# National Shellfish Sanitation Program Guide for the Control of Molluscan Shellfish 2007

#### FDA MANUAL OF INTERPRETATIONS Interpretation Number: 07-IV-@.02-101

## Guide Contents

National Shellfish Sanitation Program U.S. Food and Drug Administration Shellfish Safety Team Division of Cooperative Programs Office of Compliance

> Date: November 27, 2001 Final: May 17, 2002 Revised: August 13, 2004 Reissued: September 1, 2004

Model Ordinance Reference: Chapter IV @.02 D, E, F and G

**Key Words:** Weighted 90<sup>th</sup> Percentile, Adverse Pollution Condition, Systematic Random Sampling, Estimated 90<sup>th</sup> Percentile

**Question:** What is the procedure for determining the value of the 90<sup>th</sup> percentile to be used in the analysis of sample data derived in the transition to a different MPN procedure?

**Interpretation:** A weighted 90<sup>th</sup> percentile value is calculated for each set of samples derived in the transition to a different MPN procedure.

**Rationale:** A number of states have availed themselves of the advantages afforded by the action of the 8<sup>th</sup> National Shellfish Sanitation Workshop in allowing the use of a virtual limitless combination of tubes and dilutions in MPN procedures used in support of the National Shellfish Sanitation Program (NSSP). A change in the combination of tubes and/or dilutions from those traditionally used in the NSSP alters the precision or variability of the test and thus its associated 90<sup>th</sup> percentile. When a change in MPN procedures is instituted, new data with a different 90<sup>th</sup> percentile must be phased into the existing sample database. During this phase-in period a "hybrid" 90<sup>th</sup> percentile value must be calculated and used as the variability component of the bacteriological standard against which the variability of sample data is to be compared. This "hybrid" 90<sup>th</sup> percentile value is calculated by weighting the relative contributions of each MPN method to the sample database. The resulting value is known as the weighted 90<sup>th</sup> percentile. Weighted 90<sup>th</sup> percentile values can be used equally effectively with either Adverse

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Pollution Condition (APC) or Systematic Random Sampling (SRS) regimes.

**Calculations:** The value of the weighted 90<sup>th</sup> percentile from a data set derived in the transition to a different MPN procedure is calculated in the following manner:

- a. Convert the 90<sup>th</sup> percentile values for both MPN procedures to their respective base 10 logarithmic values.
- b. Multiply the logarithmic values for each MPN procedure by the number of samples in the database examined by that procedure.
- c. Add these logarithmic values, then divide by the total number of samples examined.
- d. Take the antilog of this value.
- e. Round off conventionally to the nearest whole number.
- f. This value is the weighted 90<sup>th</sup> percentile against which sample data is compared.
- g. Recalculate the weighted 90<sup>th</sup> percentile when new data is added to the database.
- h. Once all accumulated data is from the same MPN procedure and the transition in methodologies is complete, the corresponding 90<sup>th</sup> percentile value for this MPN procedure is then used for comparing sample data.

## **Example 1**

Data was gathered for a sampling station under the APC sampling regime. The growing area which encompasses this sampling station is in the approved classification. The first ten samples in the database were examined by the traditional 5-tube, decimal dilution MPN test for **fecal coliforms**. The remaining five samples required under APC sampling were analyzed by the 12-tube, single dilution MPN test for **fecal coliforms**. The 90<sup>th</sup> percentile value for the 5-tube, decimal dilution MPN test for **fecal coliforms** is 43. The 90<sup>th</sup> percentile value for the 12-tube, single dilution MPN test is 28. The weighted 90<sup>th</sup> percentile value which results from this data will lie somewhere between the 90<sup>th</sup> percentile values of the MPN procedures used. Its proximity to either method's 90<sup>th</sup> percentile value will depend on the relative number of samples analyzed from each method. Since most of the samples in this example were derived from the 5-tube MPN test, the 90<sup>th</sup> percentile value calculated will be weighted toward 43.

To calculate the weighted 90<sup>th</sup> percentile for this data set:

- a. The 90<sup>th</sup> percentile values of 43 for the 5-tube, decimal dilution MPN test and 28 for the 12-tube, single dilution MPN test are converted to base 10 logarithms. This gives base 10 log values of 1.633 and 1.447 respectively.
- b. The base 10 log values are then multiplied by the number of samples in the database examined by each MPN procedure used. Ten of 15 samples were analyzed by the 5-tube,

decimal dilution MPN test. The remaining 5 of 15 were examined by the 12-tube, single dilution test. This gives 1.633 for the 5-tube test x 10 samples = 16.330 and 1.447 for the 12-tube, single dilution test x 5 samples = 7.235.

- c. These values are added together and the resultant divided by the total number of samples in the database being used. Thus, 16.330 + 7.235 = 23.565, 23.565/15 = 1.571.
- d. The antilog of this value is taken. In this example, the antilog of 1.571 is 37.239.
- e. The antilog value is rounded off to the nearest whole number which in this example is 37.
- f. The weighted 90<sup>th</sup> percentile for this data set is 37. Thirty-seven (37) is the 90<sup>th</sup> percentile value which cannot be exceeded more than 10% of the time by the sample station data in this data set under the APC sampling regime for this station to remain in the approved classification status. When new data is added to the database of this sampling station, the value of the weighted 90<sup>th</sup> percentile would have to be recalculated until the transition in methodologies is completed and all the data from this sampling station is derived from the same MPN procedure. At this time, the corresponding 90<sup>th</sup> percentile value of 28 for the 12-tube, single dilution MPN procedure in use will be employed in comparisons with sample data.

## Example 2

Data was derived from a sampling station under the SRS sampling regime. The growing area which encompasses this sampling station is also in the approved classification for **fecal coliforms**. The first 18 of 30 samples were analyzed using the 5-tube, decimal dilution MPN test. The remaining 12 of 30 samples were examined using a 3-tube, decimal dilution MPN test. The 90<sup>th</sup> percentile values for the 5-tube, decimal dilution test in the approved classification status is 43. That for the 3-tube, decimal dilution MPN test is 49. Again the value for the weighted 90<sup>th</sup> percentile will be somewhere between the respective 90<sup>th</sup> percentile values of both MPN methods. Its proximity to either is a function of the number of samples in the data set contributed by each MPN procedure. In this example, a somewhat greater number of samples were derived from use of the 5-tube, decimal dilution MPN test; so that, the value of the 90<sup>th</sup> percentile will be weighted in that direction also.

To calculate the weighted 90<sup>th</sup> percentile for this data set

- a. The 90<sup>th</sup> percentile values of 43 for the 5-tube, decimal dilution MPN test for **fecal coliforms** and 49 for the 3-tube, decimal dilution MPN test for **fecal coliforms** are converted to base 10 logs. This gives base 10 log values of 1.633 for the 5-tube, decimal dilution test and 1.690 for the 3-tube, decimal dilution MPN test.
- b. These base 10 log values are then multiplied by the number of samples in the database analyzed by each MPN procedure. In this example, 18 of 30 samples were examined by the 5-tube, decimal dilution MPN test: and, 12 of 30 samples were analyzed by the 3-tube, decimal dilution MPN test. This gives 1.633 for the 5-tube, decimal dilution MPN test x 18 samples = 29.394 and 1.690 for the 3-tube, decimal dilution MPN test x 12 samples = 20.280.

- c. These values are added together and the resultant divided by the total number of samples in the database being used. Thus, 29.394 + 20.280 = 49.674, 49.674/30 = 1.656
- d. The antilog of this value is determined. In this example, the antilog of 1.656 is 45.269.
- e. This antilog value is rounded to the nearest whole number which in this example is 45.
- f. The weighted 90<sup>th</sup> percentile value for this data set is 45. Forty-five (45) is the value of the 90<sup>th</sup> percentile which will be compared to the estimated 90<sup>th</sup> percentile calculated from the data in the sample data set collected under the SRS sampling regime and examined using the two different MPN methods. To remain in the approved status the estimated 90<sup>th</sup> percentile calculated from this data set must be less than or equal to the value determined for the weighted 90<sup>th</sup> percentile of the data set. Again the weighted 90<sup>th</sup> percentile will have to be recalculated as new data becomes available. This recalculation must continue until the transition in methodologies is completed and all the data from this sampling station has been derived from the same MPN procedure. At this time, the corresponding 90<sup>th</sup> percentile of 49 for the 3-tube, decimal dilution MPN procedure in use will be employed in comparisons to the estimated 90<sup>th</sup> percentiles calculated directly from the sampling data.

## Example 3

Data in this example was collected from a sampling station under the SRS sampling regime. This sampling station is in an area classified as restricted. The first 24 of the 30 samples collected were analyzed by the 5-tube, decimal dilution MPN test for **fecal coliforms**. The remaining 6 samples of the 30 collected were analyzed using a 5-tube, fivefold dilution MPN test for **fecal coliforms**. The 90<sup>th</sup> percentile value for each of these MPN procedures is 260 and 190 respectively. The value of the weighted 90<sup>th</sup> percentile for this data set will be somewhere between 190 and 260. The proximity to either value will depend on the respective number of samples analyzed by each MPN method. In this example, most of the samples were derived from the 5-tube, decimal dilution MPN test. Consequently, the 90<sup>th</sup> percentile value will be heavily weighted in that direction.

To calculate the weighted 90<sup>th</sup> percentile for this data set:

- a. The 90<sup>th</sup> percentile values of 260 for the 5-tube, decimal dilution MPN test for **fecal coliforms** and 190 for the 5-tube, fivefold dilution MPN test for **fecal coliforms** are converted to base 10 logs. This gives a base 10 logarithmic value of 2.415 for the 5-tube, decimal dilution MPN test and 2.279 for the 5-tube, fivefold MPN test.
- b. These base 10 log values are then multiplied by the number of samples in the database analyzed by each MPN procedure. In this example, the 5-tube, decimal dilution MPN was used in the analysis of 24 of the 30 samples while the 5-tube, fivefold dilution MPN was used to test the remaining 6 samples. Hence, 2.415, the log 90<sup>th</sup> percentile value for the 5-tube, decimal dilution MPN test is multiplied by 24, the number of samples tested by this MPN procedure to give 57.960; and, 2.279, the log 90<sup>th</sup> percentile value for the 5-tube, fivefold dilution MPN test is multiplied by 6, the number of samples obtained using this

MPN procedure to give 13.674.

- c. These values are added together and subsequently divided by the total number of samples analyzed by both methods. In this example, 57.960 + 13.674 = 71.634, 71.634/30 = 2.388.
- d. The antilog of this value is determined. In this example the antilog of 2.388 is 244.343.
- e. This antilog is conventionally rounded to the nearest whole number which in this example is 244.
- f. The weighted 90<sup>th</sup> percentile value for the data set is 244. Two hundred forty-four (244) is the value of the 90<sup>th</sup> percentile which will be compared to the estimated 90<sup>th</sup> percentile calculated from the data in the sample data set collected under the SRS sampling regime and examined using the two MPN methods. To remain in the restricted classification, the estimated 90<sup>th</sup> percentile calculated from the data set will have to be less than or equal to the value of the weighted 90<sup>th</sup> percentile obtained from the data set. This weighted 90<sup>th</sup> percentile value will need to be recalculated as more data becomes available and until such time as the transition in methodologies is completed and all the samples have been derived from the same MPN procedure. When this occurs, the corresponding 90<sup>th</sup> percentile of 190 for the 5-tube, fivefold dilution MPN procedure in use will be employed in comparisons to the estimated 90<sup>th</sup> percentile calculated directly from the sampling data.

## Example 4

Data in this example was collected from a sampling station under the APC sampling regime. This sampling station is in the approved classification and 5 of 15 samples in the database were tested by the 5-tube, decimal dilution MPN test for **total coliforms**. The remaining 10 samples in the database were analyzed by the 3-tube, decimal dilution MPN test for **total coliforms**. The 90<sup>th</sup> percentile value for each of these MPN tests were 230 and 330 respectively. The value of the weighted 90<sup>th</sup> percentile will be somewhere between 230 and 330. Its proximity to either value depends on the respective number of samples analyzed by each MPN procedure. In this example, the preponderance of samples were tested by the 3-tube MPN procedure. As a result, the value of the 90<sup>th</sup> percentile will be weighted more heavily toward 330.

To calculate the weighted 90<sup>th</sup> percentile for this data:

- a. The 90<sup>th</sup> percentile values of 230 for the 5-tube, decimal dilution MPN test for **total coliforms** and 330 for the 3-tube, decimal dilution MPN test for total coliforms are converted to base 10 logarithms. This gives base 10 log values of 2.362 and 2.519 respectively.
- b. These base 10 log values are then multiplied by the number of samples in the database analyzed by each MPN procedure. In this example, 5 of 15 samples in the database were analyzed by the 5-tube, decimal dilution MPN test. The remaining 10 of 15 samples were examined by the 3-tube, decimal dilution test. Thus, the base 10 log value of 2.362, the

90<sup>th</sup> percentile of the 5-tube MPN procedure for **total coliforms** is multiplied by the 5 samples tested by this MPN method to give 11.810. In addition, the base 10 log value of 2.519, the 90<sup>th</sup> percentile of the 3-tube MPN test for **total coliforms** is multiplied by the 10 samples examined by this MPN procedure to give 25.190.

- c. These values are added together and the resultant divided by the total number of samples in the database being analyzed. In this example, 11.810 + 25.190 = 37.000, 37.000/15 = 2.467.
- d. The antilog of this value is determined. For this example, the antilog of 2.467 is 293.089
- e. This antilog is rounded off to the nearest whole number which in this case is 293.
- f. The weighted 90<sup>th</sup> percentile for this data set is 293. Two hundred ninety three (293) is the value for the 90<sup>th</sup> percentile that cannot be exceeded more than 10% of the time by this sampling station under APC for it to remain in approved classification status. The value for the weighted 90<sup>th</sup> percentile must be recalculated as more data becomes available. This will continue until the transition in methodologies is completed and all samples in the database have been analyzed using the same MPN procedure. When this happens, the corresponding 90<sup>th</sup> percentile of 330 for the 3-tube, decimal dilution MPN procedure in use will be employed in comparisons with the sample data.

## **Other References:**

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## **Contact:**

U.S. Food and Drug Administration Shellfish Safety Team 5100 Paint Branch Parkway (HFS-626) College Park, Maryland 20740-3835

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