ON A STANDARD SYSTEM OF BACTERIOLOGICAL DILUTIONS.

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Proper bacteriological dilutions are necessary for correct interpretations. The system here proposed adopts the dilution scale for its standards. On a consistent system of laboratory technique, including logical methods of computing results and a natural scale of interpretation, depends the real practicability of bacteriological standards.

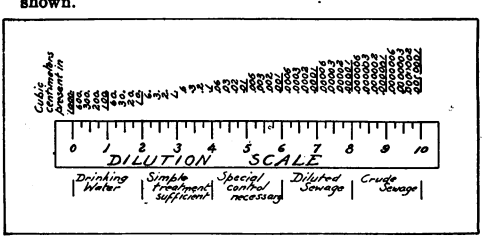
SANITARIANS generally realize the limitation imposed upon bacteriological standards through want of precision in quantitative methods. Those who have attempted to remedy results by applying the theory of probability to quantitative bacteriology have fallen into two errors. Being rather more mathematicians than bacteriologists, they have developed unnecessarily complicated expressions which practical laboratory analysts cannot understand or apply, and though arriving at a solution satisfactory to themselves, they have overlooked the most important difficulty of the bacteriologist. Their efforts aim at the determination of the number of bacteria in each particular sample, while the principal problem is to predict the condition of a source knowing the condition of the several samples. Moreover, the greatest need at present is a standard system of dilutions built on the system of interpretation to follow.

Suppose the theoretical number of bacteria per cubic centimeter were given, how much further toward interpretation is the bacteriologist? He uses such a hypothetical figure, with all its limitations, as an index by which he can think of his results in terms of experience. He associates it with actual conditions under which he expects the given bacteria will occur in given dilution quantities of the sample. Why not cancel in one bold stroke the fearful process of calculation with the doubtful mental return to judgment?

This is accomplished by the dilution scale proposed in a previous paper.* It is graphically represented in Figure I.

FIGURE I.

Dilution Scale Diagrammatically Measuring Pollution represented by presence of *B. coli* in quantities of water shown.



The practical technical principle of the dilution is taken as the basic unit of measure, and consequently it is necessary to think directly in terms of the actual steps of procedure. It is drawn to resemble the ordinary measuring rule so as to emphasize the similarity to pollution measurement. The various points on the scale represent the grades of pollution which the presence of *B. coli* in the given quantities signify. One can think of it as a rule to measure the pollution of an unknown sample; of which the quantities

* AMERICAN JOURNAL PUBLIC HEALTH, September, 1919, pages 664–667.

are tested to indicate a degree somewhere between the point representing the highest dilution showing B. coli and the lowest in which it is absent. According to the number of dilutions tested, these points can be brought as close together as desired. The scale above figured has forty divisions obtained by dividing into quarters the ten whole dilutions ranging from a condition of the purest drinking water to that of crude sewage. Though the divisions are finer than bacteriologists ordinarily apply, the given scale covers all conditions possible in practice.

By adhering to the dilutions shown on the scale and expressing results in terms of dilutions the resulting computations reduce to simple arithmetic. The mathematical reason for this is perfectly sound,* for one has in making the given dilutions solved technically the complicated relation involved. Perhaps the fact that the dilution scale has evolved in the familiar laboratory technique is more than mere coincidence, but in any case it admits of a great simplification in the interpretation of results. It requires but the adoption of a standard system of dilutions and in return offers to solve the main trouble in a very simple and practical way. While affording a much needed criterion for the distribution of dilutions, it also cuts away a maze of confusion, exposing an otherwise clear technique which an unnatural system has obscured.

First to appear on examination is the fallacy of the false economy of dilutions. It is obviously not necessary to apply every dilution on the scale in testing each sample. In reality a few dilutions are chosen, either consciously or unconsciously representing the small range or span of the scale in which the bacteriologist expects to find the deciding dilutions. But where so few are used, and the range is so great that a qualitative uncertainty may change the location of a result by a whole dilution on the scale, then that dilution is unnecessarily important, and the use of a few more tubes would greatly increase the precision. By the use of a fermentation tube battery described in another paper* it is possible rapidly to inoculate a large number of tubes and eliminate this source of error. It is, therefore, considered an integral part of the technique.

Simplicity demands more or less standard batteries, although they can be made to contain any number of tubes. This limitation on technique thus tends toward standardization and is in no way inconsistent with precision. On the other hand, since the accuracy demanded in the result is in a general way proportional to the variation expected in choosing the dilutions, such a standard number of tubes gives a standard of precision for each range or dilution span of the scale which the given dilutions must cover.

A standard number of dilutions covering a given range or dilution span on a standard dilution scale determines the quantities used for inoculation. The dilutions are evenly distributed throughout the dilution span in accordance with the dilution scale. The length of the dilution span is, therefore, inversely proportional to a precision standard. It is necessary to estimate the dilution span from the preliminary evidence at hand. It should be always chosen to give a positive and a negative tube, and is, therefore, the span between the highest dilution judged surely to be positive and the lowest surely negative. With well known waters it may be closely determined and the precision of the result will be greater, while with little known samples it is correspondingly broader and the precision is

* Science, N. S. Vol. XLIX, No. 1269, pages 400-402, April 25, 1919.

* American Journal Public Health, December, 1919, pages 904-905.

relatively lower. In this way the standard system of dilutions adapts itself to the requirements of the test and to the subsequent computations of which it becomes a part.

There only remains in applying these general considerations to establish definite values and to give concrete examples of practice. Considering the labor involved in collecting the sample and other steps in the test, less than five tubes are under ordinary circumstances insufficient, while more than ten are usually superfluous. For practical reasons, therefore, the battery of ten tubes was selected. It contains two samples of five tubes each, though where greater precision is desired all ten may be used for the same sample by making duplicate series, the average of which gives doubled accuracy.

For purposes of illustration typical examples of the application of the system to common dilution spans are given. Suppose the bacteriologist knows that the 10 cc. tube of a sample will give a positive test and the 1 cc. tube will be negative. Five dilutions at intervals of a quarter dilution will cover the span as follows:

Example of one dilution span.

Dilution	2.0	2.25	2.5	2.75	3.0
Cubic centimeters	10.	6.	3.	2.	1.

The two dilution span will ordinarily be found most convenient with unknown drinking waters. The intervals are, therefore, half dilutions as in the following example:

Example of two dilution span.

Dilution	2.0	2.5	3.0	3.5	4.0
Cubic centimeters	10.	3.	1.	.3	.1

By using intervals of whole dilutions a four dilution span is covered as follows:

Example of four dilution span.

Dilution	2.0	3.0	4.0	5.0	6.0
Cubic centimeters	10.	1.	.1	.01	.001

Very seldom will it be found necessary to make dilutions cover a wider range. With ten tubes greater precision could be obtained by duplicating each series and averaging the dilution positives of both or the dilution span could be divided so as to make even intervals.

Intermediate between the two and four comes the three dilution span which divided at regular intervals gives awkward dilutions. Since, however, the middle dilution is the more important because it is more likely to contain the dilution positive, this dilution is divided as in the two dilution span while the first and third are whole dilutions.

Example of three dilution span.

Dilution	2.0	3.0	3.5	4.0	5.0
Cubic centimeters	10.	1.	.3	.1	.01

The same effect, using ten tubes, can be obtained by making each five tube series as in the two dilution span but allowing the two series to overlap one dilution, which is the middle dilution.

Example of three dilution span.

Dilution	2.0	2.5	3.0	3.5	4.0	4.5	5.0
Cubic centimeters	10.	3.	1.	.3	.1		
Cubic centimeters			1.	.3	.1	.03	.01

By thus concentrating on the middle dilution, which is judged most probable to contain the dilution positive, there is a favorable chance of reaching the precision of a two dilution span; otherwise the result will be that of a four dilution span.

There remains a very important combination which has been independently developed. The United States Treasury standard for drinking waters is defined by inoculating ten tubes each with 10 cc. of water; of which not more than two may be positive. Where quantities of sample larger than 10 cc. must be tested (which now includes the majority of good drinking waters) this test is the only practical one since more than 10 cc. cannot conveniently be put in the test tubes described in the battery. The technique does not fit exactly to the theory of the dilution scale which is built on a geometrical series. It is, however, an arithmetical

approximation and if the dilution span is not too great, sufficiently accurate if each positive tube is considered to represent a tenth dilution as follows:

Dilution equivalent of U. S. Treasury Standard method.

Dilution..	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0
Cubic Centimeters	10.	10.	10.	10.	10.	10.	10.	10.	10.	10.

While such an expedient is justified under this practical case it is not recommended where it is just as convenient to make inoculations consistent with the theory of the scale.

To show a concrete example of the actual application of the system in practical routine Figure II is described.

FIGURE II.

WATER EXAMINATION FOR B. COLI

System of fermentation tube inoculation for three types of conditions showing scale for dilutions positive, corresponding number of B. coli per c.c., and action demanded by quality represented.

TYPE I	cubic c. 10	cubic c. 1.0	cubic c. 0.1	cubic c. 0.01	Dilution Positive	B. coli per 100 c.c.	Action demanded
Drinking Water Developed supplies or passed without treatment. Routine control examinations.	−	−	−	−	<1.0	<1	Excellent.
	+	−	−	−	1.2	2.	Passed by A.E.F. Standards.
	+	+	−	−	1.4	3.	Repeat and improve treatment until passed.
	+	+	+	−	1.6	4.	
	+	+	+	+	1.8	6	
	+	+	+	+	>2.0	>10	Repeat under Type II.

TYPE II	cubic c. 10	cubic c. 3	cubic c. 1	cubic c. 3	cubic c. 1	Dilution Positive	B. coli per 100 c.c.	Action demanded
Water Sources Proposed for Lyster bag treatment. Routine preliminary examinations.	−	−	−	−	−	<2.0	<10.	Repeat under Type I.
	+	−	−	−	−	2.0	10	Suitable for Lyster Bag Treatment
	+	+	−	−	−	2.5	30	
	+	+	+	−	−	3.0	100	
	+	+	+	+	−	3.5	300	
	+	+	+	+	+	>4.0	>1000.	Repeat under Type III.

TYPE III	cubic c. 1	cubic c. 0.3	cubic c. 0.1	cubic c. 0.03	cubic c. 0.01	Dilution Positive	B. coli per 100 c.c.	Action demanded
Special Sources requiring special authority for use and special treatment and control.	−	−	−	−	−	<3.0	<100	Repeat under Type II.
	+	−	−	−	−	3.0	100	
	+	+	−	−	−	4.0	1000.	Only to be used after treatment recommended and controlled by special authority.
	+	+	+	−	−	4.5	3000.	
	+	+	+	+	−	5.0	10000.	
	+	+	+	+	+	>6.0	>100000.	

Note:
< Signifies less than
> " " greater than

It was used at certain laboratories in France under the writer's direction as a working guide to conform to the regulations used by the Water Supply Service. Under these rules waters appeared to the officer to fall under three general classes: First, *Drinking Water* was defined as passing the United States Treasury standard on repeated tests continually made as a control. Second, *Prospective Drinking Water* tested to determine suitability, comprised practically all undeveloped sources, and preliminary examination only was needed to show whether treatment was necessary or simple treatment sufficient. If this test indicated a Class I source repeated examination automatically became necessary, while if it was worse than Class II it fell into the Third Class of *Raw Waters*, to be condemned if possible for drinking purposes. If the only available source, special treatment and control were required, the risk of unskilled supervision being too great and conditions were so exceptional that special precautions were advisable.

The laboratory tests were, therefore, standardized under three types of procedure. Preliminary samples were analized under Type II and ordinarily simple treatment in Lister bags found sufficient. These were chiefly wells and springs used in billeting areas. In more or less permanent camps there were many supplies developed by the engineers, and routine examinations of these were made under Type I. The raw water samples for these supplies sometimes were Type III, which ordinarily constitutes an emergency class. Each laboratory assistant, therefore, knew definitely what dilutions to make and consequently the results were uniform and perfectly easy to interpret on the dilution scale.

Conclusion.

If dilutions are made in conformity with a standard dilution scale and results computed in terms of dilutions, and interpreted directly according to their location on the scale, many of the present difficulties of bacteriological standards will disappear.