APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1988, p. 2996–3002
0099-2240/88/122996-07$02.00/0

Vol. 54, No. 12

Test of the Validity of the Poisson Assumption for Analysis of Most-Probable-Number Results

CHARLES N. HAAS[1]* AND BARBARA HELLER[2]

Pritzker Department of Environmental Engineering[1] and Department of Mathematics,[2] Illinois Institute of Technology, Chicago, Illinois 60616

Received 8 August 1988/Accepted 28 September 1988

A test of the validity of the Poisson assumption for sample replicates in dilution series of finite length is proposed and its properties are examined by using Monte Carlo simulation. The test is based on an examination of the number of intervals between complete sterility and complete infection in a series. The test is applied to a data set of routine influent coliform samples at the Chicago water supply intake. By this test, the data set is rejected as being drawn from a Poisson replication. Tables for direct application to a 3-dilution, 5-tube decimal series are presented, and their application is illustrated.

The interpretation of coliform most-probable-number (MPN) determinations has been discussed for over 70 years (4, 5, 7, 9, 13, 17, 19, 20, 26). The usual theories universally assume "(i) that the organisms are randomly distributed throughout the solution, and (ii) that each sample from the solution, when incubated in the culture medium, is certain to exhibit [growth] whenever the sample contains one or more organisms" (17). This allows the binomial distribution to be used to describe the number of positive tubes obtained in each dilution of the MPN test with the parameter of the binomial for each dilution given by the zero term for the Poisson distribution. Despite this understanding and the testing of such assumptions in other contexts (1, 2, 4, 21, 23, 27), only Woodward (26) has attempted to test such assumptions for coliforms. In his test, the proportion of likely tube combinations was formulated by an unspecified procedure, probably by using Bayes theorem and an assumed prior distribution for coliform densities, and this analysis has been recently criticized on theoretical grounds (17).

With the current interest in statistics of coliform enumeration and the proposed use of presence-absence tests, it has once again become important to question the distribution of coliform counts in water supplies. One strong argument for the use of the presence-absence test is the non-Poisson variability attributed to microorganisms in water samples. This argument has been based on studies of samples taken over time (8, 10, 22) and has ignored the enumeration methodology itself as a source of variability. In a previous paper (12), we have shown that the membrane filter method for total coliform enumeration often produces results on replicate samples with variability in excess of that predicted by the Poisson model for coliform counts in replicate samples. In the dilution MPN test, such excess replication error might arise from error in serial dilutions or tube-to-tube variations in recovery efficiency. In some such circumstances, the existence of non-Poisson replication variations may serve as a sentinel for inadequate technique, which could result in misestimation of the true bacterial density (9).

This report provides and validates a method for detecting such unusual replication variability in MPN count data and applies it to data from the City of Chicago. By the use of Monte Carlo simulation, this statistic will be shown to have sufficient power to detect deviations from the Poisson hypothesis while not rejecting Poisson-distributed replicates. Finally methods and a table for the routine application of this statistic to usual sampling data are presented.

* Corresponding author.

MATERIALS AND METHODS

The experimental data consisted of all routine presumptive MPN total coliform determinations (semi-daily) made by City of Chicago Department of Water personnel on Lake Michigan intake water at the Jardine plant of the City of Chicago during the years 1978 to 1980, for a total of 1,335 samples. The dilution protocol consisted of five replicate tubes at each of four volumes (10, 1, 0.1, and 0.01 ml) for each sample. A more detailed discussion of this data set has been presented elsewhere (B. Mui, M.S. thesis, Illinois Institute of Technology, Chicago, 1986).

RESULTS

From the raw data, a frequency distribution of the resultant MPN scores was then constructed. Table 1 summarizes the observed score frequencies in order of occurrence, along with the computed MPN densities, estimated by using the ordinary maximum likelihood method.

DISCUSSION

Development of a test statistic. Since the individual samples may have been drawn from a water with a fluctuating (e.g., with season) mean microorganism density, it was necessary to develop a test for consistency of the observed sample scores with an underlying hypothesis of Poisson variability among replicates within a sample. By constructing a test statistic which is conditional on the underlying distribution of microbial densities, the effect of the temporal variability of the underlying mean density is removed. It is possible that there is a seasonal effect on the between-replicate sample variability; however, this is a higher-order effect to be studied if and only if the overall deviations are shown to be significant.

In the following discussion, it is necessary to differentiate between the experimental and computed values obtained in the standard MPN multiple-dilution test. The sequence of positive tubes in any determination (such as 5-0-1-0) will be referred to as the score, while the computed number of microorganisms per 100 ml estimated by the usual maximum likelihood technique will be referred to as the density.

TABLE 1. Summary of observed frequencies of scores

Observed positive scores at vol (ml)				Frequency	MPN/100 ml
10	1	0.1	0.01		
0	0	0	0	213	0.0
2	0	0	0	200	4.47
1	0	0	0	182	1.98
3	0	0	0	146	7.77
5	0	0	0	115	23.04
4	0	0	0	111	12.73
5	1	0	0	76	32.74
4	1	0	0	42	16.85
3	1	0	0	34	10.69
2	1	0	0	26	6.83
5	2	0	0	23	48.9
5	4	0	0	15	127.56
1	1	0	0	13	4.02
4	2	0	0	11	21.56
5	2	1	0	11	69.2
2	2	0	0	8	9.3
5	3	0	0	8	78.2
5	0	1	0	7	31.24
3	2	0	0	6	13.82
5	5	1	0	6	329.06
5	4	1	0	6	168.88
5	1	1	0	6	45.29
3	0	1	0	5	10.55
0	1	0	0	5	1.82
5	5	4	0	4	1,299.3
2	3	0	0	3	11.88
5	5	5	1	3	3,476.7
4	0	1	0	3	16.54
5	5	2	0	3	493.22
3	2	1	0	2	16.94
4	3	0	0	2	27.01
4	0	2	0	2	20.69
5	5	0	0	2	231.16
5	3	2	0	2	138.42
5	5	5	0	2	2,397.9
2	0	1	0	2	6.77
1	0	1	0	2	3.99
5	0	1	1	2	42.15
5	3	1	0	2	108.6
5	1	0	1	2	44.99
4	3	4	0	1	51.87
4	5	2	0	1	55.71
4	4	2	0	1	46.48
1	1	1	1	1	6.07
1	3	1	1	1	12.51
4	4	0	0	1	33.41
5	4	4	0	1	335.12
3	3	0	0	1	17.19
4	2	1	0	1	26.38
5	3	3	0	1	172.16
2	0	2	0	1	9.11
3	1	1	0	1	13.63
1	2	0	0	1	6.12
4	1	1	0	1	21.11
5	2	2	0	1	93.22
5	4	2	0	1	216.09
5	1	2	0	1	62.49
5	5	3	1	1	1,086.4
5	5	2	1	1	699.64
5	4	0	1	1	165.77
5	5	3	0	1	793.43
5	5	4	1	1	1,723.8
5	5	5	3	1	9,178.4

The statistic used was a extension of the range-of-transition homogeneity test proposed by Stevens (24) which was originally described for infinitely long dilution series (in which total infected at low dilution and total sterile at high dilution bracketed the sequence). Each score is transformed to a canonical form by replacing all intermediate scores (positive tubes between and including 1 through 4) by *M* (for middle). The following rules are used to compute the value of the range-of-transition statistic for a four-dilution, five-tube experiment. (i) All scores of 0-0-0-0 are discarded from the analysis. (ii) The combinations 5-0-0-0, 5-5-0-0, 5-5-5-0, and 5-5-5-5 are accorded a range of transition of 0. (iii) The combinations *M*-0-0-0, 5-*M*-0-0, 5-5-*M*-0, and 5-5-5-*M* are accorded a range of transition of 1. (iv) The combinations *M*-*M*-0-0, 5-*M*-*M*-0, and 5-5-*M*-*M* are accorded a range of transition of 2. (v) The combinations *M*-*M*-*M*-0 and 5-*M*-*M*-*M* are accorded a range of transition of 3. (vi) The combinations *M*-*M*-*M*-*M* are accorded a range of transition of 4. (vii) All other combinations are accorded a range of transition of *R* (for reversal).

The theoretical distribution of the range-of-transition statistic is computed by considering each of the five replicate tubes in a given dilution as a sample from an independent identical Poisson distribution. Hence, the probability distribution of positive tubes is binomial. For any single volume (v) sampled, where u is the mean density in the sample, equations 1 to 3, respectively, give the probabilities that all, no, and some intermediate number of tubes are positive (i.e., show growth).

$$P_5 = [1 - \exp(-uv)]^5 \quad (1)$$

$$P_0 = \exp(-5uv) \quad (2)$$

$$P_M = 1 - P_5 - P_0 \quad (3)$$

In equations 1, 2, and 3, the subscripts denote the number of tubes positive at that volume sampled, and exp is the exponential function.

By direct enumeration, the following equations give the probabilities of each range of transition in a given sample (adopting the symbol Q_i to denote the probability of a given range-of-transition score given that value of u):

$$Q_0 = (P_{51}P_{02}P_{03}P_{04} + P_{51}P_{52}P_{03}P_{04} + P_{51}P_{52}P_{53}P_{04} + P_{51}P_{52}P_{53}P_{54})/D \quad (4)$$

$$Q_1 = (P_{M1}P_{02}P_{03}P_{04} + P_{51}P_{M2}P_{03}P_{04} + P_{51}P_{52}P_{M3}P_{04} + P_{51}P_{52}P_{53}P_{M4})/D \quad (5)$$

$$Q_2 = (P_{M1}P_{M2}P_{03}P_{04} + P_{51}P_{M2}P_{M3}P_{04} + P_{51}P_{52}P_{M3}P_{M4})/D \quad (6)$$

$$Q_3 = (P_{M1}P_{M2}P_{M3}P_{04} + P_{51}P_{M2}P_{M3}P_{M4})/D \quad (7)$$

$$Q_4 = P_{M1}P_{M2}P_{M3}P_{M4}/D$$

$$Q_R = 1 - Q_0 - Q_1 - Q_2 - Q_3 - Q_4 \quad (8)$$

$$\text{where } D = 1 - P_{01}P_{02}P_{03}P_{04}$$

The quantity D represents the proportion of all samples remaining after excluding samples in which all tubes in all dilutions are sterile. For each sample observed, Q_0, Q_1, Q_2, Q_3, Q_4, and Q_R are computed from the above equations. The values for each of the Q's are then summed over all samples to give the theoretical distribution of the range-of-transition statistic.

Application of the range-of-transition statistic is shown graphically in Fig. 1. From all tube scores (other than

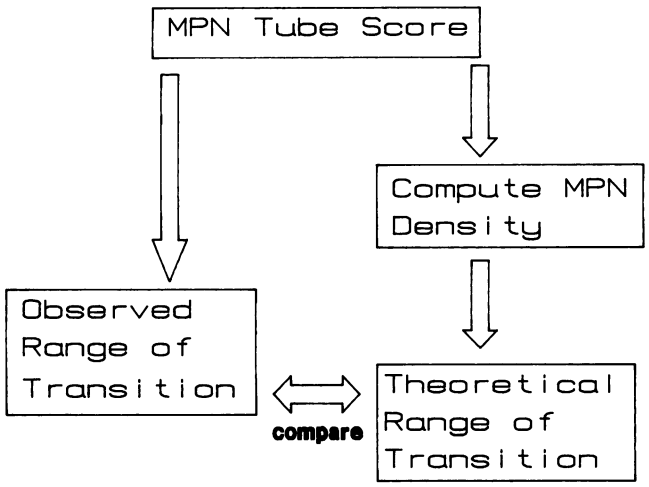

FIG. 1. Schematic procedure for computation of range-of-transition comparison.

0-0-0-0), the observed distribution of the range-of-transition statistic is computed. If the theoretical and observed distributions are different, then the null hypothesis (of underlying Poisson replication errors between replicate tubes) must be rejected.

Validation of proposed test statistic. To verify that this method does not incorrectly reject replicates which are Poisson distributed, a Monte Carlo experiment was conducted. From the distribution of MPN densities from Table 1 (comprising 1,123 nonsterile samples), 100 replicate runs of 1,123 nonsterile tube scores each were constructed by assuming the Poisson relationship [i.e., in a single tube at a given dilution, the probability of sterility is given by $\exp(-uv)$]. For each run, the theoretical distribution of the range-of-transition statistic for each of the 1,123 observations was computed from equations 4 through 8 by using as the u value the estimate from the maximum likelihood computation for that observation. The test of agreement between the theoretical and observed range-of-transition distributions was made by using a chi-square goodness-of-fit comparison. The frequencies for ranges of transition 3 and 4 were pooled to give cell categories above 5. Thus, there were 4 degrees of freedom (6 categories less 1 for the pooling less 1 for equating total numbers of observations).

Figure 2 shows that the distribution of the 100 simulation values of the computed chi-square statistic agrees with the theoretical distribution of chi-square for 4 degrees of freedom. The experimental and expected density functions were not statistically different (chi-square for the fit to the theoretical sampling distribution was 4.86, not significant at the 7 degrees of freedom for the test of significance). Furthermore, of the 100 runs, only 4 had chi-square values in excess of 9.49 (the critical value at 5% for rejection of the null hypothesis). Hence, the proposed test has adequate properties in terms of type 1 error (rejection of the null hypothesis when, in fact, it is true).

The ability of this test to detect departures from Poisson behavior was tested by assuming that the distribution between replicates was negative binomial. This distribution was chosen since it has been found to fit data from a number of potable-water microbial-frequency distributions (22) and has also been used as an alternative sampling distribution to

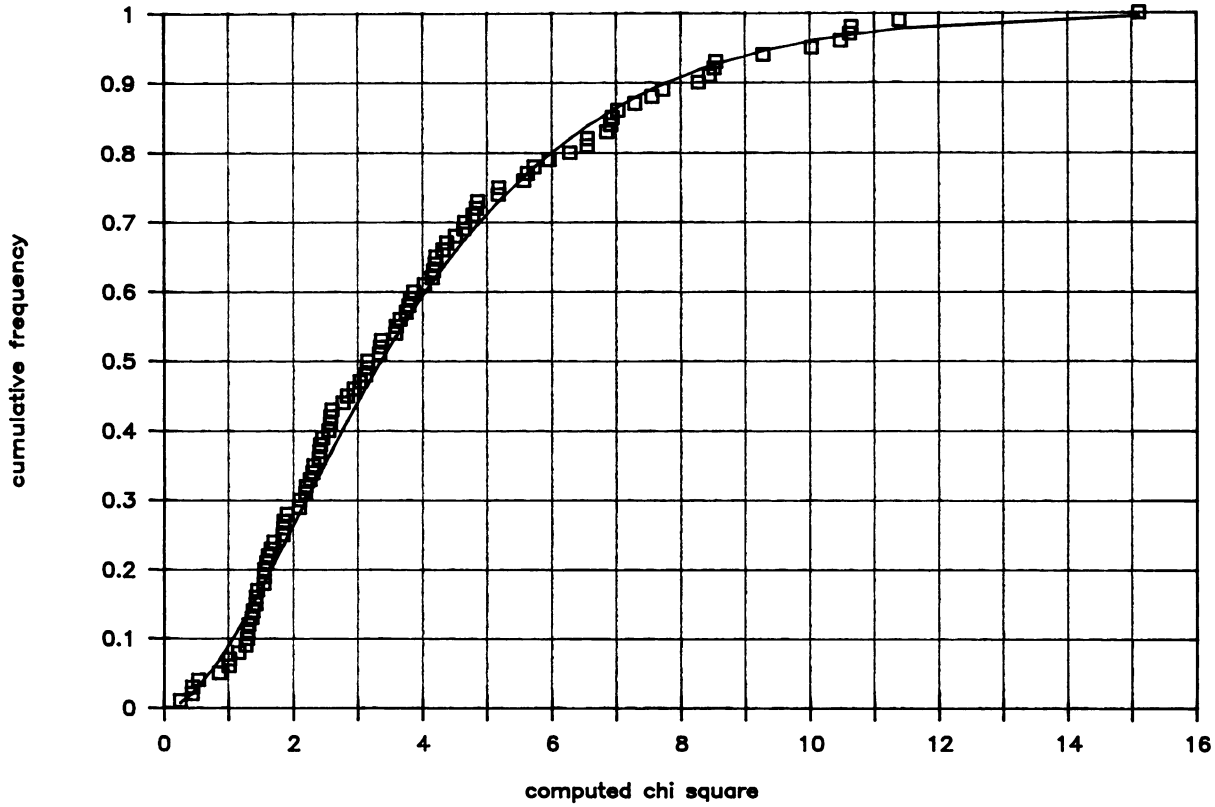

FIG. 2. Comparison of computed range-of-transition statistic from Monte Carlo simulations with exact chi-square distribution.

the Poisson for microbial counts (5, 18). Also, the negative binomial distribution results if the individual susceptibilities of replicate tubes are distributed on the basis of a gamma distribution (21). Furthermore, the negative binomial is a simple two-parameter distribution which has, as a limiting case, the Poisson distribution. The probability of a negative tube is then determined from the zero probability of the negative binomial distribution, given in equation 9, rather than the zero probability term of the Poisson distribution.

$$P(\text{sterility in a single tube}) = (1 + uv/k)^{-k} \quad (9)$$

As k approaches infinity, at a constant value of the mean, the Poisson distribution appears as a limiting case.

A single Monte Carlo run assuming negative binomial distributions was conducted by constructing a set of observations (1,123 per run) where one mean each noted in the Chicago data was used along with a fixed k value to construct the set of tube scores. For each of 20 runs at a given k value, the chi-square statistic between the theoretical and experimental range-of-transition distributions was computed. Table 2 summarizes the ability of the range-of-transition test to detect the deviance from assumed Poisson behavior (the null hypothesis of the test). At a 5% critical value for the chi-square statistic (9.49 for 4 degrees of freedom), with a k value as high as 5 (i.e., only 20% excess variation as compared with the Poisson), the type 2 error rate (acceptance of the null hypothesis when it is, in fact, false) is less than 20%. Therefore, it is concluded that this statistic has sufficient power to detect even relatively small deviations from the Poisson assumption. The power of this test is undoubtedly related to the number of samples (1,123) examined. For smaller sample sizes, the power of the test (or the range of k values at which the test adequately rejects the null hypothesis) is likely to be less.

TABLE 2. Summary of Monte Carlo tests for negative binomial trials

k value	Total no. of trials	No. of times the null hypothesis was:		Type 2 error (%)
		Rejected	Accepted	
1	20	20	0	0
3	20	20	0	0
5	20	16	4	20
10	20	3	17	85

A second aberrant distribution could arise from the necessity for more than one organism to be delivered to a tube for growth to occur. This could occur in the case of sublethal injury, or if mutual growth dependencies existed. If, for example, two cells had to be delivered to a tube for growth to occur (a two-hit model), then the probability of zero tubes would be given by: $P = (1 + uv)\exp(-uv)$. A Monte Carlo experiment with 20 replicates of the 1,123 observations showed rejection of the null hypothesis of the Poisson model in all occasions.

Application of test statistic to Chicago data. Table 3 describes the application of the proposed test to Chicago data. By using the density distribution of the actual Chicago data set, the expected distribution of the range-of-transition statistic was constructed. The test between the distribution of

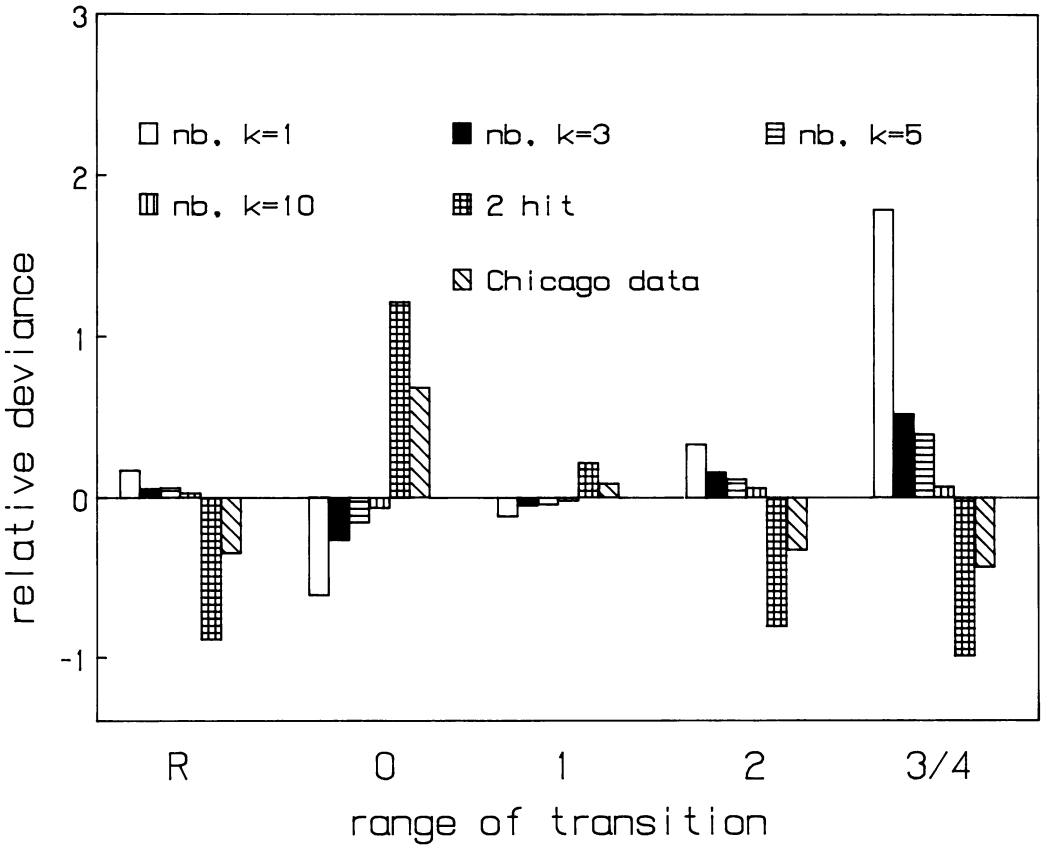

FIG. 3. Effect of nature of non-Poisson distribution on the relative deviance in the range-of-transition statistic.

TABLE 3. Test of Chicago MPN data

Range-of-transition statistic	Frequency		Chi-square[b]
	Theoretical	Observed	
R	50.39	33	6.0014
0	70.67	119	33.0521
1	715.33	779	5.6671
2	270.77	183	28.4506
3 and 4	15.84	9	2.9536

[a] R, Reversal in the range for that tube combination.
[b] The sum of the chi-square results was 76.0978.

observed versus simulated statistics was performed as per the earlier comparisons. It is obvious that, to a highly significant degree, the actual MPN data from Chicago shows deviations from the underlying null hypothesis ($P > 0.9999$).

Figure 3 presents, in graphical form, a comparison of the average deviances [defined as (observed frequency − theoretical frequency)/theoretical frequency for a given class] for each class of distributions examined in the Monte Carlo trials and for the Chicago data set. For the negative binomial distribution, there is a tendency for the larger values (as well as the reverses) of the range-of-transition statistic to increase and the smaller values of the range-of-transition statistic to decrease as the value of k decreases (and the variance becomes ever greater than the Poisson). For the two-hit model, in contrast, the smaller values of the range-of-transition statistic are more frequent than expected. The actual Chicago data set shows a pattern of deviation more in line with the two-hit model than with the negative binomial model—i.e., the 0 range of transition is more frequent than with the Poisson, and the longer ranges of transition (as well as the reverses) are less frequent than with the Poisson.

Application to quality control. The procedure developed is useful for routine quality control in laboratories conducting MPN tests (or any dilution test procedure, such as the 50% tissue culture infective dose determination in virus assay). For each MPN score, by using its associated maximum likelihood estimator for the density, the distribution of the range-of-transition statistic to be expected may be tabulated. Table 4 presents the expected value of this statistic for the common 5-tube, 3-decimal dilution test (as well as the range-of-transition statistic associated with that particular score) for the more common tube combinations. This may readily be extended to other combinations of dilutions and tubes.

As an example of the use of this table, consider the experimental series of 29 observations made on a given water supply in Table 5. By direct inspection, the observed distribution of the range of transition may be computed. The last line of Table 5 represents the theoretical distribution of

TABLE 4. Expected value of Stevens' range-of-transition statistic for the 3-decimal-dilution, 5-tube test

Score for vol (ml)			Range[a]	MPN/100 ml	Expected distribution of range for the associated MPN density				
10	1	0.1			Reverse	0	1	2	3
0	1	0	R	1.82	0.0681	0.0002	0.8500	0.0810	0.0007
1	0	0	1	1.99	0.0658	0.0003	0.8447	0.0884	0.0009
1	1	0	2	4.03	0.0455	0.0036	0.7746	0.1728	0.0035
2	0	0	1	4.47	0.0427	0.0052	0.7582	0.1896	0.0043
2	1	0	2	6.84	0.0341	0.0210	0.6669	0.2687	0.0093
3	0	0	1	7.78	0.0329	0.0305	0.6312	0.2938	0.0116
2	2	0	2	9.31	0.0323	0.0492	0.5769	0.3261	0.0155
3	0	1	R	10.57	0.0325	0.0663	0.5378	0.3448	0.0186
3	1	0	2	10.71	0.0326	0.0683	0.5338	0.3465	0.0189
4	0	0	1	12.76	0.0335	0.0966	0.4849	0.3616	0.0234
3	2	0	2	13.84	0.0340	0.1105	0.4666	0.3634	0.0255
4	0	1	R	16.58	0.0349	0.1397	0.4423	0.3538	0.0292
4	1	0	2	16.89	0.0350	0.1423	0.4414	0.3518	0.0295
4	1	1	3	21.16	0.0350	0.1645	0.4569	0.3126	0.0310
4	2	0	2	21.61	0.0349	0.1653	0.4608	0.3080	0.0310
5	0	0	0	23.12	0.0345	0.1666	0.4761	0.2923	0.0304
4	3	0	2	27.08	0.0329	0.1602	0.5242	0.2553	0.0274
5	0	1	R	31.39	0.0305	0.1435	0.5769	0.2262	0.0228
5	1	0	1	32.91	0.0296	0.1366	0.5936	0.2191	0.0211
5	1	1	2	45.62	0.0212	0.0822	0.6786	0.2087	0.0093
5	2	0	1	49.31	0.0189	0.0708	0.6872	0.2161	0.0070
5	2	1	2	69.96	0.0091	0.0439	0.6673	0.2785	0.0013
5	3	0	1	79.24	0.0063	0.0457	0.6420	0.3054	0.0006
5	2	2	2	94.35	0.0034	0.0586	0.5970	0.3409	0.0001
5	3	1	2	108.64	0.0019	0.0767	0.5576	0.3638	0.0000
5	4	0	1	129.93	0.0007	0.1070	0.5124	0.3798	0.0000
5	3	2	2	140.56	0.0005	0.1218	0.4971	0.3807	0.0000
5	4	1	2	172.38	0.0002	0.1582	0.4804	0.3612	0.0000
5	4	2	2	221.16	0.0001	0.1855	0.5201	0.2943	0.0000
5	5	0	0	239.79	0.0002	0.1875	0.5477	0.2646	0.0000
5	4	3	2	278.10	0.0002	0.1814	0.6130	0.2054	0.0000
5	5	1	1	347.67	0.0003	0.1521	0.7281	0.1194	0.0000
5	5	2	1	542.26	0.0003	0.0776	0.9020	0.0201	0.0000
5	5	3	1	917.84	0.0000	0.0883	0.9112	0.0005	0.0000
5	5	4	1	1,609.44	0.0000	0.3280	0.6720	0.0000	0.0000

[a] R, Reversal in the range for that tube combination.

TABLE 5. Example of application of range-of-transition test to a 5-tube, 3-decimal-dilution experiment data set

Score	Frequency	Range[a]	Expected frequency of range of transition				
			Reverse	0	1	2	3
1-0-0	8	1	0.5262	0.0021	6.7577	0.7070	0.0071
2-0-0	7	1	0.2987	0.0364	5.3077	1.3272	0.0300
1-1-0	4	2	0.1820	0.0114	3.0985	0.6910	0.0141
3-0-1	2	R	0.0651	0.1326	1.0755	0.6896	0.0372
0-1-0	1	R	0.0681	0.0002	0.8500	0.0810	0.0007
2-1-0	2	2	0.0683	0.0419	1.3337	0.5375	0.0187
5-0-0	3	0	0.1036	0.4999	1.4284	0.8770	0.0912
5-1-0	1	1	0.0296	0.1366	0.5936	0.2191	0.0211
Sum			1.3414	0.8640	20.4451	5.1294	0.2200

[a] R, Reversal in the range for that tube combination.

this statistic for the observed distribution of MPN densities. These two distributions are compared in Table 6.

As in the comparison of the Chicago data, it is necessary to pool adjacent categories (Reverse/0/1, 2 and 3) to produce cell counts in excess of 5 (required for the chi-square comparison). The total chi-square is below the significance level for 1 degree of freedom (2-1), and hence the null hypothesis is accepted (i.e., the assumption of Poisson replication error cannot be refuted).

Alternatives in the absence of Poisson replication errors. In the absence of a finding of Poisson replication errors between replicates, the use of the standard MPN tables is not justified. Wadley (25) has shown, in the single dilution case, that if an overdisperse distribution (including the negative binomial) characterizes the between-replicate error, the use of the Poisson assumption in the computation of the mean density produces an underestimate of the true density. If the failure to find Poisson replication errors is due to factors not influencing the average recovery (perhaps by errors in serial dilution), then it might be possible to produce revised MPN tables based on the assumption of a constant replication error distribution (e.g., negative binomial with a fixed k value). While, in principle, it might be possible to fix the form of the distribution, such as negative binomial, and estimate the unknown parameter of the distribution along with the mean, we have found that such a procedure produces erratic results even when four decimal dilutions are used in the common 5-tube protocol. An alternative approach might be the estimation of the median volume in which a microorganism is contained. Such an estimate can be constructed by nonparametric procedures without assuming a structure for the replication error (14–16).

Conclusions. It is concluded that the commonly accepted hypotheses for computation of the coliform MPN from dilution tests do not have universal validity. While there are plausible methodological (23) and statistical (3, 11) grounds for such discrepancies, this study suggests the need for a reconsideration of the MPN procedure. It is possible, for example, that the assemblage of noncoliform organisms present in samples may interfere with random error propagation in the test. It is possible that the coliforms in a water sample may have sublethal injuries which result in a distribution of their recovery probabilities in the MPN test. It is also possible that random contamination of the tubes with a growth factor or an inhibitor may cause tube-to-tube variation in coliform recovery. It is also possible that propagation of errors in decimal dilution series cause increased relative errors in higher dilution samples (23). In any event, caution in the interpretation of MPN tests is necessary until the basis for the variation observed in this study is ascertained.

Prudence dictates that until the basis for non-Poisson behavior is ascertained, a robust estimation procedure (such as interpretation by a presence-absence approach) should be followed. However, to the degree that failure to adhere to Poisson statistics may reflect methodological deficiences (for example, by the variable recovery of sublethally injured microorganisms), it is entirely possible that any numerical interpretation of MPN tests, when the current analytical procedures are used, may inadequately represent the true mean of the number of bacteria from which water samples are taken. In this regard, the observations are similar to those made in an analysis of current total coliform membrane filter procedures (12).

TABLE 6. Analysis of hypothetical 3-dilution experiment

Range-of-transition statistic	Frequency		Chi-square[a]
	Theoretical	Observed	
Reverse/0/1	22.65061	22	0.02
2 and 3	5.3494	6	0.08

[a] The sum of the chi-square results was 0.10.

ACKNOWLEDGMENTS

We thank Col. John Corey, formerly Commissioner of the Department of Water for the City of Chicago, for release of the data and Bon Mui, graduate student at the Illinois Institute of Technology, for compilation of the data.

LITERATURE CITED

1. **Armitage, P., and C. C. Spicer.** 1956. The detection of variation in host susceptibility in dilution counting experiments. J. Hyg. **54**:401–414.
2. **Chang, S. L., G. Berg, K. A. Busch, R. E. Stevenson, N. A. Clarke, and P. W. Kabler.** 1958. Application of the most probable number method for estimating concentration of animal viruses by the tissue culture technique. Virology **6**:27–42.
3. **Chase, G. R., and D. G. Hoel.** 1975. Serial dilutions: error effects and optimal designs. Biometrika **62**:329–334.
4. **Cochoran, W. G.** 1950. Estimation of bacterial densities by means of the most probable number. Biometrics **6**:105–117.
5. **Collings, B. J., and B. H. Margolin.** 1985. Testing goodness of fit for the Poisson assumption when observations are not identically distributed. J. Am. Stat. Assoc. **80**:411–418.
6. **Colwell, N. D., and M. D. Morisetti.** 1969. Microbiological techniques—some statistical aspects. J. Sci. Food Agric. **20**: 573–579.
7. **DeMan, J. C.** 1975. The probability of most probable numbers. Eur. J. Appl. Microbiol. **1**:67–78.
8. **Dempsey, K. W., and W. O. Pipes.** 1986. Evaluating relative stringencies of existing and proposed microbiological MCL's. J.

Am. Water Works Assoc. **78(11)**:47–54.
9. **Eisenhart, C., and P. W. Wilson.** 1943. Statistical methods and control in bacteriology. Bacteriol. Rev. **7**:57–137.
10. **El-Shaarawi, A. H., S. R. Esterby, and B. J. Dutka.** 1981. Bacterial density in water determined by Poisson or negative binomial distributions. Appl. Environ. Microbiol. **41**:107–116.
11. **Finney, D. J.** 1975. Statistical method in biological assay, 2nd ed. Hafner Press, New York.
12. **Haas, C. N., and B. A. Heller.** 1986. Statistics of enumerating total coliforms in water samples by membrane filter procedures. Water Res. **20**:525–530.
13. **Haldane, J. B. S.** 1939. Sampling errors in the determination of bacterial or virus density by the dilution method. J. Hyg. **39**: 289–293.
14. **Hamilton, M. A.** 1979. Robust estimates of the ED50. J. Am. Stat. Assoc. **74**:344–354.
15. **Hamilton, M. A., R. C. Russo, and R. V. Thurston.** 1977. Truncated Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. **11**:714–719.
16. **James, B. R., K. L. James, and H. Westenberger.** 1984. An efficient R-estimator for the ED50. J. Am. Stat. Assoc. **79**:164–173.
17. **Loyer, M. W., and M. A. Hamilton.** 1984. Interval estimation of the density of organisms using a serial dilution experiment. Biometrics **40**:907–916.
18. **Margolin, B. H., N. Kaplan, and E. Zeigler.** 1981. Statistical analysis of the Ames *Salmonella*/microsome test. Proc. Natl. Acad. Sci. USA **78**:3779–3783.
19. **Matuszewski, T., J. Neyman, and J. Supinska.** 1935. The accuracy of the dilution method. J. R. Stat. Soc. Suppl. **2**:63–82.
20. **McCrady, M. H.** 1915. The numerical interpretation of fermentation tube results. J. Infect. Dis. **17**:183–212.
21. **Moran, P. A. P.** 1954. The dilution assay of viruses. J. Hyg. **52**: 189–193.
22. **Pipes, W. O., P. Ward, and S. H. Ahn.** 1977. Frequency distribution for coliforms in water. J. Am. Water Works Assoc. **69**:664–668.
23. **Savage, G. M., and H. O. Halvorson.** 1941. The effect of culture environment on results obtained with the dilution method of determining bacterial populations. J. Bacteriol. **41**:355–362.
24. **Stevens, W. L.** 1958. Dilution series: a statistical test of technique. J. R. Stat. Soc. Part B **20**:205–214.
25. **Wadley, F. M.** 1950. Limitations of the "zero method" of population counts. Science **119**:689–690.
26. **Woodward, R. L.** 1957. How probable is the most probable number? J. Am. Water Works Assoc. **49**:1060–1068.
27. **Worcester, J.** 1954. How many organisms? Biometrics **10**:227–234.