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Most probable numbers of organisms: revised tables for the multiple tube method

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SUMMARY

Estimation of numbers of organisms is often made using dilution series, for example when examining water samples for coliform organisms. In this paper the most probable numbers (MPNs) are calculated for a 15-tube series consisting of five replicates at three consecutive tenfold dilutions. Exact conditional probabilities are computed to replace previous approximations.

When growth is observed in several of the tubes it is not realistic to select a single MPN. Instead a most probable range (MPR) should be reported. But using an MPR creates problems when comparison has to be made with a legislated, single-valued Standard. It is suggested that the wording of the Standards should be expressed differently when the multiple tube method is used.

INTRODUCTION

The multiple tube method was introduced in 1918 (McCrady) and has been widely used for estimating numbers of particular organisms in water and other public health specimens. The original sample is thoroughly mixed and divided into pre-determined sub-volumes (with or without dilution). These are added to media and incubated in separate tubes at temperatures appropriate to the relevant organisms. The tubes are then examined for signs of growth which indicate that at least one organism was present in that sub-volume. Wherever possible dilution levels are chosen so that at least some of the sub-volumes contain none of the relevant organisms.

The mathematical equations for estimating total numbers of organisms based on the numbers of tubes showing growth were solved approximately. This meant that the sample examined was assumed to be part of a large body of bacteriologically homogeneous water (or whatever the substance being tested). Modern computers allow us to solve the equations more accurately, without making this assumption.

The computational method has already been reported in detail, together with tables of probable numbers for the 11-tube dilution series 1×50 ml:5×10 ml:5×1 ml (Tillett & Coleman, 1985).

In this paper probable numbers for the 15-tube series 5×10 ml:5×1 ml:$5 \times 0{\cdot}1$ ml are presented.

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METHODS

If n bacteria are distributed at random among m test tubes of equal volume, the probability that $(m-j)$ tubes will not receive any of them and will thus remain sterile is given exactly by the classical occupancy theory described by David & Barton (1962). This probability,

$$p\ (j \text{ occupied} \mid n \text{ bacteria}) = \frac{1}{m^n} \frac{m!}{(m-j)!} \Delta_{j,n},$$

(where $\Delta_{j,n}$ is Stirling's number of the second kind, with initial condition: $p(1 \mid 1) = 1$ and using $p(0 \mid n) = 0$ for all n and $p(j \mid n) = 0$) for $j > n$.

Therefore, when there are five tubes of equal volume, $m = 5$ and

$$p(j \mid n) = \frac{1}{5^n} \frac{5!}{(5-j)!} \Delta_{j,n}.$$

In the dilution series:

five I-tubes of 10 ml where i tubes show growth ($i = 0$ to 5);
five J-tubes of 1 ml where j tubes show growth ($j = 0$ to 5);
five K-tubes of 0·1 ml where k tubes show growth ($k = 0$ to 5);

the probability that i, j and k tubes show growth given that there are n bacteria present in the 55·5 ml examined, with n_i, n_j and n_k of them in the I, J and K tubes respectively, is

$$p(i,j,k \mid n) = \sum_{\substack{n_i\ n_j\ n_k \\ (\text{such that} \\ n_j+n_j+n_k=n)}} \sum \sum \sum p(i \mid n_i)\, p(j \mid n_j)\, p(k \mid n_k) \times \frac{n!}{n_i!\, n_j!\, n_k!} \times \frac{50^{n_i} 5^{n_j} 0{\cdot}5^{n_k}}{55{\cdot}5^n}.$$

RESULTS

Table 1 gives probable numbers of organisms associated with the more commonly observed values of i, j and k – the numbers of tubes showing growth at the three consecutive tenfold dilutions.

The table relates to 10 ml, 1 ml and 0·1 ml dilutions, but other series can be accommodated by multiplying results by the appropriate factor, e.g. 10 if levels of 1 ml, 0·1 ml and 0·01 ml are used. Combinations not appearing in Table 1 are those for which the sum of all conditional probabilities is less than 1 % (i.e. $\Sigma_n p(i, j, k \mid) \leqslant 0{\cdot}01$). These combinations are very unlikely if the sample has been adequately agitated before dilution and subdivision. The revised MPNs, as calculated from the exact conditional probabilities, are given as numbers per 100 ml as is conventional, although only 55·5 ml are examined in this dilution series. Numbers above 50 are rounded to the nearest five and above 150 to the nearest ten. The MPNs from McCrady's method are shown alongside and have been taken from Report 71 (DOE, 1983).

When a small number of tubes out of the dilution series show growth there is a clear-cut MPN. Fig. 1 shows the relative likelihood that two to six organisms are present in the 55·5 ml examined when only two of the 10 ml tubes show a positive reaction. The probability that the volume examined contains two organisms (i.e. four per 100 ml) is nearly twice the probability of there being three present. However, when many of the tubes show growth there is no outstanding MPN. Fig.

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Table 1. *Probable numbers of organisms**

i, *j*, *k*	Revised MPN	Previous MPN	Most probable range (MPR†)
0 0 1	2	2	2
0 1 0	2	2	2
1 0 0	2	2	2
1 0 1	4	4	4
1 1 0	4	4	4
1 2 0	5	6	5
2 0 0	4	5	4
2 0 1	5	7	5
2 1 0	5	7	5
2 1 1	7	9	7
2 2 0	7	9	7–9
2 3 0	11	12	11
3 0 0	7	8	7
3 0 1	9	11	9
3 1 0	9	11	9
3 1 1	13	14	13
3 2 0	13	14	13
3 2 1	16	17	14–16
3 3 0	16	17	14–16
4 0 0	11	13	11–13
4 0 1	14	17	14–16
4 1 0	16	17	14–16
4 1 1	20	21	18–20
4 2 0	20	22	18–22
4 2 1	25	26	23–27
4 3 0	25	27	23–27
4 3 1	31	33	29–34
4 4 0	32	34	29–34
4 4 1	38	40	34–41
5 0 0	22	23	20–23
5 0 1	29	31	25–34
5 0 2	41	43	36–50
5 1 0	31	33	27–36
5 1 1	43	46	36–50
5 1 2	60	63	50–70
5 1 3	85	84	70–95
5 2 0	50	49	40–55
5 2 1	70	70	60–80
5 2 2	95	94	80–110
5 2 3	120	120	105–135
5 3 0	75	79	65–90
5 3 1	110	110	90–125
5 3 2	140	140	120–160
5 3 3	175	180	155–200
5 3 4	210	210	185–240
5 4 0	130	130	110–150
5 4 1	170	170	150–200
5 4 2	220	220	190–250
5 4 3	280	280	240–320
5 4 4	345	350	300–390

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Table 1. *Probable numbers of organisms**

i, *j*, *k*	Revised MPN	Previous MPN	Most probable range (MPR†)
5 5 0	240	240	200–280
5 5 1	350	350	290–420
5 5 2	540	540	450–660
5 5 3	910	920	750–1100
5 5 4	1600	1600	1350–1900

* Per 100 ml for the dilution series 5 × 10 ml : 5 × 1 ml : 5 × 0·1 ml when *i*, *j* and *k* tubes show growth.

† MPR range of numbers which are at least 95% as likely as the MPN.

0 0 0 implies 'none found in 55·5 ml'.

5 5 5 implies a number too large to be estimated from this series of tubes. (It is likely that 1800+ organisms are present.)

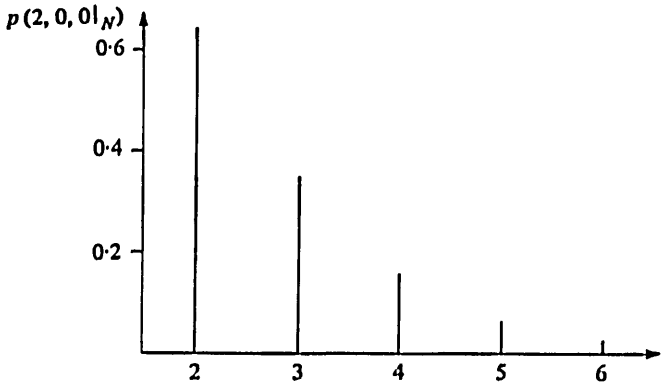

Fig. 1. Probabilities of observing growth in two of the 10 ml tubes, conditional on the presence of N bacteria (N = organisms in 55·5 ml).

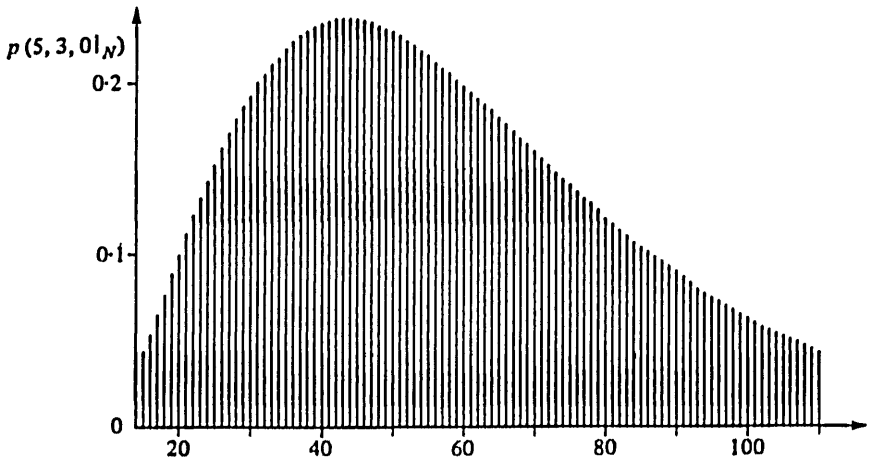

Fig. 2. Probabilities of observing growth in five of the 10 ml and three of the 1 ml tubes, conditional on the presence of N bacteria (N = organisms in 55·5 ml).

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2 shows the relative likelihood that N organisms are present. The most probable count is $N = 43$ (i.e. 75 per 100 ml rounded to the nearest five) but values of N in the range 36–51 (i.e. 65–90/100 ml) are at least 95% as likely to be the correct answer as the MPN. As more tubes show reaction the picture becomes even less clear, as illustrated by the most probable ranges (MPRs) shown in the final column of Table 1. The MPR is arbitrarily defined as the range of counts which are at least 95% as probable as the MPN.

If none of the tubes shows reaction the result can be unambiguously expressed as 'none found in a 55·5 ml sample'. If all the tubes show reaction, theoretically the MPN is infinity and the most that can be said is that there are unlikely to be fewer than 1800 organisms per 100 ml since, for counts above this value, the most probable result is 5, 5, 5.

DISCUSSION

Modern computer facilities have allowed new estimations of probable numbers of organisms from the multiple tube method. No assumption is made about the sample other than that it was examined by the standard laboratory techniques associated with dilution series, including thorough shaking/stirring before dilution.

Previous tables of MPNs have been calculated using an approximation which necessitates assuming that the sample examined is a small part of a very large bacteriologically homogeneous body. With water samples, either drinking or recreational, it is usually unrealistic to assume that the sample is part of a homogeneous water source. Recreational waters may have very variable coliform content, and drinking waters are being monitored for unexpected changes. With the original McCrady tables (1918) and many subsequent publications it was assumed that the water sample was part of a large identical body of water in order to solve the mathematical equations. In practice, whether or not this assumption is made makes little difference to the MPN, as illustrated by Table 1 in this paper and the one for the 11-tube series (Tillett & Coleman, 1985). However, two points have emerged.

First, the confidence intervals attached to some published tables (DOE, 1983; APHA, 1985) are only appropriate if the assumption about a bacteriologically homogeneous water source can be made. In such a situation the bacteria are distributed according to Poisson theory and their variance can be estimated from a single sample. If this assumption cannot be made the variability of bacterial density in the water source must be estimated by collecting multiple samples over place and time.

Secondly, the multiple tube method cannot provide a precise count of the viable organisms. Detailed computation of the probable numbers of organisms has illustrated that there is often no clear-cut MPN, and it is suggested that a Most Probable Range is a more appropriate way of reporting the results. The arbitrary definition of the MPR used in this paper is counts which are at least 95% as likely as the MPN.

The European Community Standards for drinking waters and recreational waters express bacterial levels as single-figure upper limits. This could lead to problems when comparing MPRs with such Standards. Should the whole range fall

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below the upper limit? If Standards were expressed differently according to the method used this problem would be resolved. In the United States drinking water standards are described as permitted numbers of tubes showing reaction and as counts from the membrane filtration method (APHA, 1985, p. 829).

The fact that the multiple tube method does not always give a precise result should not weigh against it. Recent water quality control trials in the Public Health Laboratories, using the methods described by Gray & Lowe (1976), demonstrated that the multiple tube method was more sensitive than membrane filtration in detecting low counts of *Escherichia coli* and often gave higher total coliform counts (Tillett, 1986).

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