EVALUATION OF LIQUID MEDIA FOR THE QUANTITATIVE DETERMINATION OF STREPTOCOCCI FROM SOIL AND WATER

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AND [illegible]

By

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INTRODUCTION

During the last decade the value of streptococci as indicators of pollution has been the subject of a great deal of work. In order to draw any conclusions from work for evaluating the validity of the organisms as indicators of pollution, it is first necessary to use a medium which will grow all of the original viable streptococci in the sample. Since a complex flora will be encountered in the sample, it is also necessary to limit the growth of bacteria other than streptococci. A fairly simple method of the results is to be desired for routine work. With these facts in mind it can be seen that a medium is needed which will allow all of the viable streptococci to grow and at the same time offer an easy method for determining the presence of streptococci in the tubes.

In order to determine the usefulness of streptococci as indicators of health hazard, it is necessary to use a medium that gives an accurate measurement of the streptococci present in the material tested. Although such methods as those reported by Kuhlmann and Delpi (1) and Litsky and Morey (2) have been used, there are no comparative

studies available to indicate the accuracy of each method. This is also true of later media which have been reported. Therefore it seemed advisable to make a comparative study of available media to determine the value of each. This thesis presents such a study.

Much literature has been published pro and con on the reliability of streptococci as indicators of pollution. Hallmann (3) studied the flora of swimming pools and found streptococci to be quite prevalent. In these studies he showed that the streptococcus indices varied directly with the bathing load and that the streptococci would not multiply in the pool as Escherichia coli will if given suitable conditions. In a number of papers various methods appeared for determining the presence of streptococci when found in the mixed flora of river water, sewage, milk, soil, or swimming pool water. Probably the greatest reason for the reluctance to adopt streptococci as indicators of pollution is the extreme difficulty of determining the streptococcus index. The presence of the coliform group may be indicated by the formation of gas from lactose. However, no such simple test is available for the streptococci. The work of VanderVelde, Hallmann, and Moore (4) showed that the streptococcus index increased when the bathers submerged their heads and did breathing exercises. Before the bathers submerged their heads a low streptococcus index was reported. While a great amount of work has

been carried out to utilize fecal streptococci as indicators of pollution, it can be seen that a reliable index for bathing waters would include buccal streptococci as well as fecal streptococci. It is with this thought in mind that a technique for determining streptococcus indices must be such that all species of Streptococcus will be included. Furthermore, these streptococci must be differentiated from the complex flora in which they occur.

Since no biochemical reaction is common to all streptococci, the presence of the organism must be determined in some other manner. The first, and perhaps most obvious method, was a direct microscopical examination of a culture of the suspected material. In this way the streptococci could be seen among the other organisms present.

Hallmann and Selgi (1) described such a method. They advised using the fermentation tubes for Escherichia coli. They allowed these tubes to stand at room temperature for one to three days after the normal incubation time of forty-eight hours at 37°C. After this period of standing the streptococci in the tubes have settled and the supernatant fluid can be decanted. The sediment is then smeared on a slide, stained, and examined for the presence of streptococci. They also stated that streptococci may be recognized macroscopically by the appearance of a heavy sediment arranged in the bottom of the tube in a fashion similar to the deposit in the macroscopic agglutination

test. However, this test is not specific and should always be confirmed by microscopic examination.

The medium used in this method is standard lactose broth. Since a wide variety of bacteria would be growing in the tubes, it is not unlikely that some of these bacteria might exhibit an inhibitory effect on the streptococci. Also, the presence of a wide assortment of bacteria would be highly competitive in the matter of available food. In either case there would be the possibility of not all of the original viable streptococci growing. If some or all of the other bacteria could be inhibited so that the streptococci would grow, the indices would become more reliable.

Heilin and Hartree (5) and Blaschke (6) described the inhibitory action of sodium azide on certain bacteria. Hartman (7) used sodium azide to prevent the growth of gram-negative bacteria while permitting the growth of streptococci. Clifton and Logan (3) presented a possible method of inhibition due to sodium azide. They showed that the sodium azide blocks the assimilation of carbohydrates by Escherichia coli. When this assimilatory process is blocked by the sodium azide the growth of the cells is inhibited.

Bryan, Devereux, Hirsch, and Corbett (9) used sodium azide in combination with brilliant green and dextrose as a preservative to decrease the proportion of bacteria other than streptococci in the microscopic and Motis tests for

streptococcic mastitis.

Snyder and Lichstein (10) and Lichstein and Snyder (11) used strengths of 1:8,000 and 1:10,000 sodium azide in blood agar to prevent the spreading growth of Proteus and thus allow the development of the streptococcal colonies. Fecal specimens were used for inoculum and it was found that gram-negative bacteria other than Proteus were inhibited.

Hallmann (12) proposed the first liquid sodium azide medium for the determination of streptococcus indices. This medium offers higher nutritional standards for streptococcus than Lactose Broth while incorporating an inhibiting agent which restricts competitive bacteria, allowing the streptococci to grow.

A liquid medium is needed for determining indices since the numbers are based on the number of organisms per one hundred milliliters of water. Since the numbers of streptococci may vary from one per one hundred milliliters to several million per one hundred milliliters, a solid medium would prove most cumbersome if used for determining indices. In the case of one streptococcus per one hundred ml. it would be difficult to prepare and use an agar concentrated enough to yield the proper strength medium after diluting with one hundred ml. of sample. The sample could be divided in smaller portions as is the custom when using liquid media. However, in routine

work agar is much more difficult to use than a liquid. In the case of very high inocula the number of agar plates used for the proper dilutions would be prohibitive for most laboratories in carrying out routine work.

Hellmann, Hetrright, and Churchill (13) demonstrated that a concentration of 1:5,000 sodium azide would inhibit gram-negative bacteria and some gram-positive rods while permitting the growth of streptococci.

Hajna and Perry (2) proposed a liquid medium designed to be selective for Streptococcus faecalis. They stated that the presence of growth and acid at 45.5°C. is almost complete evidence of the presence of Strep. faecalis. An indicator was added to indicate the presence of acid. However, since the medium is buffered it would seem that the presence of few or weak streptococci might not be sufficient to produce a great enough shift in pH for the indicator to visibly change. The fact that Strep. faecalis will grow at 45.5°C. while other species will not, also gives further selective action.

Ritter and Breece (14) presented a sodium azide broth in connection with their work on streptococci in swimming pools.

EXPERIMENTAL PROCEDURE

A medium, in order to satisfy routine determination of streptococcus indices, must:
1) be simple and inexpensive to prepare, 2) produce optimum growth of streptococci, 3) be selective for streptococci, 4) be easily checked, and 5) yield a negligible percentage of false positives. If all of these factors are satisfied the medium can be accepted without reservations.

The following media were selected for use in the experimental work of this thesis.

Lactose Broth

Beef Extract	0.3%
Peptone	0.5%
Lactose	0.5%
pH 6.8	

Mallmann's Sodium Azide Broth (11)

Tryptose	2%
Lactose	0.5%
K_2HPO_4	0.4%
KH_2PO_4	0.15%
NaCl	0.5%
Sodium Azide	0.02%
pH 6.8	

Hajna and Perry's SF Medium (15) (Difco)

Tryptone..... 2,

Dextrose..... 0.5,

KH_2PO_4..... 0.4,

K_2HPO_4..... 0.15,

NaCl..... 0.5,

Sodium Azide..... 0.05,

Brom Cresol Purple..... 0.0002,

pH 6.9

Azide Dextrose Broth Exp'l. No 394074 (Difco)

Tryptose..... 1.5,

Beef Extract..... 0.45,

Dextrose..... ~~1.5,~~ .75 %

NaCl..... ~~1.5,~~ .75 %

Sodium Azide..... 0.02,

pH 7.2

Mitter-Treece Sodium Azide Broth (14)

Proteose Peptone No. 3..... 2,

Dextrose..... 0.1,

NaCl..... 0.5,

Sodium Azide..... 0.02,

pH 6.8

Other media were considered but discarded for testing either because they were solid media or their preparation was too costly and tedious for routine work.

The media being tested were made up in the laboratory media preparation room by the personnel in charge. Preparation of media in other laboratories for routine work might be better or worse, but it is believed that the media being tested were prepared under average conditions which might occur in other laboratories. It was noted that more uniformity of batches of the same medium was obtained in the case of the prepared dehydrated lifco products than when the media was prepared from the formula in the media book.

A medium should be adaptable to all types of material receiving bacterial or fecal sterilization. Fecal streptococci have been reported in sewage, soils treated with sewage sludge, sewage contaminated rivers, and swimming pools, samples from all these sources were used for test purposes.

Double strength concentrations of all media were made so that 10 ml. of this strength were diluted with 10 ml. of the sample. Tubes of single strength media were inoculated with 1 ml., 0.1 ml., 0.01 ml., and 0.001 ml. of the sample. Five tubes of each dilution were used. All media were incubated at 37°C. except EC which was incubated at 44.5°C.

Streptococcus indices were determined by first obtaining a smear from each tube and examining the smear for the presence of Gram positive cocci in chains of three or more cells. The twenty-four hour indices were obtained from smears made after vigorously shaking the tubes which had previously been incubated for twenty-four hours. The forty-eight hour indices were obtained from a smear made of the sediment remaining after decanting the supernatant of the same tubes incubated for another twenty-four hours. The indices were determined from Hoskins' (16) table of most probable numbers per 100 ml. of sample.

The above procedure for obtaining streptococcus indices was used in all of the following experiments. The indices recorded in Tables I to VII inclusive were determined by the presence of streptococci in the stained smears only. The presence of growth or acid in the tubes was not considered in the indices reported on these tables. A discussion of the macroscopic determination of indices will follow.

In the first series of tests, samples were collected from the Red Cedar River at the Farm Lane Bridge and the Railroad Bridge. The results are presented in Table I. From an examination of the data it is evident that forty-eight hours' incubation is needed for all of the media. The Azide Dextrose Broth was consistently superior to the other three. Since a relatively low number of samples

TABLE I

Streptococcus Indices on Red Cedar River Water Collected over a Period of Eight Months

Sample	Incub. Time (Hours)	Streptococcus Indices			
		Lactose Broth 37°C.	Mallmann's Sodium Azide Broth 37°C.	SF Broth 48°C.	Azide Dextrose Broth (Difco) 37°C.
1	24	1,700	1,300	43	2,400
	48	3,400	3,400	130	8,100
2	24	430	730	340	10,000
	48	730	11,000	2,400	31,000
3	24	700	730	470	21,000
	48	1,400	24,000	7,000	49,000
4	24	700	800	400	2,400
	48	1,700	1,000	920	7,000
5	24	300	1,100	140	2,400
	48	430	1,700	230	2,400
6	24	130	730	43	1,000
	48	300	3,400	240	7,000
7	24	330	2,300	140	4,000
	48	300	3,400	230	7,000
8	24	1,100	4,000	1,700	8,400
	48	3,100	7,000	3,000	7,000
9	24	3,400	11,000	400	30,000
	48	7,300	31,000	8,200	31,000
10	24	3,700	20	440	14,000
	48	3,900	470	920	18,000
11	24	1,000	1,100	790	7,900
	48	4,700	4,400	1,200	14,000
Log Averages of 48 Hr. Indices		1,380	3,130	1,000	14,780

was used with a large range in indices, and since the dilutions were ten fold in nature, it seemed advisable to compute log averages for the indices in order to compare one medium with another. An examination of the log averages will reveal that the Azide Dextrose Broth is superior to Hallmann's Sodium Azide Broth, Lactose Broth, and MF Broth in descending order.

In the second series of tests, samples of soil which had been treated with sludge were used. One gram of soil was suspended in 99 ml. of sterile water. The soil suspension was then added to tubes of media in the manner previously described.

The data presented in Table II represent twenty-four and forty-eight hour streptococcus indices of six soil samples. An examination of these data will show that Azide Dextrose Broth is again the best with Hallmann's Sodium Azide Broth, MF Broth, and Lactose Broth in descending order.

In the third series of tests, samples were collected from swimming pools. All samples were taken while bathers were in the pools. Nothing leads to think sampling varied and the samples represent water from four public wood pools.

The data in Table III represent streptococcus indices for six swimming pool water samples. An examination of these data will reveal that the Azide Dextrose Broth is

TABLE II

Streptococcus Indices of [illegible] [illegible] [illegible] with [illegible] [illegible].

Sample	Incub. Time (Hours)	Streptococcus Indices			
		Lactose Broth 37°C.	Mallmann's [illegible] Azide Broth 37°C.	SF Broth 45°C.	Azide Dextrose Broth (Difco) 37°C.
1	24	0	0	0	2
	48	0	[illegible]	11	11
2	24	[illegible]0	110	[illegible]	[illegible]00
	48	[illegible]	790	1,[illegible]00	1,[illegible]00
3	24	110	4[illegible]0	1[illegible]0	7[illegible]
	48	4[illegible]0	700	[illegible]	1,[illegible]00
4	24	17	11	[illegible].[illegible]	11,000
	48	1[illegible]0	2[illegible]0	[illegible]7	17,000
5	24	110	2[illegible]0	1[illegible]0	7[illegible]0
	48	[illegible]	790	[illegible]0	1,[illegible]00
6	24	11	1[illegible]0	7.8	[illegible]0
	48	240	[illegible]0	1[illegible]0	1,[illegible]00
Log Averages of 48 Hr. Indices		12[illegible]	20[illegible]	102	[illegible]75

TABLE III

Streptococcus Indices of Swimming Pool Water

Sample	Incub. Time (Hours)	Streptococcus Indices			
		Lactose Broth 37°C.	Mallmann's Sodium Azide Broth 37°C.	SF Broth 45°C.	Azide Dextrose Broth (Difco) 37°C.
1	24	2	11	0	23
	48	4.5	23	4.5	33
2	24	22	350	11	1,300
	48	54	4,000	32	5,300
3	24	4.5	23	2	250
	48	7.8	450	4.5	700
4	24	64	1,300	40	2,000
	48	130	4,000	110	6,000
5	24	4.5	11	2	23
	48	17	150	10	300
6	24	17	240	4.5	1,600
	48	79	2,400	11	2,200
Log Averages of 48 Hr. Indices		24	625	4.5	892

again superior to the other tubes. Log averages of the forty-eight hour indices places Azide Dextrose Broth as the best, Hallmann's Sodium Azide Broth, Lactose Broth, and SF Broth in descending order.

In the fourth series of tests, six sewage samples were used. Tubes of the media were inoculated with quantities of sewage samples in the same manner as before. Since the streptococcus indices were much higher for sewage than in previous tests, higher dilutions of the samples had to be made.

An examination of the data in Table IV reveals that the indices for all media for any one sample were comparatively close together. However, the log averages of the forty-eight hour indices indicate that the Azide Dextrose Broth is superior to Hallmann's Sodium Azide Broth, SF Broth, and Lactose Broth in descending order.

Because a marked variation occured in the number of streptococci obtained with various media tested, it appeared that the differences might be due to the rate of growth in each of the media. Accordingly, a study was made of growth rates of these media. In order to study growth rates of the various media, a plating medium had to be selected which would allow only the streptococci to grow. This was necessary because the Lactose Broth allows all the bacteria present to grow and a mixed population would occur at all stages of development. Also, in the media

TABLE IV

Streptococcus Indices on Sewage

Sample	Incub. Time (Hours)	Streptococcus Indices			
		Lactose Broth 37°C.	Mallmann's Sodium Azide Broth 37°C.	SF Broth 45°C.	Azide Dextrose Broth (Difco) 37°C.
1	24	23,000	40,000	16,000	49,000
	48	49,000	62,000	49,000	79,000
2	24	13,000	23,000	15,000	34,000
	48	34,000	47,000	40,000	49,000
3	24	11,000	34,000	23,000	34,000
	48	40,000	49,000	35,000	62,000
4	24	130,000	330,000	230,000	330,000
	48	330,000	790,000	490,000	1,300,000
5	24	110,000	240,000	110,000	230,000
	48	230,000	330,000	240,000	490,000
6	24	28,000	130,000	22,000	220,000
	48	490,000	1,700,000	920,000	2,200,000
Log Averages of 48 Hr. Indices		116,000	200,000	140,000	264,000

containing sodium azide, although the gram negative organisms and spore-formers are inhibited from growing, they are not necessarily killed. Hence the plating medium must contain an inhibitory agent such as sodium azide. Because azide dextrose broth has not given the best results as indicated in the studies reported, a solid medium was prepared by adding 1.5 percent agar to Azide Dextrose Broth. Trial tests were made of this medium by plating samples containing streptococci in mixed populations. Colonies were picked at random to determine the presence of streptococci. All colonies which were examined proved to be pure cultures of streptococci. It was assumed, therefore, that any colonies appearing on the plates of Azide Dextrose Agar were streptococci and the total count of these colonies could be classified accordingly.

River water was used for these tests. One ml. of river water was placed in 9 ml. of the broth to be tested. Twelve tubes of each medium were inoculated in this manner. One tube was used at each time interval, thereby allowing uninterrupted incubation for all tubes up to the time for sampling. At the time for sampling one tube of each medium was removed from the incubator, thoroughly mixed, and dilutions of 1 ml. portions plated on the Azide Dextrose Agar. These plates were incubated for forty-eight hours at 37°C. and the number of streptococci per ml. of broth was determined.

The streptococcus growth rates in Lactose Broth, Mallmann's Sodium Azide Broth, and Azide Dextrose Broth are tabulated in Table V. The streptococcus indices in each medium for the original sample of water are also presented.

An examination of these data shows that the growth rates are very close together. The streptococci are in their log phase of growth in all media up to twenty-four hours. An examination of the streptococcus index for each medium will reveal that approximately nine of the streptococci placed into Lactose Broth were able to grow, seventeen were able to grow in Mallmann's Sodium Azide Broth, and sixty in the Azide Dextrose Broth. Since the growth rates are fairly comparable, the differences in total counts would likely be due to the fact that the initial numbers starting were different. If the growth rates were exactly the same in all of the media, the Azide Dextrose Broth would show a much higher count as the initial start would be represented by sixty organisms as contrasted to nine organisms for the Lactose Broth. These data thus indicate that the higher indices of the Azide Dextrose Broth are due to the fact that this medium grows out more of the viable streptococci than do the other media. These findings are very similar to those found by Darby and Mallmann (16) in their studies on lauryl tryptose broth and lactose broth for the determi-

TABLE V

Streptococci counts in Lactose Broth, Hallmann's Sodium Azide Broth, and Difco's Azide Dextrose Broth.

Time (hours)	Streptococci per milliliter of broth		
	Lactose Broth	Hallmann's Sodium Azide Broth	Azide Dextrose Broth (Difco)
0	1	0	1
2	1	0	3
4	7	19	20
6	1,200	870	1,000
8	5,000	1,000	11,000
10	540,000	970,000	1,210,000
24	80,000,000	100,000,000	90,000,000
30	80,000,000	150,000,000	180,000,000
48	80,000,000	150,000,000	300,000,000
Initial Strep. Index	920	1,700	3,000

River water used as inoculum. Streptococci per milliliter of broth determined by plating 0.1 ml. samples of each broth on Azide Dextrose Agar at time intervals.

nation of coliform organisms.

Kellerman, Petwright, and Churchill (13) stated that storage of the sodium azide medium will result in a decrease in the potency of the inhibitory powers. In order to determine if storage would affect the media when used to determine streptococcus indices a fresh batch of Azide Dextrose Broth was tested on river water along with some Azide Dextrose Broth which had been allowed to stand in the laboratory for thirty days. The data presented in Table VI indicate that there was no loss in quality. More tests of this nature would be needed to draw valid conclusions. However, even though age may not alter the inhibitory powers of sodium azide, it is generally advisable to use only a freshly prepared sodium azide medium for routine work.

Near the completion of the experimental work for this thesis Ritter and Preece (14) reported work on streptococci in swimming pools using a protease-peptone sodium azide broth. A general comparison of this medium with the other four was made using one sample each of river water, swimming pool water, sewage, and soil. Since only one sample was used, the results only give a general indication of the quality of this medium as compared to the other four.

The data from these tests are presented in Table VII. An examination of these data reveals that the Ritter-Preece

TABLE II

Comparison of Streptococcus Indices of Fresh Dilute Milk Dextrose Broth and Thirty-Day Old Dilute Milk Dextrose Broth.

Incubation Time (Hours)	Streptococcus Indices	
	Fresh Milk Dextrose	Old Milk Dextrose
24	24,000	24,000
48	48,000	48,000

TABLE VII

Streptococcus Indices of River Water Using Lactose Broth, Mallmann's Sodium Azide Broth, SF Broth, Azide Dextrose Broth, and Ritter-Treece Broth

Sample	Incub. Time (Hours)	Streptococcus Indices				
		Lactose Broth 37°C.	Mallmann's Sodium Azide Broth 37°C.	SF Broth 45°C.	Azide Dextrose Broth 37°C.	Ritter-Treece Sodium Azide Broth 37°C.
River Water	24	3,300	23	540	54,000	2,400
	48	4,900	490	540	92,000	3,300
Swimming Pool Water	24	17	240	4.5	1,600	23
	48	79	2,400	11	2,200	700
Sewage	24	11,000	34,000	23,000	34,000	24,000
	48	40,000	49,000	35,000	62,000	54,000
Soil	24	17	11	6.8	11,000	5,000
	48	130	240	27	17,000	5,400

broth is probably not as good as the bile dextrose broth. In order to draw more valid conclusions a larger number of samples would have to be used.

As was previously stated, all of the tubes recorded in the first seven tables represent the actual presence of streptococci in the tubes as determined by examination of a gram stain of a smear from each tube. This is an extremely tedious and time consuming process. Hajna and Perry (2) state that the presence of growth that will in their medium indicate, almost completely, the presence of fecal streptococci. If a test can be established of a medium which will indicate proof of the presence or absence of streptococci, then not time would be saved and the source of error reduced. For this reason in all cases except for Lactose broth the presence of growth in the tubes was recorded as well as the actual presence of streptococci as indicated by a microscopic examination.

In Table VIII are presented more representative streptococcus indices along with the corresponding indices of growth and, in the case of bile broth, indices of acid. River water, swimming pool water, sewage, and well samples were used. The indices recorded have been plotted by Madsen in order to illustrate the general trend of a given medium for a particular type of material. The results reported in this case are all forty-eight hour indices.

An examination of the data presented in Table VIII

TABLE VIII

Indices in Various Media as Indicated by the Presence of Growth, Acid and Microscopic Proof of Streptococci

Sample	Indices (48 Hour)	Lactose Broth 37°C.	Mallmann's Sodium Azide Broth 37°C.	SF Broth 45°C.	Azide Dextrose Broth 37°C.	Ritter-Treece Sodium Azide Broth 37°C.
River Water	Microscopic	5,000	540	500	80,000	3,000
	Growth		540	1,600	80,000	3,000
	Acid			350		
Swimming Pool Water	Microscopic	79	2,400	11	2,200	700
	Growth		2,400	11	2,200	700
	Acid			4.5		
Soil	Microscopic	130	240	27	17,000	5,400
	Growth		240	27	92,000	35,000
	Acid			11		
Sewage	Microscopic	45,000	50,000	200,000	280,000	250,000
	Growth		50,000	200,000	280,000	250,000
	Acid			460		

reveals some interesting information about this medium. Naturally, growth in the [illegible] broth is no criterion in determining the streptococcus index. The only method to be used with this medium is a microscopic examination. If few streptococci are present in a field, the chance of overlooking their presence is increased. Otherwise the method would be accurate if the medium would support the growth of all the original streptococci. However, the data already presented show that [illegible] fails in this respect.

In every case, growth in Mallmann's Sodium azide broth was confirmed as positive for streptococci. However, Gram positive rods were occasionally encountered in some tubes from a few samples. This being the case, it would seem probable that growth might occur due to these rods without the presence of streptococci. Also, the data already presented indicate that this medium fails to grow all of the original streptococci.

SF Broth, using river water as the inoculum, produced growth in tubes where no streptococci could be demonstrated. The bacteria responsible were Gram positive rods. Also, acid production did not accompany the presence of streptococci in the high dilutions. Since the authors claim that the presence of growth and acid is indicative of streptococci, the indices thus obtained would be considerably lower than those obtained by a microscopic examination

of the tubes. However, indices obtained merely by the presence of growth would be higher. Growth and confirmation by microscopic examination coincided when swimming pool water was used as inoculum. This is probably due to a lower incidence of the Gram positive rods in the pools. Again the acid production lagged behind actual growth and presence of streptococci. Soil or sewage when used as an inoculum produced much the same picture in Berry's EF Broth as the swimming pool water. However, some Gram positive rods were found in a few tubes. The presence of acid was determined by a visual change in color of the brom cresol purple.

Using Azide Dextrose Broth with river water, swimming pool water, and sewage as an inoculum, growth was confirmed for the presence of streptococci in all cases. However, when soil was used growth due to Gram positive rods occurred without the presence of streptococci. Occasionally, in some tubes from any type of inoculum a few Gram positive rods occurred. Therefore, there is still the possibility that if visual growth was used to indicate the presence of streptococci a few false positives might occur. A very large number of samples would have to be run, checking each tube showing growth with a microscopic examination to determine the incidence of false positives.

The Ritter-Treece Sodium Azide Broth presented the same picture as the Azide Dextrose Broth. However, since

Only one set of samples was used with the Ritter-Greece Sodium Azide Broth, very accurate conclusions can not be drawn for this medium.

SUMMARY

Five liquid media suggested for the determination of streptococcus indices were compared. The media to be tested were run in parallel on portions of each sample and streptococcus indices for each medium determined by means of a microscopic examination of all tubes showing visual growth after twenty-four and forty-eight hours. A comparison of the presence of visual growth and actual presence of streptococci was made.

The experimental work presented in this thesis showed that the Azide Dextrose Broth (Difco) produced the highest indices, hence can be considered as the best selective liquid medium at present for the growth of streptococci. For samples of river water, swimming pool water, and sewage, results of this thesis would indicate that the presence of visual growth is absolute proof of the presence of streptococci. However, using this criterion for samples of soil tested in this medium, false positives will occur and a microscopic examination must be made. It would seem advisable to check tubes showing growth with a microscopic examination if other types of samples are used or if different sources of water are to be tested. The bacteria which grow out to produce false positives are gram positive rods.

Since it is desirable to grow all of the original viable streptococci, the medium producing the highest

inlices from a single would be the superior medium. The Azide Dextrose Broth is, therefore, superior to Lactose Broth, Hallman's Sodium Azide Broth, SF Broth, and Ritter-Ireece Sodium Azide Broth.

It was found that the presence of gold was grown at 45°C. in SF Broth was not a reliable indication of the presence of streptococci.

CONCLUSIONS

1. Azide Dextrose Broth consistently produced the highest streptococcus indices when tested in parallel with the other media on the same sample.

2. The presence of visual growth was a reliable indication of the presence of streptococci in tubes of Azide Dextrose Broth when using samples of river water, swimming pool water and sewage.

3. Growth in tubes of Azide Dextrose [illegible] to show positive [illegible] from soil samples was responsible for false positives.

4. The presence of acid [illegible] in [illegible] broth is not a reliable indication of streptococci. Streptococci will grow in [illegible] producing sufficient acid to change the color of the indicator. Other organisms will produce growth without the presence of streptococci.

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