

the country is called into service, that true patriotism demands that they should observe social morality and self-control; so that, unhampered by disease, they may give to their country their best and finest efforts and splendidly uphold America's promise of Liberty: "That every nation, God willing, shall have a new birth of Freedom, and that the Government of

the people, by the people and for the people shall not perish from the Earth."

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A CRITICAL STUDY OF THE BACTERIAL COUNT IN WATER AND SEWAGE.

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THE bacterial count is one of the most generally used measures for the sanitary phases of water supply and sewage disposal questions. By means of it we gauge the pollution of a water, the strength of a sewage, the efficiency of a process of purification or disinfection, and the purity of the effluent. Doubtless we are assisted in our judgment by other indications, as, for instance, the coli test in the case of water supplies.*

All the details of the bacterial count have been subjected to considerable study and the whole procedure has been standardized, wherefore it rightly enjoys a great degree of confidence, but because of this confidence its use is often extended far beyond its scope unquestioningly, and the results obtained are taken at face value by many competent sanitary engineers.

If we inquire into the interpretation which we ordinarily almost subconsciously place upon this test, we find this to be that it represents *all the individual bacteria* in a cubic centimeter of the liquor

examined. The fact that this is not true, when known, is generally reconciled by the vague conception that the number indicated is directly proportional to the total number present.

The causes which operate to make the observed results differ from the true or desired results may be grouped as follows:

1. Those arising from mathematical considerations based on the laws of chance.
2. Those due to errors or imperfections in the manipulation and technique of the test.
3. Those due to clotting of bacteria, or their adhesion to or occlusion in solid particles, causing a number of bacteria to produce but one colony.
4. Those due to failure of bacteria to grow because of unsuitable conditions.
5. Those due to accidents, such as death of bacteria, juxtaposition causing several to appear as one colony, etc.

It will be noted that causes 3, 4 and 5 all tend to reduce the bacterial count. Cause 2, while present with the best technique, may assume very large pro-

* An article on the mathematical interpretation of the coli test by the same author appeared in the *Engineering News*, May 24, 1917.

portions in incompetent hands, and unfortunately it is usually impossible to verify the details of testing *a posteriori* so that results must be taken at their face value.

MATHEMATICS OF THE BACTERIAL COUNT.

In considering the mathematical aspects of the bacterial count, it is well to impose certain restricting conditions:

1. It will be assumed that we are dealing with a single fair sample, eliminating all consideration of periodical and secular variations.

2. It will be assumed that the sample is well mixed and that all bacteria are free to move about, so that a reasonably uniform distribution of the bacteria throughout the sample is obtained.

3. That the plate count shows all the bacteria plated and intermediate errors are eliminated.

We may consider the sample to be composed of a large number of minute droplets, each the size of a bacterium, and interspersed with these the bacteria themselves.

If we consider that a one cubic centimeter sample pipetted off for plating contains *s* such particles, of which *a* are bacteria and *β* are droplets of water, then:

The probability that any such particle is a bacterium

$$= p = \frac{a}{s} \quad \text{I}$$

the probability that any such particle is a water droplet

$$= q = \frac{\beta}{s} = 1 - p \quad \text{II}$$

Now, it can be shown that the most probable or expected number of bacteria in a sample of *n* c.c.'s or *ns* particles is:

$$\epsilon(na) = nsp, \text{ or } \epsilon(a) = sp \quad \text{III}$$

and further, that the *mean error* of this expected value

$$\epsilon(na) = \sqrt{nspq}, \text{ or } \epsilon(a) = \sqrt{\frac{spq}{n}} \quad \text{IV}$$

The proof for these formulæ can be found in the more recent text-books on probabilities.

So far we have been theoretically correct in that we divided our sample into particles of the same size as bacteria. We might have used much larger droplets, distinguishing between those containing a bacterium and those not containing one. For the expression $\epsilon(a)$ may be written

$$\epsilon(a) = \sqrt{s \cdot \frac{a}{ns} \cdot q}$$

wherein the *s*'s cancel and *q* approaches unity as the size of particles decreases, for all reasonable number of bacteria (up to many million per cubic centimeter). So we may say, without appreciable error, that

$$\epsilon(a) = \sqrt{\frac{a}{n}} \quad \text{V}$$

This holds practically even when several dilutions are made. For supposing a plating after double dilution to .0001 yields 100 colonies. This means an error of

$$\sqrt{100} \text{ or } 10 \text{ per cent.}$$

The error of sampling the first dilution would be

$$\sqrt{100 \times 100} \text{ or } 100,$$

equivalent to 1 per cent and of sampling the original,

$$\sqrt{100 \times 100 \times 100} = 1000$$

or 0.1 per cent. This would give a total mean error of sampling, including that due to dilutions of

$$\pm \sqrt{101} = \pm 10.05 \text{ or } \pm \sqrt{99} = \pm 9.95$$

In considering a series of determinations, the same reasoning applies. If N samples of n cubic centimeters each are taken, the most probable or expected number of bacteria per cubic centimeter is

$$\epsilon(Nna) = Nnsp, \text{ or } \epsilon(a) = sp \text{ as before.}$$

Similarly the expected error in the average of N determinations is

$$\epsilon(a_M) = \sqrt{\frac{a}{Nn}} \quad \text{VI}$$

From these formulæ the following rules may be formulated:

The expected error in bacteria per cubic centimeter based on a single sample is equal to the square root of the quotient obtained in dividing the number of bacteria per cubic centimeter by the size of the sample taken in cubic centimeters.

The expected error of the mean of N samples is obtained by dividing the expected error for a single sample by the square root of N .

The mean value of N determinations is equal to the expected value for a single sample.

We are interested in knowing the probability that the bacterial count on any plate will not deviate from the true value by more than a certain number of times the mean error. The criterion of Tchebycheff tells us that the probability P_T of a deviation of a variable from its expected value, not larger than λ times its

mean error, is greater than $\left(1 - \frac{1}{\lambda^2}\right)$.

$$\lambda = 3, P_T > 1 - \frac{1}{9} = 0.888$$

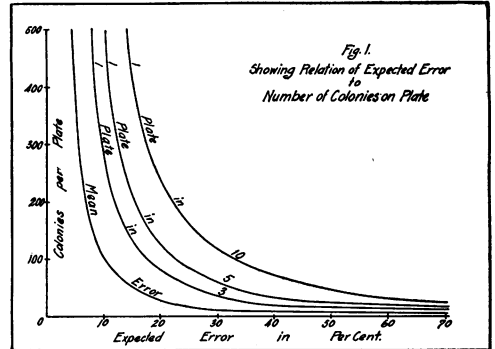
$$\lambda = 4, P_T > 1 - \frac{1}{16} = 0.937$$

$$\lambda = 5, P_T > 1 - \frac{1}{25} = 0.960$$

We are now in a position to plot the mathematical error of plating a sample of water or sewage, as well as the errors which may be expected once in five, ten times, etc. This has been done in

Figure 1. These values are subject to the restrictions enumerated at the beginning of this section.

Two conclusions can be drawn from this mathematical study and by inspection of Figure 1:



1. The size of sample or the dilution should be such as to give from 200 to 400 colonies on the plate. With larger numbers the error decreases but slowly (and the greater accuracy is more than offset by interferences, etc.); while with smaller numbers the error increases rapidly.

2. There is considerable probability that, if only one plate is used, the count will depart largely from the true value. Hence the importance of making several plates (3 to 10) from the same sample, particularly in the case of disinfection and similar studies.

MATHEMATICAL INTERPRETATION OF RESULTS.

In actual work we do not know *a priori* the number of bacteria in the liquor under investigation, but attempt to deduce this from the tests. The vagaries of a single test have been shown in the preceding section. In laboratory tests, where we are following the same sample of sewage through various processes, a series of platings should be made at each desired step in the process. In plant control or large scale studies, it is obvious that the average of say thirty daily

tests would depart much more from the true mean than would the average of thirty tests on the same sample. Let us restrict ourselves for the present to the laboratory condition.

Suppose we have made N platings of a sample. It can be proven that the *arithmetic mean* is equal (theoretically) to the expected number of bacteria, $\epsilon(a) = sp$ (Equation III).

Another important function is the dispersion. This may be indicated by δ and defined by the equation

$$\delta^2 = \frac{(a_1 - M)^2 + (a_2 - M)^2 + \dots + (a_N - M)^2}{N} \quad \text{VII}$$

where $a_1, a_2, a_3 \dots a_N$ are the number of bacteria obtained on the various plates, and M is the mean value. Now, it can be shown that δ is equal to ϵ or the mean error in a single trial. Thus we are able to approximate the number of bacteria in a sample from the average of a number of platings and the deviation of same.

A STUDY OF SOME ACTUAL RESULTS.

Below are given a few actual results to illustrate the methods of computation and the actual variations in results from the same sample of sewage due to errors of sampling, manipulation, etc.

Table I gives the results of a series of tests made by an experienced man with special care as to all details. This shows a standard deviation (dispersion) of 11 per cent or over twice the theoretical error due to sampling. Had only one test been made, and had this by chance been No. 14, the result would have been 25 per cent too high, whereas had the chance fallen on No. 4, the result would have been only 20 per cent of the correct value, and no one the wiser.

Table II gives the results of a series of tests made during routine work and shows a standard deviation of 26 per

cent and maximum variations of over 40 per cent.

Table III is added as tending to show that the number of dilutions does not affect accuracy of results greatly.

From a considerable number of such series of tests we may roughly formulate the following as the variations to be expected, not regarding occasional "flukes."

1. For careful and accurate work (200 colonies per plate):

- | | |
|------------------------------|------------|
| a. Standard Deviation | $\pm 12\%$ |
| b. Deviation (1 in 10 times) | $\pm 25\%$ |

2. For ordinary routine work (200 colonies per plate):

- | | |
|------------------------------|------------|
| a. Standard Deviation | $\pm 25\%$ |
| b. Deviation (1 in 10 times) | $\pm 50\%$ |

Now, as to tests made in the routine of plant operation and control. Obviously, in addition to the errors due to sampling and plating, as in a single sample, we here have variations in the samples themselves, which make it much more difficult to interpret results rationally.

In water supplies the variation may be periodic from season to season, as in lakes and large reservoirs, or this periodic fluctuation may have superimposed upon it very erratic changes, as those due to floods, etc., in river supplies.

In the case of sewage there are several superimposed periodic variations: a daily, a weekly and a seasonal period, and in addition a secular fluctuation due to gradually changing conditions.

The condition in which the proportion of positive to total results (that is, the probability) varies from test to test has been studied mathematically, and the series of errors thus derived is known as the Lexian or hypernormal series. It can be shown that the mean in such a series is the same, as in the usual (Bernoullian) series already discussed, but

TABLE I.

RAW SEWAGE, WEST 58TH STREET OUTFALL, JULY 2, 1918.

Incubation, 24 hrs. at 37° C. Dilution, 1/100×1/100. Controls, 0-0-0. Special Care Used.

	Count.	Deviation.	Deviation. ²
1	3,780,000	204,000	41,600,000,000
2	4,340,000	356,000	126,600,000,000
3	3,800,000	184,000	33,900,000,000
4	840,000 (omitted)		
5	4,280,000	296,000	87,600,000,000
6	3,340,000	644,000	414,000,000,000
7	3,980,000	4,000	16,000,000,000
8	3,300,000	684,000	466,000,000,000
9	4,370,000	386,000	149,000,000,000
10	4,160,000	176,000	31,000,000,000
11	4,060,000	76,000	5,760,000,000
12	3,730,000	254,000	64,500,000,000
13	3,650,000	334,000	111,200,000,000
14	5,000,000	1,016,000	1,030,000,000,000
15	Overgrowths		
			2,577,160,000,000
Av.	3,984,000		
Med.	3,980,000		

$$\text{Standard Deviation} \sqrt{\frac{2,577,160,000,000}{13}} = \pm 445,000 = \pm 11.2\%$$

$$\begin{aligned} \text{Maximum Deviation} &= +1,016,000 = +25.5\% \\ &= -684,000 = -17.1\% \end{aligned}$$

$$\text{Computed Mean Error} \sqrt{\frac{3,984,000}{1/10000}} = \pm 200,000 = \pm 5.0\%$$

$$\text{Lexian Ratio} = 2.2$$

$$\text{Coefficient of Disturbancy} = 10.0$$

TABLE II.

RAW SEWAGE, WEST 58TH STREET OUTFALL, MAY 31, 1918.

Incubation, 24 hrs. at 37° C. Dilution, 1/100×1/100. Controls 0-0-0. Routine Analysis.

	Count.	Deviation.	Deviation. ²
2a	1,800,000	20,000	400,000,000
b	2,410,000	630,000	396,900,000,000
3a	1,320,000	460,000	211,600,000,000
b	1,130,000	650,000	422,500,000,000
4a	1,050,000	730,000	532,900,000,000
b	2,040,000	260,000	67,600,000,000
5a	1,810,000	30,000	900,000,000
b	2,020,000	240,000	57,600,000,000
6	2,500,000	720,000	518,400,000,000
7	1,720,000	60,000	3,600,000,000
			2,212,400,000,000
Av.	1,780,000		
Med.	1,805,000		

$$\text{Standard Deviation} \sqrt{\frac{2,212,400,000,000}{10}} = +470,000 = \pm 26.4\%$$

$$\begin{aligned} \text{Maximum Deviation} &= +720,000 = +40.0\% \\ &= -730,000 = -41.0\% \end{aligned}$$

$$\text{Computed Mean Error} \sqrt{\frac{1,780,000}{1/10000}} = \pm 133,000 = \pm 7.5\%$$

$$\text{Lexian Ratio} = 4.2$$

$$\text{Coefficient of Disturbancy} = 30.7$$

TABLE III.

RAW SEWAGE DILUTED TO 1/100, WEST 58th STREET OUTFALL, JUNE 5, 1918.
Incubation, 24 hrs. at 37° C. Dilution, 1/100. Controls, 0-0-0.

	Count.	Deviation.	Deviation. ²
1	24,000	18,560	344,100,000
2	36,500	6,060	36,700,000
3	40,800	1,760	3,100,000
4	42,200	360	129,600
5	26,100	16,460	271,000,000
6	30,000	12,560	158,000,000
7	62,000	19,440	378,000,000
8	43,500	940	884,000
9	58,500	5,940	35,200,000
10	62,000	19,440	378,000,000
Av.	42,560		1,605,113,600
Med.	41,500		

Standard Deviation $\sqrt{\frac{1,605,113,600}{10}} = \pm 12,670 = \pm 29.8\%$
 Maximum Deviation $= +19,440 = +45.7\%$
 $= -18,560 = -43.5\%$
 Computed Error $\sqrt{\frac{42,560}{1/100}} = \pm 2060 = \pm 4.8\%$
 Lexian Ratio = 6.15
 Coefficient of Disturbancy = 29.3

the dispersion is greater. There can be many such series, depending upon how the probability varies from test to test. An extreme and very interesting case is that in which the probability varies from zero to one (*i.e.*, certainty) during the series of tests. Such a series can be readily studied both mathematically and experimentally by the black and white ball scheme, and may be regarded as a sort of standard for inconsistent results. (See No. 15, Hypernormal Mathematical Series (Lexis) in Table VI.)

Most statistical series are in the Lexian group so that the dispersion obtained is that of a Lexian series rather than of a normal or Bernoullian series. We have two criteria by which to judge the departure of the series from normal conditions:

a. *The Lexian Ratio*: which is the ratio of the dispersion computed from the actual tests to the theoretical mean error ϵ or δ_B (for a series of tests):

$$L = \frac{\delta}{\delta_B} \quad \text{VIII}$$

b. *The Charlier Coefficient of Disturbancy*: which has the advantage of eliminating the effect of the number and size of samples:

$$100p = \frac{\sqrt{\delta^2 - \delta_B^2}}{M} \times 100 \quad \text{IX}$$

In Table VI the Lexian Ratio and Coefficient of Disturbancy are given for a number of statistical series and error series for bacterial tests. Values for a subnormal, normal and hypernormal mathematical series are inserted to act as guide posts. It will be noted that the monthly data, based on one test a day, group around the Hypernormal Series, which we have characterized as a standard of inconsistency.

This state of affairs imposes upon the sanitary engineer, in addition to the precautions for the single test, the necessity of studying these various fluctuations, before any reliable judgment can be passed upon the bacterial content of the water or sewage in question.

In addition, it must be remembered that even when the sampling schedule covers the full scope of these variations, the deviations will be many times greater than in the same number of tests made on a liquor of constant composition.

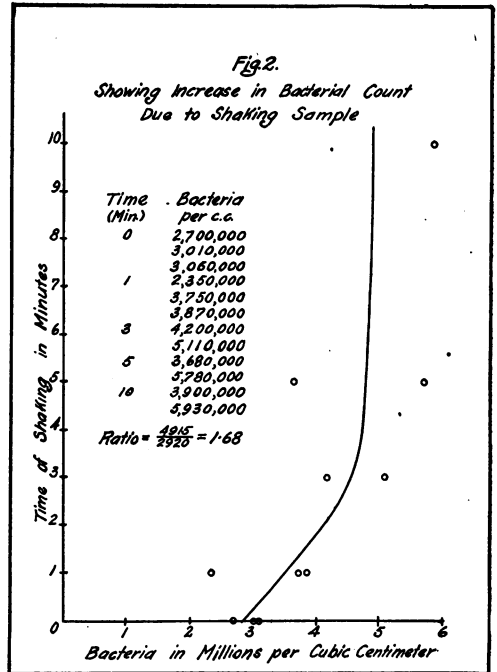
It is not intended to create the impression that an extremely elaborate series of tests should usually be made, but rather that the usual tests should be supplemented sufficiently by careful study of conditions to enable a fair judgment of their true significance to be rendered, and it should be realized that monthly averages as usually made are very far from being representative.

RELATION OF USUAL COUNT TO TOTAL BACTERIA.

The bacterial count does not represent the total number of bacteria present, even if all errors of sampling and manipulation are eliminated.

Examination of samples of sewage under the high power microscope will reveal many particles to which numerous bacteria are clinging, and there is good evidence that many more are occluded within the particles. The writer has attempted to separate or release such bacteria previous to plating by vigorously shaking the sample for ten minutes in a sterile bottle containing coarse sterilized sand. A record of a number of such tests is shown in Table IV, which shows an increase of 67 per cent over the usual method. In Figure 2 are given the results of an experiment on the effect of various periods of shaking.

But, even so, there is no record of the types which do not find the media and conditions favorable to development—such as anærobics. In investigating this question the writer made use of a method, the application of which to sewage work he believes to be novel, *i.e.*, the direct



count. The sample, after vigorous shaking with sand as above outlined, is stained with methylene blue. A drop is then placed on a blood-counting cell and counted under the high power microscope. (This method shows some promise as a means for rapidly determining the bacteria in sewage, aside from its usefulness in this case.) In one instance, where the average of ten tests by the usual method gave 3,950,000 per cc., the direct count gave 70,720,000 as the average of ten counts made by two observers. This latter figure included dead cells and probably some minute organic particles which might have been mistaken for bacteria, but the difference seems too great to explain away on any such grounds. Since making the above tests the writer's attention has been called to the fact that recently work along similar lines has been done on soil analysis, giving about twenty times as many bacteria by direct count as by the plating method.

APPLYING THE STANDARD METHOD TO SPECIAL CONDITIONS.

In applying the standard count to unusual conditions considerable caution should be used if it is desired to avoid erroneous interpretation of results. As an example, the usual method of interpreting disinfection tests suggests itself. A raw sample and a disinfected sample are plated and incubated at 37° C. for 24 hours. The raw count is 2,000,000, the disinfected count, 20,000. The plates are returned to the incubator and counted again at the end of 48 and 72 hours. The raw will remain approximately constant, but the disinfected sample may give counts of 60,000 and 100,000 respectively. Apparently some of the bacteria were merely stunned or weakened by the disinfectant and only recovered sufficiently to develop as visible colonies one or two days later. Table V gives some actual results of this kind. This is quite apart from the question of "after-growths" which are due to multiplication of the bacteria, which have not been destroyed, in the water itself. Other cases where the literal use of standard methods will not yield the desired results will suggest themselves to the reader.

CONCLUSIONS.

It has been shown, that for platings of a uniform sample:

a. The expected error in a series of bacterial counts is given by the formula:

$$\epsilon(a_M) = \sqrt{\frac{a}{Nn}}$$

b. That consistent with convenience, from 200 to 400 colonies per plate give the greatest accuracy.

c. That for ordinary work three, and for accurate work, five or ten platings should be made of each sample, a single plating being unreliable.

d. A study of Table VI shows that with sufficient care the error in determining the bacteria in a single sample can be made to approach the normal error of sampling, putting such determinations in a class with other reliable statistical data.

Further study of Table VI shows that the results of a series of bacterial samples taken from day to day are extremely inconsistent. The criteria show this inconsistency to be due both to errors in the single sample, which can be eliminated to a large extent, and to the fact that the samples were not properly apportioned to the variations in the water or sewage, which source of error can be reduced by study of these variations. In these days of standards for filterable water, drinking water, etc., would it not be well to inquire into the nature of the rule which we are using for our measurements, both to determine its actual length and establish its accuracy?

It has further been shown that usual methods do not give the total number of bacteria present, due to clotting and unsuitable conditions for their growth. Rather perfunctory tests indicate that the number developed by plating is not over 10 per cent of those present, but a review of governing conditions will convince that no constant proportion can exist.

It has also been shown that under unusual conditions the standard method gives distorted results, and that for special work modifications to reduce these distortions seem advisable.

TABLE IV.

RELATION OF THE BACTERIAL COUNTS BY THE USUAL METHODS AND AFTER SHAKING SAMPLES FOR TEN MINUTES.

Test.	Usual Method.	After Shaking Ten Minutes.
25a	2,170,000	2,640,000
b		3,970,000
c	1,360,000	2,700,000
26a	1,980,000	3,200,000
b	2,180,000	3,050,000
c	4,370,000	1,130,000
27a	2,980,000	2,770,000
b	2,060,000	3,640,000
c	1,450,000	3,310,000
28a	2,110,000	4,820,000
b	1,890,000	5,200,000
c	1,330,000	5,700,000
29a	3,280,000	4,110,000
b	2,500,000	3,920,000
c	2,000,000	2,180,000
31a	3,310,000	8,070,000
b	2,970,000	6,490,000
c	4,370,000	7,860,000
Av.	2,490,000	4,153,000

$$\text{Ratio} = \frac{4,153,000}{2,490,000} = 1.67$$

Above tests made on crude sewage at West 58th Street, Cleveland, Ohio, July, 1918.

TABLE V.

BACTERIOLOGY OF SEWAGE. THE EFFECT OF LENGTH OF INCUBATION ON RAW AND STERILIZED SEWAGE COUNTS.

	Raw Sewage.			Sterilized.		
	1st Day. No. 25	2d Day.	3d Day.	1st Day.	2d Day.	3d Day.
A.	2,250,000	2,080,000	2,300,000	60,000	90,000	150,000
	2,140,000	1,850,000	2,010,000	40,000	40,000	50,000
	3,900,000	3,900,000	4,000,000	20,000	30,000	50,000
	<u>2,763,000</u>	<u>2,610,000</u>	<u>2,770,000</u>	<u>40,000</u>	<u>53,300</u>	<u>83,300</u>
	No. 26					
B.	1,980,000	2,270,000		60,000	60,000	
	2,180,000	2,980,000		80,000	200,000	
	4,370,000	5,570,000		80,000	150,000	
	<u>2,840,000</u>	<u>3,610,000</u>		<u>73,000</u>	<u>137,000</u>	
	No. 27					
C.	2,980,000	3,480,000	4,300,000	200,000	330,000	00,000
	2,060,000	2,410,000	2,920,000	90,000	300,000	310,000
	1,450,000	1,730,000	2,020,000	80,000	240,000	290,000
	<u>2,163,000</u>	<u>2,540,000</u>	<u>3,080,000</u>	<u>123,000</u>	<u>290,000</u>	<u>300,000</u>
	1,360,000	1,900,000	1,900,000	0	0	0
	2,170,000	4,010,000	3,620,000	0	0	0
				0	10,000	30,000
	<u>1,765,000</u>	<u>2,960,000</u>	<u>2,760,000</u>	<u>0</u>	<u>3,300</u>	<u>10,000</u>
	Averages of A, B and C.					
	2,763,000	2,610,000	2,770,000	40,000	53,000	83,300
	2,840,000	3,610,000		73,000	137,000	
	2,163,000	2,540,000	3,080,000	123,000	290,000	300,000
	<u>2,589,000</u>	<u>2,920,000</u>	<u>2,925,000</u>	<u>78,700</u>	<u>160,000</u>	<u>191,000</u>
	1	1.13	1.13	1	2.04	2.43

TABLE VI.

COMPARISON OF THE CHARACTERISTICS OF ERROR SERIES IN BACTERIOLOGICAL TESTS WITH THOSE OF COMMON STATISTICS AND MATHEMATICAL SERIES.

Description.	Lexian Ratio.	Disturbancy Coefficient.
1. Subnormal mathematical series (Poisson).....	.98	$\sqrt{-1}$
2. Selected mortality series (life insurance).....	1.00	0.0
3. Normal mathematical series (Bernoulli).....	1.00	0.0
4. Birth series (a).....	15.50	4.07
5. Birth series (b).....	12.50	4.50
6. Fortunate series of ten plates on crude sewage.....	1.45	4.60
7. Marriage series.....	4.40	5.70
8. Careful series of fourteen plates on crude sewage.....	2.22	10.00
9. Ordinary series of ten plates on crude sewage.....	3.26	15.50
10. Ordinary series of ten plates on crude sewage.....	3.53	25.00
11. Ordinary series of ten plates on disinfected sewage.....	6.15	29.00
12. Daily tests for one month on crude sewage.....	5.30	41.00
13. Daily tests for one month on crude sewage.....	8.27	47.60
14. Daily tests for one month on crude sewage.....	9.00	49.00
15. Hypernormal mathematical series (Lexis).....	7.40	50.80
16. Daily tests for one month on Lake Erie water.....	29.00	124.00
17. Daily tests for one month on filtered water.....	10.85	178.00

NOMENCLATURE AND FORMULÆ.

- α = Number of bacteria per cubic centimeter.
- β = Number of arbitrarily small particles of water per cubic centimeter.
- s = Total number of particles per cubic centimeter, $s = \alpha + \beta$.
- p = Probability of drawing one bacterium, $p = \frac{\alpha}{s}$.
- q = Probability of not drawing one bacterium, $q = 1 - p$.
- n = Number of cubic centimeters in each sample tested.
- e = Expected Value— $e(a)$ = expected value of a , etc.
- ϵ = Expected Error— $\epsilon(a)$ = expected error of a , etc.
- N = Number of determinations.
- λ = Size of error in terms of ϵ .
- M = Mean value.
- $a_1 a_2 a_3$ etc. = Number of bacteria per cubic centimeter in the individual samples of a series.

- δ = Standard deviation.
- L = Lexion ratio.
- ρ = Charlier coefficient of disturbancy.

$e(a_M) = sp = a.$

$\epsilon(a) = \sqrt{\frac{\alpha}{n}}$ for a single test.

$= \sqrt{\frac{\alpha}{Nn}}$ for N tests.

$M(a) = \frac{\alpha_1 + \alpha_2 + \alpha_3 \dots \alpha_n}{N}$

$\delta^2 = \frac{(\alpha_1 - M)^2 + (\alpha_2 - M)^2 + \dots + (\alpha_N - M)^2}{N}$

$L = \frac{\delta}{\epsilon}$

$100\rho = \sqrt{\frac{\delta^2 - \epsilon^2}{M}} \times 100.$



Annual Meeting, A. P. H. A., Chicago, Ill., December 9-12
 Headquarters, Hotel Morrison
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