APPLICATION OF STATISTICS TO PROBLEMS IN BACTERIOLOGY

I. A MEANS OF DETERMINING BACTERIAL POPULATION BY THE DILUTION METHOD

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INTRODUCTION

Some time ago the authors had occasion to study the effect of bacteria on the curing of meat. As a part of the work it was necessary to determine the number of bacteria present in the pickle at different stages of the cure. When plates were made of the pickle in the usual manner, surprisingly low counts were obtained, as compared with the number estimated by direct microscopic observations of wet preparations. While the latter indicated from 500,000 to 1,000,000 organisms per cubic centimeter, the plate count obtained from the same material was 10,000 or less. The low values obtained with plating were at first attributed to an unfavorable medium; consequently a solid medium was made by adding agar to clarified pickle. The counts obtained with the use of this medium were approximately the same as those obtained with use of standard beef extract agar. These results led us to believe that a considerable portion of the usual flora of curing pickle could not develop on solid media. Attempts were then made to determine the number of bacteria present by means of the direct count. For this purpose a modification of the Breed and Brew (1925) method was used. This was not satisfactory because of the high salt content of the pickle. The salt not only brought about a precipitation of the dye, but also, in crystallizing, obscured many of the organisms. Furthermore, the salt prevented proper fixation of bacteria on the slide, so that

attempts to remove the salt by washing resulted in the removal of most of the bacteria. Because of the great variety of organisms present, many of which were actively motile, and because of the presence of débris, methods making use of a counting chamber could not be used.

These difficulties forced us to consider the dilution method as a means of evaluating the bacterial population.

We were at once confronted with the following problems: (1) calculation of the number of bacteria present from the number of tubes showing growth in the various dilutions, (2) evaluation of the accuracy of the data thus obtained, and (3), determination of the variation in accuracy with the number of tubes used in each dilution. A careful review of the literature was made in an attempt to find answers to the questions involved. We found that many investigators had advanced equations that would enable us to calculate the most probable number of organisms from the number of tubes that showed growth, and some had published tables to aid in the calculation. We were, however, unable to find any satisfactory solution of the problem of variation in accuracy with the number of tubes used in each dilution, or, what would have been more desirable, a method for the determination of the number of tubes that must be used to get a specified accuracy. We therefore deemed it necessary to reconsider the entire problem. This is the first of a series of articles dealing with the various problems involved in the dilution method of determining a bacterial population.

HISTORY

The use of dilution methods in bacteriology dates back to the early days of the science. About 1875 Pasteur obtained pure cultures of bacteria by diluting the original inoculum during several successive transfers to a suitable culture medium. Later Miquel, Brefeld, and Lister (Kolle, Kraus and Uhlenhuth (1930)) obtained pure cultures by inoculating small amounts of diluted bacterial suspension into a series of tubes of medium.

For many years bacteriologists have been using dilution methods to give some idea of the number of organisms in the material

examined. This method consists in diluting the material to be examined, usually in powers of 10, and inoculating equal volumes of the diluted material into liquid media. If growth occurs from the inoculation of 1 cc. of a 1:100 dilution and not from a 1:1000 dilution, the number of organisms present in the original material is said to be between 100 and 1000 per cubic centimeter. On the basis of chance, however, it might be possible to have only 50 organisms per cubic centimeter, or more than 1000 per cubic centimeter in the original material and still get growth from the 1:100 dilution and not above that in a single test. A presumptive coli test, utilizing varying amounts of inoculum, has been used in a similar way in the bacteriological analysis of water to give an approximate idea of the quality of the water.

This was essentially the basis of the method introduced by Phelps (1908) to estimate the *B. coli* content of water from presumptive test data. In his method it is assumed that the reciprocal of the highest dilution which shows growth represents the most probable number of organisms present. His method was adopted by a Committee on Standard Methods of Water Analysis of the American Public Health Association (1920). In case "skips" occurred, that is, a positive presumptive test from a dilution higher than one which was negative, the result taken was the reciprocal of the dilution next higher than the smallest one giving a positive test.

A more accurate method of interpreting dilution data has been supplied by McCrady (1915). In developing his equations he begins with the proposition that there is only one organism for each 100 cc. in the sample. He stated that this one organism must obviously be contained in one of the 1 cc. volumes and that the probability of not getting the organism when a single cubic centimeter is removed from the 100 cc. volume is 0.99. The following quotation from his article (p. 185) shows how he has developed his equation.

> Now suppose 2 *B. coli* are in the sample. The probability of each organism's not being contained in the 1 cc. withdrawn for the fermentation test has been shown to be (0.99). Then, by the principle just il-

lustrated, the probability of neither organism's appearing in this 1 cc. is equal to the product of the separate probabilities, or (0.99) (0.99) = 0.9801. And if a great number of such samples were examined, about 98.01 per cent of the results would be "0/1 in 1 cc."

In general if V represents the number of volumes in the sample, and x the number of *B. coli* in the sample, and one volume is withdrawn, the probability that this volume will contain no *B. coli* is given by

$$\left[\frac{V-1}{V}\right]^x$$

Thus when 1 cc. of the sample is withdrawn for the test, V becomes 100 and the formula becomes $\left[\frac{99}{100}\right]^x$. When a 10 cc. quantity is withdrawn, V becomes 10 (there are ten 10 cc. volumes in the sample), and the formula becomes 0.9^x.

With this reasoning as a foundation for his later work, McCrady developed the equations from which it is possible to calculate the most probable number of organisms per cubic centimeter from data obtained by inoculating a series of tubes with the same dilution or from several series of tubes inoculated with several different dilutions. For the special case where a series of tubes are inoculated with 10 cc., a second with 1 cc., and a third with 0.1 cc., the following formula is given:

$$(p+q)(\log 0.9) + (r+s)(\log 0.99) + (t+u)(\log 0.999) = \frac{p(\log 0.9)}{1-0.9^x} + \frac{r(\log 0.99)}{1-0.99^x} + \frac{t(\log 0.999)}{1-0.999^x}$$

In this equation, p, r, and t represent the number of tubes showing growth in the different series and q, s, and u represent the number of tubes showing no growth in the corresponding series of dilutions. To simplify the use of the method, McCrady (1918) has solved the equation for all possible combinations for several special cases, including those when 5 and 10 tubes are used in each dilution. These solutions have been put into tables so that the method may be used without tedious calculation.

While McCrady's tables and equations may be regarded as solving the problem of calculating, from dilution data, the most probable number of bacteria present, they do not offer a solution

to the more general problem of variation in accuracy with the number of tubes used. In attempting to solve this problem with McCrady's equations, we find that the mathematics become very cumbersome. McCrady's equations are each limited to a special case, so that to solve them one must resort to rather tedious calculation. A more general solution whereby all cases could be evaluated from a single equation and a single table is very desirable. We have accordingly approached the problem from a different angle and have worked out tables somewhat analogous to those of McCrady.

On the basis of minor assumptions, Wolman and Weaver (1917) have simplified McCrady's formula. Their equations are convenient for the calculation of the most probable number of bacteria, but they do not enable one to evaluate the accuracy of the data.

Various methods of interpreting dilution data have been considered in a series of articles by Wells (1918; 1919; 1921), and in an article by Wells and Wells (1922). Their treatment does not lead to results that can be used in the general solution of the problem.

The methods advocated by Wells have been objected to by several investigators. Notable among these is Cairns (1918).

More general considerations of the dilution method have been contributed by Stein (1922), Greenwood and Yule (1917), Fisher (1925), and Reed (1925). All of these men have shown that the number of tubes showing no growth when inoculated with a fixed quantity of a single dilution is equal to e^{-ax}, where x represents the number of organisms per cubic centimeter in that dilution, and a represents the volume of the dilution used for the inoculation.

Stein not only made use of this exponential relationship to interpret dilution data, but he attempted to simplify its application by graphical means. He also showed by graphical means how the accuracy of the method varied with the number of tubes used. These considerations were, however, limited to a few special cases.

Greenwood and Yule considered not only the special case of a

single dilution inoculated into a series of tubes but also the general case of several dilutions inoculated into a series of tubes. Although they give the equation from which the most probable number of organisms can be calculated from experimental data involving several dilutions and several tubes in each, they have made no attempt to simplify the solution of this general case. Since the general equation is rather involved, it is necessary to simplify the solution by means of tables in order to make it applicable for practical use. Greenwood and Yule show how the accuracy of dilution data can be evaluated. Here again, simplification is needed for general application.

Their equation for the general case is as follows:

$$(a_1n_1 + a_2n_2 + \ldots\ldots a_nn_n) = \frac{a_1m_1}{1 - e^{-a_1x}} e^{-a_1x} + \frac{a_2m_2}{1 - e^{-a_2x}} e^{-a_2x} \ldots\ldots \frac{a_nm_n}{1 - e^{-a_nx}} e^{-a_nx}$$

In this equation x is the most probable number of bacteria per cubic centimeter that will give n_1 negative and m_1 positive results when N_1 tubes are inoculated with a_1 cc. each, and will give n_2 negative and m_2 positive results when N_2 tubes are inoculated with a_2 cc. each, etc.

Reed's contribution is very helpful in the special cases where one tube in each of several dilutions or where several tubes of the same dilution are used, but his solution is not extended to the more general problem of several tubes in each of several dliutions.

The dilution method has been used by Cunningham (1915), by Cutler (1919a), and by Cutler, Crump, and Sandon (1922) to evaluate the number of protozoa in soil. In the first two of these publications the dilution data were interpreted by the method of Phelps (1908), while Cutler, Crump, and Sandon used a table calculated by Fisher (1925). This table by Fisher is useful only for a very special case, so that it is not of any material value in general application. Fisher's table appears to be calculated from the exponential function e^{-x}.

A theoretical consideration of the dilution method has also been contributed by Clark (1927), who applied the method to determine the numbers of bacteriophage in a suspension.

After a careful review of the literature on the subject we still

feel it is necessary to reconsider the entire problem. General as well as special equations should be developed that are based on the reasoning used by Stein (1922), Greenwood and Yule (1917), Fisher (1925), and Reed (1925). These equations should be solved for all the special cases that are in common use, and the solutions should be arranged in tabular form, as McCrady did for his equations. Furthermore, tables should be made available which will aid in the solution of any special case not in common use.

A more detailed consideration of the accuracy of the method is needed. The calculation of the accuracy should be simplified so that anyone using the dilution method will be able to determine, to a reasonable degree, the limits of accuracy of his data. This must be made simple enough so that it can be applied generally.

The mathematics involved in the dilution method should also be applied to other problems in bacteriology, such as the determination of the percentage of insects that may be infected with certain viruses, or the interpretation of data obtained when a series of animals are injected with a single dilution or several dilutions of a given pathogenic bacterium or virus. It is also desirable to investigate the effect produced on dilution data if we accept the theory that single cells cannot develop.

A consideration of these and other probability problems will be published in a series of articles on the subject. It has been deemed necessary to verify by experimental data some of the mathematical considerations, and data thus obtained will also be presented in this series.

This first paper is confined to the development of equations to be used for the evaluation of bacterial populations by the dilution method. Tables are included which aid in the solution of these equations, as well as special solutions which simplify their general application.

THEORY

In order to determine the number of bacteria in a sample of liquid material by the dilution method it is necessary to dilute

the material to such an extent that when a sample is removed, bacteria may or may not be present. The problem at hand, then, is to determine the probability of getting bacteria in a certain sample. This probability will depend upon the number of organisms present.

A number of earlier investigators have shown how this probability is related to the number of organisms present. It is believed desirable, however, to develop these relationships from fundamental reasoning so that it will be possible for those not familiar with Poisson's Series to understand the derivation of the equations without having to make a special study of the mathematics on which the Poisson's Series is based. An understanding of the derivation of the equations is necessary for their proper application.

There are several ways in which the bacterial population can be determined by the dilution method. One may determine the number present by inoculating a large number of tubes of media with an equal volume of the sample to be tested, and determine the number present by the percentage of tubes that show growth. Or one may inoculate a series of tubes of media with a given volume, another set with a smaller volume, and a third set with a still smaller volume, and then determine the number of organisms present by the number of tubes showing growth in the different series.

We are interested, therefore, in several cases: (1) the probability of getting growth in a single tube, when it is inoculated with a definite volume of the sample; (2) the probability of getting a certain number showing growth out of a series of tubes, all of which are inoculated with the same volume of a given sample; (3) the probability of getting a certain combination of tubes showing growth out of several series of tubes when the tubes in each series are inoculated with different sized samples.

Case I we can designate as a single tube of a single dilution; case II, as several tubes of a single dilution; and case III, as several tubes of each of several dilutions.

CASE I

To calculate the probability that an organism will or will not be contained in a certain sample, let us assume that a large volume (N cc.) of the material to be sampled be at hand, and that in this material there are x organisms per cubic centimeter. Let us assume further, that the volume of an organism is v cc. (unit volume), that all organisms have the same volume, and that the water present be divided up into particles of the same unit volume as a bacterium. If we imagine now that we remove one of these particles, and let P equal the probability that our selection will not be an organism, then

$$P = \frac{\frac{N}{v} - Nx}{\frac{N}{v}}$$

This will be true because $\frac{N}{v}$ represents the total number of particles in a volume N. Nx is the number which are bacteria, and $\frac{N}{v} - Nx$, the number which are water. The probability Q that the selection will be an organism is $\frac{Nx}{\frac{N}{v}}$. These expressions can be simplified to $P = 1 - vx$; $Q = vx$.

Let us assume that enough of these small particles are removed so that the aggregate is 1 cc. The total probability will be expressed by the binomial $[(1-vx) + vx]^{\frac{1}{v}}$. The first term of the binomial will be $(1 - vx)^{\frac{1}{v}}$, which represents the probability that no organisms will be contained in a 1 cc. sample.

$$P = (1 - vx)^{\frac{1}{v}}.$$

This can be simplified as follows:

$$\ln P = - x - \frac{vx^2}{2} - \frac{v^2x^3}{3} - \frac{v^3x^4}{4} \cdot \cdot \cdot \cdot \cdot$$

Since v is very small, the second and following terms will be very small in comparison with the first term as long as relatively small values of x are considered.

Therefore

$$\ln P = -x$$

or

$$P = e^{-x} \tag{1}$$

If instead of taking out 1 cc., we remove a cc., then

$$P = (1 - vx)^{\frac{a}{v}}$$

$$\ln P = a\left[-x - \frac{vx^2}{2} - \frac{v^2x^3}{3} \cdot \cdot \cdot \cdot \cdot\right]$$

$$= -ax$$

or

$$P = e^{-ax} \tag{2}$$

and

$$Q = 1 - e^{-ax} \tag{3}$$

CASE II

If several tubes are to be inoculated with the same dilution the number of tubes which show growth will depend upon the number of organisms present and upon the element of chance.

If n tubes are inoculated, the total probability will be expressed by the binomial $[e^{-ax} + (1 - e^{-ax})]^n$. By expanding this binomial, the following series is obtained:

$$(e^{-ax})^n + n(e^{-ax})^{n-1}(1 - e^{-ax}) + \frac{n(n-1)}{2!}(e^{-ax})^{n-2}(1 - e^{-ax})^2 \cdot \cdot \cdot \cdot \cdot$$

The first term of this series gives an expression for the probability that none of the tubes will show growth, the second term for the probability that only one of the tubes will show growth, etc.

If we let p equal the number of tubes that show growth and q the number that show no growth, in which case $p + q = n$, the series becomes:

$$(e^{-ax})^q + n(e^{-ax})^{q'}(1 - e^{-ax})^{p'} + \frac{n(n-1)}{2!}(e^{-ax})^{q''}(1 - e^{-ax})^{p''} \ldots\ldots$$

If we let P be the probability, then the following equation will hold for any combination of p and q:

$$P = \frac{(p+q)!}{p!\,q!}(e^{-ax})^q(1 - e^{-ax})^p \tag{4}$$

If p, q, and a are kept constant in this equation, P will vary only with x. From the equation and the nature of the problem, it is evident that there must be a maximum value of P corresponding to some particular value of x. This optimum value of x can be found by differentiating equation (4), which gives the following:

$$\frac{dP}{dx}\frac{1}{P} = -qa + \frac{pa\,e^{-ax}}{1 - e^{-ax}}$$

By putting the derivative equal to 0, it is possible to find the value of x that corresponds to the maximum value of P. If we let $\bar{x}$ be the optimum or most probable value of x, its value may be found by substituting $\bar{x}$ for x in the above equation, when the derivative is placed equal to 0. The resulting equation simplifies to

$$\bar{x} = \frac{1}{a}\ln\frac{n}{q} \tag{5}$$

In the case of several tubes in a single dilution, the most probable number of organisms per cubic centimeter is given by the natural logarithm of the ratio of the total number of tubes to those that show no growth. Changing from base e to base 10, the expression becomes

$$\bar{x} = \frac{2.3026}{a}\log\frac{n}{q} \tag{6}$$

In deriving this equation, it has been assumed that in bacterial suspensions, all values of x are equally likely to occur between

0 and a number determined by the maximum bacterial population that can occur in a sample. By means of this formula it is easy to calculate the most probable number of organisms present in a sample from the number of tubes that show no growth. This can also be obtained by means of equation (5) and table 1 (appendix). From equation (5), we have

$$\frac{q}{n} = e^{-ax}$$

The function e^{-ax} then gives the fraction of tubes which show no growth. By means of a table showing values of e^{-x} for different values of x, it is possible to determine the most probable number of organisms present corresponding to any value of $\frac{q}{n}$. Such tables have been calculated by other investigators (L. von Bortkewitsch (1898) and H. E. Soper (1915)). The table of Bortkewitsch is carried out to only four places and that of Soper to six places. Whereas these tables may be adequate for the calculation of the most probable number from dilution data, we found that they did not suffice for the calculation of the frequency distribution of experimental values that might be obtained on a single suspension. Such calculations are essential in order to show a convenient relationship between the accuracy of the data and the number of tubes used in each dilution. For this reason we found it necessary to calculate these tables to nine decimal places. They are included in the appendix. Values of the logarithm of $(1 - e^{-x})$ are included in this table to simplify the solution of other problems which will be discussed later.

Examples: Suppose that in an experiment 0.100 of the tubes inoculated with a cc. each showed no growth, then from the table we find that the value of x corresponding to $e^{-x} = 0.100$ is 2.30. This number is therefore the most probable number of organisms present in the size sample used for the inoculation.

By means of this table it is also possible to determine the percentage of tubes showing growth which are inoculated with different sized samples. Let us assume that a liquid contained 0.650 organism per cubic centimeter, or 65 organisms in a 100 cc.

sample. In this case there will be 0.0065 organism in 0.01 cc., 0.065 organism in 0.1 cc., and 0.65 organism in 1 cc. Referring to the table, we see that for

$x = 0.0065$	$e^{-x} = 0.9935$
$x = 0.065$	$e^{-x} = 0.9371$
$x = 0.650$	$e^{-x} = 0.5222$
$x = 6.500$	$e^{-x} = 0.0015$

This means that for every 10,000 tubes inoculated with each of these dilutions, on the average, 15 of those inoculated with 10 cc., 5222 of those inoculated with 1.0 cc., 9371 of those inoculated with 0.1 cc., and 9935 of those inoculated with 0.01 cc. would show no growth; or if 100 tubes were inoculated with each dilution, one would expect that all the tubes receiving 10 cc., 48 receiving 1 cc., 6 receiving 0.1 cc., and 1 receiving 0.01 cc. would show growth. If a person wished to determine the most probable number of organisms by the result of inoculating a series of tubes with a single dilution, the best size of inoculum to use would in that case be 1 cc.

When a person inoculates a series of tubes with a single dilution and finds the percentage which show growth, he will also be able, by means of this table, to determine what size inoculum would have produced growth in 50 per cent of the tubes.

CASE III

In this case several dilutions are used, and several tubes are inoculated with each dilution.

For this development the following terms will be used:

w_1, $(1 - w_1)$ = the probability of a success and failure respectively, in a sample of size a_1,
w_2, $(1 - w_2)$ = the probability of a success and failure respectively, in a sample of size a_2,
w_3, $(1 - w_3)$ = the probability of a success and failure respectively, in a sample of size a_3,
n_1 = the number of samples of size a_1 that are taken,
n_2 = the number of samples of size a_2 that are taken,
n_3 = the number of samples of size a_3 that are taken,
p_1, q_1 = the number of failures and successes obtained out of n_1 trials.
p_2, q_2 = the number of failures and successes obtained out of n_2 trials,
p_3, q_3 = the number of failures and successes obtained out of n_3 trials.

The total probability will therefore be the product of several binomials, and by expanding each of them and multiplying the expanded forms together, the different terms in the product will be the probability of getting any set of combinations of failures and successes, as

$$p_1q_1;\ p_2q_2;\ p_3q_3;\ \ldots\ldots$$

The general formula for the probability of any one of these terms will then be:

$$P = \frac{(p_1+q_1)!}{p_1!\,q_1!}(w_1)^{q_1}(1-w_1)^{p_1}\frac{(p_2+q_2)!}{p_2!\,q_2!}(w_2)^{q_2}(1-w_2)^{p_2}\frac{(p_3+q_3)!}{p_3!\,q_3!}(w_3)^{q_3}(1-w_3)^{p_3}\ldots\ldots \quad (7)$$

This equation is obtained by multiplying together several equations, as (4), each being derived from a different binomial.

Now if the probabilities w_1, w_2, w_3, etc., are functions of x, it is possible to find the most probable values of x by differentiating the above equation with respect to x and putting the derivative equal to zero. This equation can be differentiated most readily by taking the logarithms of both sides, thus:

$$\ln P = \ln\frac{(p_1+q_1)!}{p_1!\,q_1!} + \ln\frac{(p_2+q_2)!}{p_2!\,q_2!} + \ln\frac{(p_3+q_3)!}{p_3!\,q_3!}\ldots\ldots + q_1\ln w_1 +$$

$$q_2\ln w_2 + q_3\ln w_3\ldots\ldots + p_1\ln(1-w_1) + p_2\ln(1-w_2) + p_3\ln(1-w_3)\ldots\ldots$$

Differentiating and substituting $n_1 - p_1$ for q_1, etc., we get

$$\frac{dP}{dx}\frac{1}{P} = d\frac{\ln w_1}{dx}\left[n_1 - \frac{p_1}{1-w_1}\right] + d\frac{\ln w_2}{dx}\left[n_2 - \frac{p_2}{1-w_2}\right] + d\frac{\ln w_3}{dx}\left[n_3 - \frac{p_3}{1-w_3}\right]\ldots\ldots$$

Now if x represents the percentage of a certain kind that are present in a unit, and a_1, a_2, a_3, etc., represent the number of these units that are selected, then

$$w_1 = (1-x)^{a_1};\ w_2 = (1-x)^{a_2};\ w_3 = (1-x)^{a_3};\ \text{etc.}$$

and

$$\frac{d\ln w_1}{dx} = \frac{-a_1}{1-x},\ \frac{d\ln w_2}{dx} = \frac{-a_2}{1-x},\ \frac{d\ln w_3}{dx} = \frac{-a_3}{1-x},\ \text{etc.}$$

Substituting these values in the above differential equation, putting it equal to zero, and factoring out all common multipliers, we get:

$$a_1\left(n_1 - \frac{p_1}{1 - w_1}\right) + a_2\left(n_2 - \frac{p_2}{1 - w_2}\right) + a_3\left(n_3 - \frac{p_3}{1 - w_3}\right) = 0$$

$$\frac{p_1 a_1}{1 - w_1} + \frac{p_2 a_2}{1 - w_2} + \frac{p_3 a_3}{1 - w_3} \ldots\ldots = a_1 n_1 + a_2 n_2 + a_3 n_3 \ldots\ldots \quad (8)$$

This is the same type of equation as that developed by Greenwood and Yule (1917), but is a little more general. With this formula a_1, a_2, a_3, etc., n_1, n_2, n_3, etc., are constants for any given experiment. p_1, p_2, p_3, etc., can be determined by experimental data. Since w_1, w_2, w_3, etc., are functions of x, it is then possible to solve for x the most probable value of that variable. The solution of the equation can be simplified if tables are worked out that give the relationship between x and w_1, w_2, w_3, etc.

To apply this to the problem of determining the most probable number of bacteria per cubic centimeter, let us assume that a set of tubes are inoculated with various amounts of the sample, one set inoculated with 10 cc. each, another set with 1 cc. each, and a third set with 0.1 cc. in each tube. In this case,

$w_1 = e^{-10x}$	$w_2 = e^{-x}$	$w_3 = e^{-\frac{x}{10}}$
$a_1 = 10$	$a_2 = 1$	$a_3 = 0.1$

Let us assume that ten tubes are used in each set, Then

$$n_1 = n_2 = n_3 = 10$$

The general formula then becomes

$$\frac{10\, p_1}{1 - e^{-10x}} + \frac{p_2}{1 - e^{-x}} + \frac{p_3}{10\left(1 - e^{-\frac{x}{10}}\right)} = 111$$

in which p_1 = the number of tubes receiving 10 cc. that show growth,

p_2 = the number of tubes receiving 1 cc. that show growth,

p_3 = the number of tubes receiving 0.1 cc. that show growth.

In the appendix, table 2, will be found values of $\frac{1}{1 - e^{-x}}$ for

the different values of x. With the aid of these tables it is an easy matter to solve the equation by trial and error by selecting the value of $\frac{1}{1-e^{-10x}}, \frac{1}{1-e^{-x}}, \frac{1}{1-e^{-\frac{x}{10}}}$, that will make the left hand side of the equation equal to 111. The values of x corresponding to these values of $\frac{1}{1-e^{-x}}$, etc., will be the most probable number of organisms per cubic centimeter.

To demonstrate the method of calculation, a specific example is included.

Suppose in an actual experiment 160 tubes were inoculated with each of a series of dilutions. Suppose that the following results were obtained:

DILUTION	NUMBER SHOWING GROWTH	NUMBER SHOWING NO GROWTH
10^4	160	0
10^5	160	0
10^6	158	2
10^7	61	99
10^8	8	152
19^9	0	160

The critical dilutions are therefore 10^6, 10^7, 10^8. The most probable number of organisms present in the 10^7 dilution can be obtained from the general equation:

$$\frac{a_1p_1}{1-e^{-a_1x}}+\frac{a_2p_2}{1-e^{-a_2x}}+\frac{a_3p_3}{1-e^{-a_3x}}=a_1n_1+a_2n_2+a_3n_3$$

In this experiment, a_1 is 10, a_2 is 1, and a_3 is 0.1; $n_1 = n_2 = n_3 = 160$; $p_1 = 158$, $p_2 = 61$, $p_3 = 8$. Substituting these values in the above we get

$$\frac{10\cdot158}{1-e^{-10x}}+\frac{1\cdot61}{1-e^{-x}}+\frac{8}{10\left(1-e^{-\frac{x}{10}}\right)}=160\ (10+1+0.1)$$

$$\frac{1580}{1-e^{-10x}}+\frac{61}{1-e^{-x}}+\frac{8}{10\left(1-e^{-\frac{x}{10}}\right)}=1776.0$$

An approximate idea of the value of x can be obtained by considering the middle dilution in which the proportion of tubes

showing no growth is $\frac{99}{160} = 0.6188$. From table 1 (appendix) the value of $e^{-x} = 0.6188$ is found to correspond to a value of $x = 0.4800$. By obtaining the reciprocals of $1 - e^{-10x}$, $1 - e^{-x}$, and $1 - e^{-\frac{x}{10}}$ from table 2 (appendix) and substituting in the above equation, we can obtain a more accurate value of x. Substituting the proper reciprocals in the left hand side of the equation and solving, we get

1776.898 for $x = 0.4650$
1774.598 for $x = 0.4700$
1770.194 for $x = 0.4800$

By interpolation, we find that the function becomes

1776.00 for $x = 0.4669$

In the case of the ten tubes inoculated with each of the dilutions this equation has been solved for all the combinations that are likely to occur. Table 3 (appendix) shows the most probable number of organisms which correspond to each of the combinations.

When 10 tubes are used in each of these dilutions, and when $a_1 = 10$ cc., $a_2 = 1$ cc., and $a_3 = 0.1$ cc., the general equation (7) becomes

$$P = \frac{10!}{p_1!\,q_1!}\frac{10}{p_2!\,q_2!}\frac{10}{p_3!\,q_3!}\left(e^{-10x}\right)^{q_1}\left(e^{-x}\right)^{q_2}\left(e^{-\frac{x}{10}}\right)^{q_3}\left(1-e^{-10x}\right)^{p_1}\cdot\left(1-e^{-x}\right)^{p_2}\cdot\left(1-e^{-\frac{x}{10}}\right)^{p_3}$$

Taking the log to the base 10 of both sides of the equation, we get

$$\log P = \log\frac{10!}{p_1!\,q_1!} + \log\frac{10!}{p_2!\,q_2!} + \log\frac{10!}{p_3!\,q_3!} - x\left(10q_1 + q_2 + \frac{q_3}{10}\right)\log e +$$

$$10p_1\log\left(1-e^{-10x}\right) + p_2\log\left(1-e^{-x}\right) + p_3\log\left(1-e^{-\frac{x}{10}}\right)$$

By means of this equation it is possible to solve for the value of P for any given value of x and p_1, p_2, and p_3. This has been done for all the combinations where P has a value greater than 0.01 per cent. These values show how often a certain combination can be expected if the number of organisms in the solution are as indicated by the most probable values of x. These frequencies

are helpful in interpreting data. If certain combinations are found to occur more often than indicated by these values of P, one may become suspicious that something is wrong either with the technic or with the medium.

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TABLE OF EXPONENTIAL FUNCTIONS

x	e^{-x}	$\log(1-e^{-x})$
.0001	.999,900,005	5.999,978,285
.0002	.999,800,020	6.300,986,569
.0003	.999,700,045	6.477,056,112
.0004	.999,600,080	6.601,973,135
.0005	.999,500,125	6.698,861,435
.0006	.999,400,180	6.777,021,196
.0007	.999,300,245	6.844,946,046
.0008	.999,200,320	6.902,916,280
.0009	.999,100,405	6.954,047,092
.0010	.999,000,500	6.999,782,871
.0011	.998,900,605	7.040,119,990
.0012	.998,800,720	7.078,920,695
.0013	.998,700,845	7.113,661,092
.0014	.998,600,980	7.145,824,065
.0015	.998,501,124	7.175,765,579
.0016	.998,401,279	7.203,772,593
.0017	.998,301,444	7.230,079,823
.0018	.998,201,619	7.254,881,699
.0019	.998,101,804	7.278,341,087
.0020	.998,001,999	7.300,595,773
.0021	.997,902,203	7.321,763,365
.0022	.997,802,418	7.341,945,044
.0023	.997,702,643	7.361,228,493
.0024	.997,602,878	7.379,508,982
.0025	.997,503,122	7.397,397,254
.0026	.997,403,377	7.414,408,887
.0027	.997,303,642	7.430,777,599
.0028	.997,203,916	7.446,550,161
.0029	.997,104,201	7.461,768,423
.0030	.997,004,496	7.476,455,478
.0031	.996,904,800	7.490,688,711
.0032	.996,805,115	7.504,455,292
.0033	.996,705,439	7.517,797,550
.0034	.996,605,773	7.530,740,826
.0035	.996,506,118	7.543,308,249
.0036	.996,406,472	7.555,521,005
.0037	.996,306,836	7.567,398,527
.0038	.996,207,211	7.578,958,698
.0039	.996,107,595	7.590,218,008
.0040	.996,007,989	7.601,191,692
.0041	.995,908,394	7.611,893,857
.0042	.995,808,808	7.622,337,591
.0043	.995,709,232	7.632,535,057
.0044	.995,609,666	7.642,497,579
.0045	.995,510,110	7.652,235,718
.0046	.995,410,564	7.661,759,337
.0047	.995,311,028	7.671,077,666
.0048	.995,211,502	7.680,199,348
.0049	.995,111,985	7.689,132,493
.0050	.995,012,479	7.697,884,720
.0051	.994,912,983	7.706,463,196
.0052	.994,813,497	7.714,874,667
.0053	.994,714,020	7.723,125,497
.0054	.994,614,554	7.731,221,692
.0055	.994,515,097	7.739,168,927
.0056	.994,415,651	7.746,972,570
.0057	.994,316,214	7.754,637,704
.0058	.994,216,788	7.762,169,148
.0059	.994,117,371	7.769,571,480
.0060	.994,017,964	7.776,849,018
.0061	.993,918,567	7.784,005,910
.0062	.993,819,180	7.791,046,072
.0063	.993,719,803	7.797,973,240
.0064	.993,620,436	7.804,790,973
.0065	.993,521,079	7.811,502,664
.0066	.993,421,732	7.818,111,552
.0067	.993,322,394	7.824,620,729
.0068	.993,223,066	7.831,033,148
.0069	.993,123,750	7.837,351,636
.0070	.993,024,444	7.843,578,811
.0071	.992,925,146	7.849,717,432
.0072	.992,825,859	7.855,769,892
.0073	.992,726,582	7.861,738,568
.0074	.992,627,314	7.867,625,741
.0075	.992,528,056	7.873,433,598
.0076	.992,428,808	7.879,164,240
.0077	.992,329,570	7.884,819,687
.0078	.992,230,342	7.890,410,879
.0079	.992,131,124	7.895,912,682
.0080	.992,031,915	7.901,353,967
.0081	.991,932,717	7.906,727,313
.0082	.991,833,528	7.912,034,462
.0083	.991,734,350	7.917,277,017
.0084	.991,635,181	7.922,456,526
.0085	.991,536,023	7.927,574,481
.0086	.991,436,874	7.932,632,323
.0087	.991,337,735	7.937,631,441
.0088	.991,238,607	7.942,573,178
.0089	.991,139,488	7.947,458,829
.0090	.991,040,379	7.952,289,650
.0091	.990,941,280	7.957,066,851
.0092	.990,842,190	7.961,791,604
.0093	.990,743,111	7.966,465,044
.0094	.990,644,042	7.971,088,268
.0095	.990,544,982	7.975,662,340
.0096	.990,445,933	7.980,188,287
.0097	.990,346,893	7.984,667,109
.0098	.990,247,864	7.989,099,771
.0099	.990,148,844	7.993,487,210
.0100	.990,049,834	7.997,830,337
.0105	.989,554,933	8.019,911,248
.0110	.989,060,279	8.039,006,327
.0115	.988,565,872	8.058,203,040
.0120	.988,071,713	8.076,578,085
.0125	.987,577,800	8.094,198,500
.0130	.987,084,135	8.111,123,496
.0135	.986,590,716	8.127,405,578
.0140	.986,097,544	8.143,091,521
.0145	.985,604,619	8.158,223,172
.0150	.985,111,940	8.172,838,122
.0155	.984,619,507	8.186,970,263
.0160	.984,127,320	8.200,376,562
.0165	.983,635,379	8.213,905,941
.0170	.983,143,686	8.226,762,616
.0175	.982,652,237	8.239,243,457
.0180	.982,161,032	8.251,369,718
.0185	.981,670,075	8.263,160,698
.0190	.981,170,362	8.274,634,336
.0195	.980,688,895	8.285,807,122
.0200	.980,198,673	8.296,694,289
.0205	.979,708,696	8.311,798,990
.0210	.979,218,965	8.317,667,183
.0215	.978,729,477	8.327,778,159
.0220	.978,240,235	8.337,654,199
.0225	.977,751,237	8.347,305,866
.0230	.977,262,484	8.356,743,022
.0235	.976,773,975	8.365,974,895
.0240	.976,285,710	8.375,010,131
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.0255	.974,822,379	8.401,014,692
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.0265	.973,848,044	8.417,504,179
.0270	.973,361,243	8.425,513,958
.0275	.972,874,684	8.433,374,806
.0280	.972,388,367	8.441,092,095
.0285	.971,902,294	8.448,670,862
.0290	.971,416,464	8.455,963,978
.0295	.970,930,878	8.463,431,920
.0300	.970,445,534	8.470,623,123
.0305	.969,960,432	8.477,693,682
.0310	.969,475,573	8.484,647,519
.0315	.968,990,956	8.491,488,371
.0320	.968,506,582	8.498,219,796
.0325	.968,022,450	8.504,845,189
.0330	.967,538,560	8.511,367,787
.0335	.967,054,911	8.517,790,682
.0340	.966,571,505	8.524,116,829
.0345	.966,088,340	8.530,349,053
.0350	.965,605,416	8.536,490,058
.0355	.965,122,734	8.542,542,431
.0360	.964,640,293	8.548,508,652
.0365	.964,158,094	8.554,391,188
.0370	.963,676,137	8.560,192,033
.0375	.963,194,419	8.565,113,735
.0380	.962,712,941	8.571,558,131
.0385	.962,231,705	8.577,127,382
.0390	.961,750,709	8.582,623,387
.0395	.961,269,954	8.588,048,013
.0400	.960,789,439	8.593,403,065
.0405	.960,309,165	8.598,690,241
.0410	.959,829,130	8.603,911,283
.0415	.959,349,335	8.609,067,651
.0420	.958,869,781	8.614,161,026
.0425	.958,390,466	8.619,192,857
.0430	.957,911,390	8.624,164,576
.0435	.957,432,554	8.629,077,592
.0440	.956,953,957	8.633,933,230
.0445	.956,475,600	8.638,732,792
.0450	.955,997,482	8.643,477,531
.0455	.955,519,603	8.648,168,659
.0460	.955,041,962	8.652,807,348
.0465	.954,564,561	8.657,394,733
.0470	.954,087,399	8.661,931,898
.0475	.953,610,474	8.666,335,665
.0480	.953,133,787	8.670,859,861
.0485	.952,657,339	8.675,252,662
.0490	.952,181,130	8.679,599,312
.0495	.951,705,158	8.683,900,748
.0500	.951,229,425	8.688,157,880
.0505	.950,753,929	8.692,371,590
.0510	.950,278,671	8.696,542,732
.0515	.949,803,650	8.700,672,139
.0520	.949,328,867	8.704,760,616
.0525	.948,854,321	8.708,808,948
.0530	.948,380,012	8.712,817,895
.0535	.947,905,941	8.716,788,197
.0540	.947,432,107	8.720,720,574
.0545	.946,958,509	8.724,615,725
.0550	.946,485,148	8.728,474,329
.0555	.946,012,024	8.732,297,049
.0560	.945,539,136	8.736,084,528
.0565	.945,066,484	8.739,837,393
.0570	.944,594,071	8.743,556,244
.0575	.944,121,891	8.747,241,695
.0580	.943,649,947	8.750,894,325
.0585	.943,178,240	8.754,514,678
.0590	.942,706,769	8.758,103,313
.0595	.942,235,534	8.761,660,766
.0600	.941,764,534	8.765,187,558
.0605	.941,293,769	8.768,684,199
.0610	.940,823,239	8.772,151,185
.0615	.940,352,946	8.775,589,000
.0620	.939,882,887	8.778,998,118
.0625	.939,413,063	8.782,378,998
.0630	.938,943,474	8.785,732,092
.0635	.938,474,119	8.789,057,839
.0640	.938,005,000	8.792,356,667
.0645	.937,536,114	8.795,628,997
.0650	.937,067,463	8.798,875,237
.0655	.936,599,047	8.802,095,788
.0660	.936,130,864	8.805,291,039
.0665	.935,662,916	8.808,461,374
.0670	.935,195,203	8.811,607,157
.0675	.934,727,722	8.814,728,770
.0680	.934,260,474	8.817,826,571
.0685	.933,793,460	8.820,900,891
.0690	.933,326,680	8.823,952,081
.0695	.932,860,133	8.826,980,474
.0700	.932,393,820	8.829,986,398
.0705	.931,927,740	8.832,970,172
.0710	.931,461,992	8.835,931,478
.0715	.930,996,278	8.838,872,519
.0720	.930,530,896	8.841,791,699
.0725	.930,065,747	8.844,689,942
.0730	.929,600,830	8.847,567,539
.0735	.929,136,146	8.850,424,769
.0740	.928,671,694	8.853,261,911
.0745	.928,207,474	8.856,079,234
.0750	.927,743,486	8.858,877,003
.0755	.927,279,731	8.861,655,479
.0760	.926,816,207	8.864,414,917
.0765	.926,352,914	8.867,155,566
.0770	.925,889,855	8.869,877,548
.0775	.925,427,026	8.872,581,465
.0780	.924,965,427	8.875,261,418
.0785	.924,502,060	8.877,935,102
.0790	.924,039,924	8.880,585,388
.0795	.923,578,020	8.883,218,286
.0800	.923,116,346	8.885,834,103
.0805	.922,654,904	8.888,432,785
.0810	.922,193,691	8.891,014,811
.0815	.921,732,710	8.893,580,297
.0820	.921,271,959	8.896,129,447
.0825	.920,811,438	8.898,607,610
.0830	.920,351,147	8.901,179,525
.0835	.919,891,087	8.903,680,841
.0840	.919,431,256	8.906,166,593
.0845	.918,971,655	8.908,636,966
.0850	.918,512,284	8.911,092,143
.0855	.918,053,143	8.913,532,301
.0860	.917,594,231	8.915,957,615
.0865	.917,135,549	8.918,369,831
.0870	.916,677,097	8.920,764,393
.0875	.916,218,873	8.923,146,198
.0880	.915,760,877	8.925,513,838
.0885	.915,303,111	8.927,867,460
.0890	.914,845,574	8.930,207,228
.0895	.914,388,265	8.932,533,298
.0900	.913,931,185	8.934,845,822
.0905	.913,474,334	8.937,144,951
.0910	.913,017,711	8.939,430,833
.0915	.912,561,316	8.941,703,612
.0920	.912,105,150	8.943,963,432
.0925	.911,649,211	8.946,210,432
.0930	.911,193,500	8.948,444,753
.0935	.910,738,017	8.950,666,529
.0940	.910,282,762	8.952,875,894
.0945	.909,827,735	8.955,072,980
.0950	.909,372,934	8.957,257,918
.0955	.908,918,362	8.959,430,083
.0960	.908,464,016	8.961,591,854
.0965	.908,009,898	8.963,741,102
.0970	.907,556,007	8.965,876,942
.0975	.907,102,343	8.968,004,762
.0980	.906,648,904	8.970,119,422
.0985	.906,195,693	8.972,222,781
.0990	.905,742,708	8.974,314,958
.0995	.905,289,950	8.976,396,066
.1000	.904,837,418	8.978,466,217
.1050	.900,324,585	8.998,588,324
.1100	.895,834,134	9.017,725,428
.1150	.891,366,143	9.035,965,194
.1200	.886,920,436	9.053,384,125
.1250	.882,496,901	9.070,049,320
.1300	.878,095,430	9.086,019,987
.1350	.873,715,911	9.101,348,676
.1400	.869,358,235	9.116,082,039
.1450	.865,022,292	9.130,262,049
.1500	.860,707,976	9.143,926,269
.1550	.856,415,176	9.157,108,540
.1600	.852,143,788	9.169,839,575
.1650	.847,893,703	9.182,147,193
.1700	.843,664,816	9.194,059,821
.1750	.839,457,020	9.205,591,320
.1800	.835,270,211	9.216,772,142
.1850	.831,104,284	9.227,618,634
.1900	.826,959,134	9.238,148,680
.1950	.822,834,658	9.248,378,767
.2000	.818,730,753	9.258,324,131
.2050	.814,647,315	9.267,998,881
.2100	.810,584,245	9.277,416,099
.2150	.806,541,440	9.286,587,951
.2200	.802,518,797	9.295,525,776
.2250	.798,516,217	9.304,240,096
.2300	.794,533,601	9.312,740,809
.2350	.790,570,848	9.321,037,134
.2400	.786,627,860	9.329,137,713
.2450	.782,704,537	9.337,050,658
.2500	.778,800,783	9.344,783,585
.2550	.774,916,496	9.352,343,702
.2600	.771,051,584	9.359,737,643
.2650	.767,205,949	9.367,971,878
.2700	.763,379,493	9.373,052,381
.2750	.759,572,121	9.380,984,823
.2800	.755,783,740	9.387,774,576
.2850	.752,014,253	9.394,426,720
.2900	.748,263,566	9.400,946,076
.2950	.744,531,586	9.407,337,217
.3000	.740,818,220	9.413,604,468
.3050	.737,123,373	9.419,751,974
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.3150	.729,788,873	9.431,703,229
.3200	.726,149,036	9.437,514,274
.3250	.722,527,352	9.443,141,912
.3300	.718,923,732	9.448,824,177
.3350	.715,338,086	9.454,329,365
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.3500	.704,688,089	9.470,280,962
.3550	.701,173,442	9.475,419,192
.3600	.697,676,326	9.480,472,157
.3650	.694,196,650	9.485,442,239
.3700	.690,734,330	9.490,331,714
.3750	.687,289,279	9.495,142,771
.3800	.683,861,409	9.499,877,513
.3850	.680,450,636	9.504,537,958
.3900	.677,056,874	9.509,126,045
.3950	.673,680,039	9.513,643,639
.4000	.670,320,046	9.518,092,541
.4050	.666,976,810	9.522,474,477
.4100	.663,650,250	9.526,791,109
.4150	.660,340,280	9.531,044,047
.4200	.657,046,820	9.535,234,834
.4250	.653,769,785	9.539,364,965
.4300	.650,509,095	9.543,435,878
.4350	.647,264,667	9.547,448,964
.4400	.644,036,421	9.551,405,565
.4450	.640,824,276	9.555,306,976
.4500	.637,628,151	9.559,154,452
.4550	.634,447,967	9.562,949,204
.4600	.631,283,645	9.566,692,402
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1.050	.349,937,749	9.812,954,495
1.100	.332,871,084	9.824,209,765
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1.200	.301,194,211	9.844,356,494
1.250	.286,504,797	9.853,391,058
1.300	.272,531,793	9.861,814,018
1.350	.259,240,260	9.869,677,371
1.400	.246,596,963	9.877,027,367
1.450	.234,570,288	9.883,905,316
1.500	.223,130,160	9.890,348,261
1.550	.212,247,974	9.896,389,529
1.600	.201,896,518	9.902,059,206
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1.750	.173,773,943	9.917,098,887
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2.200	.110,803,158	9.948,997,912
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2.500	.082,084,998	9.962,802,468
2.550	.078,081,666	9.964,692,452
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2.650	.070,651,213	9.968,178,736
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2.900	.055,023,220	9.975,421,137
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3.300	.036,883,167	9.983,678,973
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3.450	.031,745,636	9.985,989,463
3.500	.030,197,383	9.986,683,352
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3.850	.021,279,736	9.990,658,580
3.900	.020,241,911	9.991,118,858
3.950	.019,254,702	9.991,556,235
4.000	.018,315,639	9.991,971,872
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4.100	.016,572,675	9.992,742,271
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4.200	.014,995,577	9.993,438,181
4.250	.014,264,233	9.993,760,515
4.300	.013,568,558	9.994,066,907
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4.550	.010,567,204	9.995,386,301
4.600	.010,051,835	9.995,612,455
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4.700	.009,095,277	9.996,031,898
4.750	.008,651,695	9.996,226,268
4.800	.008,229,747	9.996,411,078
4.850	.007,828,377	9.996,586,802
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5.250	.005,247,519	9.997,715,031
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5.600	.003,697,864	9.998,391,061
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5.800	.003,027,555	9.998,683,155
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5.900	.002,739,445	9.998,808,642
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8.800	.000,150,733	9.999,934,533
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9.000	.000,123,410	9.999,946,400
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9.200	.000,101,040	9.999,956,117
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9.700	.000,061,283	9.999,973,384
9.750	.000,058,295	9.999,974,686
9.800	.000,055,452	9.999,975,917
9.850	.000,052,747	9.999,977,092
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9.950	.000,047,727	9.999,979,271
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TABLE OF RECIPROCALS

x	$\frac{1}{1-e^{-x}}$	x	$\frac{1}{1-e^{-x}}$	x	$\frac{1}{1-e^{-x}}$	x	$\frac{1}{1-e^{-x}}$		$\frac{1}{1-e^{-x}}$		$\frac{1}{1-e^{-x}}$
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.0002	5000.499 90	.0019	526.815 947	.0036	278.278 077	.0053	189.179 686	.0070	143.357 753	.0087	115.443 253
.0003	3333.833 35	.0020	500.500 166	.0037	270.770 578	.0054	185.685 635	.0071	141.345 689	.0088	114.137 096
.0004	2500.500 03	.0021	476.690 651	.0038	263.658 211	.0055	182.318 640	.0072	139.389 516	.0089	112.860 292
.0005	2000.500 04	.0022	455.045 637	.0039	256.910 581	.0056	179.071 895	.0073	137.486 935	.0090	111.611 861
.0006	1667.166 71	.0023	435.282 800	.0040	250.500 333	.0057	175.939 071	.0074	135.635 776	.0091	110.390 868
.0007	1429.071 45	.0024	417.340 967	.0041	244.402 780	.0058	172.914 276	.0075	133.833 982	.0092	109.196 418
.0008	1250.500 06	.0025	400.500 208	.0042	238.595 624	.0059	169.992 014	.0076	132.079 604	.0093	108.027 656
.0009	1111.611 18	.0026	385.115 601	.0043	233.058 497	.0060	167.167 166	.0077	130.370 794	.0094	106.883 762
.0010	1000.500 08	.0027	370.870 595	.0044	227.773 093	.0061	164.434 934	.0078	128.705 800	.0095	105.763 949
.0011	909.591 000	.0028	357.643 090	.0045	222.722 597	.0062	161.790 839	.0079	127.082 958	.0096	104.667 466
.0012	833.833 433	.0029	345.327 827	.0046	217.891 687	.0063	159.230 683	.0080	125.500 666	.0097	103.593 591
.0013	769.730 876	.0030	333.833 583	.0047	213.266 349	.0064	156.750 533	.0081	123.957 465	.0098	102.541 632
.0014	714.785 830	.0031	323.080 903	.0048	208.833 733	.0065	154.346 695	.0082	122.451 902	.0099	101.510 926
.0015	667.166 791	.0032	313.000 266	.0049	204.582 040	.0066	152.005 701	.0083	120.982 519	.0100	100.500 833
.0016	625.500 133	.0033	303.530 578	.0050	200.500 416	.0067	149.754 289	.0084	119.548 319		
.0017	588.735 435	.0034	294.617 930	.0051	196.578 856	.0068	147.559 390	.0085	118.147 767		
.0100	100.500 833	.1000	10.508 331 9	1.000	1.581 976 70	.0550	18.686 401 2	.5500	2.363 786 22	5.500	1.004 103 54
.0105	95.738 970 2	.1050	10.032 557 9	1.050	1.538 314 21	.0555	18.522 642 7	.5550	2.347 816 08	5.550	1.003 902 62
.0110	91.410 007 6	.1100	9.600 073 79	1.100	1.498 960 65	.0560	18.361 809 2	.5600	2.332 138 84	5.600	1.003 711 57
.0115	87.457 480 0	.1150	9.205 233 40	1.150	1.463 350 60	.0565	18.203 823 1	.5650	2.316 746 21	5.650	1.003 529 93
.0120	83.834 333 3	.1200	8.843 330 87	1.200	1.431 012 75	.0570	18.048 609 8	.5700	2.301 630 72	5.700	1.003 357 19
.0125	80.501 041 7	.1250	8.510 413 84	1.250	1.401 551 11	.0575	17.896 096 1	.5750	2.286 785 11	5.750	1.003 192 94
.0130	77.424 160 2	.1300	8.203 137 91	1.300	1.374 630 52	.0580	17.746 212 3	.5800	2.272 202 42	5.800	1.003 036 74
.0135	74.575 199 1	.1350	7.918 653 94	1.350	1.349 965 37	.0585	17.598 891 7	.5850	2.257 875 89	5.850	1.002 888 21
.0140	71.929 738 1	.1400	7.654 519 97	1.400	1.327 310 81	.0590	17.454 068 9	.5900	2.243 799 01	5.900	1.002 746 97
.0145	69.466 725 6	.1450	7.408 630 76	1.450	1.306 455 68	.0595	17.311 680 7	.5950	2.229 965 48	5.950	1.002 612 64
.0150	67.167 916 6	.1500	7.179 161 95	1.500	1.287 216 91	.0600	17.171 666 3	.6000	2.216 369 21	6.000	1.002 484 91
.0155	65.017 420 7	.1550	6.964 524 32	1.550	1.269 435 00	.0605	17.033 966 9	.6050	2.203 004 31	6.050	1.002 363 41
.0160	63.041 050 0	.1600	6.763 327 60	1.600	1.252 970 35	.0610	16.898 852 5	.6100	2.189 865 11	6.100	1.002 247 90
.0165	61.107 435 5	.1650	6.574 349 71	1.650	1.237 700 21	.0615	16.765 287 2	.6150	2.176 945 07	6.150	1.002 138 04
.0170	59.324 950 8	.1700	6.396 512 76	1.700	1.223 516 25	.0620	16.634 198 5	.6200	2.164 241 87	6.200	1.002 033 55
.0175	57.643 322 9	.1750	6.228 861 57	1.750	1.210 322 51	.0625	16.505 207 9	.6250	2.151 747 36	6.250	1.001 934 18
.0180	56.057 055 5	.1800	6.070 547 44	1.800	1.198 033 62	.0630	16.378 265 5	.6300	2.139 457 54	6.300	1.001 839 58
.0185	54.555 595 7	.1850	5.920 813 24	1.850	1.186 573 44	.0635	16.253 322 8	.6350	2.127 367 57	6.350	1.001 749 80
.0190	53.133 162 2	.1900	5.778 981 71	1.900	1.175 873 82	.0640	16.130 332 9	.6400	2.115 472 75	6.400	1.001 664 32
.0195	51.783 676 2	.1950	5.644 444 83	1.950	1.165 873 58	.0645	16.009 250 5	.6450	2.103 768 56	6.450	1.001 583 02
.0200	50.501 666 6	.2000	5.518 655 56	2.000	1.156 517 64	.0650	15.890 031 6	.6500	2.092 250 57	6.500	1.001 505 70
.0205	49.282 196 1	.2050	5.395 120 11	2.050	1.147 756 29	.0655	15.772 633 5	.6550	2.080 914 53	6.550	1.001 432 16
.0210	48.120 797 6	.2100	5.279 391 88	2.100	1.139 544 55	.0660	15.657 014 7	.6600	2.069 756 30	6.600	1.001 362 22
.0215	47.013 419 5	.2150	5.169 065 66	2.150	1.131 841 61	.0665	15.543 135 2	.6650	2.058 771 87	6.650	1.001 295 69
.0220	45.956 379 8	.2200	5.063 773 08	2.200	1.124 610 38	.0670	15.430 956 3	.6700	2.047 957 33	6.700	1.001 232 42
.0225	44.946 319 4	.2250	4.963 178 59	2.250	1.117 817 05	.0675	15.320 439 6	.6750	2.037 308 91	6.750	1.001 172 25
.0230	43.980 177 5	.2300	4.866 975 84	2.300	1.111 430 76	.0680	15.211 548 5	.6800	2.026 822 94	6.800	1.001 115 01
.0235	43.055 149 8	.2350	4.774 884 44	2.350	1.105 423 29	.0685	15.104 248 0	.6850	2.016 495 86	6.850	1.001 060 57
.0240	42.168 666 6	.2400	4.686 647 46	2.400	1.099 347 07	.0690	14.998 503 1	.6900	2.006 324 21	6.900	1.001 008 80
.0245	41.318 368 1	.2450	4.602 028 89	2.450	1.094 443 44	.0695	14.894 280 4	.6950	1.996 304 63	6.950	1.000 949 53
.0250	40.502 083 3	.2500	4.520 811 66	2.500	1.089 425 48	.0700	14.791 547 1	.7000	1.986 433 86	7.000	1.000 912 71
.0255	39.717 811 2	.2550	4.442 795 59	2.550	1.084 694 77	.0705	14.690 271 6	.7050	1.976 708 72	7.050	1.000 868 16
.0260	38.963 705 1	.2600	4.367 796 10	2.600	1.080 232 75	.0710	14.590 444 4	.7100	1.967 126 16	7.100	1.000 825 78
.0265	38.238 057 3	.2650	4.295 642 41	2.650	1.076 022 27	.0715	14.491 971 8	.7150	1.957 683 15	7.150	1.000 785 48
.0270	37.539 288 8	.2700	4.226 176 38	2.700	1.072 047 50	.0720	14.374 196 8	.7200	1.948 376 80	7.200	1.000 747 14
.0275	36.865 929 8	.2750	4.159 251 44	2.750	1.068 293 73	.0725	14.299 144 5	.7250	1.939 204 27	7.250	1.000 710 67
.0280	36.216 619 0	.2800	4.094 731 44	2.800	1.064 747 35	.0730	14.204 712 9	.7300	1.930 162 80	7.300	1.000 675 99
.0285	35.590 094 2	.2850	4.032 489 81	2.850	1.061 395 71	.0735	14.111 566 4	.7350	1.921 249 73	7.350	1.000 643 00
.0290	34.985 175 2	.2900	3.972 408 69	2.900	1.058 227 06	.0740	14.019 679 6	.7400	1.912 462 44	7.400	1.000 611 62
.0295	34.400 763 4	.2950	3.914 378 19	2.950	1.055 230 45	.0745	13.929 026 3	.7450	1.903 798 39	7.450	1.000 581 78
.0300	33.835 833 2	.3000	3.858 295 90	3.000	1.052 395 69	.0750	13.839 582 7	.7500	1.895 255 13	7.500	1.000 553 39
.0305	33.289 426 8	.3050	3.804 065 85	3.050	1.049 713 28	.0755	13.751 324 1	.7550	1.886 830 23	7.550	1.000 526 38
.0310	32.760 647 8	.3100	3.751 598 45	3.100	1.047 174 36	.0760	13.664 227 4	.7600	1.878 521 37	7.600	1.000 500 70
.0315	32.248 656 7	.3150	3.700 809 84	3.150	1.044 770 64	.0765	13.578 269 7	.7650	1.870 326 27	7.650	1.000 476 27
.0320	31.752 666 6	.3200	3.651 621 21	3.200	1.042 494 36	.0770	13.493 429 2	.7700	1.862 242 71	7.700	1.000 453 03
.0325	31.271 939 0	.3250	3.603 958 83	3.250	1.040 338 29	.0775	13.409 683 5	.7750	1.854 268 51	7.750	1.000 430 92
.0330	30.805 750 2	.3300	3.557 753 24	3.300	1.038 295 62	.0780	13.327 169 7	.7800	1.846 401 57	7.800	1.000 409 90
.0335	30.353 537 9	.3350	3.512 939 21	3.350	1.036 360 02	.0785	13.245 394 5	.7850	1.838 606 06	7.850	1.000 389 90
.0340	29.914 597 9	.3400	3.469 455 35	3.400	1.034 525 39	.0790	13.164 810 4	.7900	1.830 981 35	7.900	1.000 370 88
.0345	29.488 382 1	.3450	3.427 243 84	3.450	1.032 786 46	.0795	13.085 240 6	.7950	1.823 424 13	7.950	1.000 352 78
.0350	29.074 345 1	.3500	3.386 250 15	3.500	1.031 137 65	.0800	13.006 665 9	.8000	1.815 966 21	8.000	1.000 335 57
.0355	28.671 972 3	.3550	3.346 422 77	3.550	1.029 574 14	.0805	12.929 067 8	.8050	1.808 605 82	8.050	1.000 319 20
.0360	28.280 777 7	.3600	3.307 713 17	3.600	1.028 091 28	.0810	12.852 428 2	.8100	1.801 341 13	8.100	1.000 303 63
.0365	27.900 301 8	.3650	3.270 075 33	3.650	1.026 684 69	.0815	12.776 729 3	.8150	1.794 170 36	8.150	1.000 288 81
.0370	27.530 111 2	.3700	3.233 465 90	3.700	1.025 350 24	.0820	12.701 954 5	.8200	1.787 091 79	8.200	1.000 274 72
.0375	27.169 792 5	.3750	3.197 843 67	3.750	1.024 084 15	.0825	12.628 086 3	.8250	1.780 103 75	8.250	1.000 261 32
.0380	26.818 956 0	.3800	3.163 169 66	3.800	1.022 882 67	.0830	12.555 108 6	.8300	1.773 204 59	8.300	1.000 248 57
.0385	26.477 234 2	.3850	3.129 406 94	3.850	1.021 742 40	.0835	12.483 005 3	.8350	1.766 392 72	8.350	1.000 236 45
.0390	26.144 275 5	.3900	3.096 520 46	3.900	1.020 660 11	.0840	12.411 761 0	.8400	1.759 666 57	8.400	1.000 224 90
.0395	25.819 747 2	.3950	3.064 476 96	3.950	1.019 632 72	.0845	12.341 360 3	.8450	1.753 024 62	8.450	1.000 213 94
.0400	25.503 333 2	.4000	3.033 244 78	4.000	1.018 657 36	.0850	12.271 788 3	.8500	1.746 465 37	8.500	1.000 203 51
.0405	25.194 732 9	.4050	3.002 793 88	4.050	1.017 731 29	.0855	12.203 030 5	.8550	1.739 987 38	8.550	1.000 193 58
.0410	24.893 660 4	.4100	2.973 095 71	4.100	1.016 851 95	.0860	12.135 072 7	.8600	1.733 589 22	8.600	1.000 184 13
.0415	24.599 843 7	.4150	2.944 123 01	4.150	1.016 016 91	.0865	12.067 857 3	.8650	1.727 269 50	8.650	1.000 175 15
.0420	24.313 023 7	.4200	2.915 849 91	4.200	1.015 223 86	.0870	12.001 502 1	.8700	1.721 026 87	8.700	1.000 166 61
.0425	24.032 953 3	.4250	2.888 251 67	4.250	1.014 470 64	.0875	11.935 862 3	.8750	1.714 860 00	8.750	1.000 158 58
.0430	23.759 397 1	.4300	2.861 304 78	4.300	1.013 755 19	.0880	11.870 968 7	.8800	1.708 767 59	8.800	1.000 150 75
.0435	23.492 130 6	.4350	2.834 986 76	4.350	1.013 075 57	.0885	11.806 809 0	.8850	1.702 748 38	8.850	1.000 143 40
.0440	23.230 939 0	.4400	2.809 276 17	4.400	1.012 429 94	.0890	11.743 370 7	.8900	1.696 801 15	8.900	1.000 136 40
.0445	22.975 618 3	.4450	2.784 152 52	4.450	1.011 816 56	.0895	11.680 641 6	.8950	1.690 924 66	8.950	1.000 129 75
.0450	22.725 972 0	.4500	2.759 596 26	4.500	1.011 233 79	.0900	11.618 610 0	.9000	1.685 117 74	9.000	1.000 123 42
.0455	22.481 813 5	.4550	2.735 588 67	4.550	1.010 680 06	.0905	11.557 264 3	.9050	1.679 379 24	9.050	1.000 117 40
.0460	22.242 963 6	.4600	2.712 111 86	4.600	1.010 153 90	.0910	11.496 593 2	.9100	1.673 708 02	9.100	1.000 111 67
.0465	22.009 251 2	.4650	2.689 148 70	4.650	1.009 653 90	.0915	11.436 585 6	.9150	1.668 102 97	9.150	1.000 106 23
.0470	21.780 512 8	.4700	2.666 682 79	4.700	1.009 178 76	.0920	11.377 230 8	.9200	1.662 563 01	9.200	1.000 101 05
.0475	21.560 773 2	.4750	2.644 698 43	4.750	1.008 727 20	.0925	11.318 518 0	.9250	1.657 087 09	9.250	1.000 096 12
.0480	21.337 333 1	.4800	2.623 180 56	4.800	1.008 298 03	.0930	11.260 437 0	.9300	1.651 674 17	9.300	1.000 091 43
.0485	21.122 598 2	.4850	2.602 114 76	4.850	1.007 890 14	.0935	11.202 977 6	.9350	1.646 323 22	9.350	1.000 086 97
.0490	20.912 246 4	.4900	2.581 487 18	4.900	1.007 502 45	.0940	11.146 130 0	.9400	1.641 033 27	9.400	1.000 082 73
.0495	20.706 145 0	.4950	2.561 284 54	4.950	1.007 133 94	.0945	11.089 884 4	.9450	1.635 803 33	9.450	1.000 078 69
.0500	20.504 166 4	.5000	2.541 494 08	5.000	1.006 783 65	.0950	11.034 231 2	.9500	1.630 632 46	9.500	1.000 074 85
.0505	20.306 188 3	.5050	2.522 103 55	5.050	1.006 450 67	.0955	10.979 161 3	.9550	1.625 519 73	9.550	1.000 071 20
.0510	20.112 092 9	.5100	2.503 101 20	5.100	1.006 134 14	.0960	10.924 665 4	.9600	1.620 464 22	9.600	1.000 067 73
.0515	19.921 767 2	.5150	2.484 475 71	5.150	1.005 833 23	.0965	10.870 734 7	.9650	1.615 465 03	9.650	1.000 064 43
.0520	19.735 102 3	.5200	2.466 216 21	5.200	1.005 547 15	.0970	10.817 360 5	.9700	1.610 521 31	9.700	1.000 061 28
.0525	19.551 993 8	.5250	2.448 312 22	5.250	1.005 275 20	.0975	10.764 534 1	.9750	1.605 632 18	9.750	1.000 058 29
.0530	19.372 340 9	.5300	2.430 753 71	5.300	1.005 016 63	.0980	10.712 246 9	.9800	1.600 796 78	9.800	1.000 055 45
.0535	19.196 046 8	.5350	2.413 530 96	5.350	1.004 770 80	.0985	10.660 491 2	.9850	1.596 014 36	9.850	1.000 052 74
.0540	19.023 018 3	.5400	2.396 634 65	5.400	1.004 537 07	.0990	10.609 258 7	.9900	1.591 284 06	9.900	1.000 050 17
.0545	18.853 165 2	.5450	2.380 055 79	5.450	1.004 314 84	.0995	10.558 541 5	.9950	1.586 605 10	9.950	1.000 047 73

PROBABILITY TABLE A

Code			X	P	Code			X	P	Code			X	
10	10	10	100.0	100.0	10	10	0	2.40	3.4	10	9	0	1.70	5.30
10	10	9	23.0	38.7	10	9	9	6.07	0.01	10	8	7	3.10	0.01
10	10	8	16.2	20.2	10	9	8	5.26	0.04	10	8	6	2.76	0.14
10	10	7	12.0	26.2	10	9	7	4.58	0.27	10	8	5	2.49	0.56
10	10	6	9.18	25.0	10	9	6	3.96	0.85	10	8	4	2.21	1.64
10	10	5	7.02	24.2	10	9	5	3.46	2.25	10	8	3	1.96	4.50
10	10	4	5.42	23.4	10	9	4	2.96	4.80	10	8	2	1.71	8.46
10	10	3	4.28	21.7	10	9	3	2.63	8.57	10	8	1	1.50	10.83
10	10	2	3.49	17.5	10	9	2	2.28	11.77	10	8	0	1.30	7.21
10	10	1	2.75	10.2	10	9	1	1.97	11.02	10	7	6	2.19	0.01
10	7	5	1.95	0.20	10	6	3	1.25	0.59	10	4	5	1.073	0.01
10	7	4	1.74	0.80	10	6	2	1.09	2.73	10	4	4	0.943	0.07
10	7	3	1.53	2.57	10	6	1	0.933	8.42	10	4	3	0.818	0.48
10	7	2	1.33	6.17	10	6	0	0.792	10.73	10	4	2	0.700	2.26
10	7	1	1.16	10.01	10	5	5	1.30	0.02	10	4	1	0.589	7.97
10	7	0	1.01	9.33	10	5	4	1.15	0.14	10	4	0	0.493	14.99
10	6	6	1.75	0.01	10	5	3	1.02	0.91	10	3	4	0.773	0.02
10	6	5	1.57	0.08	10	5	2	0.872	3.30	10	3	3	0.662	0.24
10	6	4	1.41	0.11	10	5	1	0.742	8.94	10	3	2	0.561	1.60
10	6	3	1.25	0.59	10	5	0	0.622	12.58	10	3	1	0.474	6.93
10	3	0	.399	17.67	10	0	2	.314	0.15	9	5	1	.372	0.86
10	2	4	.631	0.01	10	0	1	.268	1.44	9	5	0	.341	1.78
10	2	3	.534	0.12	10	0	0	.231	7.95	9	4	3	.408	0.02
10	2	2	.456	0.99	9	8	0	.499	0.03	9	4	2	.365	0.22
10	2	1	.388	5.30	9	7	1	.488	0.02	9	4	1	.324	1.13
10	2	0	.329	18.14	9	7	0	.435	0.17	9	4	0	.290	3.56
10	1	3	.442	0.04	9	6	2	.474	0.06	9	3	3	.362	0.04
10	1	2	.376	0.50	9	6	1	.425	0.23	9	3	2	.324	0.26
10	1	1	.317	3.71	9	6	0	.381	0.56	9	3	1	.288	1.77
10	1	0	.275	15.14	9	5	2	.416	0.10	9	3	0	.255	6.23
9	2	3	.316	0.03	8	6	0	.270	0.11	8	2	2	.210	0.14
9	2	2	.264	0.26	8	5	1	.267	0.11	8	2	1	.188	1.24
9	2	1	.253	2.19	8	5	0	.242	0.46	8	2	0	.169	7.12
9	2	0	.223	9.53	8	4	2	.266	0.04	8	1	2	.167	0.11
9	1	2	.249	0.19	8	4	1	.240	0.33	8	1	1	.166	1.46
9	1	1	.221	1.84	8	4	0	.217	1.47	8	1	0	.147	9.31
9	1	0	.193	9.03	8	3	2	.239	0.08	8	0	2	.166	0.06
9	0	2	.217	0.07	8	3	1	.214	0.77	8	0	1	.146	0.85
9	0	1	.191	0.79	8	3	0	.193	3.73	8	0	0	.128	6.41
9	0	0	.164	5.02	8	2	3	.233	0.01	7	6	0	.212	0.02
7	5	1	.209	0.06	7	2	0	.133	5.49	6	4	0	.139	0.32
7	5	0	.191	0.16	7	1	2	.149	0.06	6	3	2	.153	0.01
7	4	2	.208	0.01	7	1	1	.132	1.10	6	3	1	.138	0.20
7	4	1	.188	0.12	7	1	0	.116	8.98	6	3	0	.123	1.35
7	4	0	.171	0.68	7	0	2	.132	0.04	6	2	2	.137	0.03
7	3	2	.188	0.03	7	0	1	.116	0.80	6	2	1	.122	0.15
7	3	1	.169	0.35	7	0	0	.101	7.92	6	2	0	.107	4.12
7	3	0	.152	2.44	6	5	0	.155	0.06	6	1	2	.121	0.06
7	2	2	.167	0.04	6	4	2	.171	0.00	6	1	1	.106	0.87
7	2	1	.150	0.78	6	4	1	.155	0.04	6	1	0	.092	8.73
6	0	2	.106	0.04	5	2	0	.086	3.36	4	3	0	.080	0.43
6	0	1	.092	0.63	5	1	2	.099	0.03	4	2	1	.080	0.16
6	0	0	.078	3.77	5	1	1	.086	0.58	4	2	0	.068	2.16
5	5	0	.128	0.04	5	1	0	.073	8.28	4	1	2	.080	0.01
5	4	1	.127	0.02	5	0	2	.085	0.01	4	1	1	.068	0.50
5	4	0	.114	0.14	5	0	1	.072	0.79	4	1	0	.056	5.21
5	3	1	.113	0.08	5	0	0	.060	12.10	4	0	2	.067	0.02
5	3	0	.100	0.79	4	5	0	.106	0.02	4	0	1	.056	0.76
5	2	2	.113	0.01	4	4	0	.093	0.06	4	0	0	.045	14.62
5	2	1	.099	0.28	4	3	1	.092	0.03	3	4	0	.076	0.04
3	3	2	.086	0.00	3	0	0	.032	18.64	1	2	1	.038	0.02
3	3	1	.075	0.02	2	4	0	.062	0.02	1	2	0	.029	0.53
3	3	0	.064	0.23	2	3	0	.051	0.11	1	1	1	.028	0.12
3	2	1	.064	0.08	2	2	0	.041	3.95	1	1	0	.019	4.76
3	2	0	.053	1.53	2	1	1	.040	0.02	1	0	1	.019	0.94
3	1	2	.064	0.01	2	1	0	.030	6.01	1	0	0	.110	34.58
3	1	1	.053	0.31	2	0	2	.040	0.01	0	2	0	.018	0.21
3	1	0	.043	7.00	2	0	1	.030	0.41	0	1	1	.018	0.04
3	0	2	.052	0.02	2	0	0	.020	24.13	0	1	0	.009	3.05
3	0	1	.042	0.51	1	3	0	.038	0.04	0	0	1	.009	0.26

This table indicates the number of bacteria per cubic centimeter based on the number of tubes showing growth which have been inoculated with dilutions as outlined. Ten tubes are to be inoculated with 10 cc. each, ten tubes with 1 cc. each, and ten tubes with 0.1 cc. each. The code number is made up of the number of tubes showing growth in each case, the first number of the code representing the number of tubes showing growth that are inoculated with 10 cc. each, the second those inoculated with 1 cc. each, and the last those inoculated with 0.1 cc. each. The column labeled *X* then gives the most probable number of bacteria per cubic centimeter in the material used for inoculation. The column labeled *P* gives the percentage of times that the code would be obtained if an infinite number of determinations were made of a solution containing the number of organisms indicated by *X*.

Example: Suppose that a culture is inoculated into broth in a series of 10 dilutions in steps of 10. Assume that 9 of the tubes inoculated with 10^{-7} cc., that 3 of the tubes inoculated with 10^{-8} cc. and that none of the tubes inoculated with 10^{-9} cc. show growth. The code will then be 9 3 0. Referring to the table, *X* is found to be 0.255. This means that the most probable number of bacteria in the 10^{-8} cc. dilution was 0.255 bacteria per cubic centimeter, or that the most probable number in the original solution was 0.255 times 10^8 or 25,500,000. If this experiment were repeated an infinite number of times on a solution containing this number, this result would be obtained 6.23 per cent of the time.

If 5 tubes are used in each dilution instead of 10, then multiply the code obtained by two, and refer to the table, as above. In this case, however, the column labeled *P* does not apply.