ON THE STATISTICAL INTERPRETATION OF SOME BACTERIOLOGICAL METHODS EMPLOYED IN WATER ANALYSIS.

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(With 3 Charts.)

We were recently consulted by an officer serving on the Western Front as to the significance attaching to ordinary bacteriological methods of gauging the potability of waters. He wished to know what was the probability that a given water supply did not contain more than a certain proportion of bacteria in the unit volume, it having been found that particular samples tested showed no growth while, perhaps, larger samples had done so, or that so many out of a series of samples of the same size had given positive results. Having obtained what seemed to us a reasonable solution of the particular problem proposed, we thought that the results might interest other officers and bacteriologists who have to do similar work. A survey of the criteria actually used by bacteriologists when they form an opinion as to the purity of waters seems to us to emphasise the need for some discussion.

So far as we can learn, the standard method is to find the minimum quantity of water from which a culture of the organism in question is obtained, usually at a single trial. It is also generally held that a useful indicator of pollution is furnished by the *B. coli* group; the difference between such highly refined techniques as that of the Metropolitan Water Board's experts and the rapid Field Service method introduced by Lieut.-Colonel P. S. Lelean is that the former envisage a carefully defined and limited group of organisms, while the latter merely determines the presence of such as ferment lactose, when grown in MacConkey's bile salt broth, within 24 hours. It will be remembered that Houston

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has expressed positive results in terms of the numbers of tubes containing "flaginacs," *i.e.* organisms which:

(1) give greenish fluorescence (fl) in neutral-red broth,
(2) acid and gas (ag) in lactose-peptone,
(3) indol (in) in broth,
(4) acidity (ac) and clotting of litmus-milk.

Whether this rigorous examination really excludes many organisms which, although passing the lactose fermentation test, ought not to be admitted into the *B. coli* fold, is a question we are incompetent to answer.

With respect to standards, Dr Savage, who has worked in the Metropolitan Water Board's laboratory, writes that any deep well or spring water which contains *B. coli* (rigidly defined by such methods as that above detailed) in a sample of 100 c.c. or less should be regarded with great suspicion. In the case of surface supplies and shallow wells, he writes: "If no *B. coli* are present in 50 c.c., the water may probably be safely passed as satisfactory, as far as conditions actually present are concerned." "For rivers used as sources of drinking water, without artificial purification, similar standards are applicable[1]."

Colonel Lelean's standard is not defined. In practice he appears to have used 7 tubes containing respectively 20, 15, 10, 5, 2, 1 and $\frac{1}{2}$ c.c. of water to be tested[2]. The total volume of water so used is said to have been 50 c.c. (in reality, as it appears, $53\frac{1}{2}$ c.c.) and the tests "giving all negatives were recorded as having fractors in 75 c.c. instead of 50 c.c., while the all-positive results were given a value of fractors in $\frac{1}{4}$ c.c. instead of $\frac{1}{2}$ c.c." This novel statistical approximation does not, however, seem to have been uniformly employed by Colonel Lelean, for the number 50, and also the number 25, is frequently to be found in the column of his tables headed "Minimal number of c.c. containing lactose fractors." As no single tube of his series contained either 50 or 25 c.c. it is not obvious how these results were reached.

Nor is Colonel Lelean the only writer from the perusal of whose lucubrations the student may rise with some sense of bewilderment. Professor Hewlett[3] states that for the examination of an ordinary

[1] Savage, *The Bacteriological Examination of Water Supplies*, pp. 185–6. London, 1906.

[2] Bacteriological Examination of Waters in the Field. *Journ. Royal Army Medical Corps.* Sept. 1914.

[3] *Manual of Bacteriology*, 5th Edition. London, 1914.

drinking water he usually employs five tubes with 1 c.c. of the water in each, two tubes (double strength) with 10 c.c. in each, and one tube (double strength) with 25 c.c." (p. 584). Then later he remarks (p. 587) "The detection and enumeration of *B. coli* are regarded by all as perhaps the most important part of water examination. The number of *B. coli* is estimated from the amounts of water that have been added to the tubes of media, which, however, assumes that the organism is regularly distributed throughout the sample, and this must so far as possible be ensured by thorough mixing. The results generally come out fairly concordantly, though irregularities exceptionally occur which can only be obviated by making duplicate sets of cultures. It is better to state the result as "*B. coli* present in ... c.c. of water" rather than to say that so many *B. coli* are present, though as a matter of fact the latter statement is approximately correct. Adopting the writer's method for *B. coli* (p. 584), if *none* of the tubes contains *B. coli*, we say that "*B. coli* is absent from 50 c.c."; if the 25 c.c. tube contains *B. coli*, but not the remainder, "*B. coli* is present in 25 c.c. but not in less, and so on."

"If nothing is known about the water, the following standards may be adopted:

(*a*) *Waters of good quality.* *B. coli* absent in 50 c.c. of the water.

(*b*) *Waters of medium quality.* *B. coli* present in 50 c.c. but absent in 25 c.c.

(*c*) *Waters of poor quality.* *B. coli* present in 50 c.c. and 25 c.c., but absent in 10 c.c.

(*d*) *Waters of suspicious quality.* *B. coli* present in 50 c.c., 25 c.c. and 10 c.c., but absent in 1 c.c.

(*e*) *Waters unfit for drinking.* *B. coli* present in 1 c.c. or less."

In following paragraphs there is some qualification of the standards here laid down, it being pointed out that in upland surface waters a high degree of contamination may only be due to pollution by the excreta of animals and therefore not dangerous, while in the case of spring or deep well water *B. coli* should be absent from at least 50 c.c.

In the passage cited from p. 587 it is not explained how "the number of *B. coli* is estimated from the amounts of water that have been added to the tubes of media" even on the assumption of regular distribution, nor how any amount of stirring can ensure regular results, any more than stirring up the tickets in a bag containing equal numbers of black

and white cards can ensure that one will always draw five black and five white out of ten. It is not clear how duplicate sets of cultures can obviate irregularities, when two or more sets may give different results. The student will agree that when he fails to get a positive in any one of the author's series of tubes "*B. coli* is absent from 50 c.c." But if the 25 c.c. tube gives a positive but none of the remainder, he may legitimately object to the statement "*B. coli* is present in 25 c.c. but not in less," seeing that what has really been found is that *B. coli* is present in one lot of 25 c.c. but not in another—the lot made up of five 1 c.c. tubes and two of 10 c.c. The concluding words of the paragraph "and so on" also open up a vista of possible questions. If one of the 10 c.c. tubes gives a positive and the other a negative, how is the result to be stated? If one, or two, or more of the 1 c.c. tubes give positives, but not the remainder, what conclusion is to be drawn? Passing then to the standards laid down under heads (*a*)—(*e*), how is the student to determine standard (*b*) from the author's series of tubes? If *B. coli* is "present in 50 c.c." in his series, it is most likely to be present in the 25 c.c. tube and is therefore not absent from 25 c.c.: if present in the 25 c.c. tube, it is also very probably present in one of the others which total to 25 c.c. and this makes the case worse. Standard (*b*) cannot therefore be determined from the given series of tubes. Standard (*c*) also fails: if none of the 10 c.c. or 1 c.c. tubes give a positive, the actual result is, as already pointed out, "*B. coli* present in one sample of 25 c.c. but not in another." If either of the 10 c.c. tubes give a positive, one cannot state that *B. coli* is absent from 10 c.c. Precisely similar criticisms apply to (*d*). If *B. coli* is absent from all the 1 c.c. tubes, it is absent from 5 c.c., not from 1 c.c. If any one of the 1 c.c. tubes give a positive, it is not "absent from 1 c.c."

The train of reasoning followed in the treatise of Colonel Beveridge and Major Wanhill is somewhat different. These authors employed the usual MacConkey medium and they classified waters in practically the same way as Savage, *e.g.* they held that the absence of *B. coli* from 100 c.c. indicated a very pure water, while presence in 100 but absence from 50 c.c. "is an indication of a good water, which has been polluted in some way, by animal or by human excreta, but in such small amount, or at such a distant period that there would not be much danger attaching to the use of such water for a town supply, if filtered" (p. 152). They further observed that "For temporary camps, if inspection could reveal the presence of no polluting agency and it could be surmised that the excreta of sheep or cattle were the cause, the presence of *B. coli* in

10 c.c. or less might be allowed, this number being often found in moorland streams, where human contamination is unlikely[1]."

Beveridge and Wanhill also provide an arithmetical illustration of the method of determining the density of bacilli in the source from an examination of samples each of the same volume. They say that if ten tubes each inoculated with 1 c.c. of water yielded three positive and seven negative results the sample may be considered to contain *B. coli* in every 3 c.c. of water, adding: "this is a rough estimate and is not mathematically accurate." We may observe that it was this somewhat delphic utterance which induced an officer on water duty to submit to us the problem which was the starting point of the present investigation.

Before detailing our investigation, we may emphasise certain considerations which were, no doubt, present in the minds of the various authors cited but seemed to them too trivial to state.

The fact that a given volume of water tested contains no bacilli, or none which will grow, does not prove that the source of supply is sterile, the point is merely that the greater the volume tested with negative results the smaller is likely to be the population of organisms existing in the supply; none of the writers has attempted to provide a scale of bacterial densities corresponding to the increase of the minimum quantity of water found sterile on examination. We think, indeed, that the tenor of the passages cited creates a presumption that the authors' criterion really is that sources *shown by other methods or found from practical experience* to be safe or to be unsafe have *usually* been found to give sterile readings when samples of the assigned size have been tested. This would explain, for instance, the lower standard adopted in the case of moorland waters. This is undoubtedly a reasonable attitude of mind enough, but it is necessary to remark that the process is not wholly satisfactory, since two observers both testing the same source on, say, the basis of a sample of 100 c.c. might obtain the one a positive, the other a negative result, so that the one would reject and the other pass the supply. Further, no criterion is provided of the increase in accuracy of prediction attained when two, three or more samples of 100 c.c. all give sterile readings.

The object of this paper is to provide such criteria or at least to indicate the method by which they may be obtained in any given case. The actual technique employed is so different in detail in different

[1] Beveridge and Wanhill, *The Sanitary Officer's Handbook of Practical Hygiene*, 2nd Edition. London, 1912.

cases (thus the Metropolitan Water Board use tubes with volumes in decreasing geometrical progression, 100 c.c., 10 c.c., 1 c.c., etc., while Colonel Lelean had tubes of diminishing volume but not diminishing uniformly), that it is not possible to provide a table of standards which will be of use to all observers. Such tables can be drawn up, with the help of general formulae obtained, but, as the arithmetic if simple is laborious, we have confined ourselves to the provision of a few illustrative examples.

SECTION I. PRELIMINARY PROPOSITIONS.

If in the water from which samples of, say, 1 c.c. each are drawn there exist B bacilli in all in a total volume of W c.c. of water, then, the distribution of bacilli being assumed to be random, the probable numbers of c.c. with 0, 1, 2, 3,... bacilli in each are given by the binomial expansion of

$$\left(\frac{W-1}{W}+\frac{1}{W}\right)^B \quad \text{.....(1).}$$

Since B and W are both very large indeed, (1) becomes by a well-known transformation originally given by Poisson[1]:

$$e^{-\lambda}\left(1+\lambda+\frac{\lambda^2}{2!}+\frac{\lambda^3}{3!}\text{.....}\right) \quad \text{.....(2)}$$

where $\lambda=\frac{B}{W}$. The problem then reduces itself to that of determining the appropriate value of λ and the probable reliability of its determination.

SECTION II. CASE OF A SINGLE TEST.

We first consider the case of a sample of N c.c. having been taken and found sterile.

The chance of this happening for a given value of λ, is, by (2), $e^{-N\lambda}$.

Now it is reasonable to assume that all values of λ from 0 to some upper limit w are *a priori* equally probable (by analogy with Bayes' postulate) so that the chance of λ being within the range $\lambda \pm \frac{1}{2}d\lambda$ is $d\lambda/w$ and the chance that, λ being within the range, the event happens is $e^{-N\lambda}$, so that the complete chance of λ not exceeding some assigned value κ is:

$$P=\frac{\int_0^\kappa e^{-N\lambda}.d\lambda}{\int_0^w e^{-N\lambda}.d\lambda}=\frac{1-e^{-N\kappa}}{1-e^{-Nw}}.$$

[1] *Recherches sur la Probabilité d. Jugements, etc.*, p. 190 and p. 206.

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e^{-Nw} diminishes very rapidly as w increases and we may put $w = \infty$ so that the required chance is

$$P = 1 - e^{-N\kappa} \quad \text{.....(3).}$$

Consequently if we desire to assign an upper limit to the probable density of bacilli in a source of supply a sample of N c.c. from which has proved sterile we have the following simple method. Suppose we take as our standard the limit corresponding to odds of 99 to 1.

Then, from (3)

$$e^{-N\kappa} = \cdot 01,$$

and the odds are 99 to 1 that κ is not greater than

$$-\frac{\log \cdot 01}{N \log e}.$$

Looking at the matter from the standpoint of the frequency distribution, we may say that the frequency distribution of bacilli per c.c. in waters which give a negative result on testing N c.c. is

$$y = N \, . \, e^{-N\lambda}.$$

The maximum is at zero and the curve tails off rapidly towards the higher densities. The actual curve for $N = 100$ is shewn in Fig. 1.

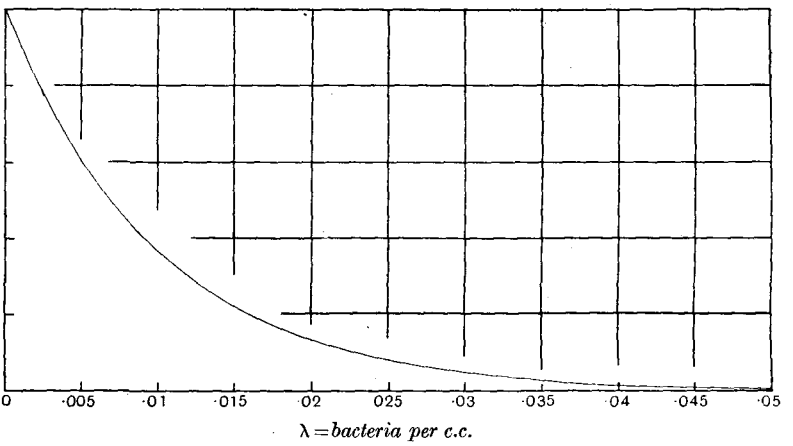

Fig. 1. Frequency distribution of bacterial densities when 100 c.c. give a negative result. The area of the curve is 10 squares.

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SECTION III. CASE OF TWO TESTS.

We now take the instance of two tubes having been used with respectively n and m c.c. of water in each. Should both prove negative, we reach the previous result putting $n + m = N$. If the n c.c. tube is negative and the m c.c. tube positive then from (2) by similar reasoning we have for the chance that λ does not exceed κ:

$$P = \frac{\int_0^\kappa e^{-\lambda n}(1 - e^{-\lambda m})\, d\lambda}{\int_0^w e^{-\lambda n}(1 - e^{-\lambda m})\, d\lambda},$$

putting $w =$ infinity, as before, we have

$$P = 1 - \left(\frac{n+m}{m} e^{-n\kappa} - \frac{n}{m} e^{-(n+m)\kappa}\right) \quad \ldots\ldots\ldots\ldots\ldots\ldots(4).$$

Taking again some particular value of odds, such as 99 to 1, (4) enables us to find a value of κ.

It is to be noted that the method breaks down if *both* samples give positive results for

$$\frac{\int_0^\kappa (1 - e^{-n\lambda})(1 - e^{-m\lambda})\, d\lambda}{\int_0^w (1 - e^{-n\lambda})(1 - e^{-m\lambda})\, d\lambda},$$

vanishes for $w = \infty$, so that no upper limit is assignable to λ.

The problem of this section may also be studied from the point of view of curve fitting. We have, writing $m + n = N$,

$$y = \frac{Nn}{m} e^{-\lambda n}(1 - e^{-\lambda m}) \quad \ldots\ldots\ldots\ldots\ldots\ldots(5),$$

for the distribution of λ, since the total area is

$$\int_0^\infty (1 - e^{-\lambda n})(1 - e^{-\lambda m})\, d\lambda = \frac{m}{Nn}.$$

The curve extends from 0 to ∞, and differentiating (5) with respect to λ and equating to zero we find for the mode

$$\lambda = \log \frac{N}{n} \cdot \frac{1}{m \log e} \quad \ldots\ldots\ldots\ldots\ldots\ldots(6).$$

Now

$$\int_0^\infty x^n e^{-x\kappa} \cdot dx = \frac{n}{\kappa} \int_0^\infty x^{n-1} \cdot e^{-x\kappa} \cdot dx = \ldots = \frac{n!}{\kappa^{n+1}}$$

(integrating successively by parts and noticing that the first part

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vanishes), so that the successive moments of (5) are determinate. We have:

$$\text{mean} = \frac{N+n}{nN} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(7),$$

$$\mu_2 = \frac{N^2+n^2}{n^2 . N^2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(8),$$

$$\mu_3 = \frac{2\,(N^3+n^3)}{N^3 n^3} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(9).$$

This curve therefore has positive skewness for all finite values of n, *i.e.* the long tail of the distribution extends to high values of λ.

Again if we write λ_1 for the modal value found in (6), the chance that the true bacterial density does not exceed the modal value is:

$$1 - \frac{Nn}{m}\int_{\lambda_1}^{\infty} (e^{-\lambda n} - e^{-\lambda N})\, d\lambda \quad \ldots\ldots\ldots\ldots\ldots\ldots(9\,a),$$

integrating and putting λ_1 equal to (6), (9 a) becomes:

$$1 - \frac{1}{mN}\left(\frac{n}{N}\right)^{\frac{n}{m}} (N^2 - n^2) \quad \ldots\ldots\ldots\ldots\ldots\ldots(10).$$

If $m = \rho n$, $N = (\rho + 1)\, n$, (10) becomes:

$$1 - \frac{1}{\rho\,(\rho+1)\, n^2}\left\{\frac{1}{(\rho+1)}\right\}^{\frac{1}{\rho}} \{(\rho+1)^2 n^2 - n^2\} = 1 - \frac{\rho+2}{(\rho+1)^{\frac{\rho+1}{\rho}}} \ldots(11).$$

If ρ becomes very large the fraction approximates to unity and most of the area lies beyond the mode. This is reasonable, for the information that a second sample of infinite size gave a positive result adds nothing to our knowledge and (4) reduces to the case of the last section, for which, as λ cannot be negative, the mode is at $\lambda = 0$.

As an illustration of the forms that frequency distributions may take, the curves have been calculated (1) for the case in which 100 c.c. gives a positive result and 50 c.c. a negative, (2) for the reciprocal case in which 100 c.c. gives a negative but 50 c.c. a positive—an inconsistent but perfectly possible result. The two curves are shewn together in Fig. 2. Both distributions are very skew. The respective modes are $\lambda_1 = 0{\cdot}011$ and $\lambda_1 = 0{\cdot}0081$.

The question naturally arises, what is the probability of an inconsistence such as that assumed in the second illustration? If n c.c. have given a negative result what is the probability that a subsequent sample of m c.c. from the same water will also give a negative, or on the other

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hand a positive? If n c.c. have given a negative, the probability that λ lies within the limits $\kappa \pm \frac{1}{2}d\kappa$ and that a second sample of m c.c. will then give a negative is $ne^{-N\kappa}\,d\kappa$, where $N = m + n$ as above. For

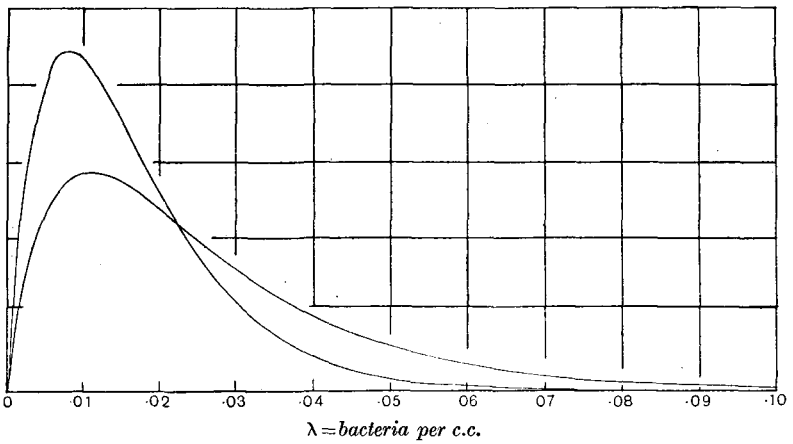

Fig. 2. Frequency distributions of bacterial densities for (1) 100 c.c. +, 50 c.c. −, the curve with the lower maximum, (2) 100 c.c. −, 50 c.c. +, the curve with the higher maximum. The area of each curve is 10 squares.

all values of λ the required probability is therefore the integral of this from 0 to ∞ or:

$$\left.\begin{array}{c}\text{chance that } n \text{ c.c. having given a negative, a second}\\ \text{sample of } m \text{ c.c. will also give a negative}\end{array}\right\} = \frac{n}{N}.$$

If $n = 100$, $m = 50$, the chance is $\frac{2}{3}$ or the chance of an inconsistence no less than $\frac{1}{3}$. If $m = n$, the chance is $\frac{1}{2}$. The results emphasise our previous remarks on the necessity of remembering that all results are subject to considerable fluctuations of sampling.

SECTION IV. CASE OF SEVERAL TESTS.

We first examine the case of N tubes, each containing the *same* quantity of water, which is taken as unity, n of the tubes being sterile while m give positive results.

If follows directly from (2) that the most probable value of λ is given by

$$\frac{N-m}{N} = e^{-\lambda} \text{ or } \lambda = \frac{1}{\log e}\log\frac{N}{N-m} = 2{\cdot}302585\log\frac{N}{N-m} \quad ...(12).$$

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The value so obtained is, however, subject to wide fluctuations of sampling as we shall now show.

Calling $\frac{N-m}{N}$, x, we have

$$\sigma_x^2 = \frac{1}{N} \cdot \frac{N-m}{N} \cdot \frac{m}{N}.$$

If $$z = \log \frac{1}{x}, \qquad dz = -\frac{1}{x}\,dx, \qquad (dz)^2 = \frac{1}{x^2}\,(dx)^2,$$

and consequently

$$\sigma_z^2 = \frac{1}{x^2}\,\sigma_x^2 = \frac{m}{N\,(N-m)}.$$

And finally the "probable error" of λ determined from (12) is:

$$1{\cdot}553068\sqrt{\frac{m}{Nn}} \qquad \text{.....(13).}$$

As will be seen from the probable errors calculated as illustrations in the columns headed 4 and 10 of Table I the fluctuations are such that it seems better to resort to the method adopted in the previous sections.

TABLE I.

Probable number of bacilli per 1000 *c.c. for a given number of blanks out of a given number of test tubes* (1 *c.c. in test*). *If* 10 *c.c. are placed in each tube instead of* 1, *divide all figures by* 10.

No. of blanks	Out of 2	3	4	5	6	7	8	9	10
1	693	1098	1386 ±1345	1609	1792	1946	2079	2197	2303 ±1473
2	—	405	693 ± 777	916	1098	1253	1386	1504	1609 ± 982
3	—	—	287 ± 448	510	693	847	981	1098	1204 ± 750
4	—	—	—	223	405	560	693	811	916 ± 602
5	—	—	—	—	182	336	470	588	693 ± 491
6	—	—	—	—	—	154	287	405	510 ± 401
7	—	—	—	—	—	—	134	251	357 ± 322
8	—	—	—	—	—	—	—	118	223 ± 246
9	—	—	—	—	—	—	—	—	105 ± 164

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We have accordingly for the chance (P) that λ does not exceed some value κ

$$P = \frac{\int_0^{\kappa} e^{-\lambda n}(1-e^{-\lambda})^m\, d\lambda}{\int_0^{w} e^{-\lambda n}(1-e^{-\lambda})^m\, d\lambda} \quad \text{.....(14).}$$

Expanding the brackets, the numerator and denominator can be integrated as before and an equation obtained for the value of x corresponding to a given value of P. For small numbers of tubes there is no difficulty, though the laboriousness of the work rapidly increases with the number of tubes. Alternatively, writing

$$e^{-\lambda} = x, \qquad d\lambda = -\frac{dx}{x},$$

substituting and assuming w to be infinite (14) becomes

$$\frac{\int_{e^{-\kappa}}^{1} x^{n-1}(1-x)^m\, dx}{\int_0^1 x^{n-1}(1-x)^m\, dx} = 1 - \frac{\int_0^{e^{-\kappa}} x^{n-1}(1-x)^m \,.\, dx}{\frac{(n-1)!\,m!}{(m+n)!}} \quad \text{.....(15).}$$

If now we require the value of κ which corresponds to some assigned probability, we can rewrite (15) as

$$\frac{\int_0^{x} x^{n-1}(1-x)^m \,.\, dx}{\frac{(n-1)!\,m!}{(m+n)!}} = 1 - P \quad \text{.....(16).}$$

Integrating the numerator of (16) by parts, (taking x^{n-1} as the direct integrand so as to keep the terms all positive) it becomes

$$\frac{x^n}{n}\left\{(1-x)^m + \frac{m}{n+1}x(1-x)^{m-1} + \frac{m(m-1)}{(n+1)(n+2)}x^2(1-x)^{m-2}\ldots\right\}$$
$$\text{.....(17).}$$

The series converges fairly rapidly and in rough practice it will be found that a sufficiently good approximation is often reached by putting the bracket equal to unity and solving for x from

$$\frac{x^n}{n} = \frac{(n-1)!\,m!}{(m+n)!}(1-P) \quad \text{.....(18).}$$

As the sum within the bracket cannot exceed unity, this approximation underestimates x and therefore over-estimates λ, an error on the safe side.

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A point of some interest is to consider the problem suggested by (9 *a*) of the last section taking the modal value from (12), *i.e.* putting

$$x = \frac{n}{n+m}$$

(17) then becomes

$$\frac{\left(\frac{n}{n+m}\right)^n}{n}\left\{\left(\frac{m}{m+n}\right)^m + \frac{m}{n+1}\left(\frac{n}{n+m}\right)\left(\frac{m}{m+n}\right)^{m-1} + \frac{m(m-1)}{(n+1)(n+2)}\left(\frac{n}{n+m}\right)^2\left(\frac{m}{m+n}\right)^{m-2} + \ldots\right\} \ldots\ldots(19),$$

and in the important special case of $m = n$

$$\frac{(\frac{1}{2})^{2n}}{n}\left\{1 + \frac{n}{n+1} + \frac{n(n-1)}{(n+1)(n+2)} + \frac{n(n-1)(n-2)}{(n+1)(n+2)(n+3)}\cdots\right\} \ldots\ldots\ldots(19\,a).$$

The series in (19 *a*) is readily summed. Call it $F(n)$. Then

$$\begin{aligned}\frac{2n!}{n!\,n!} \cdot F(n) &= \frac{(2n)!}{n!\,n!} + \frac{(2n)!}{(n+1)!\,(n-1)!} + \frac{(2n)!}{(n+2)!\,(n-2)!} + \ldots \\ &= C_n{}^{2n} + C_{n+1}{}^{2n} + C_{n+2}{}^{2n} \ldots \\ &= \frac{1}{2}\overset{2n}{S}_0 C_r{}^{2n} + \frac{1}{2}C_n{}^{2n} \\ &= \frac{1}{2}\cdot 2^{2n} + \frac{1}{2}\frac{(2n)!}{n!\,n!}.\end{aligned}$$

$$\therefore\ F(n) = \frac{1}{2}\left\{\frac{2^{2n}}{(2n)!}\cdot n!\,.\,n! + 1\right\}.$$

$$\therefore\ (19\,a) = \frac{(n-1)!\,n!}{2n!\,2} + \frac{1}{2^{2n+1}n} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(19\,b).$$

Substituting in (15) we have

$$P = 1 - \left(\frac{1}{2} + \frac{(2n)!}{n!\,n!}\cdot\frac{1}{2^{2n+1}}\right) \ldots\ldots\ldots\ldots\ldots\ldots(19\,c).$$

Applying Stirling's theorem to the third term it becomes $\frac{1}{2}\frac{1}{\sqrt{\pi n}}$, which is zero when n is infinite, and then the mode divides the frequency into equal parts.

For usual values of n the approximation is slow. Thus

n	Value of (15)	n	Value of (15)
1	·2500	100	·4718
2	·3125	500	·4874
10	·4119		

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Reverting for a moment to the integral in the denominator of (16), we may note that its value can be approximated to by Laplace's method of putting

$$\int y \,.\, dx = Y \int e^{-t^2} \frac{dx}{dt} \,.\, dt.$$

Y being the maximum value of the function within the required range, in this case the value for $x = \dfrac{n-1}{n+m-1}$, if x is written $\dfrac{n-1}{n+m-1} + \vartheta$, t^2 is obtained as a function of ϑ and then ϑ as a function of t by reversion of series.

The result to a first approximation only is that the integral transforms to

$$\frac{(n-1)^{n-1}(m-2)^m}{(n+m-1)^{n+m-1}} \int e^{-t^2} \frac{\sqrt{2}}{(n+m-1)\left(\dfrac{1}{m+1} + \dfrac{m}{(m+2)^2}\right)^{\frac{1}{2}}} dt.$$

This method of treatment is, of course, well known. We can, however, employ the method of the last section and investigate the properties of $y = x^{n-1}(1-x)^m$, or the distribution of $e^{-\lambda}$, where the range is from 0 to 1.

The mode is at

$$x = \frac{n-1}{n+m-1} = \frac{n-1}{N-1} \qquad \text{.....(19 } d\text{)}$$

and, the moments about the start of the curve being simply successive B functions we easily find

$$\text{mean} = \frac{n}{N+1} \qquad \text{.....(20),}$$

$$\mu_2 = \frac{n(m+1)}{(N+1)^2(N+2)} \qquad \text{.....(21),}$$

$$\mu_3 = \frac{2n[2n^2 - 3n(N+1) + (N+1)^2]}{(N+1)^3(N+2)(N+3)} \qquad \text{.....(22).}$$

A comparison of the mean and mode shews again that the curve is skew for all finite values of n and m greater than zero. The transformed curve, $y = y_0 e^{-\lambda n}(1 - e^{-\lambda})^m$ is, of course, also very skew, cf. the two distributions shewn in Fig. 3.

Finally we have the case of samples of different sizes.

Thus if N_1 samples each of a_1 c.c. have given n_1 negative and m_1 positive results, N_2 samples each of a_2 c.c. have given n_2 negative and

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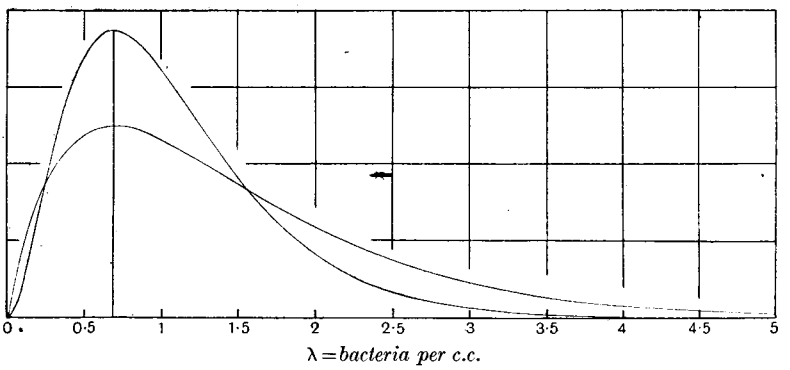

Fig. 3. Chart shewing frequency distributions of bacterial densities for 1 positive out of 2 and 2 positives out of 4 (1 c.c. in each test). The area of each curve is 10 squares.

m_2 positive results and ... N_n samples of a_n c.c. have given n_n and m_n negative and positive results, (14) becomes:

$$P = \frac{\int_0^\kappa [e^{-\lambda a_1 n_1}(1-e^{-\lambda a_1})^{m_1} \cdot e^{-\lambda a_2 n_2}(1-e^{-\lambda a_2})^{m_2} \dots e^{-\lambda a_n n_n}(1-e^{-\lambda a_n})^{m_n}]\, d\lambda}{\int_0^w [e^{-\lambda a_1 n_1}(1-e^{-\lambda a_1})^{m_1} \cdot e^{-\lambda a_2 n_2}(1-e^{-\lambda a_2})^{m_2} \dots e^{-\lambda a_n n_n}(1-e^{-\lambda a_n})^{m_n}]\, d\lambda} \quad \dots\dots\dots(23).$$

This expression does not lend itself to simple treatment in the general case but in any particular case it may furnish a solution without much difficulty. The arithmetic may however prove almost unworkable. In an illustration given in Section V only the most probable values of the bacillary density have been approximately determined by an artifice. If the tests are not repeated, only one test being made with a tube of each size, the expression simplifies.

On the general question of the best series of sizes of tubes to use, when the waters to be tested may present so great a range between purity and the reverse that the use of different sizes seems desirable, we may make a few remarks. One obvious condition, strangely overlooked, is that the size of any one sample should be greater than the sum of the sizes of the smaller samples. Otherwise the observer is simply asking for "inconsistencies" in his results. A geometrical series fulfils the required condition, and the Metropolitan Water Board have actually used a geometrical series with the ratio 10 (0·01, 0·1, 1, 10, 100 c.c.). This ratio seems rather a high one. A geometrical series seems also a natural one to use as the chance of an inconsistence is the same

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at every point of the series: r being the (ascending) ratio of the series, the chance of an inconsistence between any adjacent pair of samples (the larger giving a negative, the smaller a positive) is $1/(r+1)$.

The various expressions found above enable us to solve all the problems proposed and in the following section we provide a few arithmetical examples of their use.

SECTION V. NUMERICAL ILLUSTRATIONS.

(*a*) *The case of a blank sample.*

If 50 c.c. were tested and found sterile we have from (3)

$$e^{-50\kappa} = 1 - P \text{ or } -50\kappa \log e = \log(1-P).$$

For $P = \cdot5$ this gives $\kappa = \cdot01386$.
,, $P = \cdot99$,, ,, $\kappa = \cdot09210$.
,, $P = \cdot999$,, ,, $\kappa = \cdot13816$.
,, $P = \cdot9999$,, ,, $\kappa = \cdot18420$.

So that the chances are even that the source does not contain more than 14 bacilli per litre. It is 99 to 1 that there are not more than 92 per litre, 999 to 1 that there are not more than 138 per litre and 9999 to 1 that there are not more than 184 per litre.

Had 150 c.c., *e.g.* a sample of 100 and a sample of 50 both proved sterile, substitution of 150 for 50 gives for the corresponding values per litre, 5, 31, 46, 62.

(*b*) *One sample is sterile, the other not.*

Suppose 100 c.c. are positive, 50 c.c. sterile. Then using (4) the equation to be solved is

$$1{\cdot}5e^{-50\kappa} - {\cdot}5e^{-150\kappa} = 1 - P.$$

And we reach:

P	Bacilli per litre	P	Bacilli per litre
·5	21	·999	146
·99	100	·9999	192

(*c*) *Repeated Tests with Tubes of Equal Volumes.*

As a first illustration we take Beveridge and Wanhill's example of 10 samples, each 1 c.c., 7 of which are sterile and 3 show growth.

Here $n = 7$, $m = 3$, $N = 10$ so that by (19 D) and (20) the mode of the x distribution is at ·66667 and the mean at ·63637. That is a source with a density of 405 bacilli per litre (from $e^{-\lambda} = \cdot66667$) is

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the most probable state. Owing, however, to the marked skewness of the distribution, this result is of little service, we must solve (16) for different values of $1 - P$.

If we wish to find the density corresponding to $P = \cdot5, \cdot9, \cdot99$ we have

$$120x^7 - 315x^8 + 280x^9 - 84x^{10} = \begin{cases} \cdot5 \\ \cdot1 \\ \cdot01. \end{cases}$$

Thus, taking the last case, we have

$$f(x) = 120x^7 - 315x^8 + 280x^9 - 84x^{10} - \cdot01,$$
$$f'(x) = 840x^6 - 2520x^7 + 2520x^8 - 840x^9,$$

and by successive approximation we find that ·29716 is very nearly a root.

Hence from $e^{-\lambda} = \cdot29716$ we reach 1213 bacilli per litre: the odds are 99 to 1 that the density of the source does not exceed this value.

The densities corresponding to an even chance and to odds of 9 to 1 are similarly found to be 439 per litre and 802 per litre respectively.

In Table II we give examples of the significance of one or more sterile tubes in a series of 2–4 samples.

TABLE II.

Tables illustrating the significance of one or more blanks in two, three, or four tests on 1 *c.c. If the samples are of n c.c., not* 1 *c.c., divide the numbers of bacilli per litre by n.*

Two tests.

	If the number of blanks is	
The probability is	1	2
0·75 that the bacilli per litre exceed	690	140
0·50 ,, ,, ,, ,,	1230	350
0·25 ,, ,, ,, ,,	2050	690
0·01 ,, ,, ,, ,,	5300	2300
Most probable number per litre	690	nil

Three tests.

	If the number of blanks is		
The probability is	1	2	3
0·75 that the bacilli per litre exceed	990	400	95
0·50 ,, ,, ,, ,,	1580	690	230
0·25 ,, ,, ,, ,,	2390	1120	460
0·01 ,, ,, ,, ,,	5700	2830	1540
Most probable number per litre	1100	410	nil

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Four tests.

The probability is	If the number of blanks is 1	2	3	4
0·75 that the bacilli per litre exceed	1220	610	210	70
0·50 ,, ,, ,, ,,	1840	950	490	170
0·25 ,, ,, ,, ,,	2670	1410	780	350
0·01 ,, ,, ,, ,,	5990	3170	1960	1150
Most probable number per litre	1390	690	290	nil

As stated at the outset, divergences in the numbers of tubes forming different workers' series are too great to allow us to calculate any single table of general service. But the arithmetical examples should suffice to enable any bacteriologist to construct a table covering his own series.

(*d*) *Tubes of different volumes, test not repeated.*

A series of tubes of different volumes is used by several observers, *e.g.* as already mentioned Lelean, and Hewlett. Comment has already been made on the inconvenient character of the series used in each of these cases. That used by the Metropolitan Water Board is a simple geometrical series with a ratio of 10, viz. 100, 10, 1, 0·1, 0·01 c.c. The ratio is high, but it must be admitted that this simplifies the work of calculating the theoretical significance of the results; we have already pointed out that a geometrical series seems the right series to use.

Writing down equation (23) in its simplified form, where $n_r = 0$ and $m_r = 1$ or conversely, expanding, and retaining only the first power of x in the resulting equation we find the following approximate numbers of bacilli per litre:

	100 +, rest −	100 +, 10 +, rest −	100 +, 10 +, 1 +, rest −	100 +, 10 +, 1 +, 0·1 +
$P = 0·5$	72	720	7260	78940
$P = 0·99$	424	4245	42820	470150
Most probable numbers	23	230	2310	24540

The values of λ are nearly, it will be noticed, but not quite, a geometric series.

(*e*) *Repeated tests with tubes of different volumes.*

This involves the determination of the limiting density from (23). As an illustration we take the following series from one of the Reports of the Metropolitan Water Board.

Size of Sample	Source *A* Negative	Source *A* Positive	Source *B* Negative	Source *B* Positive
100 c.c.	308	30	312	21
10 c.c.	333	5	327	6
1 c.c.	336	2	329	4
·1 c.c.	338	0	333	0
·01 c.c.	338	0	333	0

It seemed difficult to obtain in this case even fair approximations to the values of the bacillary density for given values of P. But the following method gives good approximations to the most probable values. Taking the expression

$$y = y_0 e^{-\lambda(a_1n_1+a_2n_2+\ldots)} (1 - e^{-\lambda a_1})^{m_1} (1 - e^{-\lambda a_2})^{m_2} \ldots,$$

$$\frac{1}{y}\frac{dy}{d\lambda} = -(a_1n_1 + a_2n_2 + \ldots) + \frac{a_1m_1}{1 - e^{-\lambda a_1}} e^{-\lambda a_1} + \frac{a_2m_2}{1 - e^{-\lambda a_2}} e^{-\lambda a_2} + \ldots$$
$$= 0$$

for the most probable value. For Series A we find this gives

$$-34503{\cdot}18 + 3000 \frac{e^{-100\lambda}}{1 - e^{-100\lambda}} + 50 \frac{e^{-10\lambda}}{1 - e^{-10\lambda}} + 2 \frac{e^{-\lambda}}{1 - e^{-\lambda}},$$

or writing $e^{-\lambda} = x$

$$2 \frac{x}{1 - x} + 50 \frac{x^{10}}{1 - x^{10}} + 3000 \frac{x^{100}}{1 - x^{100}} = 34503.$$

Clearly x is near unity, as the value on the right is so large. Substitute accordingly $z = 1 - x$ for x and expanding this becomes

$$2 \frac{1 - z}{z} + 50 \frac{1 - 10z + 45z^2}{10z - 45z^2} + 3000 \frac{1 - 100z + 450z^2}{100z - 450z^2} = 34503.$$

Or, ignoring z^2

$$37{,}555z = 37,$$
$$z = 0{\cdot}000985,$$
$$x = 0{\cdot}999015,$$
$$\lambda = 0{\cdot}965 \text{ per litre.}$$

For Series B we find to a similar approximation $\lambda = 0{\cdot}838$ per litre, or the water from Source B is most probably slightly purer than that from Source A, though there is no practical difference.

As a glance at the two series suggests, moreover, they are not consistent with each other. Source B, if the better water, should give fewer positives in the small tubes as well as the large. Actually it gives much fewer in the large tube, but more in the small ones. Using the above values of λ, we find for the theoretical as against the actual distributions of positives:

Size of sample	Source *A*		Source *B*	
	Actual	Calculated	Actual	Calculated
100	30	31·7	21	27·0
10	5	3·3	6	2·8
1	2	·3	4	·2

It looks as if we were not dealing with a mere chance distribution from water essentially of constant character, but with occasional slight contaminations of the source.

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