"The Quantitative and Qualitative Bacteriological Analysis of the Manhattan City Water. "

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I Introduction.

It has only been within the past few years that scientists have discovered the value of bacteriological examination of drinking water. There have been terrible epidemics of water borne contagious diseases, but only since the advent of bacteriology has it been known how these diseases were spread.

The great importance of the bacterial analysis of drinking water is now recognized and one of the first things done in case of an epidemic of certain diseases is to examine the water used.

Water analysis should be made not only during epidemics but at frequent intervals to determine whether the water is potable.

The B. coli communis is a common intestinal organism and when found in the water it is an indication that the water is contaminated with sewage material. The normal habitat of this bacillus is in the mucus membrane of the intestine of man and animal. In itself it is not harmful when introduced into the alimentary canal, but it is evident that if this intestinal organism can gain access to the water it will also be possible for the pathogenic intestinal organisms to enter it. For this reason water found to contain B. coli communis should not be used for drinking purposes unless purified.

II. Technique.

a. When a sample of water is to be examined the collecting recepticle should first be thoroughly sterilized. If this is not done the water will be contaminated and the test will not be accurate.

b. If the sample is to be taken from a tap the faucets should be opened for at least five minutes so that the water which stands in the pipes will be removed before the collection is made.

c. If taken from a pond or lake the collecting recepticle should be held at least one foot below the surface of the water before the stopper is removed. This is done to remove the danger from surface contamination.

d. The water should be examined at once if possible and if not, it should be kept on ice. If the water is allowed to stand, some of the bacteria may die, others may multiply, therefore the test will not be accurate.

The qualative tests are very important as it is frequently necessary to determine the number of organisms per c.c. As a general rule good drinking water should not contain over 2,000 organisms per c.c.

III. Methods Used in the Examination of the Manhattan City Water.

The samples of water were collected from taps with one exception. The water was allowed to run for five minutes and then the sample was collected in a sterile flask.

When the water was collected from the reservoirs on Blue Mont a sterile bottle was introduced one foot below the surface before the stopper was removed.

a. Quantitative Analysis. The number of bacteria contained in one centemeter of water can be estimated by the use of agar plate cultures.

The tubes of sterile agar were melted and after cooling sufficiently so that the bacteria which were introduced into the media would not be killed, they were inoculated with a given sample of water. The tubes were inoculated with 1/10, 1/20, 1/100 c.c. of the given sample respectively. The mixture was thoroughly shaken and then poured into sterile Preti dishes. After the inoculated agar became

congealed the plates were then placed in the incubator for forty eight hours. At the end of this time the colonies were counted and from the number of colonies the number of bacteria per c.c. of water determined.

b. Qualitative Analysis. In qualitative analysis the colonies first must be isolated. This is done by making sub-cultures from all the different colonies found on the agar plates. With a sterilized platinum needle a portion of the colony is inoculated into a tube of steril bouillon and this is incubated for 24 hours. From this culture inoculations can be made in all the special medea.

IV. Special Tests for Various Organisms.

a. Test for B. coli communis. Inoculate a straight tube of glucose agar with a sample of the water. If after 48 hours bubbles of gas have formed in the medium it is an indication of B. coli cummunis. These gas bubbles are formed chiefly by the evolution of carbon dioxide and hydrogen. From the given sample of water inoculations should then be made into fermentation tubes of glucose,lactose and saccharose bouillon to estimate the amount and kinds of gases.

2. Another common test is conducted with Lactos-Litmus Agar. If red colonies are found after the water has been inoculated it will indicate B. Coli communis . This organism ferments lactose with the production of acid and gas; therefore, the multiplication of the bacteria with the consequent accidity of the medium immediately surrounding the colony will cause the litmus to turn red.

B. Typhosus, because this organism has no effect upon milk sugar, will be indicated by the presence of blue colonies.

b. Tests for B. Typhoses.

1. Phenol Method.

Prepare six plates of carbolized gelatine and inoculate

with 1/10, 1/30, 1/100 c.c. of water respectively. The B. Typhosis is one of the few species of organisms which will develop in the presence of a weak solution of phenol. Incubate for 48 hours at 22° C. The organisms if present will develop while other species of bacteria will be inhibited in growth.

2. Stoddart has also devised a method based on the fact that the typhoid bacillus spreads throughout a medium which is soft, while the colon bacillus retains its usual discrete colonies. A Special gelatine agar mixture is employed, and incubated at 35° C.

The following method(a) can be used to distinguish between B. coli communis and B. Typhosis: To 10 c.c. of the culture in ordinary peptone broth of the organism under examination, and which has been growing for 24 hours, add 1 c.c. of a solution of potassium or sodium nitrate and then a few drops of concentrated sulphuric acid. If indol is present, a rose to deep red coloration is produced, thus indicating the presence of indol. On applying this test to B. coli communis a positive reaction is obtained, while the B. Typhosis gives a negative result.

V. Quantitative Results.

Sample 1: This sample of water was collected from the city water tap at Mr. Harrison's store on Moro Street between ninth and tenth. As soon as the water was collected it was put on ice and examined two hours later.

1. Hewelletl's Manual of Bacteriology, P. 383.
2. Journal Path. and Bact., IV, 1897, P. 429.

(a) Fraenkland Micro-organisms in water 272.

Apr. 10, 1908.

Series plate Cultures	1/10 c.c.water inoculated	1/20 c.c.water inoculated	1/100 c.c.water inoculated
	No. per c.c.	No. per c.c.	No. per c.c.
Agar 1	210	170	230
Agar 2	190	220	203
Agar 3	180	195	209
Average No.	193	195	209

Average No. 199 bacteria per c.c.

April 10, 1908

Sample 2: Water collected at 810 Pierre Street. after the heavy spring rains had begun. Was examined two hours after it was collected. It was collected from a tap after the water had been allowed to run for five minutes.

April 17, 1908

Series Plate Cultures	1/10 water inoculated	1/20 water inoculated	1/100 water inoculated
	No. per c.c.	No. per c.c.	No. per c.c.
Agar 1	520	515	525
Agar 2	495	505	510
Agar 3	470	465	480
Average No.	495	495	505

Average No. 498 bacteria per c.c.

April 17, 1908

Sample 3: Water collected from 810 Pierre Street after the rain had continued for two weeks. Collected under the same conditions as sample 2.

May 1, 1908

Series plate Cultures	1/10 water inoculated	1/20 water inoculated	1/100 water inoculated
	No. per c.c.	No. per c.c.	No. per c.c.
Agar 1	990	970	980
Agar 2	1005	995	985
Agar 3	1010	1015	1013
Average No.	1005	993	992

Average No. 997 bacteria per c.c.

May 1, 1908

Sample 4: Water collected from tanks on Blue Mont. Examined immediately.

May 23, 1908.

Series plate Cultures	1/10 water inoculated	1/20 water inoculated
	No. per c.c.	No. per c.c.
Agar 1	200	200
Agar 2	205	200
Average No.	202	200

Average No. 201 bacteria per c.c.

May 23, 1908.

Sample 5: Water collected at the corner of tenth and Blue Mont Ave. from a tap. It was examined immediately.

May 23, 1908.

Series plate Cultures	1/10 water inoculated	1/20 water inoculated
	No. per c.c.	No. per c.c.
Agar 1	602	615
Agar 2	600	585
Agar 3	590	600
Average No.	597	600

Average No. bacteria per c.c. 598

May 23, 1908.

VI. Qualitative Results.

Sub-Cultures made from water collected April 17 after 48 hours. Water collected at 810 Pierre Street.

Bacillus I.

L.Milk. Acid.

Bouillon. Thick, white surface growth also sediment in bottom.

Glucon Agar. Smooth, white,surface growth. No gas bubbles.

Gelatine. Heavy white growth. Liquified slowly.

Sub-Cultures from water collected May 1,after three days.

Bacillus II.

Bouillon. Heavy, rough, white surface growth . Sediment.

Glucose Agar. No bubbles. Heavy surface growth.

L. Milk. Coagulated and turned a cream color.

Glucose agar tubes were inoculated from all the different colonies which appeared on the ager plate; cultures made from each sample of water. In every instance the absence of gas formation was noted. These results clearly indicate that the water did not contain coli communis or other gas producing intestinal bacteria.

From the microscopical examinations a great number of spores and short rod shaped organisms, both motile and non-motile were found.

VII. Tests for Pathogenesis.

Method 1: The Virulence of Peptone- Culture. 1. Take 1 percent of peptone and ½ percent of salt and add to a sample of water. After incubating at 37° C. for 24 hours, inoculate intra-peritoneally 2 c.c. of the culture into a guinea-pig. If this does not kill the guinea-pig within 48 hours it is indicated that the water does not contain any harmful bacteria.

Method 2: Make a bouillon culture from the water and incubate for 24 hours. Inoculate 1 c.c. into a guinea-pig intra - peritoneally. If the colon bacillus is present the death of the guinea-pig will take place in from 24 to 72 hours.

VIII. Results of Tests for Pathogenesis.

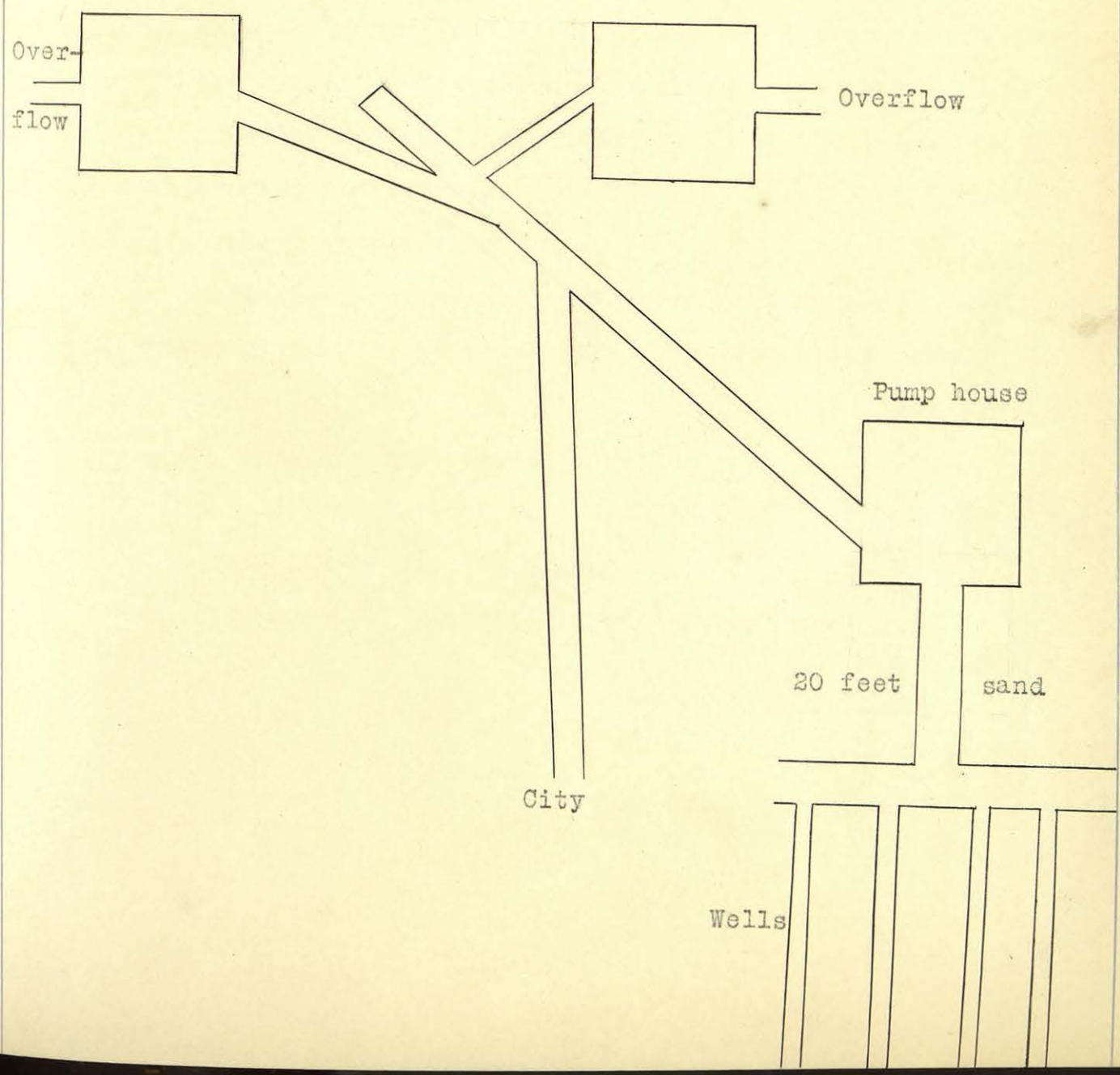
A guinea-pig was inoculated on April 18 with 2 c.c. of a 24 hour peptone culture of water collected on April 17th. The inoculation had no effect on the animal.

On May 2 a guinea-pig was injected intraperitoneally with 1 c.c. of a 24 hour bouillon culture made from water collected May 14 at 810 Pierre Street. For the first four days the inoculation had no effect. After that a local lession formed and gradually spread over the ventral portion of the body. On May 13 the guinea-pig died. Owing to the period intervening between the injection and the death of the animal the interpretation of the result would not indicate the presence of harmful organisms.

1. Lewelletl's Manual of Bacteriology, P. 386
2. Novys Method, P. 439 .

IX. Working Details of the Manhattan City Water Plant.

There are four wells 14 feet deep at the bank of the river. From these wells the water goes through 20 feet of coarse sand to the tank below the pump-house. From there it is pumped to the reservoirs on the top of Blue Mont. There are two tanks each having the capacity of ¾ million gallons. Both of these are kept full except when being cleaned. Then one is shut off and the water drained out. After the water reaches the reservoirs it is not filtered again.



X. Conclusion. The quantitative bacteriological analyses showed the following summarized results:

April 10, 08, Tap water, 199 bacteria per cc .
" 17, 08, " " , 495 " " "
May 1, 08, " " , 997 " " "
" 23, 08, " " , 598 " " "
" 23, 08, Reservoir, , 201 " " "

The variation in the number of organisms collected at different intervals may be explained by variations in environmental conditions. The counts made on April 17 and May 1 show the effects produced on the bacterial content by the warm weather and spring rains which occurred at that time. The period of time since the proper cleansing of the reservoirs would also influence the number of bacteria present in the water.

In conclusion, we venture to suggest that at the present time the Manhattan City water is reasonably pure in quality because of the following reasons:

1. B. coli communis is not present.

2. Animal inoculation, according to Navy's method, shows the absence of pathogenic or harmful organisms.

3. The number of bacteria is relatively low.