

STATISTICAL CONSIDERATIONS IN USING THE TWELVE-TUBE MPN TEST FOR ROUTINE MONITORING OF SHELLFISH WATERS

by

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The first consideration in the decision to use a 12-tube single dilution test instead of a 5-tube 3-dilution test is the degree of confidence in the sample result. Table I shows a comparison of the upper one-sided confidence limits for the 5-tube 3-dilution test, the 3-tube 3-dilution test, and the 12-tube single dilution test. The precision of the single dilution test is very poor when the samples are likely to be all negative or all positive. When all the tubes are positive, the estimated density is infinite. When all are negative, the upper 95% confidence on the density is 25 organisms per 100 ml. When 1 or 2 tubes are positive, the upper confidence limits exceed those for the 5-tube 3-dilution test. However, in the range of 3-11 positive tubes, the confidence is better for the 12-tube single dilution test than for the 5-tube 3-dilution test.

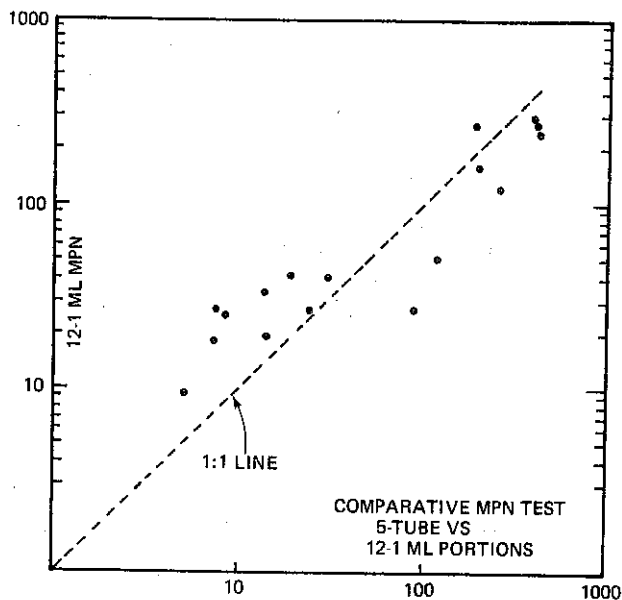
For routine monitoring, we are interested only in determining whether the true density is under 70 organisms per 100 ml, and the confidence limits obtained by the 12-tube single dilution test appear to be satisfactory.

Mr. Santo Furfari evaluated some comparative data from quintuplicate analyses using five 5-tube 3-dilution MPN tests and five 12-tube 1 ml dilution tests. Figure 1 shows a plot of the results for the 5-tube 3-dilution test against the results for the 12-tube single dilution test. The correlation between the two tests was quite good. Low counts by the 5-tube MPN test are slightly over-estimated by the 12-tube single dilution test. The crossover is at an MPN value of 30-40 organisms per 100 ml, exactly the point at which the confidence limits cross over.

TABLE I Confidence Limits Using a 12-tube single dilution, 5-tube 3-dilution and a 3-tube 3-dilution Assay: (Assuming the stated MPN value could be obtained by the 3-dilution test)

# Positive	MPN value (organisms per 100 ml)	Upper 95% Confidence by 5-tube 3-dilution	Upper 95% Confidence by 3-tube 3-dilution	Upper 95% Confidence by 12-tube single dilution
0	<8.7	indeterminate	indeterminate	25
1	8.7	23	31	41
2	18	48	64	58
3	29	78	103	75
4	41	110	146	94
5	54	144	192	115
6	70	187	248	140
7	88	235	313	171
8	110	294	391	210
9	139	372	494	263
10	179	479	636	350
11	248	663	882	550
12	>248	indeterminate	indeterminate	indeterminate

FIGURE 1 Comparison of 12-Tube Single MPN Test and 5-Tube 3-Dilution MPN Test



As Mr. Furfari states in his communication to Mr. James Redman, the next question that arises is the rejection criteria. The criteria under the current standard are 70 organisms per 100 ml median value, and a limit of not more than 10% of the samples to exceed a 230 MPN value for the 5-tube test or 330 MPN for the 3-tube test. However, these allowances are the upper limit of a two-sided 95% confidence limit on the median value, not really the 90th percentile limits expected by sampling variability of the test itself.

The median value for the standard would remain at 70 organisms per 100 ml since the expected value is the same for the 12-tube single dilution test and the 5-tube 3-dilution test. With 12 tubes and a true water quality of 70 organisms per 100 ml, or 6 out of 12 tubes positive, the binomial distribution gives the probability of obtaining at least the specified number of tubes positive. These probabilities are shown in Table II. Thus, if the true water quality is 70, we would expect that between 8 and 9 tubes would be positive 90% of the time. The exact 90th percentile would be at 8.78 tubes, which corresponds to an MPN value of 132. This would be the MPN value allowed by sampling variability alone. The present upper

TABLE II Probabilities of obtaining at least the specified number of Positive Tubes in a 12-tube 1 ml test if the true water quality is 70 organisms per 100 ml

# Tubes Positive	Probability
1	.9998
2	.9968
3	.9807
4	.9270
5	.8062
6	.6128
7	.3872
8	.1938
9	.0730
10	.0193
11	.0032
12	.0002

90th percentile limit allows for additional variabilities which arise because of changing conditions in the water. The 90th percentile for the present 5-tube 3-dilution test is 187 and the allowed 90th percentile is 230 for a 5-tube 3-dilution test. The allowable 90th percentile is a factor of 1.22 times the 90th percentile based on sampling variability alone. If this same factor is applied to an MPN of 132, the expected 90th percentile of the 12-tube single dilution test, the recommended 90th percentile for the 12-tube single dilution test would be an MPN of 160 or between 9 and 10 tubes positive. In Mr. Furfari's communication to Mr. James Redman he recommended a 90th percentile limit of 9 tubes positive, arrived at by a slightly different analysis. A 90th percentile limit of 9 out of 12 tubes positive would appear to be satisfactory and allow for sufficient variation in addition to sampling variability.

REFERENCES

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