak of typhoid fever during the 1924-1925 oyster harvest season the Report of the Committee on Sanitary Control of the Shellfish Industry in the United States was submitted to the Surgeon General, U.S. Public Health Service, giving rise to the current NSSP. The criteria for shellfish growing waters were stated in this report as "the waters should ordinarily not show the presence of Bacillus coli in 1 cc amounts, tests for B. coli being made in 10 cc, 1 cc, and 0.1 cc amounts, according to the Standard Methods". From 1928 to 1944, according to Hunt

(1977), although studies recommended that E. coli should be the index of pollution for both shellfish and shellfish waters, the NSSP disregarded the recommendation as unsatisfactory.

In 1946, based on studies performed by many researchers, the first official shellfish growing area microbiological standard was published in the Manual of Recommended Practice for Sanitary Control of the Shellfish Industry. The standard stated that "the medium bacteriological content of samples of water ... shall not show the presence of organisms of the coliform group in excess of 70/100 mL of water ...". This switched the indicator organism from B. coli to TC.

A NSSP Microbiology Task Force met in Washington D.C. in 1973 to review shellfish growing area standards, criteria, and methodology. The Task Force concluded that "the FC group was scientifically and logistically superior to the (total) coliform or fecal streptococci indicator groups as a microbiological indicator of fecal pollution in estuarine waters" (Hunt, 1977). A study group was then formed by FDA to collect and analyze both total and FC data nationwide, with the recognition that total/FC ratios would vary according to distance from pollution source, dilution, degree of treatment, and possibly other factors. The results of the study showed that the 70 TC level was equivalent to 14 FC/dL and that there was good correlation between total and FC data. Based on this study, FDA proposed a FC criterion in 1974. Then, the current water quality criteria for shellfish growing waters using either total or FC values were developed by the NSSP. Detailed description on this most current criteria is given in Section 3.