.07 Systematic Random Sampling Monitoring Strategy

NSSP guidance documents provide the public health principles supporting major components of the NSSP and its Model Ordinance, and summaries of the requirements for that component. NSSP Model Ordinance requirements apply only to Interstate commerce although most states apply the requirements intrastate. For the most up to date and detailed listing of requirements, the reader should consult the most recent edition of the *Model Ordinance.*

The first critical control point in preventing food-borne illness from shellfish consumption is identifying shellfish growing areas of acceptable sanitary quality. The completion of a sanitary survey is of paramount importance in making the distinction between acceptable and unacceptable growing areas, and is the key to accurate growing area classification as approved, conditionally approved, restricted, conditionally restricted, or prohibited. A sanitary survey is required under the National Shellfish Sanitation Program's (NSSP) *Model Ordinance* for each growing area prior to its approval by the state as a source of shellfish for human consumption or as a source for shellfish to be used in a depuration or relay operation. The principal components of a sanitary survey are: (1) identification and evaluation of the pollution sources that may affect the areas, (2) an evaluation of the meteorological factors, (3) an evaluation of hydrographic factors that may affect distribution of pollutants throughout the area, and (4) an assessment of water quality. For an in depth discussion of the sanitary survey, see the NSSP Guidance Document, *Sanitary Survey and the Classification of Growing Waters* (ISSC/FDA, 2002).

The NSSP in its *Model Ordinance* allows the Authority to classify a growing area using either a total or fecal coliform standard as part of its sanitary survey. The two standards are believed to afford the same level of public health protection. The NSSP *Model Ordinance* also recognizes two distinct water quality monitoring strategies to obtain total coliform or fecal coliform monitoring data: the adverse pollution condition strategy to be used for initial classification and for monitoring; and, the systematic random sampling strategy that can be used only for monitoring if no input from point source pollution is present.

Total coliform or fecal coliform monitoring data collected under either the adverse pollution condition or the systematic random sampling strategy and the data collected for initial classification are reported as MPN values. An MPN or most probable number is a statistically derived estimate of the number of bacteria per unit volume of water sampled. The value of the MPN is determined from the combination of positive and negative results obtained from a series of fermentation tubes used in a particular laboratory test. A complete discussion of the MPN test can be found in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1985).

NSSP water quality standards for growing area classification have two components. The first component establishes a median MPN value. The second component intended for use with data collected under uniform conditions represents the variability inherent in the testing procedure and a small allowance for some additional variability peculiar to the changing conditions in the water being sampled. The original NSSP "variability factor" for the total coliform group, an MPN of 230 per 100 milliliters of sample was developed to include 90% of the samples collected under uniform conditions in which the only sources of variability operational are due to the test procedure and the allowance for some additional variability arising from changing conditions in the water being sampled. Therefore, if only these two sources of variability are active in the sample data, then no more than 10% of the samples derived under these conditions will exceed an MPN value of 230 per 100 milliliters of sample when the 5-tube, decimal dilution MPN procedure is used. This is referred to in the NSSP as the 10% criteria where no more than 10% of the samples should exceed the variability factor established for the standard and the testing procedure being used.

This same type of reasoning has been applied to both the total and fecal coliform groups and appropriate 10% criteria developed for the MPN test employed (330 and 140 MPN per 100 milliliters for the total coliform group tested by the 3-tube, decimal dilution and the 12-tube, single dilution MPN procedures, respectively, and 43, 49 and 28 MPN per 100 milliliters for the fecal coliform group tested by the 5 and 3 -tube, decimal dilution and 12-tube, single dilution MPN procedures, respectively. Because these variability factors were derived for use with data sets collected under uniform conditions, they do not address wide swings in water quality that result from changing environmental conditions driven by random pollution events such as runoff carried pollutants following rainfall. Therefore, the 10% criteria is not considered sufficient to protect public health when shellfish are taken from growing area waters adversely affected by known meteorological or hydrological events, that occur intermittently, and are shown to degrade water quality.

While many growing area waters may meet the NSSP median value and 10% criteria, some shellfish growing area sampling stations still display a considerable level of variation in the MPN sample results. Sampling data of this type may indicate that the shellfish growing areas are intermittently polluted during adverse pollution conditions and pose a risk to the shellfish consuming public. The NSSP has never intended to place a growing area that is polluted 10% of the time in the approved classification. The dilemma facing the Authority, therefore, is how to distinguish between the inherent variation of the MPN test and the variability resulting from intermittent environmental conditions that degrade water quality. When environmental events (such as rainfall) produce unfavorable effects on water quality, the data may contain data points that vary widely from the median value of the established classification. Such a data set would probably contain upper outliers that represent periods when the shellfish may be exposed to significantly greater quantities of pollution. In this situation, the determination of NSSP conformity to the established classification standard for a set of growing water samples from a particular station may become an arbitrary function of the mechanics of sampling (timing and/or frequency) rather than an actual characteristic of the growing area. Use of a statistical method, the estimated ninetieth percentile, will detect these random pollution events that may cause a data set to be skewed because of a few high MPN values.

When shellfish water sampling data collected following intermittent pollution events are combined with data collected under normal conditions, variability is increased. The estimated ninetieth percentile will reflect this increased variability. Therefore, use of the estimated ninetieth percentile will protect against the potential public health problems that may result when shellfish are consumed from growing waters that are adversely affected by intermittent pollution events and improperly classified.

The method for calculating the ninetieth percentile for use in evaluating growing water bacteriological data was suggested by the Georgia Department of Natural Resources, as an addendum to Interstate Shellfish Sanitation Conference (ISSC) in issue 8109. The ISSC adopted the systematic random sampling monitoring strategy and the method recommended for calculating the ninetieth percentile at its 1989 ISSC Annual Meeting.

Water Quality Assessment

In the adverse pollution condition monitoring strategy, the water quality standards for both total and fecal coliforms use the 10% criteria, the variability portion of the standard to adjust for the inherent variability of the MPN testing procedure in data with uniform bacterial densities. In the systematic random sampling strategy, the application of the water quality standard employs the variability portion of the standard to detect the impact of intermittent environmental events on water quality above and beyond those attributed by the MPN testing procedure alone.

A field sampling and data analysis design that employs a systematic random sampling plan for routine monitoring assumes that a statistically representative cross section of all meteorological, hydrographic, or other pollution events will be included in the data set. Therefore, all shellfish growing area data collected under the systematic random sampling plan are used to determine compliance with the appropriate total coliform or fecal coliform water quality standard. This sample collection and data analysis design may be applied only to growing areas that are affected by randomly occurring pollution events triggered by rainfall and runoff and that meet the standard for the approved or restricted classification. This sampling strategy may also be used to monitor growing areas where water quality is influenced by seasonal water uses or where harvesting is controlled by seasonal resource management restrictions. In this situation, monitoring must be done during the season when the growing waters are open. Systematic random sampling is not intended to nor should it be applied to areas impacted by point source pollution.

The systematic random sampling monitoring strategy and data analysis design presumes that if intermittent, unfavorable changes in water quality occur, they will be revealed in the bacteriological sampling results. These unfavorable sampling results will contribute to the variability of the data set. Data sets displaying high levels of variability will consequently exhibit an elevated estimated ninetieth percentile. The Authority's option to use the systematic random sampling strategy is, therefore, contingent upon acceptance of the estimated ninetieth percentile as the statistic used to measure the variability of the data set. Also required is that timing of monitoring runs be preplanned far in advance so that effects of random nonpoint pollution events will be captured if they occur. This statistic, along with the geometric mean of the data set, can be used when evaluating each sampling station for compliance with the NSSP water quality standards.

An example of an acceptable systematic sampling plan is one that documents a pre-established sampling schedule in the growing area central file. Monthly or bimonthly sampling regimes are acceptable and the schedule is maintained so there is no avoidance of unfavorable conditions. A reasonable attempt must be made to collect samples on the pre-established days regardless of navigational conditions. Field sampling crews, however, are not required to take unnecessary risks to sample on any particular day. The sampling plan must address unsafe sample collection (boating) conditions by designating an alternate sampling day or by allocating extra sampling days in the schedule that may be used when needed.

If the growing area is to be used year-round for harvesting, the random sampling plan should stipulate the collection of samples throughout the year. If the growing area is intended to be approved for direct harvest for only part of the year, the random sampling plan would need only to address that period when the area is available for harvest. The only exception to this obligation in a random sampling regime is that the Authority will require sampling during a particular tidal condition, if that condition unfavorably impacts the water quality of the growing area.

Estimating the Ninetieth Percentile

Use of the systematic random sampling strategy involves calculating the estimated ninetieth percentile of the data. This statistic measures variability in the data and should not be exceeded by random pollution events if the growing area is properly classified. When the Authority elects to employ the systematic random sampling strategy, the following guideline must be used to calculate the estimated ninetieth percentile.

The estimated ninetieth percentile must be obtained using the following equation:

Est. 90th percentile value = Antilog $[(S_{log})1.28^{A} + x_{log}]$

Where

S_{log} = base 10 logarithmic standard deviation.

x_{log} = base 10 log mean

[A] The value 1.28 is obtained from the standard normal distribution

Other:

* For the purpose of mathematical calculations, MPN values that signify the upper or lower range of sensitivity for that test shall be increased or decreased one significant number. (MPN counts are reported in the form of two significant numbers.) For example, an MPN value of 'less than 2' shall be decreased by one to 1.9 to indicate the lower level of sensitivity of the five tube, decimal dilution MPN test. In a similar manner, 2.9 shall be used to indicate the MPN value of 'less than 3' for the three tube, decimal dilution MPN test. Therefore it would follow that a MPN value of 1700 shall be used to indicate the MPN value 'greater than 1600' for the five tube MPN test.

* Logarithms may be rounded to three decimal places.

* Antilogs of log MPN calculations may be rounded to the ***next lower integer*** (zero decimal places) [example - antilog (0.556) = 3]

* The standard deviation of the log MPN data shall be calculated in the following manner:

$$S_{log} = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

Application of the Guideline:

Example 1

(1) Convert MPN values to base 10 logarithms.

Obs	MP	Log_{10}	Obs	MPN	Log_{10}
1	2.9	0.462	16	3.6	0.556
2	2.9	0.462	17	3.6	0.556
3	2.9	0.462	18	3.6	0.556
4	2.9	0.462	19	9.1	0.959
5	2.9	0.462	20	9.1	0.959
6	2.9	0.462	21	9.1	0.959
7	2.9	0.462	22	9.1	0.959
8	2.9	0.462	23	9.1	0.959
9	3.6	0.556	24	9.1	0.959
10	3.6	0.556	25	23	1.362
11	3.6	0.556	26	23	1.362
12	3.6	0.556	27	23	1.362
13	3.6	0.556	28	43	1.633
14	3.6	0.556	29	43	1.633
15	3.6	0.556	30	460	2.663

(2) Calculate Geometric Mean and Standard Deviation.

Median - 3.6

Percentage greater than 43 - 3.3 %

Geometric Mean (Antilog x_{log}) - (Antilog 0.834) or 6

Log Standard Deviation (S_{log}) - 0.506

(3) Calculate Estimated 90th Percentile using above equation.

Est. 90^{th} = Antilog [(S_{log})1.28 + x_{log}]

= Antilog [(0.506)1.28 + 0.834]

Est. 90^{th} = Antilog [1.482] or 30

(4) Interpret.

The geometric mean of the data set is less than 14 and the estimated 90th percentile is less than 49 (three tube, decimal dilution test). This station meets the NSSP fecal coliform water quality standard for the approved classification.

<u>Example 2</u>

(1) Convert MPN values to base 10 logarithms.

Obs	MPN	Log_{10}	Obs	MPN	Log_{10}
1	1.9	0.279	16	2.0	0.301
2	1.9	0.279	17	4.5	0.653
3	1.9	0.279	18	4.5	0.653
4	1.9	0.279	19	7.8	0.892
5	1.9	0.279	20	7.8	0.892
6	1.9	0.279	21	7.8	0.892
7	1.9	0.279	22	11	1.041
8	1.9	0.279	23	11	1.041
9	2.0	0.301	24	23	1.362
10	2.0	0.301	25	23	1.362
11	2.0	0.301	26	23	1.362
12	2.0	0.301	27	23	1.362
13	2.0	0.301	28	33	1.519
14	2.0	0.301	29	540	2.732
15	2.0	0.301	30	1700	3.230

(2) Calculate Geometric Mean and Standard Deviation.

Median - 2.0

Percentage greater than 43 - 6.6 %

Geometric Mean (Antilog x_{log}) - (Antilog 0.788) or 6

Log Standard Deviation (S_{log}) - 0.737

(3) Calculate Estimated 90th Percentile using above equation -

Est. 90^{th} = Antilog [(S_{log})1.28 + x_{log}]

= Antilog [(.737)1.28 + .788]

Est. 90^{th} = Antilog [1.731] or 53

(4) Interpret.

While this station's geometric mean is less than 14, the standard deviation that resulted from the high values in this data set, would lead one to conclude that water quality may have been adversely affected by storm water runoff or another intermittent pollution event. The estimated 90th percentile was 53 (greater than 43 - for the five tube, decimal dilution MPN test). Therefore this station ***would not meet*** the NSSP fecal coliform water quality standard for the approved classification.

References

1. U.S. Food and Drug Administration (FDA). 1965. National Shellfish Sanitation Program Manual of Operations, Part I. FDA, Washington, D.C., p. 11, footnote 6.
2. Interstate Shellfish Sanitation Conference. 2002. Sanitary Survey and the Classification of Growing Waters. *In* ISSC (ed.), NSSP Guide for the Control of Molluscan Shellfish. Interstate Shellfish Sanitation Conference, Columbia, S.C.
3. American Public Health Association. 1985. *Standard Methods for the Examination of Water and Wastewater*, 16th Ed. American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington D.C.