FURTHER STUDIES ON MEDIA FOR THE COLIFORM BACTERIA

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Eleanor Laverne Gilmore

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THESIS

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by

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CONTENTS

INTRODUCTION

The following studies were initiated by the questions arising from the use of lauryl sulfate tryptose broth as a presumptive medium for the isolation of coliform organisms from water supplies.

Inasmuch as lauryl sulfate tryptose broth has been accepted by the Committee on Standard Methods for the Examination of Water and Sewage of the American Public Health Association, it is imperative that its properties in regard to the growth of coliform organisms be fully evaluated. If a better formulation can be developed it should be done before the present formula becomes standard procedure.

One of the first questions to arise has been cost. Lauryl sulfate tryptose broth costs approximately four times more than standard lactose broth. Laboratories where marked economy in operation is necessary, may hesitate to use this medium unless they are fully convinced that a cheaper medium could not be substituted successfully. A number of people have asked whether it would be possible to reduce the concentration of tryptose from two percent to one percent thus reducing the cost by half. To date the answer has been that the labor costs in the use of this medium would be less in laboratories where positive pre-

sumptive tests are encountered. In addition, media costs would not be materially different in that confirmatory media would be unnecessary. This is true, but if a reduction can be made without reducing the efficiency of the medium, it should be done. Further research is necessary to determine the effect of various concentrations of tryptose in the medium.

Another question presented has been that of minimal numbers used in comparative studies of lauryl sulfate tryptose broth and standard lactose broth. In the original work by Darby and Hallmann (2) the minimal numbers used were from 40 to 50 per ml. This is not a minimal number when compared to the coliform concentration in water supplies where the coliform index is less than one per 100 ml. Further research is necessary when the coliform concentration is approximately one or less per ml. This still would certainly be a much nearer approach. A lower concentration would be difficult to evaluate because a higher incidence of negative tests would be encountered.

Still another question arises concerning the relative growth rates of Escherichia coli and Aerobacter aerogenes. The original studies of Darby and Hallmann (2) were made with E.coli and no tests were carried out with A. aerogenes. Inasmuch as both organisms are present in water and are both

considered representatives of the coliform group, data should be obtained on growth rates in lauryl sulfate tryptose broth and standard lactose broth.

Along with studies planned to answer the above questions, it was also thought advisable to determine the value of adding sodium chloride to both lauryl sulfate tryptose broth and standard lactose broth. Only tryptose broth was tested in the studies on media for the coliform organisms by Darby and Hallmann (2).

HISTORICAL REVIEW

In 1941 Darby and Mallmann (13) presented a new medium, lauryl sulfate tryptose lactose broth, for the isolation of coliform organisms from water supplies. They reported that the use of a formula containing tryptose, sodium chloride, and phosphate buffer grew out more members of the coliform group present in the water as indicated by the higher colon indices obtained with this medium. Further, they reported that the addition of sodium lauryl sulfate in concentrations of 1:5000 prevented the growth of gram-positive lactose fermenting bacteria without any appreciable inhibition of the growth of the coliform group. They recommended that the medium be used in place of standard lactose broth, A. P. H. A. (31) in the presumptive test. They also recommended that the medium be used in place of brilliant green bile lactose broth as a confirmatory medium.

McCrady (17), in a summation of comparative studies made by a number of laboratories distributed throughout the United States, reported that lauryl sulfate tryptose broth gave a higher incidence of true presumptive tests than did standard lactose broth. He reports that a summation of results obtained from both unfinished and finished waters showed that lauryl sulfate tryptose broth gave 56 to 58 percent less primary gas positives than standard lactose broth. This would indicate that the statement by Darby and Mallmann was correct in that lauryl sulfate tryptose broth

allowed the growth of greater numbers of coliform organisms initially present in the waters examined. McCrady also showed from the collective studies that lauryl sulfate tryptose broth yielded far less false presumptives as measured by the final identification on eosin methylene blue agar in the completed test.

Levine (16) in 1941 demonstrated that lauryl sulfate tryptose broth gave a higher precentage of true presumptives than did standard lactose broth.

Perry and Hajna (20) in a comparative study of E. C. medium and lauryl sulfate tryptose broth also found the latter medium as well as E. C. medium to be superior to standard lactose broth in the number of true presumptive tests obtained.

Hupp (9) found on Indianapolis plant effluent that lauryl sulfate tryptose broth gave no false positives whereas false positives were frequently encountered in standard lactose broth.

STANDARD LACTOSE BROTH

24 Hrs.	48 Hrs.	Total	Confirmed	Completed
5	46	51	5	0

TRYPTOSE LACTOSE BROTH WITH SODIUM LAURYL SULFATE

24 Hrs.	48 Hrs.	Total	Confirmed	Completed
0	0	0	0	0

"The above comfirmed results from lactose broth did not pass the completed test because of their failure to

grow in eosin methylene blue agar."

The Committee on Standard Methods for the Examination of Water and Sewage reported to the Laboratory Section the following recommendation for the use of lauryl sulfate tryptose broth.

"In these standard tests lauryl sulfate tryptose broth may be substituted for lactose broth in the examination of all waters except final filtered, treated and filtered-treated waters. It may be substituted for lactose broth also in the examination of final, filtered, treated and filtered-treated waters provided the laboratory worker has amply demonstrated by correlation of positive completed tests (isolations of coliform organisms) secured through the use of lauryl sulfate tryptose broth with those secured through the use of lactose broth, in the examination of such waters, that the substitution results in no reduction from the density of coliform organisms indicated by the standard procedure using lactose broth."

The report was approved by the Section and recommended for inclusion in the next edition of Standard Methods.

At present this medium is in routine use in the municipal water plants of Michigan for the determination of coliform indices in raw water without confirmation by brillant green bile broth or eosin methylene blue agar.

PROCEDURE

The cultures used in this study were Escherichia coli, Aerobacter aerogenes and Pseudomonas aeruginosa. A 15 to 17 hour culture grown on a plain agar slant was used in seeding the various media.

In order to obtain minimal numbers, organisms were transferred from a 24 hour agar slant culture to a tube of sterile saline solution. Sufficient organisms were added until the first opalescent turbidity which was visible to the naked eye was obtained. In the case of E. coli the approximate number at this density is 50,000,000 per ml. The dilution was then further diluted by decimal dilutions of 1 to 100 and 1 to 10,000 until the count would be approximately one or less per ml. The last dilution was made by transferring into a flask containing 99 ml. of the medium used instead of sterile saline. One ml. samples of this broth in each flask was plated immediately to obtain the initial counts. The flasks were placed in the 37°C incubator and one ml. samples were plated at 2, 4, 6 and 24 hour intervals in appropriate dilutions. All plate counts were made after 48 hours incubation at 37°C.

Care was taken in preparation and sterilization of the media. An adjustment by the colorimetric method was made of all the media to a pH of 6.8.

The selection of the pH 6.8 was made because Darby and Hallmann (2) found that the greatest increase in growth occurred at this pH.

In the first series of experiments a comparison of the growth rate in various percentages of tryptose was made. Bactotryptose in concentrations of 0.5, 1, 1.5 and 2 percent was used in a medium containing phosphate buffers, sodium chloride and lactose in the same concentrations as used in lauryl sulfate tryptose broth. One ml. portions containing E. coli were plated and incubated.

In the second series one a nd two percent tryptose concentrations were selected for comparison with lauryl sulfate tryptose broth. The same technique as previously mentioned were employed. Twenty nine sets of determinations were made with E. coli, 25 sets with A. aerogenes and 18 sets with Ps. aeruginosa. A large number of separate determinations were made to eliminate variabilities obtained in individual sets of determinations.

In the next series, instead of measuring the effectiveness of the media through the determination of total numbers of organisms, the first appearance of gas fermentation and rates of gas production were used as measurements.

Minimal numbers of organisms were obtained in the same manner as for the studies on the growth rates by plating. However, the dilutions were carried out entirely in sterile

physiologyical salt solution instead of using media for the final dilution. Ten ml. of the desired dilution was seeded into fermentation tubes containing double strength media and the tubes were read at the end of 13 hour and 24 hour periods.

In the first series, one percent tryptose broth, two percent tryptose broth and two percent lauryl sulfate tryptose broth was compared. Due to the variability in the rate of gas production, 37 sets of fermentation tubes of E. coli and 29 sets of A.aerogenes were seeded.

In the next series these same media were compared with standard lactose broth to determine the rate of growth and the amount of gas produced.

To determine the influence of lauryl sulfate in the stimulation of growth, two ml. of a 5 percent solution of sodium lauryl sulfate was added to standard lactose broth. This is the same amount of sodium lauryl sulfate that is added to lauryl sulfate tryptose broth. Standard lactose broth was used as a control.

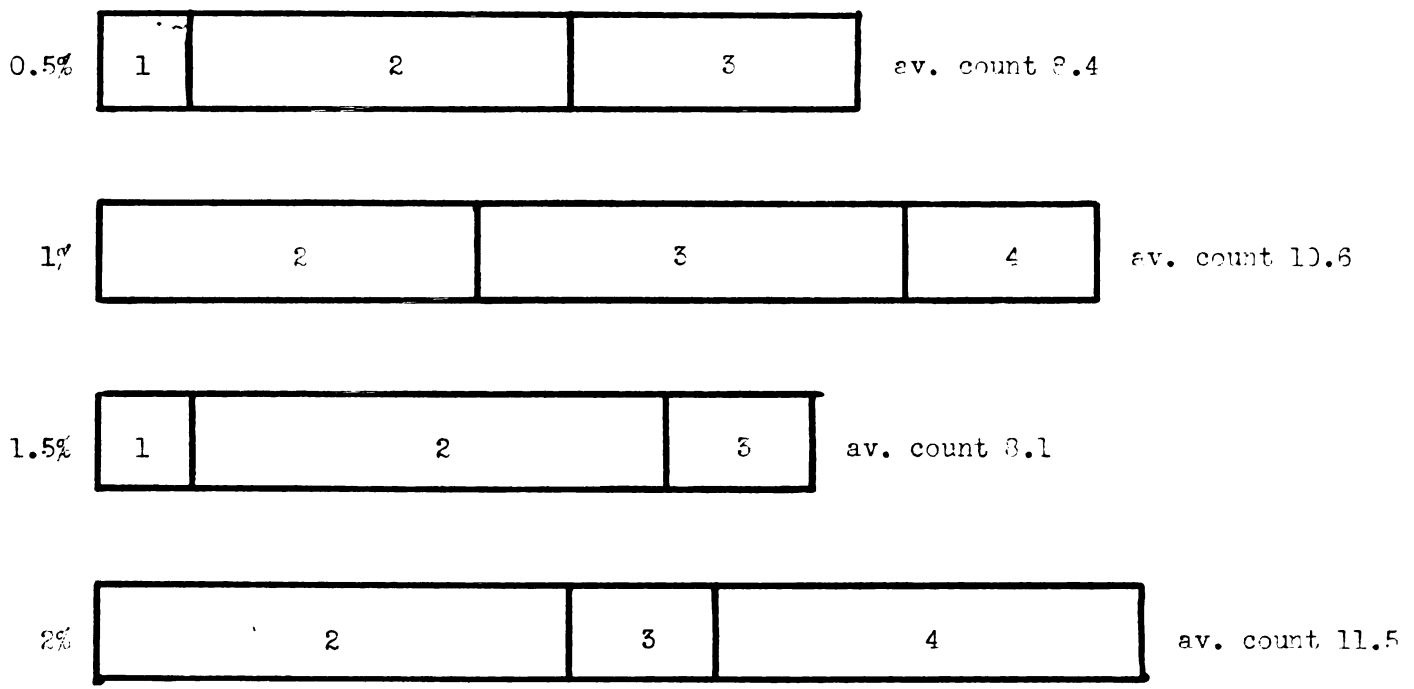
RESULTS

In the first series of experiments involving the various percentages of tryptose, a 0.5 percent concentration of tryptose broth showed consistently lower growth rate as compared to the other concentrations of tryptose. The 2 percent concentration has proved to be the most favorable for the growth of the organisms used in this study. However, the 1.5 percent tryptose broth gave plate counts closely approximating the counts for the 2 percent tryptose broth as may be seen in graphs I, II, III and IV.

The six hour plate count has been found to be a more reliable indication of the growth rate of an organism in tryptose media than the 24 hour plate count. The trend of the 6 and 24 hour counts in a series, frequently does not coincide as may be noted from the graphs and tables dealing with plate counts. For example, lauryl sulfate tryptose broth shows a higher count in 6 hours than standard lactose broth, whereas the latter medium may show the higher count in 24 hours. These differences in plate counts would indicate that the organisms has changed growth phases from the logarithmic growth phase to the negative growth acceleration. Thus having reached the negative growth acceleration phase 24 hour count is less reliable. Tryptose broth stimulates the growth of the viable cells to a greater extent than did standard lactose broth which fact is more evident by the 6 hour plate counts.

The bacterial population of the flasks varied at differ-

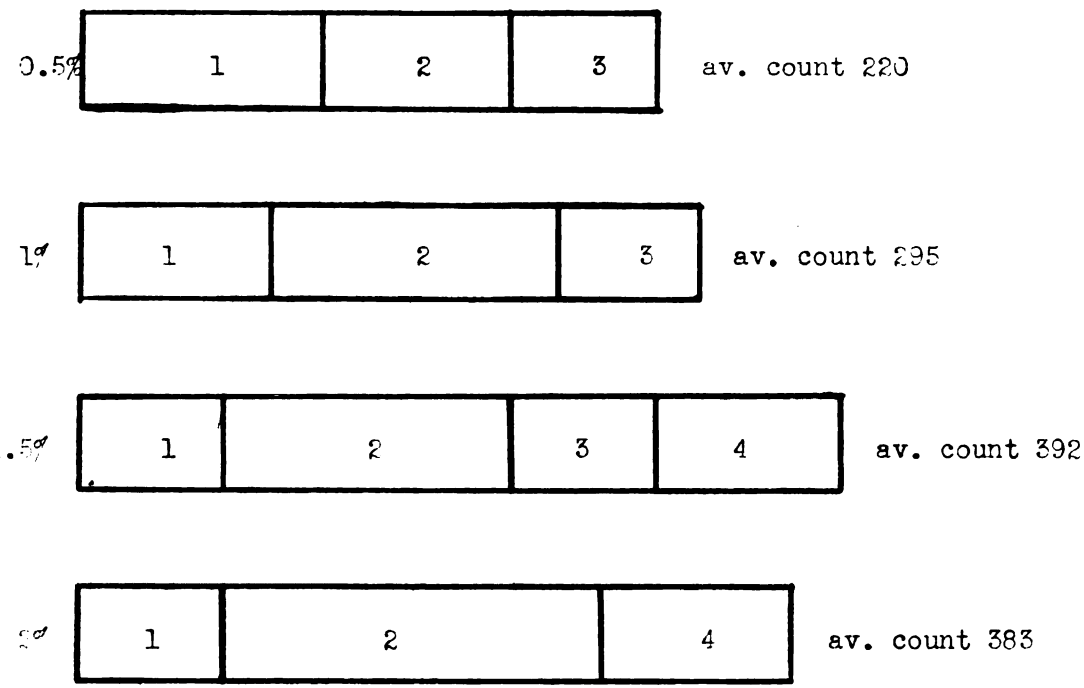
Graph I Comparison of various concentrations of tryptose by bacterial counts using Escherichia coli.



Frequency Ranges. 1: 0-5, 2: 6-10, 3 :11-15, 4: 16-20

2 HOUR PERIOD

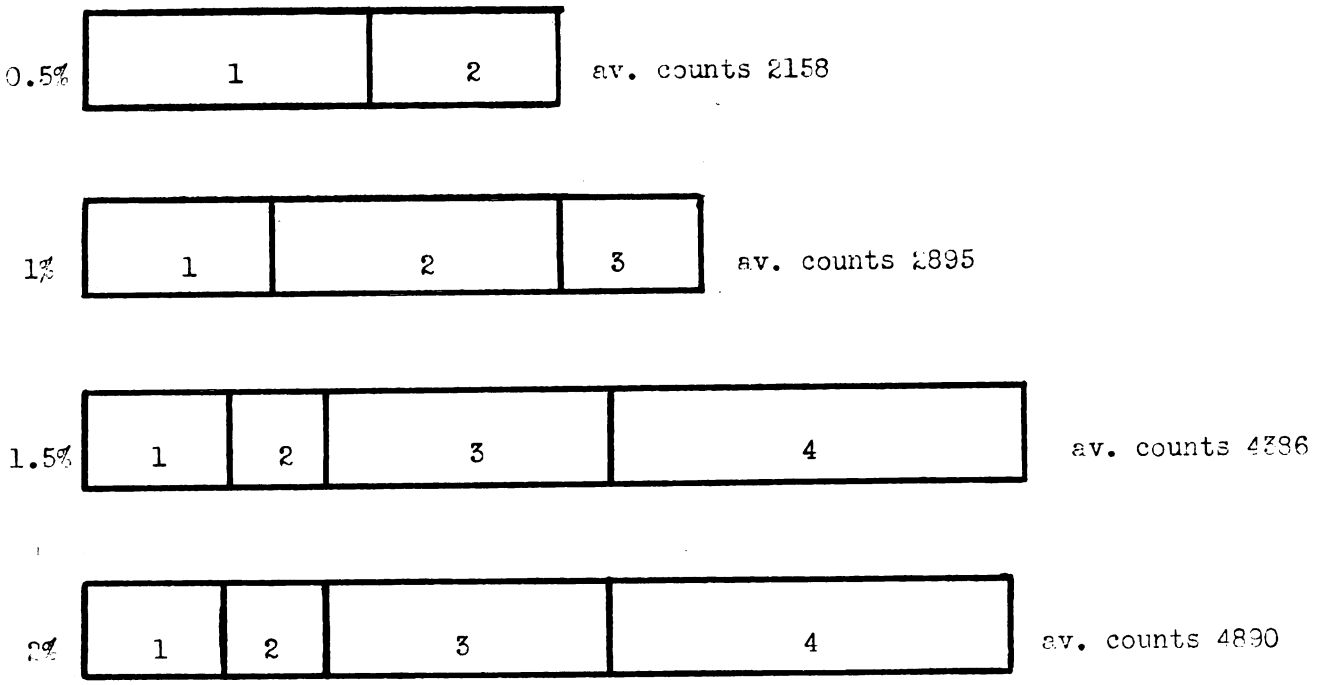
Graph II Com parison of various concentrations of tryptose by bacterial counts using Escherichia coli.



Frequency Ranges. 1:0-250, 2:251-500, 3:501-750, 4:751+

4 HOUR PERIOD

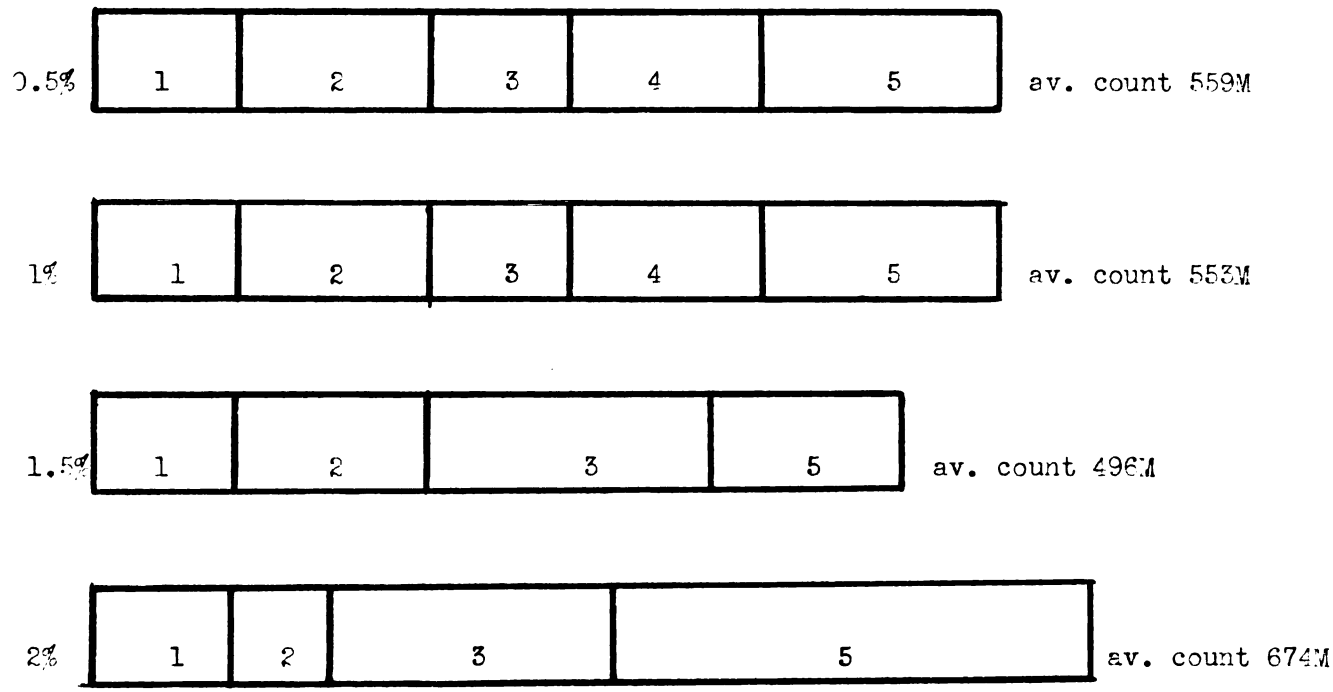
Graph III Comparison of various concentrations of tryptose by bacterial counts using Escherichia coli.



Frequency Ranges. 1:0-2500, 2:2501-5000, 3:5001-7500, 4:7501/

6 HOURS PERIOD

Graph IV Comparison of various concentrations of tryptose by bacterial counts using Escherichia coli.



Frequency Ranges. 1:0-100, 2:101-500, 3:501-1000, 4:1001-1500, 5:1501

24 HOUR PERIOD

ent time intervals in the same series and for different series. A critical survey of the data in the tables listed in the appendix show definite trends but are better understood graphically. Many methods of graphing the data were tried but in every instance only a three dimensional graph would give the desired picture. Because three dimensional graphs are impossible to depict clearly on a flat surface, other means had to be devised.

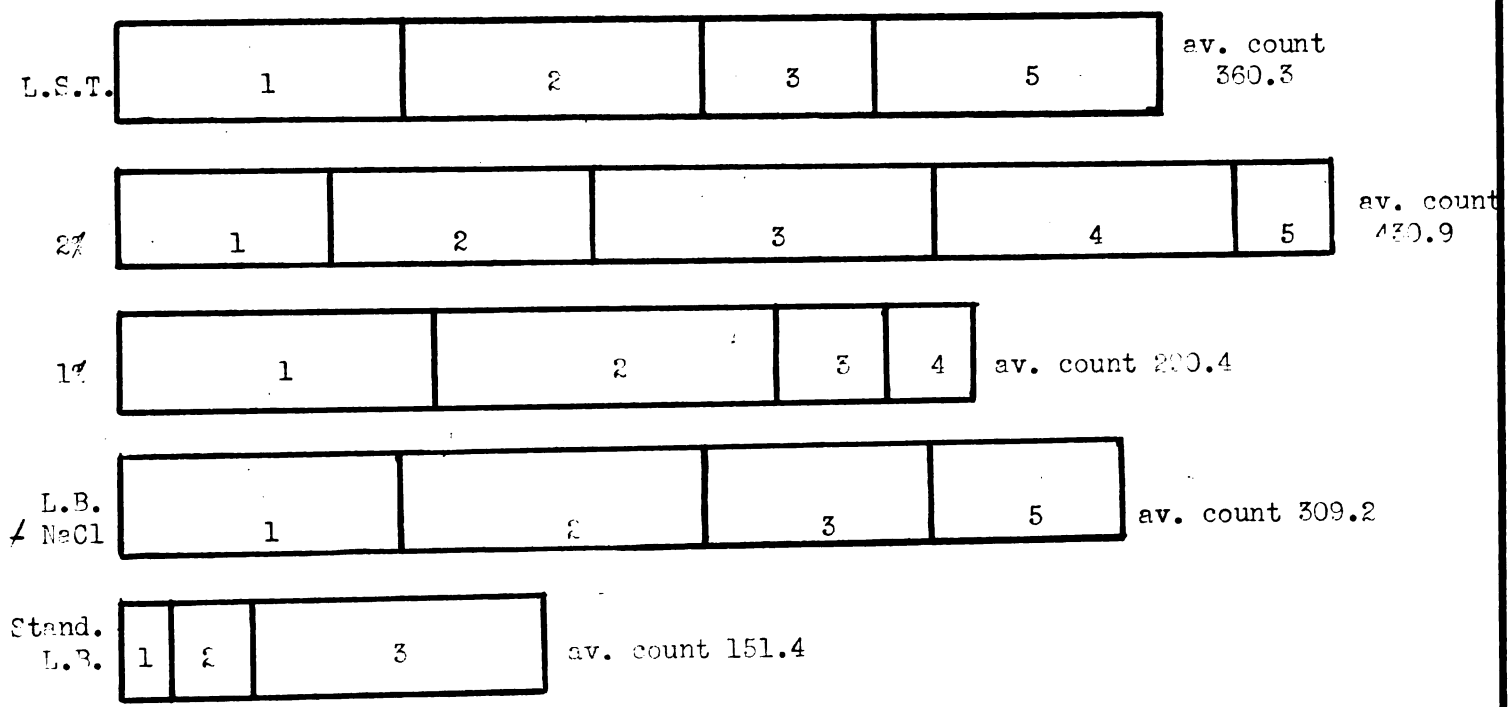
The graphs used were obtained as follows: Bacterial counts for each time interval were arranged serially from the lowest to the highest counts. The figures were classified into four or five representive groups as for example in Graph I, the first group included counts ranging from 0 to 5, the second group 6 to 10, the third group 11 to 15, and the fourth group 16 to 20. The number of plate counts falling into each group were recorded. Block graphs were made based upon the number of samples appearing in each group. Because it was desired to show which medium gave the highest bacterial counts, increased importance was given the frequencies in the higher brackets. Thus in Graph I, 0 to 5 was given a value of one unit; 6 to 10, two units; 11 to 15, three units; 16 to 20, four units. Therefore, a medium with a high count frequency would give a wider block than one with a low count frequency. If the counts varied considerably, the block graph would still show the extent of the high counts by the width of the block.

Averages of the counts were also used, although the writer is aware of the danger of averaging widely divergent bacterial counts. However, the averages agree fairly well with the block graphs. In justification of averaging the counts, it may be stated that to a very large extent the counts varied less than 25 percent from the mean. Such data can be averaged without danger of obtaining answers not representative of the growth rates.

In a second series of experiments where one percent tryptose and two percent tryptose were compared with L.S.T. (lauryl sulfate tryptose) broth graphs V, VI, VII, and VIII indicate that two percent tryptose broth is slightly more efficient than L.S.T. broth. However, the difference is negligable. Because these two broths contain the same ingredients with the exception of sodium lauryl sulfate being added to L.S.T. broth, the plate counts should approximate each other.

In another series of experiments, sodium lauryl sulfate was tested for its stimulating or inhibitory property. It was found that sodium lauryl sulfate, when added to standard lactose broth, occasionally showed a slight inhibitory action but when used with tryptose, a very slight stimulation action was noticed. This is shown in the Appendix in Tables VIII and IX. Therefore, the difference between L.S.T. broth and two percent tryptose broth may be attributed to experimental error. Lauryl sulfate tryptose broth is unquestionably a better medium than one percent tryptose broth

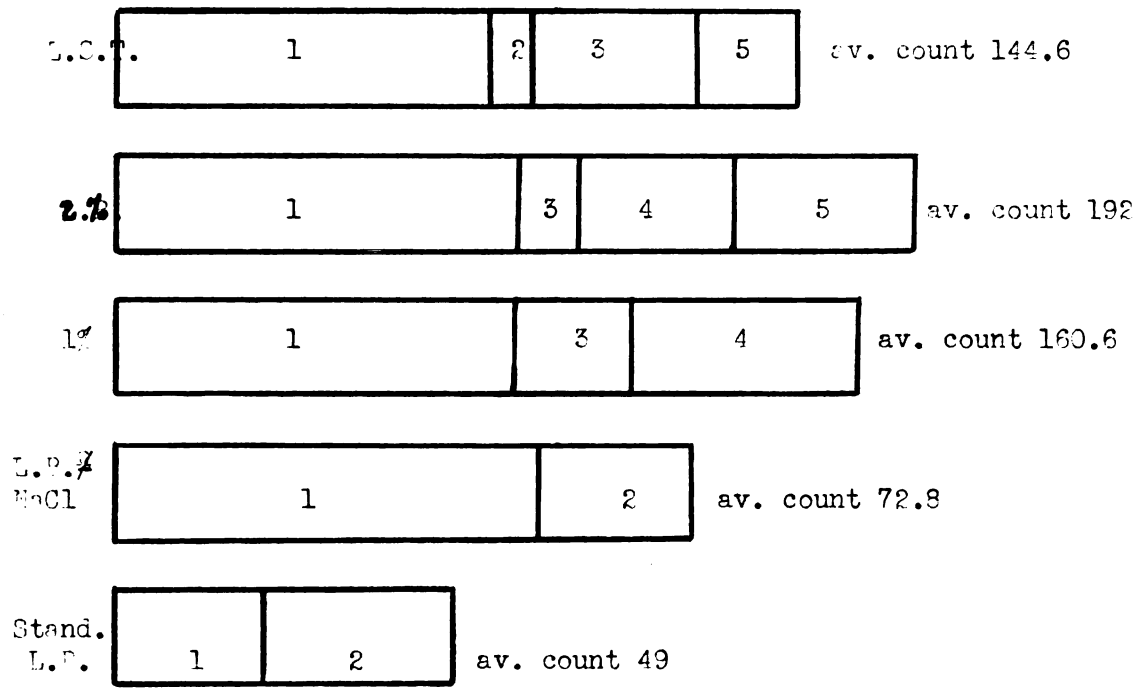
Graph V Comparison of tryptose media and lactose media by bacterial counts using Escherichia coli.



Frequency Ranges. 1:0-250, 2:251-500, 3:501-750, 4:751-1000, 5:1001+

6 HOUR PERIOD

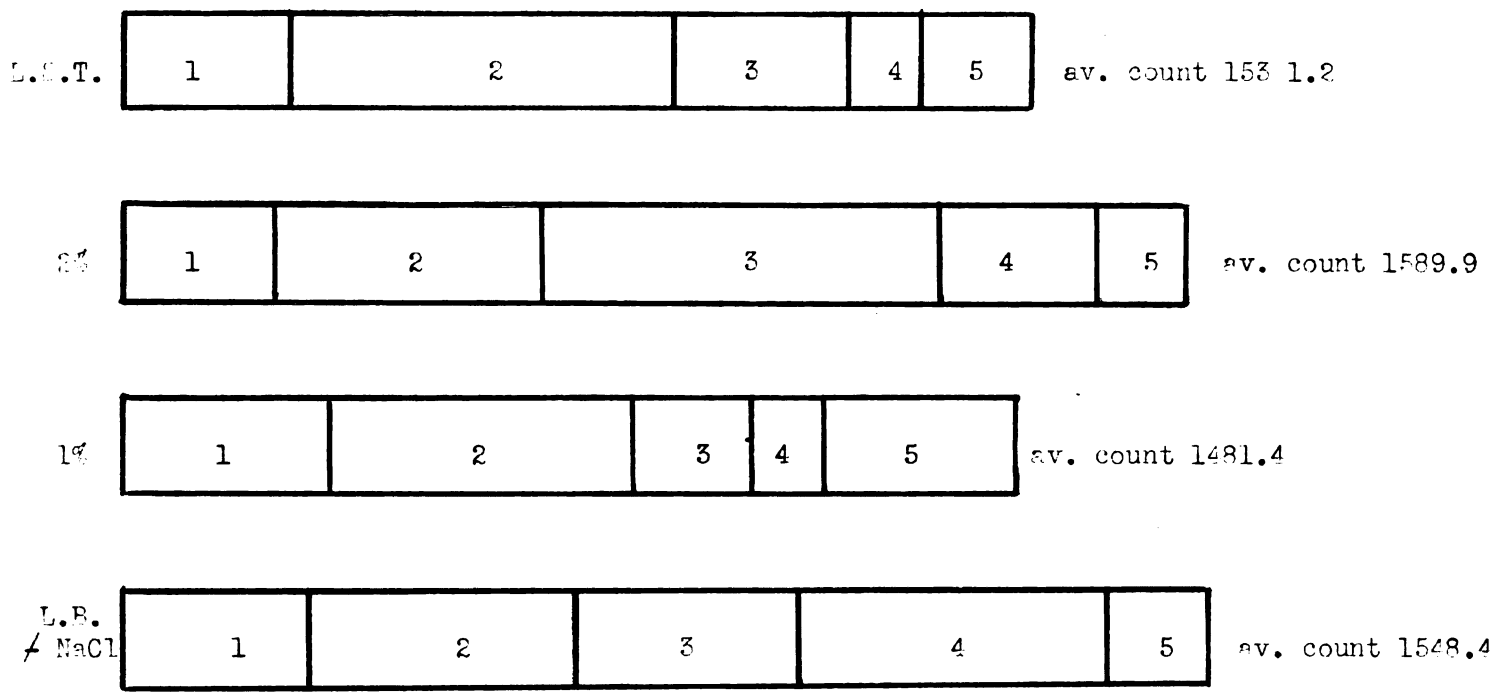
Graph VI Comparison of tryptose media and lactose media by bacterial counts using Escherichia coli.



Frequency Ranges. 1:0-250, 2:251-500, 3:501-750, 4:751-1000, 5:1001/

24 HOURS

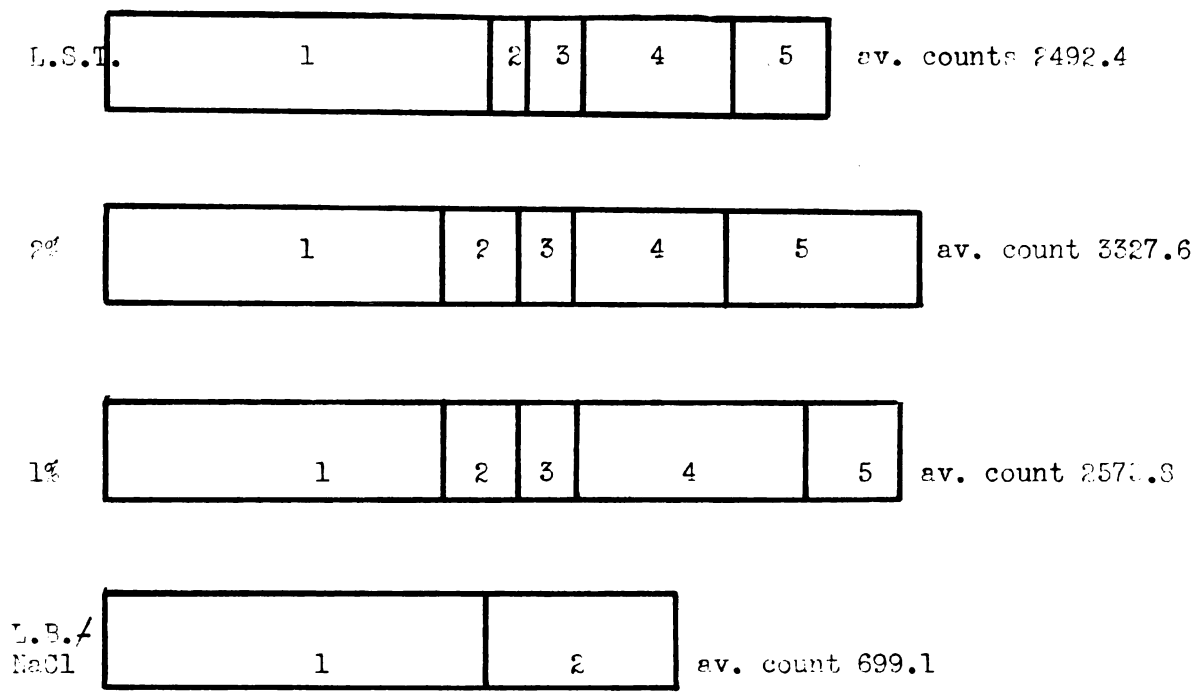
Graph VII Comparison of tryptose media and lactose media by bacterial counts using Aerobacter aerogenes.



Frequency Ranges. 1:1000, 2:1001-2000, 3:2001-3000, 4:3001-4000, 5:4001/

6 HOUR PERIOD

Graph VIII Comparison of tryptose media and lactose media by bacterial counts using Aerobacter aerogenes.



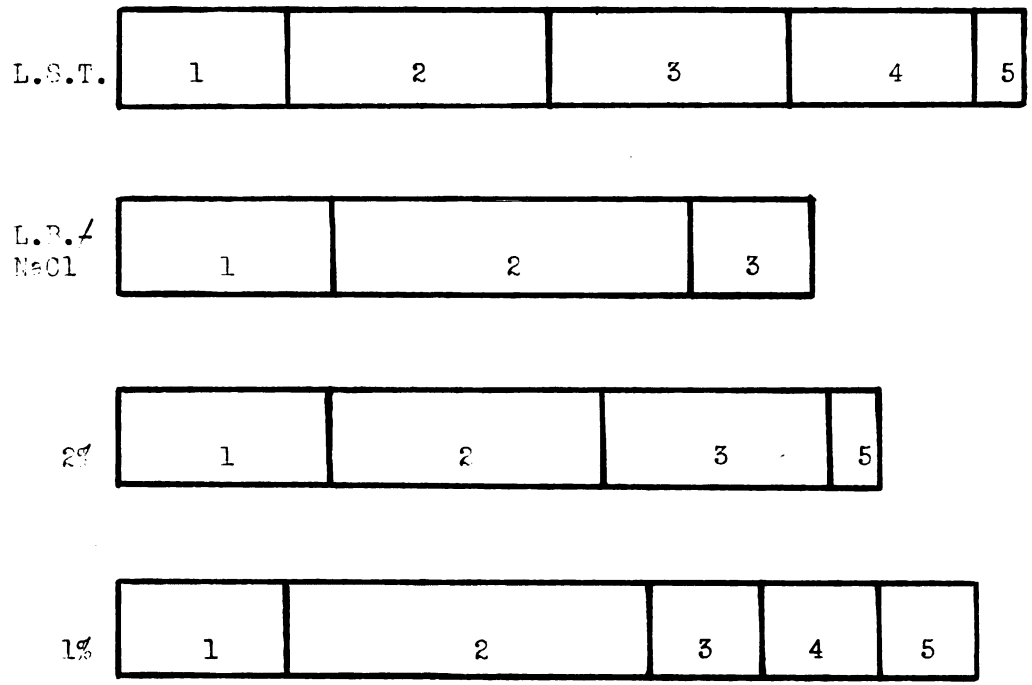
Frequency Ranges. 1:0-1000, 2:1001-5000, 3:5001-10000, 4:10001-15000, 5:15001/

24 HOUR PERIOD

for all organisms studied as may be shown by plate counts and fermentation reactions.

Fermentation studies reveal L.S.T. broth to be the superior medium for both Escherichia coli and Aerobacter aerogenes. More gas was produced in L.S.T. broth than in one percent or two percent tryptose broth. A bubble of gas would often appear in all three of these media in thirteen hours, whereas cloudiness was the only indication of growth in standard lactose broth. At the end of the 24 hour period, as much as 100 percent gas was present in L.S.T. fermentation broth, whereas, the amount of gas in standard lactose broth seldom exceeded 50 percent. Fewer negative tubes were encountered with L.S.T. broth than with standard lactose broth. It is possible that these negative tubes were not seeded due to the minimal numbers present rather than failure of growth. Tables IV and VI in the Appendix give the initial count along with the gas production. These tables were graphed in the aforementioned manner using seven ranges of frequencies. The first range includes all tubes which either failed to grow or produced growth without gas formation. The second range included all tubes containing a bubble of gas; the third range, 10 percent gas; the fourth range, 25 percent gas; the fifth range, 50 percent gas; sixth range, 75 percent gas; and the seventh range 100 percent gas. Graphs IX, X, XI and XII clearly show the fermentation rates of E.coli and A. aerogenes.

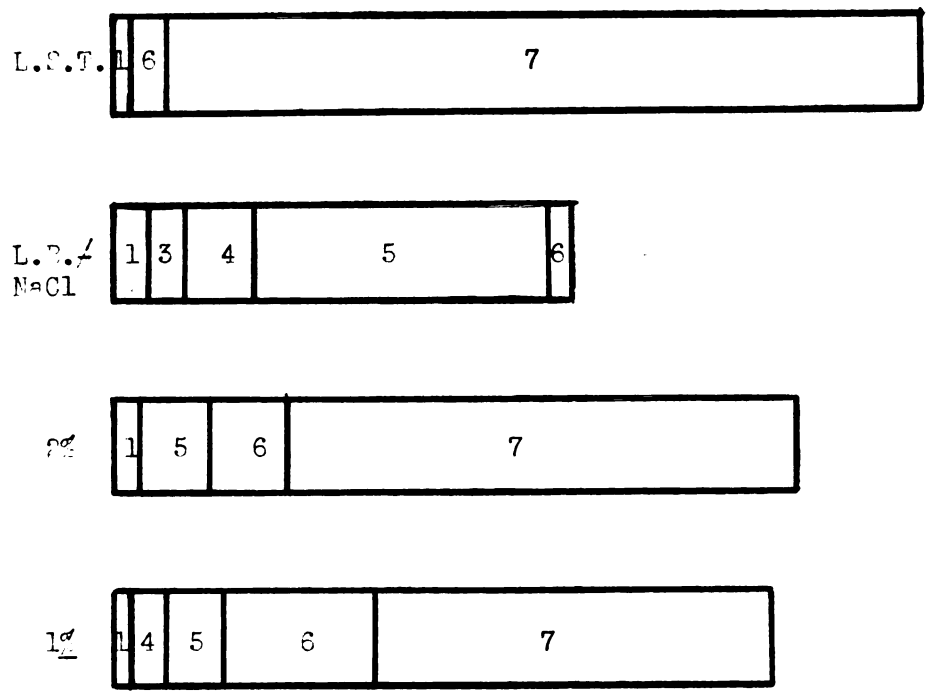
Graph IX Comparison of fermentation media showing amount of gas production for *Escherichia coli*.



Frequency Ranges. 1:no gas, 2:a bubble of gas, 3:10% gas, 4:25% gas, 5:50% gas, 6:75% gas, 7:100% gas

13 HOUR PERIOD

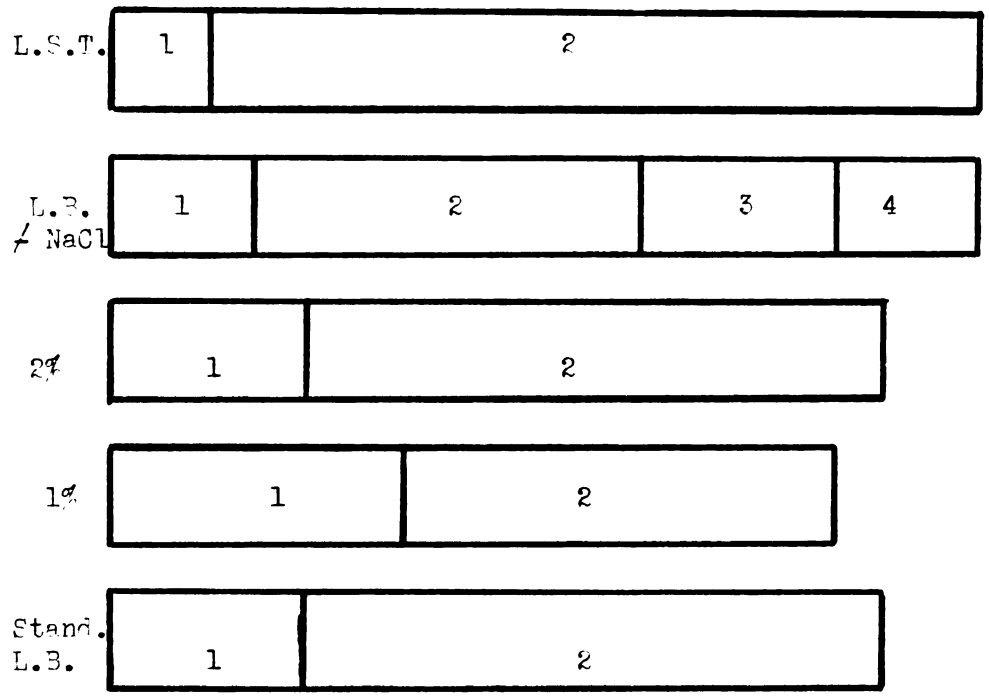
Graph X Comparison of fermentation media showing the amount of gas production for Escherichia coli.



Frequency Ranges. 1:no gas, 2:a bubble of gas, 3:10% gas, 4:25% gas, 5:50% gas, 6:75% gas, 7:100% gas

24 hour period

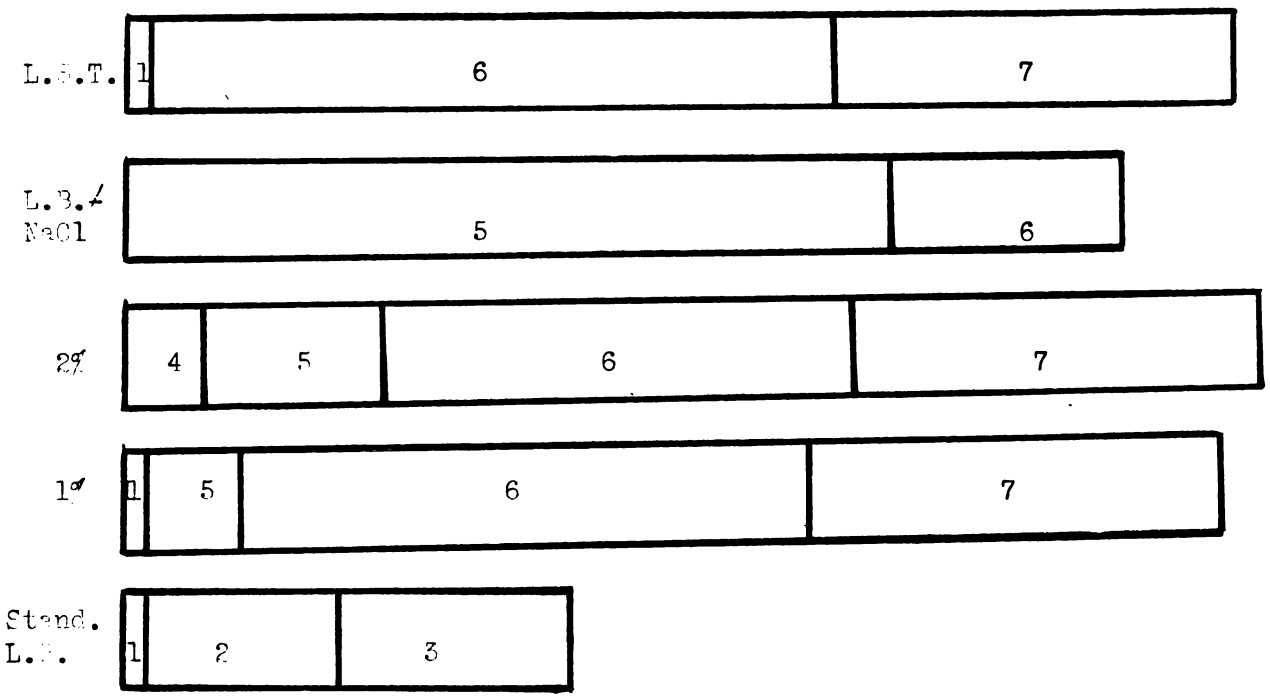
Graph XI Comparison of the fermentation media showing amount of gas production for Aerobacter aerogenes.



Frequency Ranges . 1:no gas, 2:bubble of gas, 3:10% gas, 4:25% gas, 5:50% gas, 6:75% gas, 7:100% gas.

13 Hours

Graph XII Comparison of fermentation media showing the amount of gas production for Aerobacter aerogenes.



Frequency Ranges. 1:no gas, 2:bubble of gas, 3:10% gas, 4:25% gas, 5:50% gas, 6:75% gas, 7:100% gas.

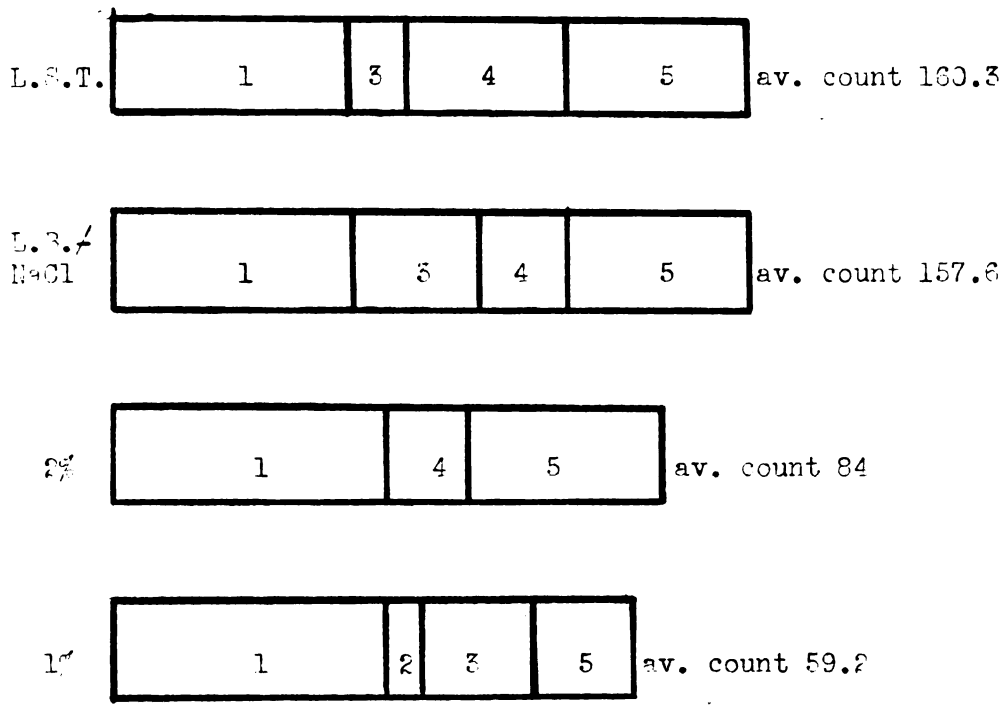
24 HOUR PERIOD

A. aerogenes did not produce as much gas as did E. coli in any of the fermentation reactions. The plate counts for A. aerogenes were considerably higher than those of E. coli thus it would be logical to assume that the fermentation rates, would correspond.

Sodium chloride was found to be definitely a stimulating agent for the growth of organisms. When added to standard lactose broth, sodium chloride almost doubled the plate counts of the standard lactose broth. At times these counts equaled those of L.S.T. broth. The rate of growth and amount of gas produced increased noticeably as shown by the fermentation reactions. However, at no time did the gas production equal that of L.S.T. broth. This stimulating property of sodium chloride is considered to aid materially to the value of tryptose broth.

In the studies involving Pseudomonas aeruginosa, it was found that this organism gave approximately the same reactions described above. However, the growth rate was considerably slower than that of either E. coli or A. aerogenes. The growth rate seldom exceeded that of E. coli under 24 hour period. Graphs XIII and XIV show the rates of growth for the 6 and 24 hour periods and more detailed growth rates may be found in the Appendix, Table X.

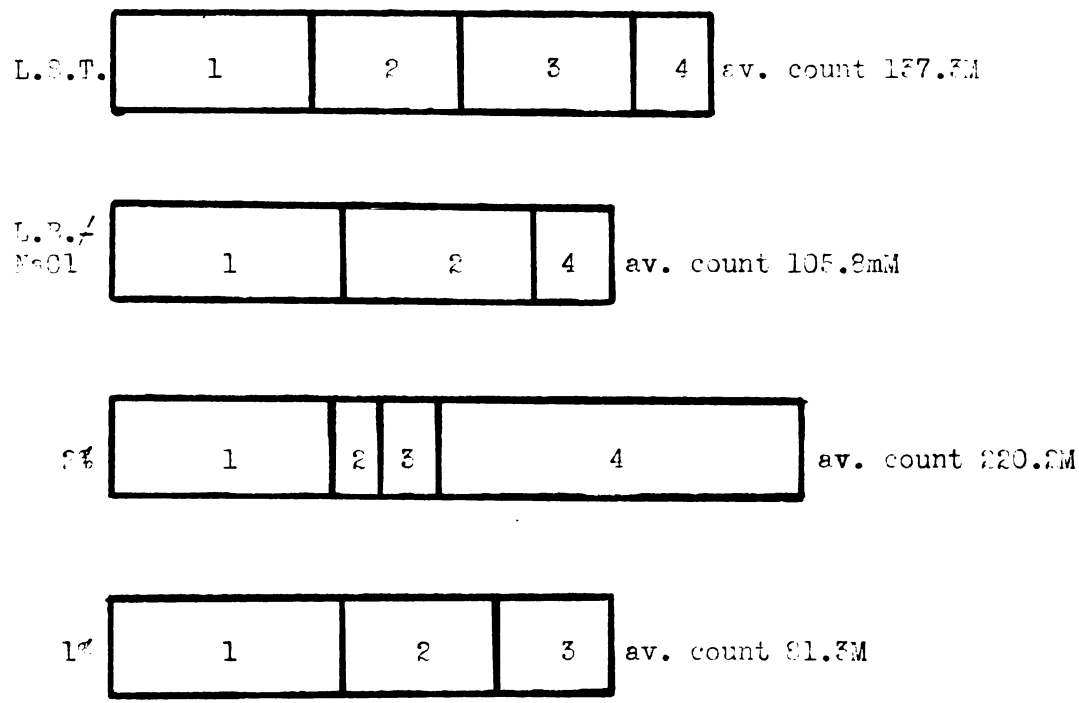
Graph XIII Comparison of tryptose media and lactose media by bacterial counts using Pseudomonas aeruginosa.



Frequency Ranges. 1:0-100, 2:101-200, 3:201-300, 4:301-400/

6 HOURS

Graph XIV Comparison of tryptose media and lactose media by bacterial counts using Pseudomonas aeruginosa.



Frequency Ranges. 1:0-100, 2;100-250, 3:250-500, 4:500/

24 HOUR PERIOD

DISCUSSION

A good diagnostic medium should stimulate the growth of viable, dormant cells as rapidly as possible. After growth and development has begun, the organism should reproduce in such numbers as to be recognized by their physiological action upon the medium. To date, lauryl sulfate tryptose broth and E.C. medium are the only media that stimulate rapid growth in a short period of time. The composition of these two media is practically the same with both containing a tryptose base.

In order for an organism to grow and reproduce it must go through a number of phases in the life cycle. Buchanan (25) defined the first four phases as follows:

1. Initial stationary phase - the number of bacteria remain constant or nearly so.
2. Lag phase or positive growth acceleration phase - the average rate of growth per organism increases with the time.
3. Logarmithmic growth phase - the rate of growth per organism is constant in a geometric ratio.
4. Negative growth acceleration - the average rate of growth per organism decreases.

Reproduction begins after an organism has overcome its stationary phase by adjustment to environment and satisfying

its food requirements. The cell undergoes fission and the two young cells begin growth. These young cells are quite susceptible to adverse conditions during this period as demonstrated by Huntington and Winslow (8). Many viable cells fail to reproduce when transferred from one environment to another, particularly a radically different environment because of the inability of adjustment. The coliform organisms from fecal material are not normal inhabitants of water supplies. These organisms become proportionately attenuated with the length of time they remain in the water, due to such factors as inadequate food supply and temperature conditions. Then too, the water greatly dilutes the number of organisms per ml. These factors must be taken into consideration when choosing a good diagnostic medium.

Kahn (25) found that an increase in the number of organisms caused a shortening of the lag phase, when comparatively small numbers were used for seeding. This is true as observed from the tables in the appendix. A relatively small seeding remained in the stationary phase for about four hours after which a noticeable increase in growth was observed. In some experimental studies by Salter (25) on the rate of growth of E.coli in peptone water, the first three hours of a nine hour period showed considerably slower growth than the last three hours. In the latter period there was always a lengthening of the generation time which would indicate

that this time included a part of the negative growth phase.

At the onset of the period of maximum reproduction there is a marked increase in metabolic activity with greater heat production, oxygen consumption, and a production of carbon dioxide and ammonia. The metabolic activity apparently declines before multiplication ceases to take place at a maximal rate. Since this is true, lauryl sulfate tryptose broth evidently stimulates the rapid growth of viable cells to maximum reproduction and increased metabolic activity much more quickly than standard lactose broth.

Although, all concentrations of tryptose broth studied increase the growth rate of an organism to a greater extent than standard lactose broth, a two percent tryptose concentration is recommended for Standard Methods. These studies show that 1.5 percent tryptose grow out the organisms almost as well as 2 percent tryptose but maximum growth is obtained with this former concentration. Laboratories where economy is of much concern are urged not to reduce cost by using a 1.5 percent concentration. Because lauryl sulfate tryptose broth eliminates false presumptives, labor and media costs are automatically cut. This would be especially true in laboratories testing treated and filtered waters. Under no conditions is a concentration below 1.5 percent to be used.

Pseudomonas aeruginosa is an organism antagonistic to Escherichia coli and is usually associated with it when is-

olated from water supplies. Ps. aeruginosa outgrows E. coli but only over a longer period of time. Growth is slowed up through the 24 hour period but after 48 hours, there is a decided increase in the growth rate over that of E. coli. Here again the advantage in using a medium which stimulates the rapid growth of E. coli and A. aerogenes can be observed.

The addition of sodium lauryl sulfate to a medium produces a very slight stimulating effect upon the coliform organisms. Cowles (1) found that sodium lauryl sulfate exerted a selective action for the coliform organisms and inhibited other gram-positive lactose fermenting organisms. Perry and Hajna (20) presented some data to show the superiority of E. C. medium over lauryl sulfate tryptose broth. In E. C. medium bile salts have been substituted for lauryl sulfate. Bile salts also inhibit the gram-positive organisms but have been proved to be inhibitory to the coliform organisms as well. To date, no literature is available concerning the inhibitory or stimulating property of lauryl sulfate. From this study it is evident that lauryl sulfate tryptose broth is the more desirable medium.

Sodium chloride has never been added to standard lactose broth because it makes the medium hypertonic and it is, therefore, reasoned that a large number of the organisms would fail to make an adjustment in this new type of environment. The marked increase in growth as demonstrated by these studies show that the organisms not only made an adjustment

but were also stimulated to increased growth rates. Because of this marked increase in the growth rate a 0.5 percent concentration of sodium chloride in standard lactose broth is recommended for Standard Methods.

The experimentation carried out for these studies is not considered complete. More work should be done with mixed cultures of E. coli and A. aerogenes as well as with several antibiotic organisms common to polluted waters.

SUMMARY

1. The growth rates in 1.5 percent tryptose and 2 percent tryptose broth approximate each other.
2. A concentration of 1.5 percent tryptose should not be substituted for a 2 percent concentration.
3. Two percent tryptose broth is recommended for Standard Methods.
4. Lauryl sulfate tryptose broth is superior to standard lactose broth.
5. Lauryl sulfate tryptose broth stimulates fermentation reactions more rapidly than standard lactose broth.
6. Escherichia coli grows more rapidly than Pseudomonas aeruginosa during the first 24 hours.
7. Sodium lauryl sulfate has little, if any, stimulating or inhibitory action upon the coliform organisms.
8. Sodium chloride shows marked stimulating properties in a0.5 percent concentration.
9. The addition of sodium chloride to standard lactose broth is recommended for Standard Methods.

REFERENCES

(1) Cowles, Philip B. A Modified Lactose Broth for Use in the Presumptive Test. J. Am. Water Works Assoc. 30:5, 979 (1940)

(2) Darby, C. W. and Mallmann, W.L. Studies on Media for Coliform Organisms. J. Am. Water Works Assoc. 31:4, 689-706 (1939)

(3) Green, Glenn Studies in Bacterial Counts. 20th Annual Conference on Water Purification. Oct. (1940)

(4) Greer, Frank E. The Sanitary Significance of Lactose Fermenting Bacteria Not Belonging to the B. coli Group. J. Infect. Dis. 42: 6, 525-536. (1928)

(5) Greer, Frank E. The Sanitary Significance of Lactose Fermenting Bacteria Not Belonging to the B. coli Group. J. Infect. Dis. 42: 6, 568-574. (1928)

(6) Griffin, A. M. and Stuart, C. A. An Ecological Study of the Coliform Bacteria. J. Bact. 40: 1, 83-100 (1940)

(7) Hajna, A. A. and Perry, Alfred C. Comparative Study of Presumptive and Confirmative Media for Bacteria of the Coliform Group and for Fecal Streptococci. Am. J. Public Health 33: 5, 550-556 (1943)

(8) Huntington, Evelyn and Winslow, C. - E.A. A Cell Size and Metabolic Activity of Various Phases of the Bacteria Culture Cycle. J. Bact. 33: 123. (1927)

(9) Hupp, E. Memorandum From Indianapolis Water Co. January 8, 1940)

(10) Janzig, A. C. and Montank, I. A. The Elimination of False Presumptive Tests. J. Am. Water Works Assoc. 20: 5, 684-695. (1928)

(11) Johnson, B. R. and Levine, Max Characteristics of coli - like Microorganisms from the Soil. J. Bact. II: 4, 379-401. (1917)

(12) Kahn, Reuben L. The Differentiation of Lactose Fermenting Anaerobes from B. coli. J. Bact. III: 6, 561-564 (1918)

(13) Ibid. Examination of B. coli in Water, Am. J. Public Health. IX: 5, 333-336 (1919)

(14) Ibid. A Simplified Confirmatory Test for B. coli. J. Bact. III: 6, (1918)

(15) Levine, Max. Bacteria Fermenting Lactose and Their Significance in Water Analysis. Iowa State Coll of Agri and Mech Arts. IX: 31, 5-119 (1921)

(16) Levine, Max F. Determination and Characterization of Coliform Bacteria from Chlorinated Waters. Am. J. Public Health 31: 351-8 (1941)

(17) McCrady, M. H. A Practical Study of Lauryl Sulfate Tryptose Broth for Detection of the Presents of Coliform Organisms in Water. 33: 1199-1207 (1943)

(18) Mallmann, W. L. and Darby, C. W. Uses of a Lauryl Sulfate Tryptose Broth for the Detection of Coliform Organisms. Am. J. Public Health. 31: 2, 127-134 (1941)

(19) Parr, Leland W. Coliform Bacteria. Bact. Reviews. 3: 1, 1-48 (1939)

(20) Perry, C. A. and Hajna A. A. Further Evaluation of E. C. Medium for the Isolation of Coliform Bacteria and Escherichia coli. Am. J. Pub. Health. 34: 735-8 (1944)

(21) Perry, C. A. and Jahna A. A. A Modified Eykman Medium. J. Bact. XXVI: 4, 419-429 (1933)

(22) Ritter, Cassandra The Presumptive Test in Water Analysis J. Am. Water Works Assoc. 24: 3, 413-424 (1932)

(23) Ruchhoft, Kallas, Chinn, Coulter Coli-aerogenes differentiation in Water Analysis. J. Bact. XXI: 6, 407-440 (1931)

(24) Salle, A. J. The Differentiation of the Coli and Aerogenes Groups of Bacteria. J. Am. Water Works Assoc. 21: 1, 71-78 (1929)

(25) Salter, Raymond C. Observations on the Rate of Growth of B coli XVIII: 42, 260-84 (1920)

(26) Stark, C. N. and Stark, P. The Relative Thermal Death Rates of Young and Mature Bacterial Cell. J. Bact 18: 333 (1929)

(27) Stuart, C. A. Sanitary Significance of the Coliform Bacteria in Water. New England Water Works. J. LV: 355-362 (1941)

(28) Stuart, C. A. Mickle, Friend Lee, Borman, Earle K. Suggested Grouping of Slow Lactose Fermenting Coliform Organisms. Am. J. Public Health. 30: 5, 499-508 (1940)

(29) Tonney, Fred O and Noble, Ralph E. The Relative Persistence of B. Coli and B. aerogenes in Nature. J. Bact. XXII: 6, 433-446 (1931)

(30) Ibid. Colon-Aerogenes Types of Bacteria as Criteria of Fecal Pollution. 24: 9, 1267-1280 (1932)

(31) Year Book. Report of Standard Methods Committee for Water and Sewage to Laboratory Section. A.P.H.A. (1943)

(32) Young, C. C. and Greenfield, M. Observation on the viability of the Bacterium Coli Group Under Natural and Artificial Conditions. Michigan Dept. Health. No. 12: 1-5 (1923)

APPENDIX

TABLE I

Plate Counts for Escherichia coli

HOURS	0.5% T	1% T	1.5% T	2% T	L.B.-NaCl
Initial	10	5	7	8	2
2	5	11	6	6	5
4	79	129	130	122	169
6	984	1460	2235	2362	1797
24	1650M	1778M	850M	1800M	381M
Initial	8	12	8	6	11
2	13	10	9	6	8
4	94	105	138	234	245
6	1050	1511	1860	3213	2600
24	952M	1080M	1522M	1588M	401M
Initial	12	11	23	17	13
2	11	13	10	17	15
4	182	197	306	500	568
6	1460	2288	4000	5750	5080
24	1270M	889M	698M	892M	473M
Initial	11	11	12	18	10
2	10	18	8	10	16
4	298	295	293	236	248
6	1570	2150	2890	1180	1210
24	120M	130M	230M	190M	80M
Initial	11	7	10	13	11
2	9	7	5	6	6
4	547	540	392	179	337
6	4440	5910	2330	1500	1200
24	50M	100M	90M	90M	20M
Initial	3	1	0	4	4
2	3	8	13	15	12
4	182	283	559	902	802
6	2286	2730	6540	11654	9779
24	36M	67M	65M	81M	29M
Initial	5	7	10	4	6
2	6	6	4	9	7
4	178	170	203	413	492
6	1226	2540	4000	8000	5080
24	28M	54M	39M	78M	22M

Plate Counts for Escherichia coli (cont'd)

HOURS	0.5% T	1% T	1.5% T	2% T	L.B.-NaCl
Initial	10	11	7	5	12
2	10	12	10	23	15
4	296	445	1115	480	518
6	4254	4572	11239	5461	6096
24	370M	330M	480M	680M	90M

TABLE II

Plate Counts for Escherichia coli

HOURS	1½ T	2½ T	L.S.T.	L.B.-NaCl
Initial	1	1	0	2
2	0	0	8	0
4	0	0	2	0
6	133	552	189	181
24	4M	9M	12M	4M
Initial	2	0	0	0
2	0	0	0	0
4	1	1	3	0
6	30	40	50	10
24	1184T	1080T	1089T	462
Initial	0	0	0	0
2	1	0	1	0
4	2	4	4	0
6	140	120	60	40
24	19M	16M	11M	3M
Initial	0	0	0	0
2	0	0	0	0
4	2	3	0	2
6	80	40	100	80
24	19M	11M	16M	3M
Initial	1	1	0	0
2	0	0	0	1
4	9	14	1	11
6	92	311	135	314
24	13M	13M	11M	3M
Initial	0	1	1	3
2	0	0	0	0
4	9	0	4	3
6	138	354	132	151
24	21M	11M	20M	3M
Initial	0	1	0	2
2	0	0	1	3
4	15	25	22	24
6	431	927	495	476
24	24M	23M	9M	6M

Plate Counts for Escherichia coli (cont'd)

HOURS	1½ T	2% T	L.S.T.	L.B.-NaCl
Initial	1	0	1	0
2	0	0	1	2
4	5	3	3	35
6	126	227	189	459
24	14M	9M	22M	6M
Initial	0	1	0	0
2	0	0	0	0
4	1	1	1	1
6	0	100	100	200
24	9840T	1080T	5970T	3240T
Initial	1	1	0	0
2	0	0	0	0
4	3	0	0	2
6	400	700	200	0
24	5590T	11750T	5780T	3080T
Initial	1	2	0	0
2	0	0	0	0
4	2	1	2	1
6	700	400	500	0
24	10500T	85700T	10500T	4450T
Initial	1	0	3	2
2	0	1	0	1
4	11	7	8	9
6	447	1677	1030	298
24	11M	13M	8M	2M
Initial	6	4	2	3
2	6	1	7	6
4	9	22	16	15
6	151	155	139	218
24	13M	14M	9M	6M
Initial	8	3	0	8
2	5	4	3	3
4	18	3	10	15
6	93	116	96	141
24	20M	15M	10M	6M

Plate Counts for Escherichia coli (cont'd)

HOURS	1½ T	2% T	L.S.T.	L.B.-NaCl
Initial	2	5	14	5
2	5	4	0	2
4	9	20	10	16
6	176	117	115	133
24	6M	2M	2M	8M
Initial	3	1	6	3
2	2	2	3	11
4	8	13	9	49
6	90	85	110	569
24	-	-	-	-
Initial	2	5	5	5
2	2	2	10	6
4	6	18	33	99
6	61	89	54	100
24	-	-	-	-
Initial	0	2	0	0
2	0	0	0	0
4	17	3	23	14
6	229	525	282	497
24	21M	8M	40M	4M
Initial	0	1	1	0
2	0	2	0	0
4	33	16	9	18
6	499	425	326	224
24	9M	0M	15M	1M
Initial	0	0	4	0
2	1	0	0	0
4	9	102	99	29
6	944	919	1634T	513
24	16M	16M	10M	0M
Initial	2	1	0	0
2	0	0	0	0
4	12	4	10	12
6	344	338	409	574
24	4M	21M	6M	6M

Plate Counts for Escherichia coli (cont'd)

HOURS	1% T	2% T	L.S.T.	L.B.-NaCl
Initial	2	2	1	0
2	0	1	0	0
4	13	12	12	18
6	438	298	379	358
24	11M	5M	1M	2M

Plate Counts for Escherichia coli

HOURS	1% T	2% T	L.S.T.	L.B.	L.B.-NaCl
Initial	1	0	0	1	0
2	0	1	0	0	0
4	21	28	10	4	43
6	205	860	495	107	476
24	623M	625M	534M	204M	335M
Initial	0	0	0	0	0
2	2	1	2	0	2
4	13	26	33	9	9
6	451	536	588	154	582
24	938M	996M	652M	297M	425
Initial	0	0	0	0	0
2	2	0	3	0	0
4	16	20	25	3	46
6	389	490	332	62	1214
24	667M	1038M	714M	498M	477M
Initial	3	0	0	1	0
2	1	3	1	2	2
4	86	36	30	24	34
6	717	899	1290	357	1585
24	890M	868M	468M	0M	224M
Initial	1	0	0	0	0
2	0	0	0	0	2
4	3	17	12	0	10
6	569	532	85	83	243
24	773M	1174M	1013M	249M	350M

TABLE III

Plate Counts for Aerobacter aerogenes

HOURS	1% T	2% T	L.S.T.	L.B.-NaCl
Initial	1	0	0	0
2	1	1	2	2
4	21	5	22	26
6	8255	12582	696	12392
24	12065T	11430T	8255T	3112T
Initial	0	2	0	3
2	0	0	1	0
4	32	8	18	22
6	9652	762	9775	9843
24	10160T	15875T	14600T	2620T
Initial	1	0	0	0
2	3	3	4	0
4	22	41	19	20
6	15875	22225	1334	1016
24	19050T	27300T	4445T	3160T
Initial	0	0	0	1
2	2	0	11	2
4	59	47	48	52
6	18615	1143	22225	1932
24	11430T	9525T	18415T	2780T
Initial	0	0	0	0
2	5	2	1	0
4	23	26	6	38
6	7239	1651	635	3048
24	6420T	13335T	12700T	3280T
Initial	1	2	0	0
2	10	0	3	0
4	195	111	232	85
6	4500	3000	6200	4700
24	OM	OM	OM	10M
Initial	0	0	0	1
2	1	5	0	5
4	66	129	144	94
6	1600	2800	1000	2600
24	150M	250M	200M	OM

Plate Counts for Aerobacter aerogenes (cont'd)

HOURS	1½ T	2½ T	L.S.T.	L.B.-NaCl
Initial	1	0	0	1
2	1	3	2	4
4	45	122	49	61
6	1300	2000	1500	1800
24	140M	210M	170M	60M
Initial	0	0	0	0
2	2	1	0	0
4	94	54	42	64
6	1900	1600	1400	2700
24	170M	240M	90M	50M
Initial	0	0	0	0
2	0	0	0	2
4	35	16	45	32
6	8000	2000	1000	3000
24	140M	250M	390M	50M
Initial	1	1	1	1
2	3	5	0	0
4	59	84	56	70
6	1111	2180	1446	108
24	23M	22M	7M	10M
Initial	0	0	2	1
2	2	3	o	1
4	66	57	67	98
6	1118	1758	1035	1822
24	15M	24M	8M	6M
Initial	0	1	0	1
2	0	3	0	0
4	12	19	23	17
6	387	476	1005	70
24	30M	18M	41M	1M
Initial	0	2	0	1
2	0	0	0	0
4	7	18	21	15
6	381	102	305	685
24	19M	13M	28M	12M

Plate Counts for Aerobacter aerogenes (cont'd)

HOURS	1% T	2% T	L.S.T.	L.B.-NaCl
Initial	0	1	1	1
2	2	3	3	0
4	17	17	18	23
6	76	1651	1843	1308
24	20M	29M	38M	4M
Initial	0	0	1	0
2	2	1	0	2
4	3	5	5	4
6	176	279	173	118
24	14M	17M	16M	8M
Initial	1	1	1	1
2	1	1	0	0
4	15	12	13	6
6	292	362	234	233
24	9M	21M	18M	5M
Initial	2	2	0	1
2	0	1	1	1
4	21	10	37	19
6	360	241	231	237
24	8M	10M	7M	4M
Initial	1	0	1	1
2	1	1	0	0
4	7	16	14	4
6	157	292	191	123
24	8M	29M	7M	3M
Initial	0	1	2	1
2	0	0	2	1
4	18	33	17	17
6	411	391	444	267
24	16M	10M	14M	6M
Initial	0	0	1	0
2	0	1	1	3
4	23	26	44	16
6	1260	1890	2560	2020
24	800M	640M	631M	390M

Plate Counts for Aerobacter aerogenes (cont'd)

HOURS	1½ T	2% T	L.S.T.	L.R.-NaCl
Initial	0	0	1	0
2	2	4	0	2
4	19	38	54	39
6	2470	2140	2050	2140
24	1106M	377M	533M	332M
Initial	0	1	0	0
2	2	0	1	0
4	20	21	16	7
6	1430	2190	1500	660
24	729M	2131M	567M	388M
Initial	0	0	0	0
2	4	0	2	0
4	46	52	40	43
6	2610	4840	3250	3990
24	789M	985M	539M	656M
Initial	0	0	1	0
2	0	0	0	0
4	38	53	72	22
6	3310	3520	5050	2080
24	1035M	1341M	602M	532M

TABLE IV

Fermentation of Escherichia coli --- 13 hours

Sample No.	L.S.T.	L.B.-NaCl	2% T	1% T	Initial Count
1	≠	≠	-	≠	0
2	≠	≠	-	-	0
3	10%	-	-	-	0
4	-	≠	-	-	0
5	≠	≠	10%	≠	2
6	≠	≠	10%	-	2
7	10%	≠	10%	20%	1
8	10%	≠	5%	10%	0
9	≠	≠	-	5%	0
10	25%	-	-	30%	0
11	20%	5%	5%	≠	1
12	10%	5%	2%	≠	1
13	≠	Growth	Growth	≠	3
14	10%	≠	≠	≠	1
15	Growth	-	-	5%	0
16	10%	-	Growth	≠	3
17	50%	5%	30%	20%	1
18	≠	-	Growth	-	8
19	≠	5%	≠	≠	3
20	≠	-	≠	≠	2
21	5%	≠	3%	≠	8
22	-	-	≠	3%	1
23	-	≠	≠	≠	0

Fermentation of Escherichia coli --- 13 hours (cont'd)

Sample No.	L.S.T.	L.B.-NaCl	2% T	1% T	Initial Count
24	20%	-	≠	≠	2
25	15%	≠	≠	40%	3
26	10%	≠	≠	15%	0
27	15%	≠	10%	≠	2
28	Growth	≠	Growth	≠	0
29	≠	≠	≠	≠	0
30	-	Growth	≠	Growth	1
31	≠	Growth	≠	≠	2
32	Growth	-	Growth	Growth	3
33	≠	≠	≠	≠	0
34	≠	≠	Growth	≠	0
35	Growth	Growth	Growth	Growth	0
36	Growth	≠	≠	Growth	0
37	≠	-	≠	≠	0

TABLE V

Fermentation of Escherichia coli --- 24 hours

Sample No.	L.S.T.	L.B.-NaCl	2% T	1% T
1	95%	45%	-	80%
2	100%	30%	-	-
3	90%	-	-	-
4	-	30%	-	-
5	90%	30%	85%	75%
6	95%	30%	95%	-
7	97%	40%	80%	85%
8	100%	30%	60%	80%
9	100%	50%	-	80%
10	100%	-	-	80%
11	80%	20%	100%	85%
12	90%	50%	50%	50%
13	85%	35%	100%	90%
14	100%	25%	75%	60%
15	75%	-	-	50%
16	100%	10%	95%	45%
17	100%	45%	100%	55%
18	85%	-	85%	-
19	100%	50%	50%	80%
20	100%	-	50%	100%
21	100%	40%	90%	95%
22	-	-	100%	25%
23	-	10%	90%	100%

Fermentation of Escherichia coli --- 24 Hours (cont'd)

Sample No.	L.S.T.	L.B.-NaCl	2% T	1% T
24	95%	-	100%	50%
25	100%	30%	100%	100%
26	100%	15%	90%	90%
27	100%	10%	100%	85%
28	100%	25%	100%	60%
29	95%	60%	30%	90%
30	60%	40%	75%	75%
31	100%	30%	95%	90%
32	100%	50%	85%	25%
33	100%	20%	100%	60%
34	100%	15%	100%	100%
35	100%	30%	45%	75%
36	80%	25%	70%	65%
37	100%	-	95%	100%

TABLE VI

Fermentation of Aerobacter aerogenes --- 13 Hours

Sample No.	L.S.T.	L.B.-NaCl	2% T	1% T	Initial Count
1	≠	≠	≠	≠	2
2	≠	≠	≠	≠	35
3	-	-	-	Growth	0
4	≠	≠	10%	≠	16
5	Growth	≠	≠	Growth	21
6	≠	≠	≠	≠	8
7	Growth	≠	≠	≠	28
8	≠	≠	≠	≠	34
9	≠	≠	≠	≠	22
10	Growth	≠	≠	≠	23
11	Growth	≠	Growth	≠	7
12	≠	≠	≠	≠	3
13	≠	≠	≠	≠	5
14	≠	Growth	≠	≠	1
15	≠	≠	≠	≠	26
16	Growth	≠	≠	Growth	8
17	Growth	Growth	≠	Growth	13
18	Growth	Growth	Growth	Growth	17
19	Growth	Growth	Growth	Growth	2
20	Growth	Growth	Growth	Growth	18

TABLE VII

Fermentation of Aerobacter aerogenes --- 24 Hours

Sample No.	L.S.T.	L.B.-NaCl	2% T	1% T
1	5%	10%	20%	25%
2	5%	10%	5%	25%
3	-	-	-	+
4	15%	5%	20%	35%
5	5%	10%	15%	20%
6	40%	20%	15%	20%
7	5%	10%	25%	25%
8	20%	15%	15%	15%
9	15%	15%	25%	30%
10	5%	10%	10%	10%
11	10%	5%	2%	+
12	30%	20%	25%	10%
13	15%	2%	10%	25%
14	10%	2%	10%	10%
15	30%	10%	10%	10%
16	5%	5%	10%	25%
17	10%	5%	30%	5%
18	10%	5%	7%	5%
19	+	+	+	+
20	+	5%	2%	10%

TABLE VIII

Fermentation of Aerobacter aerogenes --- 13 hours

Sample No.	L.S.T.	L.B.-NaCl	Stand L.B.	2% T	1% T	L.B. & L.S.	Initial Count
1	-	≠	Growth	Growth	-	Growth	0
2	-	≠	-	-	-	Growth	0
3	≠	-	Growth	-	≠	Growth	0
4	≠	≠	Growth	≠	Growth	Growth	0
5	-	-	Growth	≠	Growth	Growth	0
6	-	-	Growth	≠	-	Growth	0
7	≠	Growth	Growth	-	≠	Growth	1
8	Growth	-	Growth	-	-	Growth	3
9	≠	5%	Growth	≠	≠	Growth	0

TABLE IX

Fermentation of Aerobacter aerogenes --- 24 hours

Sample No.	L.S.T.	L.B.-NaCl	Stand L.B.	2% T	1% T	L.B. & L.S.
1.	65%	50%	35%	75%	60%	15%
2	65%	50%	30%	20%	80%	15%
3	60%	60%	45%	100%	80%	10%
4	70%	45%	25%	60%	50%	25%
5	100%	40%	10%	100%	65%	10%
6	65%	40%	40%	100%	70%	10%
7	100%	50%	40%	60%	80%	25%
8	65%	50%	40%	50%	60%	10%
9	80%	65%	45%	75%	60%	10%

TABLE X

Plate Counts for Pseudomonas Aeruginosa

HOURS	1% T	2% T	L.S.T.	L.B.-NaCl
Initial	0	2	0	1
2	0	0	1	0
4	2	0	2	0
6	4	3	9	7
24	17M	15M	2M	10M
Initial	3	0	0	1
2	0	1	0	0
4	1	0	2	1
6	5	14	24	11
24	19M	17M	4M	12M
Initial	0	3	0	0
2	2	2	1	0
4	1	3	3	0
6	11	14	18	3
24	22M	8M	3M	11M
Initial	0	0	0	0
2	0	2	0	1
4	64	2	8	7
6	18	30	37	28
24	0M	1M	2M	0M
Initial	1	0	0	0
2	1	1	1	0
4	2	2	2	149
6	23	24	36	32
24	0M	1M	3M	0M
Initial	0	0	0	0
2	0	2	2	0
4	3	1	2	1
6	11	19	53	21
24	1M	6M	1M	3M

Plate Counts for Pseudomonas Aeruginosa (cont'd)

HOURS	1% T	2% T	L.S.T.	L.B.-NaCl
Initial	0	0	2	1
2	1	2	1	1
4	1	4	4	2
6	37	65	67	50
24	2M	0M	0M	1M
Initial	1	0	0	0
2	1	4	15	85
4	0	4	2	5
6	17	18	35	16
24	0M	1M	1M	2M
Initial	0	0	1	0
2	1	9	29	24
4	0	1	7	2
6	12	17	19	34
24	0M	0M	0M	1M
Initial	4	3	2	0
2	0	0	0	4
4	8	3	6	8
6	200	0	200	200
24	201M	56M	184M	205M
Initial	0	1	1	2
2	2	2	3	2
4	32	27	40	43
6	100	500	1200T	1200T
24	207M	597M	223M	407M
Initial	3	1	1	1
2	0	0	2	2
4	9	9	6	7
6	0	300	200	300
24	248M	643M	218M	455M
Initial	2	0	1	1
2	0	1	1	0
4	5	6	5	11
6	200	0	300	400
24	170M	276M	193M	615M

Plate Counts for Pseudomonas Aeruginosa (cont'd)

HOURS	1% T	2% T	L.S.T.	L.B.-NaCl
Initial	0	1	0	2
2	0	1	0	0
4	3	2	1	3
6	7	9	10	14
24	6M	504M	167M	81M
Initial	2	0	0	0
2	0	0	0	1
4	0	0	1	0
6	9	10	10	2
24	331M	181M	129M	3M
Initial	0	3	0	1
2	0	0	0	0
4	1	1	1	4
6	4	1	8	3
24	283	930M	155M	241M