NOTE ON ESTIMATING BACTERIAL POPULATIONS BY THE DILUTION METHOD

BY ROBERT DEAN GORDON

SCRIPPS INSTITUTION OF OCEANOGRAPHY, UNIVERSITY OF CALIFORNIA*

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One method of obtaining an estimate of bacterial density in a solution, is to inoculate 1-ml. portions of the solution into each of several test tubes containing sterile nutrient medium; then dilute the solution in a convenient ratio and inoculate several more test tubes with 1-ml. portions, then dilute again, and so on, for several successive dilutions. After a period of incubation, the proportions of "positives" (tubes showing growth) obtained from each of the several dilutions, are taken as indications of the density of "viable" bacteria in the original solution.

In 1933 Halvorson and Ziegler[1] published a set of tables, in which they assumed the dilution ratio to be 10:1, and supposed a total of thirty test tubes to be inoculated with three successive dilutions, ten with one dilution, ten with a 10-fold greater dilution and ten with a 100-fold greater dilution than the first. If respectively p_1, p_2 and p_3 tubes, out of the ten inoculated, show growth, their tables show a corresponding estimate of the mean density of viable bacteria in the middle dilution. For more details, their booklet[1] may be consulted.

Dr. C. E. ZoBell, of the Scripps Institution of Oceanography, in experimenting with these tables found them to be unsatisfactory and unreliable, and asked the writer to examine them. It was found that the estimates were obtained in accordance with R. A. Fisher's "criterion of maximum likelihood," which is shown to be of very questionable significance in this type of problem. In the Bayes-Laplace theory of inverse probability the estimates are modal values. Since the estimates are actually stated as three figure numbers (e.g., 2.53/ml., 43,500/ml., etc.), it seems evident that the most reasonable simple type of estimate is the *geometric mean*. The writer has developed formulas to obtain this value together with formulas for the standard deviation of its logarithm, to serve as an indication of its proportionate accuracy. It is expected to simplify these formulas to a greater degree than at present, but they are now in form which can be used for computation.

The notation of the writer is not the same as that of Halvorson and Ziegler. It is as follows:

n_{10} = number of test tubes showing growth, out of the ten inoculated with the highest concentration of the solution.

n_1 = number of test tubes showing growth, out of the ten inoculated with the middle dilution.

$n_{0.1}$ = number of test tubes showing growth, out of the ten inoculated with the highest (100-fold) dilution.

$\bar{\rho}$ = geometric mean estimate of the density of viable bacteria in the solution, corresponding to the middle dilution (i.e., to n_1).

x = an integer $\geqq x_0$ where $x_0 = 1110 - 100n_{10} - 10n_1 - n_{0.1}$.

$N = n_{10} + n_1 + n_{0.1}$.

γ = "digamma 1" = $-0.577216\ldots$.

γ' = "trigamma 1" = $+1.644934\ldots$.

$\log_e 10 = 2.302585\ldots$.

Δ = Boolean difference operator; operates on x.

H_0 = operator on x_0.

$$= (1 + E + \ldots + E^{99})^{n_{10}}(1 + E + \ldots + E^{9})^{n_1}$$

where E = Boolean operator = $(1 + \Delta)$;

$$= 1 + P_1E + P_2E^2 + \ldots + P_rE^r.$$

With this notation, the following formulae hold:[2]

$$\log_e\bar{\rho} = \gamma + \log_e 10 - \frac{H_0\Delta^N\left\{\frac{\log_e x_0}{x_0}\right\}}{H_0\Delta^N\left\{\frac{1}{x_0}\right\}}. \tag{13}$$

$$\sigma^2_{\log\rho} = -(\log_e\bar{\rho})^2 + [\gamma + \log_e 10]^2 + \gamma' - 2[\gamma + \log_e 10]\cdot\frac{H_0\Delta^N\left\{\frac{\log_e x_0}{x_0}\right\}}{H_0\Delta^N\left\{\frac{1}{x_0}\right\}} + \frac{H_0\Delta^N\left\{\frac{(\log_e x_0)^2}{x_0}\right\}}{H_0\Delta^N\left\{\frac{1}{x_0}\right\}}. \tag{16}$$

For use in these formulas, the following auxiliary formulas have been obtained:

$$(-1)^N\Delta^N\left\{\frac{1}{x}\right\} = \frac{N!(x-1)!}{(x+N)!}. \tag{19}$$

$$(-1)^N\Delta^N\left\{\frac{\log_e x}{x}\right\} = \frac{N!(x-1)!}{(x+N)!}\log_e x + \sum_{p=N}^{\infty}\frac{(-1)^{p-N+1}}{x^{p+1}}\cdot\frac{p!}{p-N!}\cdot\left(1 + \frac{1}{2} + \frac{1}{3} + \ldots + \frac{1}{p}\right)B^{(-N)}_{p-N}. \tag{21}$$

$$(-1)^N \Delta^N \left\{ \frac{\log_e x}{x} \right\} = \frac{N!(x-1)!}{(x+N)!} \log_e(x+N) \qquad (21a)$$

$$- \sum_{p=N}^{\infty} \frac{1}{(x+N)^{p+1}} \cdot \frac{p!}{(p-N)!} \left((1 + \frac{1}{2} + \frac{1}{3} + \ldots + \frac{1}{p} \right) B_{p-N}^{(-N)}$$

where $B_{p-N}^{(-N)}$ are Bernoulli numbers of degree $p - N$ and order $(-N)$; cf., L. M. Milne-Thompson, "Calculus of Finite Differences" (1933), page 129. We have tabulated most of these numbers which we need.

For the coefficients P_ν of the operator

$$H_0 = 1 + P_1 E + P_2 E^2 + \ldots + P_\nu E^\nu + \ldots .$$

we have the direct formula

$$P_\nu = \sum_{r=0}^{.01\nu} \sum_{s=0}^{.10-10r} (-1)^{r+s} C_r^{n_{10}} C_s^{n_1} C_{\nu-100r-10s+n_{10}+n_1-1}^{\nu-100r-10s} \qquad (30)$$

where C_a^b is the binomial coefficient. Also

$$P_{99n_{10}+9n_1-\nu} = P_\nu .$$

For intermediate values of the subscript ν, P_ν is given by an Hermite expansion

$$P_\nu = \left[\left(\sum_{\mu=0}^{\infty} A_{2\mu} \cdot \frac{d^{2\mu}}{d\xi^{2\mu}} \right) \frac{c}{\sqrt{\pi}} e^{-c^2(\xi-b)^2} \right]_{\xi=\nu} \qquad (33)$$

where $b = \frac{1}{2}(99n_{10} + 9n_1)$ and $c^2 = \dfrac{6}{9999n_{10} + 99n_1 + 2}$

and the first few coefficients $A_{2\mu}$ have the following values:

$$A_0 = 10^{2n_{10}+n_1}$$

$$A_2 = 0 \qquad (38)$$

$$A_4 = -\frac{10^{2n_{10}+n_1}[(10^8-1)n_{10} + (10^4-1)n_1 + 2]}{2880}$$

and

$$A_6 = 10^{2n_{10}+n_1} K_6$$

$$A_8 = 10^{2n_{10}+n_1} \left[K_8 + \frac{1}{2} K_4^2 \right]$$

. . . . . .

where

$$K_{2\mu} = \frac{B_{2\mu}}{(2\mu)(2\mu)!}\left[(100^{2\mu} - 1)n_{10} + (10^{2\mu} - 1)n_1 + 2\right]$$

and $B_{2\mu}$ is the Bernoulli number of first order, of degree 2μ (cf. Milne-Thompson, l. c.). A trigonometric (Fourier) expansion is also possible for P_r, and may prove more useful.

Mrs. Naomi Lancaster has used these formulas to make several computations of $\bar{\rho}$, shown in the following table and compared with the corresponding estimates of Halvorson and Ziegler:

ARGUMENTS			$\bar{\rho}$ COMPUTED BY US	$\bar{\rho}$ FROM HALVORSON AND ZIEGLER	% DEVIATION FROM HALVORSON AND ZIEGLER
n_{10}	n_1	$n_{0.1}$			
10	7	3	1.43	1.53	−7.0
8	5	1	0.291	0.267	+9.0
4	2	1	0.086	0.080	+7.5

The per cent deviations shown would appear to be significant in bacteriological work. For instance, if a bacteriologist desires to attribute a particular degree of certainty to the proposition that his estimate is within 20% of the true value, the knowledge that his method of arriving at the estimate could account for a bias of from 7 to 9% must certainly be of significance to him.

As a matter of fact, comparisons of direct plate counts with estimates from Halvorson and Ziegler's tables, on the same materials, corroborate the above theoretical comparisons and even indicate that in other parts of the tables, the bias may be considerably more serious.

In order to put these results into practical form for bacteriologists and others, as well as to test the theory experimentally, it would be necessary to compute tables to replace those of Halvorson and Ziegler,[1] which would require a considerable financial outlay.

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[1] Halvorson and Ziegler, *Quantitative Bacteriology*, Burgess Publishing Co., Minneapolis, Minnesota (1933).

[2] A complete discussion will be published elsewhere.