1924

Lactose fermenting bacteria in the water supplies of the Rolla Quadrangle

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LACTOSE FERMENTING BACTERIA
IN THE WATER SUPPLIES OF THE ROLLA QUADRANGLE

BY

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A THESIS SUBMITTED TO THE FACULTY OF THE
MISSOURI SCHOOL OF MINES AND METALLURGY,
IN PARTIAL FULFILLMENT OF THE WORK
REQUIRED FOR THE DEGREE OF MASTER OF
SCIENCE IN CIVIL ENGINEERING.

ROLLA, MISSOURI
1924

APPROVED BY ______________________

Thesis: Lactose fermenting bacteria in the water supplies of the Rolla quadrangle. Butler. 1924.

FOREWORD.

The author is indebted to the following for help as noted:

To Dr. F. W. Shaw, for suggestions and advice in regards this work.

To Mr. C. J. Miller, for help and advice in connection with the securing of the samples in the field, and in the preparation of the various culture media.

To Messrs. W. F. Hauck and W. Mikell, for help in securing the field samples.

TABLE OF CONTENTS.

INTRODUCTION: SCOPE OF THIS STUDY.

I. Preliminary investigations. This includes the securing of 139 water samples from water sources in the Rolla Quadrangle, and inoculating 1cc samples from these into Dunham's Lactose tubes and thereby getting lactose gas production checks on the field samples.

II. Confirmatory tests. This consisted in the isolation of lactose fermenters on Eosin-Methylene Blue Agar and checking of the cultures back through the lactose and then through dulcitol and saccharose so as to complete Jackson's Classification. 106 cultures were classified in this manner.

III. Running through a complete set of tests of pure cultures, so as to completely(as possible) identify them. 27 pure cultures were run through a complete set of tests.

THE FUNCTION OF THIS STUDY AND OF THE TESTS IS TO GAIN A DEFINITE KNOWLEDGE OF THE POTABILITY OF WATER SUPPLIES BY BACTERIOLOGICAL EXAMINATION METHODS.

Extracts from "Bacteria Fermenting Lactose and Their Significance in Water Analysis" by Max Levine. These extracts are given here as they are very useful in stating the reasons for this study of Lactose Fermenting Bacteria.

I. Characteristics of the colon group of bacteria.

The bacterial analysis of water is an indirect and quantitative one. Specific pathogenic organisms are not sought nor are they likely to be detected even in a dangerous water. It devolves upon the analyst to interpret his findings and particular emphasis is placed upon the determination of the presence of the colon group. The investigator and analyst should therefore be thoroughly acquainted with the characteristics, peculiarities and idiosyncrasies of the organisms in the group, particularly with reference to their distribution, viability, and differential reactions.

Bacterium Coli was first discovered by Emmerich from the feces of a cholera patient in 1884X. It was soon recognized as a normal inhabitant of the intestinal tract of man and of other animals. For the past three decades the XXXX colon group of bacteria has been extensively studied by bacteriologists and sanitarians especially those interested in water supply and purification. Probably as much work has been done on this as on any other group of bacteria but there is not as yet an absolute agreement as to the limitations of this group.

Extracts from "Bacteria Fermenting Lactose and Their Significance in Water Analysis" by Max Levine.

.... The Colon group will therefore be considered to include non-sporing Gram negative bacilli which ferment lactose withx the production of acid and gas and which are capable of growing aerobically..........

V. The Colon Group as an Index of Pollution.

SAFE WATER. A safe water for human consumption may be defined as one which is free from harmful constituents important among which are disease producing microorganisms. The logical and most direct procedure to determine the potability and safety of a water would be to determine the presence or absence of pathogenic bacteria but unfortunately this task is an impossible one for routine and recourse must therefore be taken to an XXXXX indirect indexx of the probable presence of harmful germs. Since the diseases transmissable through water are primarily of intestinal origin the detection of the presence of intestinal materail naturally leads to the presumption that a potential danger exists, for if such material is present it is very probable and certainly possible that intestinal XXXXX disease germs are also present.

A number of tests both chemical and bacteriological have been suggested as indicators of intestinal pollution. The Bacterial examination by reason of the large number of XXXXX bacteria present in feces and sewage and the ease with which they may be detected in water, is a particularly delicate test

Extracts from "Bacteria Fermenting Lactose and Their Significance in Water Analysis" by Max Levine.

V. Continued..... XXXX.

Three goups of bacteria have been regarded as indicators of pollution: The Colon Group, Sewage Strepticocci, and Spore Forming Anaerobes.

An organism to be considered an ideal index of fecal pollution should have the following characteristics:

1. It should be distinctively and characteristically of human or animal intestinal origin.

2. It should be absent or extremely rare in nature XXXXX outside of the intestinal tract.

3. It must be capable of easy and rapid detection.

4. Its incidence in water should bear some constant relation to the sanitary survey or our knowledge as to the probability of pollution, particularly with sewage.

5. It should be XXXXXXXXXXXXXX distinctly more viable and more resistant in water and to treatment than are the intestianl pathogens(B.Typhi, B.Dysenteriae, etc.), but not excessively so.

Such an ideal indem is not available but the general consensus of opinion among English and American bacteriologists is favorable to the employment of the colon group for this purpose.

Extracts from "Bacteria Fermenting Lactose and their Significance in water analysis". by Max Levine.

V. Continued.

Although bacteriaX of this group are not restricted in habitat to the intestinal tract of man being characteristic also of the intestinal tract of the lower animals, it is nevertheless true that there is a correlation between the quantitative incidence of at least the coli section and known pollution. The whole group is easy of detection as will be seen by the following considerations. It is more viable than Bact. typhi but yet dies off relatively quickly; colon bacilli are present in relatively large numbers in water known to be polluted but only infrequently in natural supplies. A correlation has been established between the incidence of the colon group in drinking water and the typhoid fever rate in a community. "

It might well be stated here that Bulletin 62 of the Engineering Experiment Station of the Iowa State College, which is the "Bacteria Fermenting Lactose and their Significance in Water Analysis" by Max Levine is the outstanding publication of the present day on the scientific aspect of sanitary bacteriology. With the exception of the Citrate Medium (by Koser) all other experiments of this investigation are based on this bulletin.

Culture Media Used: Reactions and their significance. pg.1.

XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

1. Lactose broth in Dunham's fermentation tubes. The Colon-Aerogenes group and the anaerobic spore formin**g** Cl Welchii are the lactose fermenters.. The positive presumptive test for pollution by sewage is to isolate Gram negative, non-sporing, lactose fermenting (with gas) rods from a sample of water. The absence of lactose XXXXXX fermenters gives the water a clear indication of non-pollution. But the finding of the above fermenters does not immediately condemn the water but throws it under XX suspicion from which it may sometimes be cleared. There are two methods of following up this test. XXX First, by identifying the organisms in order to be sure of their origin as to whether it is fecal or non-fecal. While 100% accuracy cannot be gotten along this line still the indications have a high correlation with sewage pollution. The Second and practical method has merely to do with the quantitative incidence as the presence of large numbers of the colon group is a very definite indication of an undesirable pollution.

2. Dextrose brothX (gas formation by fermentation). The colon group are also all dextrose fermenters but there are other and non-lactose gas formers that give this reaction so that lactose has the place as colon group indicator.

Culture Media Used: Reactions and their Significance,pg 2.
XXXXXXXXXX

3. Saccharose broth (Fermentation with gas production).
This reaction is considered as primary in MacConkey's classification and secondary in Jackson's and Levine's. As a differential test between the fecal and non fecal groups this has little correlation and has been replaced by other tests.

4. Dulcitol broth (Fermentation with gas production).
This reaction is secondary in Levine's and MacConkey's classifications and is primary in Jackson's as differential test. While its correlation is XXXXXXXXXXXXXXXXX better XXXXXXXXXX than that of saccharose still it is less than others and is entitled to only a secondary place. The 27 pure cultures of this present study on an early batch of dulcitol produced gas in XX only one culture, while in a repeat in a new batch about a month later 15 of the cultures produced gas but all 15 were uniformly barely 10% gas (by quantity) formers. Both batches were checked by other than the 27 pure cultures so that their contradictory nature is rather significant.

5. Salicin broth (Fermentation with gas production).
Kligler(1915) suggested salicin to replace dulcitol in the subdivision of coli like bacteria. It was shown to have a closer correlation. However the correlation of salicin with the Voges Proskauer test in the 27 pure cultures was very poor.

Culture Media Used: Reactions and their Significance,pg 3.

6. Motility. This has been assumed to be a differentail factor with B. coli as motile and B. aerogenes(non-fecal) as non-motile. Levine found only 32% of 25 cultures from man to be motile. This was lower in sewage and higher from feces of animals. The writer did not obtain satisfactory tests from his Hesse agar inoculations. The data on the 27 pure cultures was obtained by the hanging drop method. 21 out of 27 cultures being motile did not give a very close correlation with the Voges Proskauer reaction. These tests were carefully made and checked by experienced bacteriologists.

7. Gelatin liquefaction. B. Cloacae is a lactose fermenter, gelatin liquefier. None of the 27 cultures proved to be liquefiers though other cultures readily liquefied gelatin from the same batch.

8. Litmus Milk. Coagulation and Acid Formation. This is an important reaction for the identification of the colon group.. All of the 27 cultures gave the acid reaction. 11 gave complete coagulation, 9 gave partial and the remaining 7 gave no coagulation.

9. Indol. Production of Indol from Peptone. English practice rates this reaction high classing all indol formers as"typical B. coli". All 27 of the pure cultures formed indol, with check media giving some negative reactions. This gave no correlation with other reactions.

Culture Media Used: Reactions and their Significance, pg 4.

10. Ratio of Carbon Dioxide to Hydrogen gas from dextrose. The ratio of gas prodiction of CO2 to H2 in B aerogenes has been found to be 2.0 while the ratio for B coli is 1.0. This has been proved to be a very constant reaction, but quantitative measurement requires a great deal of care. If the culture stands too long the CO2 is reabsorbed. Using Smith tubes with the 27 pure cultures the writer found he had too small amounts of gas in 18 of the cultures to get gas measurement. Some of the measurements made on the remaining cultures were not satisfactory. Time did not permit the repeating of this test.

11. Methyl Red Reaction. This tests the acidity or alkalinity of an incubated culture in 0.5% dextrose-peptone di-sodium phosphate medium. . B Coli will ferment enough acid to give an acid or positive reaction. The continued growth of B aerogenes breaks down acids and liberates X alkali thus giving a negative or alkaline reaction. This ranks second only to the Voges Proskauer reaction as a differentiating medium. A weakness of this test is that it is very dependent on exact adjustment of the media. The tests of the 27 pure cultures gives this reaction as a close second to the Voges Proskauer reaction.

Culture Media Used: Reactions and their significance, pg 5.
XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX

12. Voges Proskauer (Acetyl Methyl Carbinol).

"In the products of dextrose(glucose) XXXXXXXXXXXXXXXXXX decomposition by B. Aerogenes a X crude glycol is formed which upon oxidation yields acetyl-methyl-carbibol a volatile reducing substance, which when mixed with potassium hydroxide in the presence of peptone, imparts an eosin-like coloration to the mixture on standing. XXXXX (Above is quotation from Levine). This reaction is given by B. aerogenes but not by B. coli. Levine sums the advantages of this reaction as follows: XX

1. Any peptone medium in which the organisms will grow and which contains glucose(dextrose)(in a wide range of concentration) is suitable. It is preferable,however, to have the medium as free from color as possible.

2. The reaction may be obtained after 14 to 24 hours incubation at 30 degrees or 37 degrees C.

3. The brand of peptone employed does not affect the intensity of the reaction.

A comparison of the Voges Proskauer, Methyl Red, Uric Acid, and Citrate Medium indicated that the Voges Proskauer reaction to give the best correlation with the Methyl Red a close second and the other two together and far behind.

Culture Media Used: Reactions and their Significance, pg. 6.

13. Uric Acid. B aerogenes can utilize nitrogen from uric acid, while B. coli cannot. On the 27 pure cultures this reaction gave a correlation of 18 out of 22 cultures as determined by a majority of V.P.,M.R. CITRATE,&uric acid reactions.

14. Citrate Medium(Koser, Jour. Bact., Jan., 1924). Citrate medium has been used to differentiate the coli and aerogenes types. A wide variety of tests completed by Koser seems to show an almost exact correlation between citrate utilization and both the fecal and non-fecal aerogenes bacilli. Of the soil cultures with Citrate 97.2% positive the Methyl Red positive and Voges Proskauer negative reactions were 34.7%. This would seem to show that citrate utilization shouldcontrol over the M.R. and V.P. reactions in indicating a culture to be of non-fecal origin. This would change pure cultures #10, #21, #24 & #24 from fecal to non- fecal, and would establish #16, #19, #20, & #25 as non-fecal.

15. Endo Agar (Levine). "Levine suggested a modified and simplified Endo medium which requires no adjustment of reaction and which need not be filtered. Aside from simplicity of preparation, an advantage is claimed that B. coli may be differentiated from B. aerogenes, The former possess a distinct metallic sheen, the colonies are flat and button like, and about 2 or 3 mm in diam.; whereas the latter produces considerably larger colonies which are convex and a metallic sheen is rarely observed" (pg 62, Bull. 62,Levine '21

Culture Media Used: Reactions and their Significance,pg7.
16. Eosin-Methylene Blue Agar (Levine, 1917).
Differentiation : (Table XXVII, Bulletin 62, Iowa State)
Bact coli: Size- Well isolated colonies are 2-3 mm in diam. Confluence- Neighboring colonies show little tendency to run together. Elevation- Colonies slightly raised; surface flat or slightly concave, rarely convex. Appearance by transmitted light- Dark almost black centers which extend more than ½ across the diameter of colony; internal structure of central dark portion hard to discern. Appearance by reflected light- Colonies dark, button- like, often concentrically ringed with a greenish metallic sheen. Bact. aerogenes: Size- Well isolated colonies are larger than coli; usually 4-6 mm in diameter or more. Confluence- Neighboring colonies run together quickly. Elevation- Colonies remarkably raised and markedly convex; occasionally the center drops precipately. Appearance by transmitted light- Centers deep brown; not so dark as B. coli and smaller in proportion to the rest of the colony. Striated internal structure often observed in young colonies. Appearance by reflected light- Much lighter than B. coli. Metallic sheen not observed except occasionally in depressed center when such is present.
Practically all of the 139 field samples obtained were run through this media but the differentiation was not noted in the data. "The advantages claimed for the media is the 1. Ease preparation. 2. Relative permanency. 3. Value as differentiating medium.

Pure Culture No. 1 (11-1a)

Source: Spring on rainy day.

Short Rods, Gram negative. Motile.

Gelatin stab: No liquefation.

Agar Slant: Abundant white moist growth.

Litmus Milk: Acid, no XXXXXXXXXXXX coagulation.

Gas in dextrose, lactose, dulcitol, and salicin.

CO_2 and H_2 gas in equal quantities in glucose.

Methyl Red negative.

Voges Proskauer positive.

Uric Acid negative.

Growth in Citrate medium in 3 days.

Indol positive.

Classification;

Jackson: Bact. Communis.

MacConkey: B. Coli.

Levine: Atypical E.

Bahlman & Sohn: Atypical D (Gel -).

Winslow, Kligler & Rothberg: Atypical.

Bergey: Aerobacter Archibaldi (Salicin -)

Citrate Medium: Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal

Pure Culture No. 2. (11-3).

Source: Spring, on rainy day.

Short, Gram negative, non-motile rods.

Gelatin stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid, partial coagulation.

Gas in Dextrose, lactose, Dulcitol and salicin.

Ratio of CO_2 to H_2 gas is 1.5.

Methyl Red negative.

Voges Proskauer positive.

Uric Acid negative.

Growth in Citrate medium in 3 days.

Indol positive.

Classification.

MacConkey: B. Coli.

Jackson: B. Communis.

Levine: Atypical D.

Bahlman & Sohn: Atypical D (Gel -).

Winslow, Kligler and Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal.

Pure Culture, No. 3. (407 c.lg.)

Source: Stream, two days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. No XXXXX coagulation.

Gas in dextrose, lactose, Saccharose, dulcitol & salicin.

CO_2 and H_2 gas ratio in glucose. Not enough gas in tube.

Methyl Red positive.

Voges Proskauer X negative.

Uric Acid negative.

No growth in Citrate medium in 21 days.

Indol positive.

Classification.

MacConkey: B. Neapolitanum.

Jackson: B. Communior.

Levine: B. Communior.

Bahlman and Sohn: B. Coli.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Esch. Communior. (No coagulation of Litmus Milk).

Citrate Medium: Non Fecal.

Methyl Red: XXXXX Fecal.

Voges Proskauer: Fecal.

Pure Culture, No. 4. (407aer).

Source: Stream, two days after rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid and coagulation.

Gas in dextrose, lactose, saccharose and salicin.

Twice as much CO_2 gas as H_2 gas in glucose.

Methyl Red negative.

Voges Proskauer positive.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkey: B. Aerogenes.

Jackson: B. Aerogenes.

Levine: Atypical C.

Bahlman and Sohn: B. Aerogenes.

Winslow, Kligler and Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal.

Pure Culture, No. 5. (512).

Source: Stream, two days after rain.

Short, Gram negative, motile rods.

Gelatin : No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid and coagulation.

Gas in dextroseX, lactose, saccharose and salicin.

CO_2 and H_2 gas ratio is 2.3.

Methyl Red negative.

Voges Proskauer positive.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkey: B. Aerogenes.

Jackson: B. Aerogenes.

Levine: Atypical C.

Bahlman & Sohn: B. Aerogenes.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal.

Pure Culture, No. 6. (607-2)

Source: Spring, on rainy day.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid and coagulation.

Gas in dextrose, lactose, saccharose and dulcitol.

Too small amount in dextrose(glucose) for CO_2 & H_2 gas ratio.

Methyl Red positive.

Voges Proskauer: negative.

Uric Acid negative;

No growth in Citrate medium in 21 days.

Indol positive.

Classification.

MacConkey: B Neapolitanum.

Jackson: B. Communior.

Levine: B. Communior.

Bahlman & Sohn: B. Coli.

Winslow, Kligler & Rothberg: B. Communior.

Bergey: Escherichia Communior.

Citrate Medium: Non Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal.

Pure Culture, No. 7 (608-1).

Source: Spring, on rainy day.

Short, Gram negative, non-motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. No coagulation.

Gas in dextrose, lactose, saccharose, dulcitol and salicin.

Ratio CO_2 to H_2 gas 0.2.

Methyl Red positive.

Voges Proskauer positive.

Uric Acid positive.

Growth in Citrate medium in 3 days.

Indol positive.

Classification.

MacConkey: B. Neapolitanum.

Jackson: B. Communior.

Levine: Atypical G.

Bahlman & Sohn: Atypical B.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Aerobacter oxytocum (salicin plus).

Citrate Medium: Fecal.

Methyl Red: Fecal.

Voges Proskauer: Non Fecal.

Pure Culture, No. 8 (610).

Source: Spring, on rainy day.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid, slight coagulation.

Gas in dextrose, lactose, saccharose, dulcitol and salicin.

Ratio of CO_2 and H_2 gas is 0.3.

Methyl Red positive.

Voges Proskauer positive.

Uric Acid positive.

Growth in Citrate medium in 3 days.

Indol positive.

Classification.

MacConkey: B Neapolitanum.

Jackson: B. Communior.

Levine: Atypical H.

Bahlman & Sohn: Atypical B.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Fecal.

Methyl Red: Fecal.

Voges Proskauer: Non Fecal.

Pure Culture, No. 9 (611).

Source: Spring, on rainy day.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, moist growth. White, turning brown.

Litmus Milk: Acid, slight coagulation.

Gas in dextrose, lactose, saccharose, dulcitol & salicin.

CO_2 and H_2 gas in equal amounts from dextrose(sucrose).

Methyl Red negative.

Voges Proskauer positive.

Uric Acid positive.

No growth in Citrate medium in 21 days.

Indol faintly positive.

Classification:

Mac Conkey: B. Neapolitanum.

Jackson: B. Communior.

Levine: Atypical C.

Bahlman & Sohn: B. Aerogenes.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Non Fecal.

Methyl Red: Non Fecal

Voges Proskauer: Non Fecal

Uric Acid: Non Fecal.

Pure Culture, No. 10 (612).

Source: Spring, on rainy day.

Short, Gram negative, Non-motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, White, moist growth.

Litmus Milk: Acid, no coagulation.

Gas in dextrose, lactose, dulcitol and salicin.

Too little amount of gas in tube for CO2 & H2 ratio.

Methyl Red positive.

Voges Proskauer negative.

Uric Acid negative.

Growth in Citrate medium in 4 days.

Indol positive.

Classification:

MacConkey: B. Coli.

Jackson: B Communis.

Levine: B. Coli.

Bahlman & Sohn: B. Coli.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal

Uric Acid: Non Fecal.

Pure Culture, No.11 (613-1).

Source: Spring, on rainy day.

Short, Gram negative, non-motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. No coagulation.

Gas in dextrose, lactose, dulcitol and salicin.

Too little amt. gas in dextrose for CO2 & H2 gas ratio,

Methyl Red negative.

Voges Proskauer positive.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkey: B. Aerogenes.

Jackson: B. Aerogenes.

Levine: B. Aerogenes.

Bahlman & Sohn: B. Aerogenes.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal.

Uric Acid: Non Fecal.

Pure Culture, No. 12 (701 sm).

Source; Stream, two days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, moist growth. White, turning to brown.

Litmus Milk: Acid, slight coagulation.

Gas in dextrose, lactose, saccharose, dulcitol & salicin.

No CO_2 and H_2 gas ratio in glucose. Not enough gas.

Methyl Red positive.

Voges Proskaurr negative.

Uric Acid positive.

No growth in Citrate medium in 21 days.

Indol positive.

Classification:

MacConkey: B. Neapolitanum.

Jackson: B. Communior.

Levine: Atypical B.

Bahlman & Sohn: Atypical U P C.

Winslow, Kligler & Rothberg: B. Neapolitanum.

Bergey: Escherichia Communior.

Citrate Medium: Non Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal.

Pure Culture, No. 13 (703 sm).

Source: Stream, two days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Coagulated.

Gas in dextrose, lactose, saccharose, dulcitol & salicin.

No CO_2 & H_2 gas ratio in glucose, too little gas.

Methyl Red positive.

Voges Proskauer negative.

Uric Acid negative.

No Growth in Citrate medium in 21 days.

Indol positive.

Classification:

MacConkey: B. Neapolitanum.

Jackson: B. Communior.

Levine: B. Communior.

Bahlman & Sohn: B. Coli.

Winslow, Kligler & Rothberg: B. Neapolitanum.

Bergey; Escherichia Communior.

Citrate Medium: Non Fecal.

Methyl Red: Fecal

Voges Proskauer: Fecal.

Uric Acid: Fecal.

Pure Culture, No. 14 (706 sm).

Source: Well, with force pump. Concrete curb. In barnyard.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Coagulation.

Gas in dextrose, lactose, dulcitol and salicin.

No ratio of CO_2 to H_2 gas. Too little gas in dextrose tube.

Methyl Red positive.

Voges Proskauer negative.

Uric Acid negative.

No growth in Citrate medium in 21 days.

Indol positive.

Classification:

MacConkey: B. Coli.

Jackson: B Communis.

Levine: B. Coli.

Bahlman and Sohn: B. Coli.

Winslow, Kligler & Rothberg: B. Neapolitanum.

Bergey: Escherichia Coli.

Citrate Medium: Non Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal.

Uric Acid: Fecal.

Pure Culture, No. 16 (708 sm).

Source: Stream, two days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Slight coagulation.

Gas in dextrose, lactose and saccharose.

Ratio of CO_2 to H_2 gas is 0.7.

Methyl Red negative.

Voges Proskauer positive.

Uric Acid negative.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkey: B. Aerogenes.

Jackson: B. Aerogenes.

Levine: Atypical J.

Bahlman and Sohn: Atypical D (Gel -).

Winslow, Kligler & Rothberg: Atypical.

Citrate Medium: Fecal.

Bergey: Escherichia Ichthyosmia.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal.

Pure Culture, No. 16 (709 endo).

Source: Spring, two days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Partial coagulation.

Gas in dextrose and lactose.

Not enough gas in dextrose tubes for CO_2 to H_2 ratio.

Methyl Red positive.

Voges Proskauer negative.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkeys: B. Acidi-lacti.

Jackson: B Acidi-lacti.

Levine: Atypical A.

Bahlman & Sohn: Atype U P C.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Escherichia Pseudodysenteriae (Lit Milk acid).

Citrate Medium: Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal.

Uric Acid: Non Fecal.

Pure Culture, No. 17 (710-1).

Source: Stream, two days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Coagulated.

Gas in dextrose, lactose, saccharose and salicin.

Ratio CO_2 to H_2 gas is 0.7.

Methyl Red negative.

Voges Proskauer positive.

Uric Acid positive.

Growth in Citrate medium in 3 days.

Indol Positive.

Classification.

MacConkey: B. Aerogenes.

Jackson: B. Aerogenes.

Levine: Atypical C.

Bahlman & Sohn: B Aerogenes.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal.

Uric Acid: Non Fecal.

Pure Culture, No. 18 (710-2).

Source: Stream, two days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Coagulated.

Gas in dextrose, lactose, saccharose, dulcitol & Salicin.

Ratio of CO_2 To H_2 gas is 1.0.

Methyl Red negative.

Voges Proskauer positive.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkey: B Neapolitanum .

Jackson: B. Communior.

Levine: Atypical C.

Bahlman & Sohn: B. Aerogenes.

Winslow, Kligler, & Rothberg: Atypical.

Bergey: Atypical.

Citrate Medium: Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal.

Uric Acid: Non Fecal.

Pure Culture, No. 19 (802).

Source: Spring, three days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Partial coagulation.

Gas in dextrose, lactose.

Not enough gas for CO_2 and H_2 ratio in dextrose.

Methyl Red positive.

Voges Proskauer negative.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol Positive.

Classification:

MacConkey: B. Acidi-lacti.

Jackson: B. Acidi-lacti.

Levine: Atypical A.

Bahlman & Sohn: Atypical U P C.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Pseudodysentericae, Escherichia (Acid Litmus Milk).

Citrate Medium: Non-Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal

Uric Acid: Non Fecal.

Pure Culture: 20 (805).

Source: Spring, three days after rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Coagulation.

Gas in dextrose and lactose.

Ratio CO_2 to H_2 gas is2.3

Methyl Red positive.

Voges Proskauer positive.

Uric Acid negative.

Growth in Citrate medium in 3 days.

Indol positive.

Classification:

MacConkey: B.Acidi-lacti.

Jackson: B. Acidi-lacti.

Levine: Atypical I.

Rahlman & Sohn: Atypical A.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Escherichia Neapolitanus.

Citrate Medium: Non-Fecal

Methyl Red: Fecal.

Voges Proskauer: Non Fecal.

Uric Acid: Fecal.

Pure Culture, No. 21 (807aer)

Source: Spring, three days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. No coagulation.

Gas in dextrose and lactose.

Ratio CO_2 to H_2 gas is 0.0

Methyl Red positive.

Voges Proskauer negative.

Uric Acid negative.

Growth in Citrate medium in 3 days.

Indol positive.

Classification:

MacConkey: B. Acidi-lacti.

Jackson: B Acidi-lacti.

Levine: B. acidi-lacti.

Bahlman & Sohn: B. Coli.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Escherichia Pseudodysenterieae (Lit Milk Acid).

Citrate Medium: Non-Fecal

Methyl Red: Fecal.

Voges Proskauer: Fecal.

Uric Acid: Fecal.

Pure Culture, No. 22 (Sillner).

Source: Spring, three days after a rain.

Short, Gram [illegible] negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, moist growth. White, turning to brown.

Litmus Milk: Acid. Coagulation.

Gas in dextrose, lactose and salicin.

Ratio CO_2 to H_2 gas is 1.5

Methyl Red negative.

Voges Proskauer positive.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkey: B. Acidi-lacti.

Jackson: B. Acidi-lacti.

Levine: Atypical X.

Kahlman & Sohn: B Aerogenes.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Atypical.

Citrate medium: Non-Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Non Fecal.

Uric Acid: Non Fecal.

Pure Culture, No. 23 (Sllendo).

Source: Spring, three days after a rain.

Short, Gram negative, non-motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Coagulation.

Gas in dextrose and lactose.

Not enough gas for CO_2 to H_2 ratio in dextrose.

Methyl Red positive.

Voges Proskauer negative.

Uric Acid negative.

Growth in Citrate Medium in 14 days.

Indol positive.

Classification:

MacConkey: B Acidi-lacti.

Jackson: B. Acidi-lacti.

Levine: B. Acidi-lacti.

Bahlman & Sohn: B. Coli.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Escherichia Vesiculosa.

Citrate Medium: Non-Fecal.

Methyl Red: Fecal .

Voges Proskauer: Fecal.

Uric Acid: fecal.

Pure Culture, No. XXX 24 (812).

Source: Spring, three days after a rain.

Short, Gram negative, XXX-motile rods.

Gelatin Stab; No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Partial coagulation.

Gas in dextrose, lactose, dulcitol & salicin.

Not enough dextrose gas for CO_2 to H_2 gas ratio.

Methyl Red positive.

Voges Proskauer negative.

Uric Acid negative.

Growth in Citrate medium in 3 days.

Indol positive.

Classification:

MacConkey: B. Coli.

Jackson: B. Communis.

Levine: B. Coli.

Bahlman & Sohn: B. Coli.

Winslow, Kligler & Rothberg: B. Coli:

Bergey: Escherichia Coli.

Citrate Medium: Non-Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal.

Uric Acid: Fecal.

Pure Culture, No. 25 (904).

Source: Spring, five days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid, Coagulation.

Gas in dextrose, lactose, saccharose, dulcitol & salicin.

Ratio CO_2 to H_2 gas in dextrose is 0.8

Methyl Red positive.

Voges Proskauer negative.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkey: B. Neapolitanus.

Jackson: B. Communior.

Levine: Atypical B.

Bahlman & Sohn: Atypical U P C.

Winslow, Kligler & Rothberg: B. Neapolitanus.

Bergey: Escherichia Communior.

Citrate Medium: Non-Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal.

Uric Acid: Non Fecal.

Pure Culture, No. 26 (907).

Source: Stream, five days after a rain.

Short, Gram negative, motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. No coagulation.

Gas in dextrose, lactose, Saccharose & salicin.

Ratio of CO_2 to H_2 gas in dextrose is0.3

Methyl Red negative.

Voges Proskauer negative.

Uric Acid positive.

Growth in Citrate medium in 2 days.

Indol positive.

Classification:

MacConkey: B. Coli.

Jackson: B. Coli.

Levine: Atypical F.

Rehlman & Sohn: Atypical E.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Escherichia Pseudocoloides.

Citrate Medium: Non-Fecal.

Methyl Red: Non Fecal.

Voges Proskauer: Fecal.

Uric Acid: Non Fecal.

Pure Culture, No. 27 (910).

Source: Stream, five days after rain.

Short, Gram negative, non-motile rods.

Gelatin Stab: No liquefaction.

Agar Slant: Abundant, white, moist growth.

Litmus Milk: Acid. Partial coagulation.

Gas in dextrose, lactose, saccharose, dulcitol & salicin.

Ratio of CO_2 to H_2 gas in dextrose is 0.0

Methyl Red positive.

Voges Proskauer: negative.

Uric Acid negative.

No growth in Citrate Medium in 21 days.

Indol positive.

Classification:

MacConkey: B. Neapolitanum.

Jackson: B. Communior.

Levine: B. Neapolitanum.

Bahlman & Sohn: B. Coli.

Winslow, Kligler & Rothberg: Atypical.

Bergey: Escherichia Communior (Non-motile).

Citrate Medium: XXX Fecal.

Methyl Red: Fecal.

Voges Proskauer: Fecal.

Uric Acid: Fecal.

Culture Media Used in the Experiments.

1. Agar Slant.

Weigh out $1\frac{1}{2}$ grams of agar agar; 1 gram of peptone and 0.2 gram of Na_2HPO_4.

Dissolve agar in 50 cc of tap water.

Dissolve peptone and Na_2HPO_4 in 50 cc of tap water.

When both are completely dissolved, allow to cool and filter broth thru filter paper. Then mix, filter thru cotton plug, tube, plug and sterilize in autoclave under 15 pounds pressure for 15 minutes.

2. Eosin-Methylene Blue Agar.

1. Prepare agar in the usual way and sterilize in amounts of 100 cc in flasks.

2. To 100 cc of hot fluid agar add 1 gram lactose, 2 cc of 2% Eosin, and 2 cc of $\frac{1}{2}$% Methylene Blue. Mix thoroughly. Pour in sterilized petri disher and inoculate within 24 hrs.

3. Endo Agar. (After Levine's Simplified Endo's Medium)

1. Prepare agar as in Eosin-Methylene Blue Agar.

2. To 100 cc of melted agar add 1 gram lactose, 0.5 cc of 10%(saturated) alcoholic solution of basic fuchsin, and 2.5 cc of freshly prepared 10% sodium sulphite solution. Pour into plates. Inoculate XXXXXXXXX in ordinary way.

Culture Media Used in Experiments. Page 2.

4. Hesse Agar.

Weigh out $\frac{1}{2}$ gram of agar agar, 1 gram of peptone, and 0.2 gram Na_2HPO_4. Dissolve agar in 50 cc of tap water. Dissolve peptone and salt in 50 cc of tap water. When both are completely dissolved, allow XXXXXXX broth to cool and filter thru filter thru filter paper. Then mix, filter thru cotton plug, tube, plug and sterilize in autoclave at 15 lbs. for fifteen minutes.

4a. Hesse Agar.

1. Wash agar by a 24 hour soak in water. Dissolve $\frac{1}{2}$ gram in 50 cc of water.

2. Dissolve 1 gram of peptone and 0.2 gram of Na_2HPO_4 in 50 cc of tap water. Heat at 60 degrees Centigrade until dissolved and let stand till liquid is cold. Filter thru filter paper. Add this broth to hot agar solution.

5. Lactose Broth.

Weigh out 1 gram of lactose, 1 gram of peptone and 0.2 gram of Na_2HPO_4. Dissolve in 100 cc of tap water. Cool. Filter thru filter paper. Tube and sterilize in autoclave under 15 pounds for 15 minutes.

6. Dulcitol.

Made as lactose. Substitute 0.1 gram of dulcitol for 1gram of lactose.

7. Saccharose(Sucrose).

Made the same as lactose. Substitute 1 gram of saccharose for 1 gram of lactose.

8. Salicin.

Made the same as lactose. Substitute 0.1 gram of salicin for 1 gram of lactose.

9. Dextrose(glucose).

Made the same as lactose. Substitute 1 gram of dextrose for 1 gram of lactose

10. Gelatin (For gelatin stabs).

Weigh out 12.5 grams of gelatin and 1 gram of peptone. Dissolve gelatin in 50 cc of tap water, being careful not to burn gelatin. Dissolve peptone in 50 cc of tap water.

When completely dissolved, allow broth to cool, filter through filter paper, and mix.

Take 5 cc sample and titrate with N/20 NaOH until neutral to phenol red. From the amount required, calculate the amount necessary for the remaining 95 cc, and add the same.

Filter through cotton plug, tube, plug, and sterilize 15 pounds for 15 minutes.

Place tubes in cold water after sterilization until gelatin solidifies.

Culture Media Used in the Experiments. Page 4.

11. Litmus Milk.

Dissolve 10.5 grams of Bacto dehydrated litmus milk in 100 cc of distilled water. Tube, plug and sterilize in the autoclave, 15 lbs. for 15 minutes.

12. Nutrient Broth (For Indol).

Weigh out 1 gram peptone and 0.2 gram of Na_2HPO_4 and dissolve in 100 cc of tap water. Allow to cool. Filter thru filter paper, tube, plug, and sterilize in autoclave at 15 pounds for 15 minutes.

These were inoculated from the pure cultures and incubated at 37 degrees Centigrade for 5 days.

To the 5 day culture is added 1 cc of 10% solution of H_2SO_4 and then 1cc of 1/5000 Potassium Nitrate is added so as to form a layer on the surface. If Indol is present, a red ring will develop at the junction of the nitrite and acidified peptone. This should show up within an hour.

13. Dextrose broth for Methyl Red Reaction.

Prepared the same as Dextrose (#9) but 0.5 gram instead of 1 gram of dextrose is used.

To a 5 day incubated (37 C) broth culture add a few drops of Methyl Red indicator. A yellow coloration indicates alkalinity or a negative reaction. A red coloration denotes acidity or a positive test.

Culture Media Used in the Experiments. Page 5.

14. Uric Acid Media. (Koser).

Distilled ammonia free water	1000 cc
NaCl	5 gram.
$MgSO_4$	0.2 Gram
CaCl	0.1 "
Na_2HPO_4	1.0 "
Glycerol	30.1 "
Uric Acid	0.5 "

This gives a colorless and clear medium. Tube, plug, and sterilize at 15 pounds for 15 minutes.

15. Smith Tube Dextrose Broth for CO_2 and H_2 gas ratio.

Use 1% dextrose broth as made in # 9.

16. Citrate Medium. (Koser #1).

NaCl.... 5 gram; $MgSO_4$...).2 gram; $(NH_4)H_2PO_4$..1.0 gram. 2 gram Sodium Citrate (2,77 grams Sodium Citrate 5½ H_2O). in 1000 cc distilled water. Koser states that the above to be PH 6.7 to 6.9. $(NH_4)H_2PO_4$ was not available so $(NH_4)_2HPO_4$ was substituted. Upon titration the substitute media was found to be the same as the suggested(or Std.) media(PH 6.7 to 6.9).

Koser gives a second formula for citrate medium on page 63, Journal of Bacteriology for January,1924.

HISTORY OF WATER SAMPLE NUMBER 11.

Water Sample No. 11 was taken from a very small, housed, dry-weather spring, West side of Little Beaver road and under a high ridge. In N. E. ¼ Sec. 19-37-8. Sample taken at 2:30 P. M. Saturday, November 3, 1923, after a nine hour rain in the early morning.

11-A. 1 c.c. sample inoculated into Durham's fermentation tube of lactose broth at 4 P. M. November 3, (same day). No gas at 24 hrs., 5% gas at 46 hrs. and 10% gas at 8 days.

11-B. At 2 P. M. November 5, an Eosin Methylene-Blue Agar slant was inoculated from 11-A. At 24 hrs. no growth. Later, a slight growth resembling neither B. Coli nor B. Aerogenes groups.

11-C. At 2 P. M. November 6, an Eosin Methylene-Blue Agar slant was inoculated from 11-A. This grew but later seem to be contaminated.

11-D. At 4 P. M. November 8,plant from 11-B into lactose fermentation tube. No gas at 21 hrs.,43 hrs. or 8 days.

11-E At 4 P. M. November 8,plant from 11-B into lactose fermentation tube. No gas at 21 and 43 hours, 5% at 8 days.

11-F. At 11 A. M. November 14,plant from 11-B upon agar slant. Fairly profuse growth. Gram stains show Gram negative Coli like rods. On November 19, it was noticed that the growth was contaminated. Gram stains showed two types of Bacillis and one cocci. At 4 P. M. November 19, petri dishes 11-M & N are plated from this slant.

HISTORY OF WATER SAMPLE NUMBER 11. (CONT. PG.2)

11-G. At 11 A. M. November 14, a lactose tube is inoculated from 11-B. No gas at the end of 5 days.

11-H. At 1 P. M. November 8, a lactose tube is inoculated from 11-A. 10% gas at the end of 8 days.

11-J. Same conditions and same growth as in 11-H

11-K. At 11 A. M. November 14, inoculate dulcitol tube from 11-B. 5% gas at 48 hrs and at 5 days.

11-L. At 11 A. M. November 14, inoculate saccharose tube from 11-B. 5% gas at 48 hrs and at 5 days.

11-M & N. At 4 P. M. November 19, two petri dishes plated from 11-F. Growth no.1 was concentrically ringed and almost transparent. Growth no. 2 was white and flaky. Growth no. 3 occurred in small,round, grayish colonies.

11-O. (Pure culture 11-1a). Growth no. 1 inoculated upon agar slant. Gram negative coli like rods on stains. Inoculation of November 28 on carbohydrate media showed no gas from lactose, gas from dulcitol at 43 hrs and from saccharose at 4 days. Analysis for symbiotic action on lactose started December 6 showed the following:
11-O pure, gas at 45 hrs. 2%; 11-[illegible] pure, no gas;
11-R pure, gas at 45 hrs. 10%. The mixed cultures for symbiotic action need not be included as the above shows we have isolated two lactose fermenters. On January 4, 1924 this culture was included among the pure cultures as 11-1a.

HISTORY OF WATER SAMPLE NUMBER 11. (CONT. PG.3).

11-P. (11-1b). Inoculated on an agar slant at the same time and from the same growth as 11-O. In the earlier tests this proved to be an exact duplicate of 11-O so it was discarded in favor of 11-O.

11-Q. (XXXX culture 11-2). On November 22, growth no. 2 from 11-M & N, was inoculated upon an agar slant. Repeated tests showed it to be a non gas former in lactose, dulcitol and saccharose.

11-R. (Pure culture 11-3). On November 22, growth no. 3 from 11-M & N, was inoculated upon an agar slant. 11-R and 11-O were inoculated at the same time and on the same batches of culture media. 11-R differs from 11-O in these tests in being dulcitol minus and in fermenting 10% gas in 45 hrs on lactose cultures of December 6, 1923.

Summary.

11-O (Pure culture 11-1a)	11-R (Pure culture 11-3)
Agar slant Nov.22,1923	Agar slant Nov. 22, 1923
Result of lactose plants.	Results of lactose plants.
November X 28. No gas	November 28. No gas.
Dec. 6. 2% at 43 hrs	December 6. 10% at 43 hrs.
Jan 4,1924. at 24 hrs.	Jan. 4,1924. at 70 hours.

The writer has checked all media used above by getting both positive and negative results in inoculations from all batches.

Water Sample Trips, pagel.

1. Saturday, November 3, 1923. Millar and Butler.

Rain from mid-night to 9.A. M.. Cool, but not cold.

Samples #1 to #5 were taken near Lecoma road in the A.M. from 9:00 to 11:00. 1 cc samples from these were planted in XXXXXX lactose fermentation tubes at 1:00 P.M.

Samples #6 to #17 were taken from springs and streams near Bridge School House. 1 cc samples from these were planted in lactose fermentation tubes at 4:00 P.M. Samples taken in P.M.

2. Wednesday, November 7, 1923. Millar and Butler.

No rain since Saturday, November 3(4 days).

Samples with subscript "a" were taken from the same sources as the original numbers. Samples taken; 6a,7a,9a,10a,11a, 13a,14a,15a and 16a. 1 cc from each of the above samples was planted in lactose fermentation tubes.

Water Sample Trips, Page 2.

3. Saturday, November 24,1923. Millar and Butler.
A rain of Thursday, November 22, after a week of dry weather, effected stream and spring flow very little.
A frost [redacted] occurred during the preceding week.
Samples #101 to #112 were taken in Poole Hollow and around Newburg during the morning and 1 cc samples were planted in lactose tubes at 2:00 P.M.
Samples #113 to #121 were taken down Little Beaver valley in the afternoon, and 1 cc samples were planted in lactose tubes at 5:00 P.M.

4. Monday, November 26, 1923. Butler(trip on foot).
Five days since a rain. Cool and clear. Samples taken from sources near Rolla in P.M. Plant 1 cc in lactose, 5:30 P.M.

5. Friday, November 30, 1923. Millar, Butler and Mikell.
Cool and Clear. A rain the day before was preceded by a week of dry weather. Samples #301 to #309,taken from sources near Bridge School House in the afternoon were planted (1 cc) in lacrose tubes at 5:00 P.M.

Water Sample Trips, Page 3.

6. Saturday, December1, 1923. Millar, Butler & Christopher. Rained all day Thursday, Friday was clear, while Saturday was cloudy. Samples#401 to #409 were taken on trip to Yancy Mills. Trip made in the afternoon. Samples(1 cc) were planted in lactose tubes at 5:00 P.M.

7. Saturday, December 8, 1923. Millar, Butler and Mikell. Weather of week preceding; Monday, clear; Tuesday, snow; Wednesday and Thursday , thaw; Friday, clear.
Samples #501 to #513 were taken in Mill Creek valley, south of Newburg, on all day trip. Samples(1 cc) were planted in lactose tubes at 5:00 P.M.

8. Monday, December 10, 1923. Butler and Hauck.
Rainy, following a night of rain.
Samples #601 to #613 were taken from sources around Newburg in the afternoon. Samples(1 cc) were planted in lactose 6 P.M.

Water Sample Trips, Page 4.

9. Friday, December 14, 1923. Millar and Butler.
It rained Wednesday. Ground was frosty.
Samples #701 to #713 were taken from sources North and West of Rolla as far as Cave Spring creek. Trip in the P.M.
Samples(1 cc) planted in lactose tubes at 5:00 P.M.

10. Saturday, December 15, 1923. Butler and Crutcher.
Rain, Wednesday. Cloudy. Trip in P.M.
Samples #801 to #813 were taken from sources in and around Newburg. Samples (1 cc) planted in lactose at 7:00 P.M.

11. Monday, December 17, 1923. Butler and Hauck.
No rain for five days. Ground partly frozen. Trip in P.M.
Samples #901 to #913 were taken in Little Beaver valley from sources along and north of Rolla-Newburg road.
Samples #(1 cc) planted in lactose tubes at 5:00 P.M.

RESUME OF WATER SAMPLE TRIPS.

1, full day trip. 2 double trips(return at noon)
8 half day trips. Total number of trips, 13.

Detailed Description of Water Sample Sources, Page 1.

1. #1. Love Branch at Lecoma Road just above the mouth of Deible Creek. East side Sec. 13 twp. 37 rn 8. Septic tank of Rolla sewer system, discharges 1½ miles above. Water, cloudy. Drainage area, 2 square miles.

2. #2. Mouth of Deible Creek, East half Sec. 13- 37 -8. Slightly cloudy water. Drainage area 1¾ square miles.

3. #3. Creek west of Lecoma Road, in Sec 25-37-8. Water, only slightly cloudy. Drainage area 200 acres.

4. #4. Creek west of Lecoma Road in south part of Sec 25-37-8 Water, only slightly cloudy. Drainage area 500 acres.

5. #5. Clear stream (pool) in deep hollow in north center part Sec. 12-36-8. Drainage area 200 acres.

6. #6. Stream under Frisco Ry culvert in N. E. ¼ Sec 20-37-8. Clear. Drainage area 3 square miles.

7. #7. Small dry weather spring 200 feet S. E. of Bridge School House, under a medium height ridge (Sec 17-37-8).

8. #8. Very small dry weather spring, 150 feet N.W.of Bridge School House and under flat ridge. Sec 17-37-8.

9. #9. Small housed dry weather spring 1000 ft. from a ridge and about 1000 ft west of Bridge School House. Sec 17-37-8.

10. #10. Gollohon's spring- small housed dry weather spring. S. E. ¼ Sec 18-37-8., in narrow, shallow valley.

Detailed Description of Water Sources, page 2.

11.#11. Very small housed dry weather spring, west of Little Beaver road, and under high ridge. N. E. ¼ Sec 19-37-8.

12.#12. Small spring, mid-east part Sec.19-37-8. West of Little Beaver road.

13.#13. Large(small house) dry weather spring 75 ft east of Little Beaver creek,500ft from ridge. S.E.¼ Sec.19-37-8.

14.#14.Large open dry weather spring in shallow stream basin. 80 ft East of Little Beavar creek, 500 ft from ridge. In S.W.¼ Sec 17-37-8, and 1100 ft N.W.of Bridge School House.

15.#15. Small housed dry weather spring, south of Rolla-Newburg road in S.E.¼ Sec 8-37-8. Gentle slope to ridge.

16.#16. Little Beaver creek, south of Rolla-Newburg road. Between Sections 8 & 9 twp.37-8. South of paved ford. Drainage area 2½ square miles.

17.#17. Murky stream, south of M.S.M.Experimental Mine. North half Sec 15-37-8. Drainage area, 160 acres.

18.#6a. Same source as #6.

19.#7a. Same source as #7.

20.#9a. Same source as #9.

21.#10a. Same source as #10.

22.#11a. Same source as #11.

23.#13a. Same source as #13.

24.#14a. Same source as #14.

25.#15a. Same source as #15.

26.#16a. Same source as #16.

Detailed Description of Water Sample Sources, Page 3.

27. #101. Small flowing well- 1 inch side stream from 4 inch vertical pipe under about 4 ft of head. S.E.$\frac{1}{4}$ 23-37-9. 30 ft west of stream in Poole Hollow, and 1000ft. from Frisco culvert.

28. #102.Spring near head of small hollow in west side of Poole Hollow(med.d.w.spring). N.E.$\frac{1}{4}$ Sec 23-37-9.

29. #103. Fenced off(wood rails) small dry weather spring under bluff. N.W.$\frac{1}{4}$ Sec 24-37-9. Near farm buildings.

30. #104. Large, 10 inch diameter, flowing well, near end of deep draw on east side of Poole Hollow.N.W.$\frac{1}{4}$ 24-37-9.

31. #105. Poole Hollow(clear mountain) stream. Drainage area 1$\frac{1}{2}$ square miles. S.W.$\frac{1}{4}$ Sec 13-37-9.

32. #106. Gulley stream fed by small XXXXX springs. $\frac{1}{4}$ mile draw, pastured land. S.W.$\frac{1}{4}$ Sec 13-37-9.

33. #107.Little Piney creek at intersection of East side Section 22-37-9.

34. #108. In Newburg, Frisco spring,small (housed)2 inch pipe dry weather spring. Fair bluff behind. Sec 22-37-9.

35. #109. In Newburg. Boxed Moderate dry weather spring in hollow(near Rolla-Newburg road). Fair slopes. 23-37-9.

36. #110. Very small housed dry weather spring. East bluff, 50 ft east of Rolla-Newburg road. N.W.$\frac{1}{4}$ Sec 23-37-9.

37. #111. Small walled spring near head of flat draw. N.E.Corner, Sec 14-37-9.

Detailed Description of Water Sample Sources, page 4.

38. #112. North fork of Little Beaver creek, north of main Rolla-Newburg road. Section 8-37-8.

39. #113. Same as source no.6.

40. #114. Same as source no.7.

41. #115. Same as source no.9.

42. #116. Beaver creek, above mouth of Little Beaver. Large, clear stream. N.W.¼ Sec 30-37-8.

43. #117. Little Piney Creek, above the mouth of Beaver. West line 30-37-8. Large, fairly clear stream.

44. #118. Same source as #13.

45. #119. Same source as #11.

46. #120. Small open dry weather spring, west side of Little Beaver road under a shallow bluff. S.W.Cor. Sec 18-37-8.

47. #121. Same source as #14.

48. #201. South side Sinkum Hollow. Dry weather stream bed fed by 4 or 5 bed outcrop springs. Pastured. Human camp site. Section 2-37-8.

49. #202. Filtered clear pool in upper Sinkum Hollow, fed from stream bed sources. Section 3-37-8.

50. #203. Wet weather seep spring in steep gelley bed, 80 ft. east of road. N.E.Corner Sec. 2-37-8.

51. #204. Very small dry weather spring from covered gulley, on west side of main gulley on east side of golf links. 11-37-8.

52. #205. Very small dry weather spring at foot of waste dump S.W.end Frisco cut. S.E.¼ Sec 10-37-8.

Detailed Description of Water Sample Sources, page 5.

53. #206. Rock bottom pool in stream bed fed by seep springs. Drainage area 15 acres. 100 ft south of #52(#205).

54. #207. Creek bed fed by seep springs. Drainage area, 20 acres. N.W.¼ Sec 14-37-8.

55. #208. From mid-point on embankment Frisco pond. 2-37-8.

56. #209. Frisco pond. Embankment 50 south of #208. 2-37-8.

57. #301. Same source as #17.

58. #302. Same source as #6.

59. #303. Same source as #7.

60. #304. Same source as #9.

61. #305. Same source as #11.

62. #306. Same source as #13.

63. #307. Same source as #120.

64. #308. Same source as #14.

65. #309. Same source as #15.

66. #401. Beaver creek above Sand's bridge. S½ 34-37-8.

67. #402. Moderate walled dry weather spring. On hillside ledge, west slope of hollow 200 ft west of Sand's brigge. Piped water for hydraulic ram. S½ Sec 34-37-8.

68. #403. Small dry weather spring in Vodt's hollow. Old spring house is torn down. From rock ledge under bluff. S½ Sec 33-36-8.

69. #404. Yancy Mills large walled dry weather spring. South line Sec. 32-36-8.

Detailed Description of Water Sample Sources, page 6.

70. #405. Yancy Mills pond- above millrace. S½ Sec 32-36-8.

71. #406. Moderate dry weather cavern spring under 60 ft. sloping cliff. S.E.¼ Sec 4-35-8.

72. #407. Little Piney creek below mouth of William's branch. Section 4-35-8.

73. #408. Stream north of road. East branch at S.W.Corner Section 26-36-8. Drainage area 160 acres.

74. #409. Same as source #2.

75. #501. Yelton's spring. Small dry weather housed spring. Piped 200 ft. Flat slope. Section 33-37-9.

76. #502. Clear rocky bed stream fed from filtered seep springs. Drainage area 160 acres. Sec. 4-36-9.

77. #503. Moderate housed dry weather spring, 10 ft west of Mill creek road and under 60 ft ridge. Sec 8-36-9.

78. #504. Moderate dry weather spring(flowing from betwwen tree roots) in Mill creek road. 1 to 5 slppe. Sec 17-36-9.

79. #505. Large bubbling spring, feeding a pond. North line Sec. 20-36-9.

80. #506. Moderate dry weather spring, concreted wall,under cliffs(80 ft bluff). Sec. 29-36-9.

81. #507. Small leafy dry weather spring in creek bank. Slope 1 to 5 to 30 ft ridge. Sect. 34-36-9.

82. #508. Small dry weather spring under ledge in small hollow. Sect. 29-36-9.

Detailed Description of Water Sample Sourdes, page 7.

83. #509. Mill creek above spring pond outlet. Clear. Drainage area 10 square miles. Section 17-36-9.

84. #510. Hardester hollow creek abowe Mill creek road. Clear. Fair flow. Drainage 9 sq. miles. South line 8-36-9.

85. #511. Small spring in flat ground . Ridge 100 ft away. 100 ft east of road(Mill creek). Sec 8-36-9.

86. #512. Wagner branch above Mill creek road. Drainage area 2 square miles. South line Section 29-37-9.

87. #513. Mouth of Mill creek. Clear, rapid. East line sec 20-37-9.

88. #601. Very small boxed dry weather spring. Flat slopes. East of road. Sec. 10-37-9.

89. #602. Very small dry weather spring in stream bank under road. . Sec. 10-37-9.

90. #603. Very small dry weather spring, west side of road. Flat slopes. Section 10-37-9.

91. #604. Small housed concreted spring. Flat slope. Under 60 ft ridge. South line Section 10-37-9.

92. #605. Moderate dry weather spring, bubbling up near flat (10 ft) mount. 400 ft to high ground. Sec 15-37-9.

93. #606. Clear dry weather stream. Drainage area $\frac{3}{4}$ square ~~M~~ mile. E$\frac{1}{2}$ Sec 15-37-9.

94. #607. Moderate walled dry weather spring, at edge of flat 40 ft mound. East line Sec. 21-37-9.

Description of Water Sample Sources, page 8.

95. #608. Small dry weather spring. Flat X slope. Near edge of flat 60 ft ridge. South line Sec 22-37-9.

96. #609. Small dry weather spring under steep 80 ft hill. South half section 21-37-9.

97. #610. Small XXXX housed (pipeX) dry weather spring. At stream edge, under 15 ft flat. S½ Sec 22-37-9.

98. #611. Very small piped spring. 80 ft up on side of 200 ft hill. E½ Sec 27-37-9.

99. #612. Moderate dry weather spring in side of stream, below road,& at edge of 200 ft bluff. South line Sec 22-37-9.

100.#613. Very small seep springs in XXXXX steep side gulley in 150 ft hill. South line Sec 22-37-9.

101.#701. Sinkum Hollow. Clear stream west of road. South half Sec 35-38-8. Drainage area 1 square mile.

102.#702. Wet weather seep spring in meadow, 50 ft east of stream. West half section 35-38-8.

103.#703. Clear stream. Drainage area 1 square mile. South of road. West half section35-38-8..

104.#704. Convergence of two small,clear streams, below (North of) road. Drainage 300 acres. N.E.¼ Sec 34-38-8.

105.#705. Clear rock ledge stream. Drainage area 200 acres. North half,Section 34-38-8.

106.#706. Well. Lift pump. In barnyard. Good concrete X platform. N.W.¼ Sec 27-38-8.

Detailed Description of Water Sample Sources, page 9.

107.#707. Clear rock bed stream. Drainage area ¾ square mile. S.E.¼ Sec 32-38-8.

108.#708. Fable hollow branch. Clear stream. Drainage area, 1 square mile. Sec. 28-38-9.

109.#709. Small dry weather walled spring, 150 ft S. E. of Wynn School House. South line Sec 28-38-9.

110.#710. Cave Spring Creek- at rapids- clear. Drainage area about 6 square miles. E½ Sec 33-38-9.

111.#711. Mouth of an east branch to Cave Spring creek. Clear. Drainage, 3 square miles. East line section 33-38-9.

112.#712. Cave Spring creek. Swift. Slightly cloudy. Drainage area 2½ sq. miles. West half Section 2-37-9.

113.#713. Very small dry weather spring in creek bank. N.E.Corner Section 10-37-9.

114.#801. Same source as #601.

115.#802. Same source as #602.

116.#803. Same source as 603.

117.#804. Same source as #604.

118.#805. Same source as #605.

119.#806. Same source as #606.

120.#807. Same source as #607.

121.#808. Same source as #609.

122.#809. Same source as #109.

123.#810. Same source as 612.

Detailed Description of Water Sample Sources, page 10.

124.#811. Main stream in hollow north of Newburg. Clear. Drainage 1½ sq. miles. S.E.¼ Sec 15-37-9.

125.#812. Mouth of an East branch into #811. Drains 300 acres.

126.#813. Small stream. Drains 160 acres. E½ Sec 9-37-9.

127.#901. Housed rock ledge spring,creek bottom.N.E.¼-8-37-8.

128.#902. Clear stream. Drains 2 sq.miles. N.E.¼-8-37-8.

129.#903. Small boxed dry weather spg. N½-Sec 8-37-8.

130.#904. Small spring 30 ft north of #903.

131.#905. Rock ledge stream. Drains 300 acres. N½-8-37-8.

132.#906. Moderate dry weather spring. From 10ft.diameter mound water cress.. Meadow. Creek bank. Sec 5-37-8.

133.#907. Clear rock ledge stream.Drains 300 acres. sec 5-37-8.

134.#908. Walled housed dry weather spring. Low, flat slopes. East line Section 6-37-8.

135.#909. Gravelly stream. Drains 300 acres. E.line 6-37-8.

136.#910. Same source as #1h2.

137.#911. Same source as #15.

138.#912. Mouth of dry weather stream below 5 ft falls on north bank of Little Beaver. Drains 200 acres. S.E.¼-8-37-8.

139.#913. Same source as#16.

RESUME OF WATER SOURCES.

Springs total 54. Streams, 44. Well,1. Total is 99.

RESUME OF WATER SAMPLES.

Springs, 83. Streams, 55. Well, 1. Total, 139.

Complete tests of Pure Cultures – Gram Negative Rods.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
																Litmus Milk.		Source		Weather		
Number	Name	Dextrose	Lactose	Saccharose	Dulcitol(2)	Salicin	Methyl Red	V.P.	Uric Acid	Citrate	Motility	Indol	Dulcitol(1)	Gelatin	CO_2/H Glucose	Coag.	Acid		Date-Sample 1923		Number of Days since Rain	Number.
1	11-1a	24	24	—	40	24	—	+	—	3	+	+	—	—	1.0	—	+	Spring	11/3	+	——	1
2	11-3	24	70	—	40	24	—	+	—	3	—	+	—	—	1.5	⊕	+	Spring	11/3	+	——	2
3	407d	24	24	48	60	48	+	—	—	—	+	+	—	—	N.G.	—	+	Stream	12/1	—	2	3
4	407a	24	24	24	—	48	—	+	+	2	+	+	—	—	2.3	+	+	stream	12/1	—	2	4
5	512	24	49	24	—	24	—	+	+	2	+	+	—	—	2.3	+	+	stream	12/8	—	2	5
6	607-2	48	24	24	60	—	+	—	—	—	+	+	—	—	NG	+	+	spring	12/10	+	——	6
7	608-1	48	24	24	40	24	+	+	+	3	—	+	—	—	0.3	—	+	spring	"	+	——	7
8	610	29	45	24	70	24	+	+	+	2	+	+	—	—	0.3	⊕	+	spring	"	+	——	8
9	611	24	20	24	60	24	—	+	+	—	+	⊕	—	—	1.0	⊕	+	spring	"	+	——	9
10	612	24	45	—	40	24	+	—	—	4	—	+	—	—	N.G.	—	+	spring	"	+	——	10
11	613-1	24	24	24	—	48	—	+	+	2	—	+	—	—	N.G.	—	+	spring	"	+	——	11
12	701s	24	20	24	40	24	+	—	+	—	+	+	—	—	NG	⊕	+	stream	12/14	—	2	12
13	703s	24	20	24	60	48	+	—	—	—	+	+	—	—	NG	+	+	stream	"	—	2	13
14	706s	48	20	—	60	48	+	—	—	—	+	+	—	—	NG	+	+	well	"	—	2	14
15	708s	24	48	24	—	—	—	+	—	2	+	+	—	—	0.7	⊕	+	stream	"	—	2	15
16	709e	24	24	—	—	—	+	—	+	2	+	+	—	—	NG	⊕	+	spring	"	—	2	16
17	710-1	48	20	24	—	24	—	+	+	3	+	+	—	—	0.5	+	+	stream	"	—	2	17
18	710-2	24	24	24	24	24	—	+	+	2	+	+	24	—	1.0	+	+	stream	"	—	2	18
19	802	24	24	—	—	—	+	—	+	2	+	+	—	—	N.G	⊕	+	spring	12/15	—	3	19
20	805	24	54	—	—	—	+	+	—	3	+	+	—	—	2.3	+	+	spring	"	—	3	20
21	807a	24	41	—	—	—	+	—	—	3	+	+	—	—	0.0	—	+	spring	"	—	3	21
22	811a	24	54	—	—	24	—	+	+	2	+	+	—	—	1.5	+	+	stream	"	—	3	22
23	811e	24	24	—	—	—	+	—	—	14	—	+	—	—	N.G.	+	+	stream	"	—	3	23
24	812	24	24	—	40	24	+	—	—	3	+	+	—	—	N.G.	⊕	+	stream	"	—	3	24
25	904	24	20	24	60	24	+	—	+	2	+	+	—	—	0.5	+	+	spring	12/17	—	5	25
26	907	24	41	24	—	24	—	—	+	2	+	+	—	—	0.3	—	+	stream	"	—	5	26
27	910	24	24	24	70	48	+	—	—	—	—	+	—	—	0.0	⊕	+	stream	"	—	5	27
Number	Name	Dextrose	Lactose	Saccharose	Dulcitol(2)	Salicin	Methyl Red	V.P.	Uric Acid	Citrate	Motility	Indol	Dulcitol(1)	Gelatin.	CO_2/H Glucose	Coag. Litmus Milk.	Acid	Source	Date-Sample 1923	Rainy	Number of days since Rain Weather	Number

N.B. ⊕ indicates a weak positive reaction
Dulcitol - columns 2 and 6 - repeat test used.
{Carbon Dioxide and Hydrogen Ratio Column 16}
{N.G. indicates too small a quantity of gas for test}
Column 11 gives days, Other columns hours of time.

A Study of the Cultural Reactions and Classification of the 27 Pure Cultures. To differentiate Fecal from Non-Fecal Origin by weighting various reactions.

Comparison & Correlation of Indication of Fecal Origin.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	
MacConkey	+	+	+	−	−	+	+	+	+	+	−	+	+	+	−	−	−	+	−	−	−	−	−	+	+	+	+	
Jackson	+	+	+	−	−	+	+	+	+	+	−	+	+	+	−	−	−	+	−	−	−	−	−	+	+	+	+	
Levine	X	X	+	X	X	+	X	X	X	+	−	X	+	+	X	X	X	X	X	X	−	X	−	+	X	X	+	
B & S	X	X	+	−	−	+	X	X	−	+	−	X	+	+	X	X	−	−	X	X	+	−	+	+	X	X	+	
W.K&R.	X	X	X	X	X	+	X	X	X	X	X	+	+	+	X	X	X	X	X	X	X	X	X	+	+	X	X	
Bergey	+	X	+	X	X	+	−	X	X	X	X	+	+	+	−	+	X	X	+	+	+	X	−	+	+	+	+	
Citrate	−	−	+	−	−	+	−	−	+	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	+	
M.R.	−	−	+	−	−	+	+	+	−	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	−	+	
V.P.	−	−	+	−	−	+	−	−	−	+	−	+	+	+	−	+	−	−	+	−	+	−	+	+	+	+	+	
Uric Acid	+	+	+	−	−	+	−	−	−	+	−	−	+	+	+	−	−	−	−	+	+	−	+	+	−	−	+	
Comparison restricted to Citrate; M.R.; V.P. and Uric																												
Where Indic. is 2 to 2. Space is blank. Where indic. agrees with 2 or 3 others - 1 is put in place. 0-indicates media by itself.																												
Citrate	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1		1	1			0	1	0	0		1	1	18 OK
M.R.	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1		1	1			1	1	1	1		1	1	21 OK
V.P.	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1		1	1			1	1	1	1		0	1	22 OK
Uric Acid	0	0	1	1	1	1	1	1	1	1	1	0	1	1	0		1	1			1	1	1	1		1	1	19 OK
Origin as determined by 3 media Check.	Non-Fecal	Non Fecal	Fecal	Non Fecal	Non Fecal	Fecal	Non-Fecal	Non Fecal	Non Fecal	Fecal	Non Fecal	Fecal	Fecal	Fecal	Non Fecal		Non Fecal	Non Fecal			Fecal	Non Fecal	Fecal	Fecal		Non Fecal	Fecal	
All Characters - (Media) Showing where other classifications - reverse.																												
Majority	4	3	9	7	7	10	4	3	4	7	8	7	10	10	6	4	7	5	4	4	6	7	5	9	6	4	9	
Minority	3	3	0	0	0	0	3	3	3	1	0	1	0	0	1	3	0	2	3	3	4	0	4	1	2	3	0	
Atypical	3	4	1	3	3	0	3	4	3	2	2	2	0	0	3	3	3	3	3	3	1	3	1	0	2	3	1	
Reverse	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+	−	

X denotes Atypical reactions.

The 27 Pure Cultures classified according to Levine's Classification and showing Atypical cultures.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Number	Dextrose	Lactose	Saccharose	Salicin	V.P.	M.R.	Uric.	Motility	CO/H	Glycerol	Starch	Gelatin	Gram	Pure Cultures (Total 27.)	Total Cult	Number	
1	+	+	−	+	−	+	−		0			−	−	10, 14, 24	3	1	Bact. Coli.
2	+	+	−	−	−	+	−		0			−	−	21, 23.	2	2	Bact. Acidi-lacti.
3	+	+	+		−	+	−	+	0			−	−	3, 6, 13.	3	3	Bact. Communior
4	+	+	+	+	−	+	−	−	0			−	−	27.	1	4	Bact. Neapolitanum
5	+	+	+	−	−	+	−	−	0			−	−			5	Bact. Coscoroba
6	+	+			+	−	+	−	2t	+	+	−	−	11.	1	6	Bact. Aerogenes.
7	+	+			+	−	+	+	2t	−	−	+	−			7	Bact. Cloacae.
														Total typical	10	= 37 %	
8	+	+	−	−	−	+	+	+				−	−	16, 14	2	8	Atypical "A"
9	+	+	+	+	−	+	+	+				−	−	12, 25.	2	9	Atypical "B"
10	+	+	+	+	+	+	+	+				−	−	4, 5, 9, 17, 18	5	10	Atypical "C"
11	+	+	−	+	+	−	−	−				−	−	2.	1	11	Atypical "D"
12	+	+	−	+	+	−	−	+				−	−	1	1	12	Atypical "E"
13	+	+	+	+	−	−	+	+				−	−	26.	1	13	Atypical "F"
14	+	+	+	+	+	+	+	−				−	−	7	1	14	Atypical "G"
15	+	+	+	+	+	+	+	+				−	−	8.	1	15	Atypical "H"
16	+	+	−	−	+	+	−	+				−	−	20.	1	16	Atypical "I"
17	+	+	+	−	+	−	−	+				−	−	15	1	17	Atypical "J"
18	+	+	−	+	+	−	+	+				−	−	22	1	18	Atypical "K"

Total Atypical 17 = 63 %

Discussion.

1. The CO_2–Hydrogen gas Ratio in Glucose as determined by inoculating for all cultures proved to be generally indefinite and therefore was not included.

2. In classifying, the Voges Prostaur (V.P.), Methyl Red (M.R.) Uric Acid, Motility and Gelatin reactions were considered primary and any deviation from the specified reactions caused the culture to be classed as Atypical.

3. Glycerol and Starch were not used as media as no Gelatin liquefiers occurred in the 27 pure cultures.

Combination of Tables 3 & 4 in "Colon-Aerogenes Differentiation at Cincinnati" By Bahlman & Sohn. Pgs. 421, 421 of Journal Am. Water Works Assn. March 1924

1	2	3	4	5			
					Distribution of Atypical Cultures		
Number	M. R.	V. P.	Uric Acid	Our Designation	Number of Original Cultures	Number Cultures remaining After Repeat test.	After Rejuvenated
1	+	−	−	B Coli	——	——	——
2	+	+	−	Type A	0	0	0
3	+	+	+	Type B	3	1	0
4	+	−	+	Type UPC	43	28	4
5	−	−	−	Type C	16	12	8
6	−	+	−	Type D *	14	10	7
7	−	+	+	B Aerogenes	——	——	——
8	−	−	+	Type E	69	48	30
9	±	−	−	Type F	7	0	0
10	±	+	−	Type G *	1	0	0
11	±	+	+	Type H	3	0	0
12	±	−	+	Type I	5	5	0
13	−	−	−	C. Gel liqfr.	3	3	2
14	−	+	−	D. Gel liqfr.	6	6	1
15	−	−	+	E Gel liqfr	9	4	1
16	+	−	−	B Coli liqfr	8	8	0
Total Atypical					187	125	53

1. Types D & G liquefy Gelatin in 15 days.
Types classed as Gelatin liquefiers liquefy in 5 days.

Pure Cultures as classified by Bahlman & Sohn (Cincinnati)														
				Designation					Designation					Designation
1	−	+	−	D (Gel −)	10	+	−	−	B. Coli	19	+	−	+	U.P.C.
2	−	+	−	D (Gel −)	11	−	+	+	B. Aer	20	+	+	−	Type A
3	+	−	−	B. Coli	12	+	−	+	U P C	21	+	−	−	B. Coli
4	−	+	+	B. Aer.	13	+	−	−	B. Coli	22	−	+	+	B. Aer.
5	−	+	+	B. Aer.	14	+	−	−	B. Coli	23	+	−	−	B. Coli
6	+	−	−	B. Coli	15	−	+	−	D (Gel −)	24	+	−	−	B. Coli
7	+	+	+	Type B	16	+	−	+	U.P.C.	25	+	−	+	U.P.C.
8	+	+	+	Type B	17	−	+	+	B. Aer.	26	−	−	+	Type E
9	−	+	+	B. Aer	18	−	+	+	B. Aer.	27	+	−	−	B Coli

1. B Coli = 9 = 33.3 %
2. B Aer = 7 = 26.0 %
3. U.P.C. = 4 = 14.8 %

D (Gel −) = 3 = 11.1 %
B = 2 = 7.4 %
A = 1 = 3.7 %
E = 1 = 3.7 %

Typical 59.3 %
Atypical 40.7 %

Table of the Colon-Typhoid Series, by Winslow, Kligler and Rothberg, 1919 - Shown on page 56, Elements of Water Bacteriology, by Prescott and Winslow.
Also the classification of the Pure Cultures by this table.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Number	Hexoses	Maltose	Mannitol	Xylose	Arabinose	Rhamnose	Sorbitol	Dulcitol	Lactose	Salicin	Saccharose	Raffinose	Inosite	Dextrin	Gas	V. P.	Methyl Red	Litmus Milk.	Gelatin	Indol	Lead Acetate	Motility	Pathogenic	Species Bact
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Coag	−	−	−	−	−	Aerogenes
2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	Coag	+	−	−	+	−	Cloacae
3	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	+	Coag	−	+	−	+	−	Neapolitanus
4	+	+	+	+	+	+	+	+	+	−	+	+	−	−	+	−	+	Coag	−	+	−	+	−	Communior
5	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	−	+	Coag	−	+	−	+	−	Coli
6	+	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	+	Coag	−	+	−	+	−	Acidi-lacti
7	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	al.	−	+	+	+	+	Morgani
8	+	+	+	+	+	+	+	+	−	−	−	−	+	−	+	−	+	ac.al.	−	−	+	+	+	Schottmulleri
9	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	+	ac.al.	−	−	+	+	+	Enteritidus
10	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	−	+	ac.al.	−	−	−	+	+	Suipestifer
11	+	+	+	+	+	+	+	+	−	−	−	−	−	+	−	−	+	ac.al.	−	−	−	−	+	Gallinarium
12	+	−	+	−	+	+	+	−	−	−	−	−	−	−	−	−	+	ac.al	−	−	−	−	+	Pullorum
13	+	+	+	−	+	+	+	+	−	−	−	−	−	−	+	−	+	ac.al.	−	−	−	+	+	Paratyphosis
14	+	+	+	+	−	−	+	−	−	−	−	−	−	+	−	−	+	ac.al.	−	−	+	+	+	Typhosis
15	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	−	+	ac.al	−	+	+	−	+	Dysenteriae
16	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	ac.al.	−	−	−	−	+	Shigae
17	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	al.	−	−	+	+	−	Alcaligenes.

Pure Cultures based on Dulcitol, Salicin, Lactose, Saccharose, V.P., M.R., Litmus Milk, Indol, Motility and Gelatin gives the following.

B. Neapolitanus - 4 cultures (Numbers 12, 13, 14 & 25)
B. Communior - 1 culture - Number 6
B. Coli - 1 culture - Number 24
The remaining 21 cultures (78 %) are Atypical.

It will be noted that the true pathogenic members of this series, the dysentery, typhoid and paratyphoid organisms, all fail to ferment lactose.

Bergey's Manual of Determinative Bacteriology. 1923.
Key- Tribe Bacteregae - Species of Genera Escherichia & Aerobacter

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
											Litmus Milk			Habitat				Species		
Number	Dextrose	Lactose	Saccharose	Dulcitol	Salicin	Motility	Nitrates Reduced	Indol	V.P.	Gram Stain	Coag	Acid	Acid to Alkaline	Intestinal Canal-Man	Intestines Vertebrates	Nature, Soil, etc.	Genus	Number		Number
1	+	+	—	+	+	+	+	+	—	—	+	+	—	+	+	—	Escherichia	1	Coli	1
2	+	+	—	+	+	+	+	+	—	—	—	+	—	+	Milk	—		2	Schafferi	2
3	+	+	—	—	+	+	+	⊕	—	—	+	+	—	⊕	—	—		3	paragranthali	3
4	+	+	—	+	—	+	—	—	—	—	—	—	+	+	+	—		4	Vekanda	4
5	+	+	—	+	—	+	+	—	—	—	—	—	+	—	⊕	—		5	Anata	5
6	+	+	—	—	—	+	—	⊕	—	—	—	—	+	+	—	—		6	pseudodysenterieae	6
7	+	+	—	—	—	+	+	—	—	—	—	—	+	⊕	—	—		7	grunthali	7
8	+	+	—	+	—	—	—	+	—	—	+	+	—	+	Milk.			8	Acidi-Lacti	8
9	+	+	—	—	—	—	+	+	—	—	+	+	—	—	Cheese			9	vesiculosa	9
10	+	+	—	—	—	—	—	—	—	—	+	+	—	Slimy Milk				10	healii	10
11	+	+	+	+	+	+	+	+	—	—	+	+	—	+	+	—		11	Communior	11
12	+	+	+	—	+	+	+	+	—	—	+	+	—	+	—	—		12	peseudo coloides	12
13	+	+	+	—	—	+	+	+	—	—	—	+	—	evap. Milk				13	ichthyosmia	13
14	+	+	+	—	—	+	+	⊕	—	—	—	—	+	+	+	—		14	alkalescens	14
15	+	+	+	—	—	—	+	—	—	—	—	—	+	+	+	—		15	galactophila	15
16	+	+	+	—	—	+	—	—	—	—	—	—	—	—	Milk			16	symbiotica	16
17	+	+	+	—	—	+	+	—	—	—	+	+	—	+	+	—	Escherichia	17	gastrica	17
18	+	+	+	—	—	+	+	⊕	—	—	—	—	+	+	+	—		18	plebia	18
19	+	+	+	—	—	+	+	—	—	—	+	+	—	+	+	—		19	iliaca	19
20	+	+	+	+	+	—	+	+	—	—	+	+	—	+	—	—		20	neapolitana	20
21	+	+	+	—	—	—		+	—	—	+	+	—	⊕	⊕	—		21	pseudocoscorabae	21
22	+	+	+	—	—	—		—	—	—	+	+	—	—	⊕	—		22	astheniae	22
23	+	+	+	—	—	—	+	—	+	—	+	+	—	+	+	+	Aerobacter	1	aerogenes	23
24	+	+	+	+	—	—	+	+	+	—	+	+	—	Old Milk				2	oxytocum	24
25	+	+	—	—	—	—		—	+	—	+	+	—	India Ink				3	chiense	25
26	+	+	—	+	—	+	+	+	+	—	—	—	+	+	+	—		4	archibaldi	26
27	+	+	+	+	—	+	+	⊕	+	—	+	+	—	+	+	—		5	cloacae	27
28	+	+	+	—	+	+	+	⊕	+	+	+	+	—	Ferment Dough				6	Levans	28
Number	Dextrose	Lactose	Saccharose	Dulcitol	Salicin	Motility	Nitrates Reduced	Indol	V.P.	Gram Stain	Coag	Acid	Acid to Alkaline	Intestinal Canal-Man	Intestines Vertebrates	Nature Soil, etc.	Genus	Number		Number
											Litmus Milk			Habitat				Species		

N.B. ⊕ indicates an uncommon or doubtful positive Reaction.

Detailed Tests of Isolated Cultures.
The Gram Stains of these cultures show them to be mixed. As these were tested in the same media batches as the Pure Cultures this data is retained.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Number	Name	Dextrose	Lactose	Saccharose	Dulcitol (I)	Salicin	Methyl Red	V. P.	Uric Acid	Indol	Gelatin	Litmus Milk: Coag	Litmus Milk: Acid	Source	Date of Samples 1923	Weather: Rainy	Weather: Number of days since Rain		Number
1	407cs	24	24	48	−	48	+	−	−	+	−	−	+	stream	12/1	−	2		1
2	503a	24	45	−	−	24	+	−	+	+	−	+	⊕	spring	12/8	−	2		2
3	507a	24	24	24	−	24	+	−	+	+	−	+	⊕	spring	"	−	2		3
4	510c	24	24	24	24	24	+	+	+	+	−	−	+	stream	"	−	2		4
5	511c	24	45	−	24	−	+	−	+	+	+	+	⊕	spring	"	−	2		5
6	513c	24	24	24	24	24	+	+	+	+	−	+	+	stream	"	−	2		6
7	602c	24	24	24	−	24	+	+	+	+	−	+	+	spring	12/10	+	—		7
8	603a	24	24	24	−	24	−	+	+	−	+	−	⊕	spring	"	+	—		8
9	605	24	20	24	−	24	+	+	+	+	−	−	+	Spring	"	+	—		9
10	606r	48	45	24	−	−	−	−	+	+	+	+	+	stream	"	+	—		10
11	607-1	48	24	24	−	−	+	−	−	+	−	+	⊕	spring	"	+	—		11
12	613-2	48	24	24	−	−	+	−	−	+	−	+	⊕	spring	"	+	—		12
13	613-3	24	24	24	−	24	+	+	+	−	+	+	⊕	spring	"	+	—		13
14	701r	48	24	24	−	24	−	−	+	+	+	−	⊕	stream	12/14	−	2		14
15	702-s	48	20	−	−	48	+	−	−	+	−	−	+	spring	"	−	2		15
16	704a	24	41	24	−	48	−	+	−	+	+	−	⊕	stream	"	−	2		16
17	709a	24	48	24	−	72	−	+	−	−	−	−	+	spring	"	−	2		17
18	711s	24	20	24	−	24	+	+	+	+	−	+	+	stream	"	−	2		18
19	806a	24	20	24	−	24	+	+	+	+	−	+	⊕	stream	12/15	−	3		19
20	807r	24	41	−	−	−	−	−	+	+	+	−	⊕	spring	"	−	3		20
21	908-2	24	41	24	60	24	−	+	+	+	+	+	+	spring	12/17	−	5		21
Number	Name	Dextrose	Lactose	Saccharose	Dulcitol (I)	Salicin	Methyl Red	V. P.	Uric Acid	Indol	Gelatin	Litmus Milk: Coag	Litmus Milk: Acid	Source	Date of Samples 1923	Weather: Rainy	Weather: Number of days since Rain		Number

N.B. ⊕ in column 14 indicates a decolorization process in the litmus milk so that acidity is doubtful.
Dulcitol (I), column 6 is from the same batch as Dulcitol (I) Column 14 of pure cultures.
Numbers in Columns 3 to 7 indicates in hours the age of sample when positive reaction was noted.

Data - Lactose from Original samples - Page 1.

No	Name	Source	Lact 24 hrs	Lact 48 hrs	Remarks.	No	Name	Source	Lact 24 hrs	Lact 48 hrs	Remarks.
1	1	Stream	+	+		43	117	stream	−	+	No Lact check
2	2	Stream	+	+		44	118	spring	−	−	
3	3	stream	+	+		45	119	spring	−	+	
4	4	stream	+	+		46	120	Spring	−	+	
5	5	Pool	−	+		47	121	spring	−	⊕	
6	6	Stream	+	+		48	201	seep	−	+	
7	7	Spring	+	+		49	202	stream	−	+	
8	8	Spring	⊕	+		50	203	spring	−	−	⊕ delayed
9	9	Spring	+	+		51	204	spring	−	+	
10	10	Spring	+	+		52	205	spring	+	+	
11	11	Spring	−	⊕		53	206	spring	−	−	O.K.
12	12	Spring	⊕	+		54	207	stream	+	+	
13	13	Spring	−	−	O.K.	55	208	pond	−	−	O.K.
14	14	Spring	−	−	O.K.	56	209	pond	−	+	
15	15	Spring	⊕	+		57	301	stream	−	+	
16	16	Stream	+	+		58	302	stream	−	+	
17	17	Stream	+	+		59	303	spring	−	+	No Lact. check
18	6a	stream	−	−	⊕ delayed	60	304	spring	+	+	
19	7a	Spring	−	−	⊕ delayed	61	305	spring	−	⊕	
20	9a	spring	⊕	+		62	306	spring	−	−	O.K.
21	10a	Spring	+	+		63	307	Spring	+	+	
22	11a	Spring	−	−	⊕ delayed	64	308	spring	+	+	
23	13a	spring	−	+		65	309	spring	+	+	
24	14a	spring	−	−	O.K.	66	401	stream	+	+	
25	15a	spring	+	+		67	402	spring	−	−	O.K.
26	16a	stream	+	+		68	403	spring	−	+	
27	101	spring	−	−	O.K.	69	404	spring	+	+	
28	102	spring	−	−	⊕ delayed	70	405	spring	−	−	O.K.
29	103	spring	−	+		71	406	spring	+	+	
30	104	spring	−	−	O.K.	72	407	stream	+	+	
31	105	stream	−	+		73	408	stream	−	−	O.K.
32	106	stream	Sample Lost.			74	409	stream	⊕	+	
33	107	stream	−	+		75	501	spring	−	−	⊕ delayed
34	108	spring	−	+	No Lact. check	76	502	stream	−	−	⊖ OK.
35	109	spring	−	−	O.K.	77	503	spring	−	+	
36	110	spring	−	−	O.K.	78	504	spring	−	−	⊕ delayed
37	111	Spring	+	+		79	505	spring	−	−	⊖ O.K.
38	112	stream	−	⊕		80	506	spring	+	+	
39	113	stream	+	+		81	507	spring	−	+	
40	114	spring	−	+		82	508	spring	−	⊕	⊕ delayed
41	115	spring	−	+		83	509	stream	−	−	⊖ OK.
42	116	stream	−	⊕		84	510	stream	⊕	+	

N.B. ⊕ indicates a weak positive reaction.

Data - Lactose from original Samples - Page 2.

No	Name	Source	Lact. 24 hrs.	Lact. 48 hrs.	Remarks
85	511	spring	−	+	
86	512	stream	+	+	
87	513	stream	−	+	
88	601	spring	−	+	
89	602	spring	+	+	
90	603	spring	−	+	
91	604	spring	−	+	No Lact. check
92	605	spring	+	+	
93	606	stream	+	+	
94	607	spring	+	+	
95	608	spring	−	+	
96	609	spring	−	+	No Lact. check
97	610	spring	−	+	
98	611	spring	−	+	
99	612	spring	−	⊕	No Lact. check
100	613	spring	−	+	
101	701	stream	+	+	
102	702	spring	+	+	
103	703	stream	+	+	
104	704	stream	+	+	
105	705	stream	−	−	O.K.
106	706	well	+	+	
107	707	stream	−	−	O.K.
108	708	stream	+	+	
109	709	spring	−	⊕	
110	710	stream	+	+	
111	711	stream	+	+	
112	712	stream	−	⊕	
113	713	spring	−	+	
114	801	spring	+	+	
115	802	spring	+	+	
116	803	spring	−	⊕	
117	804	spring	−	−	O.K.
118	805	spring	−	+	
119	806	stream	+	+	
120	807	spring	−	⊕	
121	808	spring	−	−	O.K.
122	809	spring	−	−	O.K.
123	810	spring	−	⊕	
124	811	stream	−	+	
125	812	stream	−	+	
126	813	stream	+	+	

No	Name	Source	Lact. 24 hrs	Lact. 48 hrs.	Remarks.
127	901	spring	−	⊕	
128	902	stream	⊕	+	
129	903	spring	−	+	
130	904	spring	−	+	
131	905	stream	+	+	
132	906	spring	−	⊕	
133	907	stream	⊕	+	
134	908	spring	−	+	
135	909	stream	−	+	
136	910	stream	+	+	
137	911	spring	+	+	
138	912	stream	+	+	
139	913	stream	+	+	

Number of Samples	139	
Number of Checks	138	
No. Lactose −	21	
No. Delayed fermenters	8	
No.- No Lactose check	7	
Total Negative	35	
Percent. Negative	25.4	
No. 24 hr Lactose (10%)	48	
No. 24 hr Lactose trace	8	
No. 48 hr +; 24 hr −	46	
24 hr. Check −	56	40.6%
48 hr Check	53	38.4%
48 hr. Lact −	29	21.%

N.B. ⊕ indicates a weak positive reaction.

Data - Jackson's Classification Page 1.

No	Name	Lactose	Sacchr	Dulcitol	Salicin	
1	1	21	17	—		B Aerogenes
2	1		23	23		B Communior (No Lact check)
3	2	21	17	—		B Aerogenes
4	2		40	23		B Communior (No Lact check)
5	3	21	17	(40)		B Communior
6	3		23	23		B Communior (No Lact. check)
7	6	21	17	(40)		B Communior
8	6		23	23		B Communior (No Lact. check)
9	10	21	17	—		B Aerogenes
10	10		23	23		B Communior (No Lact. check)
11	12	21	17	17		B Communior
12	12		23	23		B Communior (No Lact. check)
13	17	21	17	—		B Aerogenes
14	17		23	23		B Communior (No Lact. check)
15	102	—	24	—		B Aerogenes (No Lact check)
16	103	24	24	48		B Communior
17	103	24	24	—		B Aerogenes
18	105	24	—	24		B Communis
19	107	24	24	48		B Communior
20	111	24	24	24		B Communior
21	112	24	24	24		B Communior
22	113	24	24	29		B Communior
23	114	72	—	48		B Communis
24	114	48	—	—		B Acidi-Lacti
25	115	24	48	24		B Communior
26	115	24	—	24		B Communis
27	116	72	24	—		B Aerogenes
28	118	24	24	—		B Aerogenes
29	119	24	72	24		B Communior
30	121	24	48	48		B Communior
31	201	48	48	—		B Aerogenes
32	202	24	24	—		B Aerogenes
33	203	24	24	24		B Communior
34	204	48	64	—		B Aerogenes
35	205	24	24	—		B Aerogenes
36	206	72	48	48		B Communior
37	207	24	24	24		B Communior
38	208	48	24	—		B Aerogenes
39	209	24	24	24		B Communior
40	301	48	24	—		B Aerogenes
41	302	24	24	24		B Communior
42	304	24	24	24		B Communior

Data — Jackson's Classification Page 2.

No	Name	Lact.	Sacchr	Dulcitol	Salicin	
43	305	48	24	—		B Aerogenes
44	307	24	24	24		B Communior
45	308	24	24	24		B Communior
46	309	24	24	24		B Communior
47	401	24	24	72		B Communior
48	403	24	24	24		B Communior
49	404	24	24	—		B Aerogenes
50	406	24	24	48		B Communior
51	406	24	24	—		B Aerogenes
52	407	72	24	—	48	B Aerogenes
53	409	24	24	—		B Aerogenes
54	503	24	—	—	24	B Acidi-lacti
55	506	24	24	48	48	B Communior
56	507	24	24	—	24	B Aerogenes
57	508	48	24	—	24	B Aerogenes
58	510	24	24	24	24	B Communior
59	511	24	24	24	—	B Communior
60	512	24	24	48	24	B Communior
61	513	48	24	24	24	B Communior
62	601	24	24	—	24	B Aerogenes
63	602	24	24	—	24	B Aerogenes
64	603	24	24	48	24	B Communior
65	605	24	24	24	24	B Communior
66	606	24	—	—	48	B Acidi Lacti
67	607	24	24	24	—	B Communior
68	608	24	24	24	24	B Communior
69	610	24	24	24	24	B Communior
70	611	24	—	—	48	B Acidi-lacti
71	613	24	—	—	—	B Acidi-lacti
72	613	24	24	—	—	B Aerogenes
73	602	24	24	48	—	B Communior
74	611	24	24	—	24	B Aerogenes
75	408	48	48	—		B Aerogenes
76	511	45	—	24	—	B Communis
77	603	24	24	—	24	B Aerogenes
78	605	24	24	—	24	B Aerogenes
79	607	24	24	—	—	B Aerogenes
80	701	24	24	—	24	B Aerogenes
81	702	20	—	—	48	B Acidi-Lact.
82	704	41	24	—	48	B Aerogenes
83	709	48	24	—	72	B Aerogenes
84	711	20	24	—	24	B Aerogenes
85	806	20	24	—	24	B Aerognes.

Data - Jackson's Classification Page-3.

No	Name	Lact	Sacchr	Dulcitol	Salicin	
86	807	41	—	—	—	B Acidi-lacti
87	909	41	24	60	24	B Communior
88	11	54	—	40	24	B Communis
89	407	24	48	60	48	B Communior
90	512	48	24	—	24	B Aerogenes
91	611	20	24	60	24	B Communior
92	612	45	—	40	24	B Communis
93	701	20	24	40	24	B Communior
94	703	20	24	60	48	B Communior
95	706	20	—	60	48	B Communis
96	708	48	24	—	—	B Aerogenes
97	709	24	—	—	—	B Acidi-Lacti
98	710	20	24	—	24	B Aerogenes
99	710	24	24	24	24	B Communior
100	802	24	—	—	—	B Acidi-Lacti
101	805	54	—	—	—	B Acidi-lacti
102	811	54	—	—	24	B Acidi-lacti
103	812	24	—	40	24	B Communis
104	904	20	24	60	24	B Communior
105	907	41	24	—	24	B Aerogenes
106	910.	24	24	70	48	B Communior.

Summary.

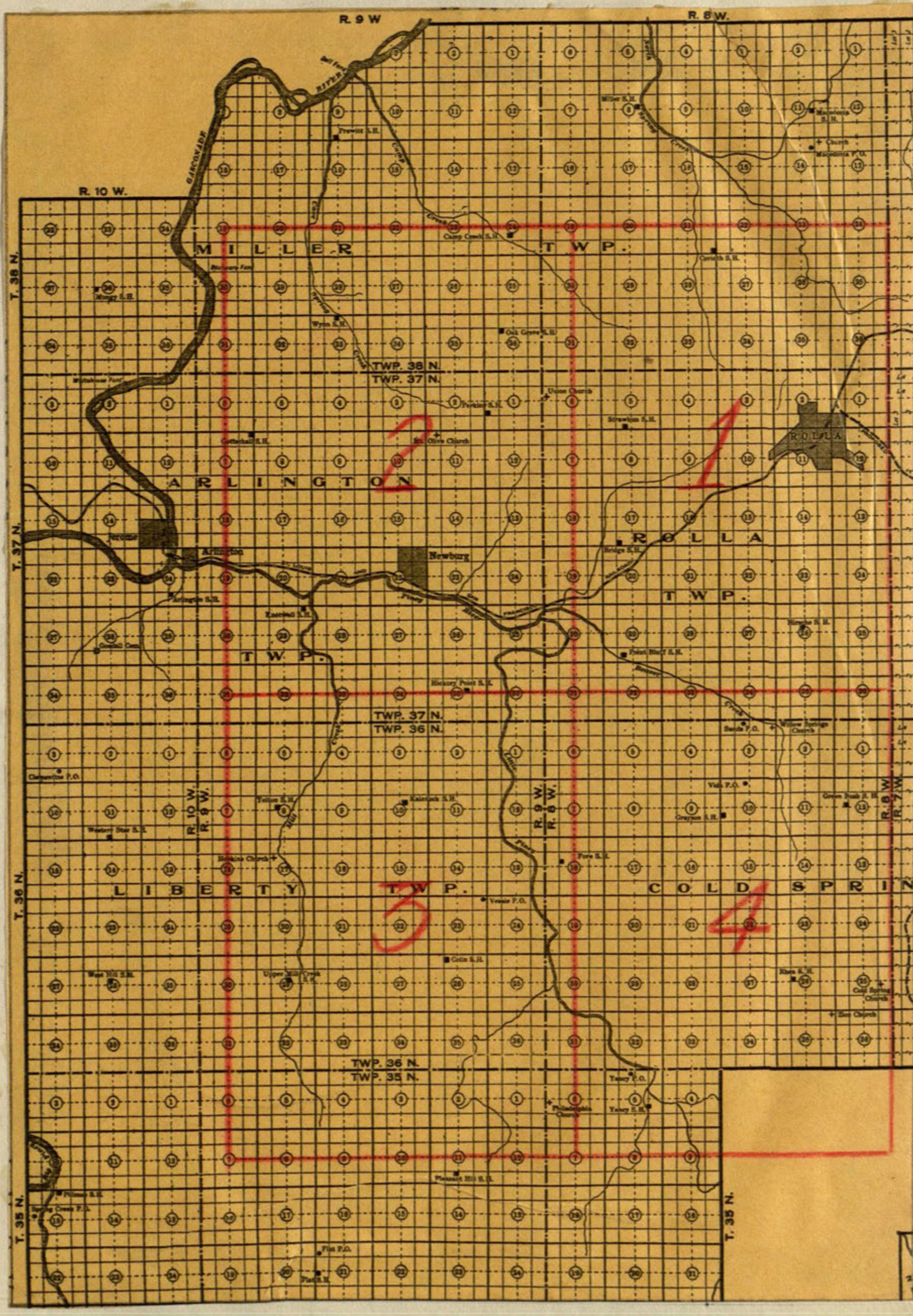
106 Cultures (where two organisms from the same sample gave identical reactions only one was included to make the above total).

1. Number of samples without Lactose check. 8
2. Number of samples B. Communior 42
3. Number of samples B. Aerogenes 37
4. Number of samples B. Acidi-lacti 11
5. Number of samples B. Communis 8

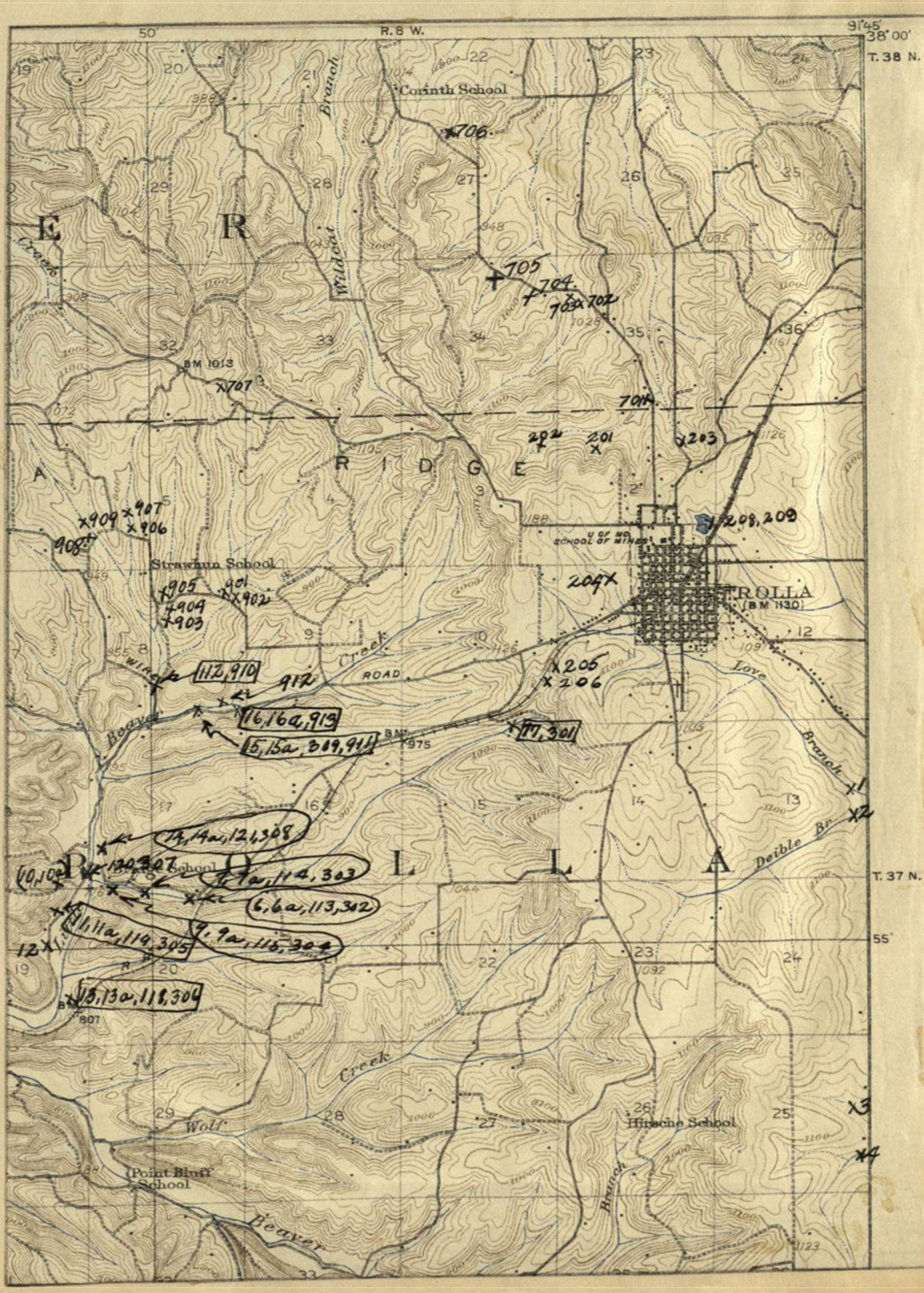
Key to Jackson's Classification

	Lactose	Saccharose	Dulcitol.	
1.	+	+	+	B. Communior
2.	+	—	+	B. Communis
3.	+	+	—	B. Aerogenes
4.	+	—	—	B. Acidi-lacti.

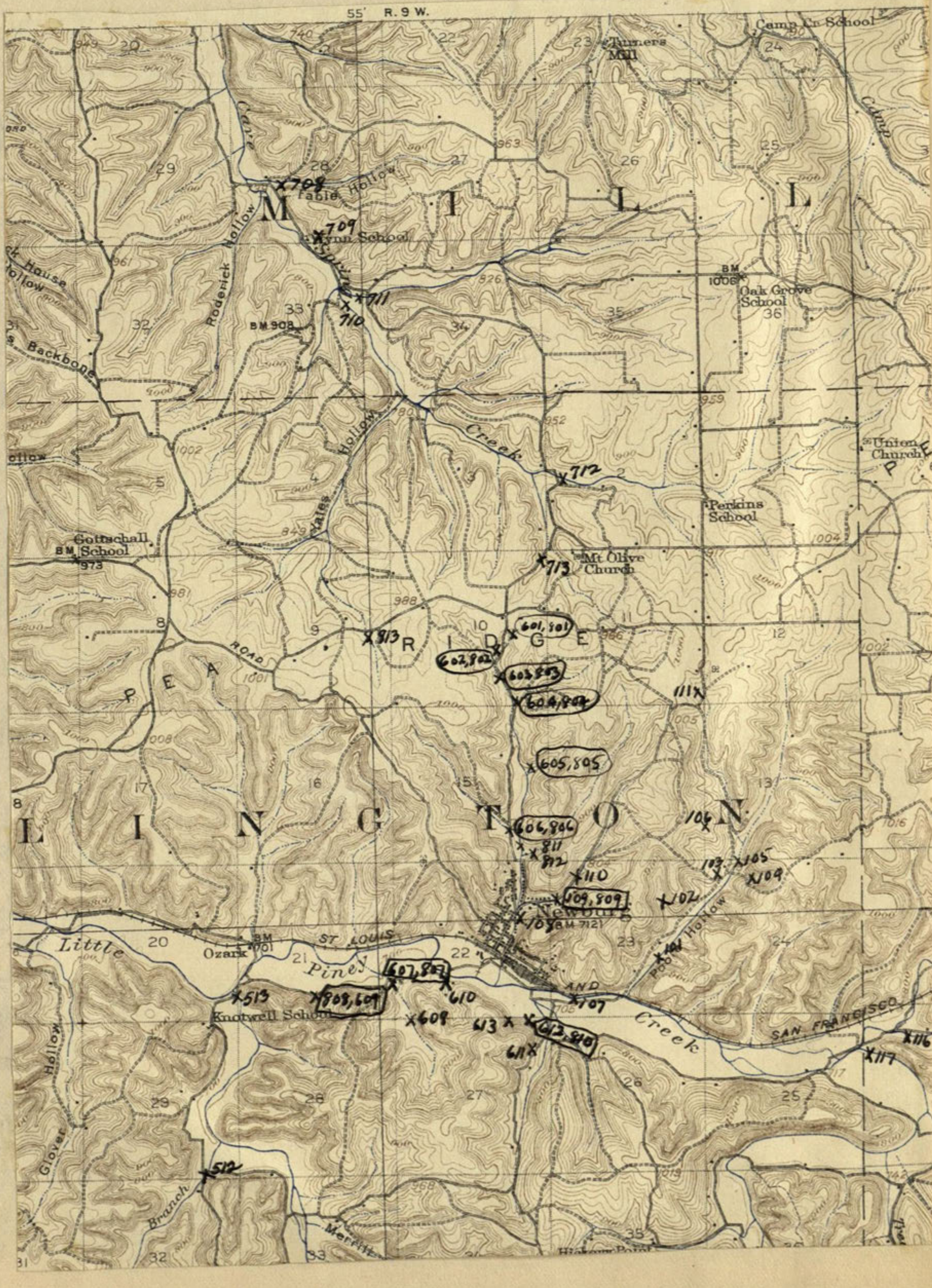
Map of Rolla Quadrangle, Showing Location of the four Sections of the Topographic Map, Shown on Pages 76to 79



Section 1 of Map, Showing Location of Water Sample Sources.



Section 2, of Map, Showing Location of Water Sample Sources.



Section 3, of Map, Showing Location of Water Sample Sources.

Section 4 of Map, Showing Location of Water Sample Sources.

Explanation of Tables. Page 1.

Table 1. Complete tests of the 27 pure cultures.

This table gives the number, and the name of the original sample with its source, the date of securing the sample and the weather conditions. It gives all the reactions to the various media, noting the time at which the first positive reactions were noted.

Table 2. A study of reactions and classifications of the 27 pure cultures. Here a comparison is made for fecal and non-fecal indications as determined in the various classifications. A determination of origin from the 4 reactions of Methyl Red, Voges Proskauer, Uric Acid, and Citrate media shows 13 as non-fecal, 10 as fecal and 4 indeterminate. This is based on a majority agreement from the above media. In the case of 11 cultures there is perfect correlation of the four media. Voges Proskauer leads the high correlation, determining 22 out of the 23 reactions; Methyl Red determines 21; Uric acid, 19; and citrate medium, 18. This is a high correlation in each case. In the cases where the 4 reactions agree the classifications also have a high correlation showing the cultures giving the same to be strongly typical. 10 out of the 11 cultures giving perfect agreement in the media also give perfect correlation with all classifications. Only in 3 cases out of the 27 does the classifications help overrule the indication of the 4 media.

Explanation of the Tables. Page 2.

Table 3. Pure cultures in Levine's Classification(also atyp.)

The Voges Proskauer(V.P.), Methyl Red(M.R.), Uric Acid, motility and gelatin reactions were considered as primary and any deviation from the specified reactions caused the culture to be classed as atypical. As shown in the table, 10 cultures are XXtypical while 17 cultures are classed as atypical and are divided into 11 groups.

According to Levine(pg 37 Bulletin 62 Iowa State College) this large variation is to be anticipated. "A very serious objection to such classifications as those of MacConkey, Bergey and Deehan, and Jackson is their extreme flexibility and complexity ;for as the number of fermentable substances or other characters observed increases, the number of XXXXXX "varieties" increases geometrically (approaching infinity) and soon produces a most unweildly scheme. Thus for eight characters there are 256 possible combinations or"varieties". This number rises to 1,024 with 10 characters and to 65,536 when 16 tests are considered." This does not apply closely to Levine'S Classification for ordinarily the characters are constant and the classification covers a large group of X organisms. But there is known to exist(especially on first isolations) small groups of atypical reacting organisms. A special study is made of this by Rahlman and Sohn(Journal Americam Water Works Assn., March,1924)

Explanation of Tables. Page 3.

Table 4. By Bahlman and Sohn in " Colon-aerogenes differentiation at Cincinnati" Journal Am. W. W. Assn., March 1924. Atypical cultures were given especial study by these men and the results obtained after repeat and XX rejuvenated tests are noted in the tables. Only 4 reactions are used; V.P., M.R., Uric Acid, and Gelatin liquefaction. Of the 27 cultures; XXXXX9 are typical B.coli and 7 are typical B. aerogenes. This makes 59.3% typical.

Table 5. Winslow, Kligler & Rothberg, 1919.(Jour. Bact.IV,429).

The 27 pure cultures based on dulcitol,salicin,lactose, saccharose, V.P., M.R., Litmus Milk, indol, motility and gelatin reactions, give 6 typical cultures and 21 atypical.

Table 6. Species X of genera Escherichia and Aerobacter of the tribe bactereae, according to Bergey's Manual of Determinative Bacteriology, 1923.

Table 7. Detailed tests of 21 isolated cultures. These cultures were carried along in parallel tests with the same media batches as the 27 pure cultures. At a late test they were to be definitely or doubtfully contaminated, but they are retained in the data for their value as check tests.

Explanation of Tables.   Page 4.

Tables 8 and 9. Lactose Gas Data from 139 Water Samples. The data is summarized at the bottom of Table 9.

Tables 10, 11 & 12. Data on 106 cultures according to Jackson's Classification. Due to the rather low value of correlation of media used in this classification as compared with Methyl Red, Voges Proskauer, and Uric Acid this data is not as important as is the more complete data of the 27 pure cultures.

Table 13. Map of the Rolla Quadrangle.

Tables 14, 15, 16 & 17 Sectional maps of part of Rolla Quadrangle, Topographic Maps showing the location of the various water sample sources. The data on location of springs given in this thesis may be of future value in connection with special studies, so all data of this nature has been included.

SUMMARY OF THE TOTAL NUMBER OF MEDIA TUBES AND PLATES INOCULATED AND STUDIED IN CONNECTION WITH THIS THESIS.

Agar Slants(plain) 84; Eosin-Methylene Blue Agar, 170; Endo agar, 40; Hesse Agar, 83; Lactose tubes, 380; Dulcitol tubes, 170; Saccharose(Sucrose) tubes,138; Salicin tubes, 94; Dextrose(Glucose), 54; Gelatin Stabs,54; Litmus Milk,54; Indol(broth), 108; Methyl Red(broth),54; Uric Acid, 54; Smith Tube Dextrose, 29; Citrate, 29.

TOTAL TUBES AND PLATES INOCULATED IS 1565.

CONCLUSIONS:

1. As to potability of water by classifications.

Potability of water is determined largely by the absence of sewage contamination. Usually, lactose fermenting bacteria of the colon-aerogenes group are sought for in testing for sewage contamination. The absence of lactose fermenters is considered as direct evidence of potability. The presence of lactose fermenters of the colon-aerogenes group, however, does not directly indicate sewage pollution, as B. Aerogenes is widely distributed in nature and rarely is of fecal origin.

The classifications of MacConkey, Jackson, Levine, Dahlman and Sohn, Winslow Kligler & Rothberg, and Bergey's Manual, all attempt to classify organisms of this group and to relate these classes to their habitat. The author has been unable to classify MacConkey's types according to habitat so has given that classification no further study. Jackson classes the dulcitol gas formers, B. communis and B. communior as of fecal origin. Taking as of fecal origin the majority indicators of the V.P., MR., Uric Acid, and Citrate reactions(and vice versa for non-fecal),Jackson's classification gives 61% correct results, out of 23 of the pure cultures. Bergey's Manual gives a greatly modified classification of this colon-aerogenes group and as there is no backing of a large number of tests and of the corresponding statistical data, the author could make out very little differential value for this grouping.

1. As to potability of water by classifications. (Cont). The 10 reactions of Winslow, Kligler & Rothberg gave only 6 typical organisms out of the 27 pure cultures. Levine's classification and Xthat used by Bahlman and Sohn are both based on statistical studies and are the only XXXXXX classifications of present day use. However, their benefit is limited by the number of atypical cultures which appear from the plating method of isolation. Levine with five reactions, M.R., V.P., Uric Acid, Motility and Gelatin liquefaction gives 17(63%) atypical organisms out of the 27 pure(plated) cultures. Bahlman and Sohn with M.R., V.P., Uric Acid and Gelatin liquefaction gives 11(41%) atypical organisms out of the 27 pure cultures. It might be noted here that all classifications (where typical) and all media gave identical origin indications in 10 out of the 27 cultures. Five were shown to be of fecal and five of non-fecal origin. Considering the lack of indication (often amounting to more than 50%) due to the atypical reactions the author believes that he has as valuable an indicator by taking a majority indication of the four XXXXXX reactions, M.R., V.P., Uric Acid and Citrate medium,and letting that determine the origin(fecal or non-fecal) of the culture.,

CONCLUSIONS:

2. Differential value of the tests.

Large group studies by Levine, Koser and others in connection with Methyl Red, Voges Proskauer, Uric Acid and Citrate reactions have shown them to be of primary value as differential tests(for fecal or non-fecal origin). At present motility, indol production and gas production from carbohydrates are in a secondary place with respect to differential value.

In 23 out of the 27 pure cultures the M.R., V.P., Uric Acid and Citrate reactions gave majority indicators for either fecal or non-fecal origin(in the 4 other cultures the indications were 2 and 2). Citrate gave the correct (majority) indication in 18 cultures; Uric Acid,in 19 cultures; Methyl Red,in 21 cultures;and Voges Proskauer, in 22 cultures. The almost perfect correlation of the M.R. and V.P. reactions fits in very well with studies of recent tests. Very likely about 75% of the 23 cultures given ~~[illegible]~~ here are of the origins indicated. This leaves the field of fecal and non-fecal organisms of this group very loosely indicated. When,,added to this it is seen that there is practically no differential test for the organisms of this group that are of human and lower animal fecal origin, then the problem is truly a difficult one.

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