A STUDY OF SOLID MEDIA WITH PARTICULAR REFERENCE TO TECHNIQUES OF EVALUATING CONSTITUENTS

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A STUDY OF SOLID MEDIA WITH PARTICULAR REFERENCE TO TECHNIQUES OF EVALUATING CONSTITUENTS

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INTRODUCTION

In all bacteriological techniques which use solid nutrient media, the media involved should be those which are best suited to grow the organisms or organism in question. This is important when the bacteria to be grown are fastidious in nature and especially important when the medium is used for purposes of enumerating the numbers of viable organisms in any substrate. There are many instances where the quality of the substrate, such as milk, water, or food is determined by the bacterial count. In these cases the medium used should be one which will most accurately measure the total number of viable organisms in the substance tested. Yet in many cases the nutrient solid medium in use is not the most efficient simply because no concentrated effort has been made to test the comparative value of the medium.

The problem of determining the efficiency of the existing media formulae or of new formulae is one of great complexity. The problem is relatively simple when liquid media are being evaluated because here one can use growth curves and generation time as a basis for evaluation. The method most generally used for solid media is one where the ability of a particular medium to grow out the viable organisms from any source is compared to the ability of another medium to do the same. Since this

involves the plating method of enumerating organisms the errors involved are those which are inherent in the plating procedure.

In 1902 De M. Gage and Adams compared plate counts from various classes of water on agar made with Merck's peptone and Witte's peptone. They also compared the relative development of pure cultures of bacteria on standard gelatine, Laurence agar, and Nahrstoff agar. They used water suspensions from fresh cultures, water suspensions from cultures which had been kept two days on ice, and water suspensions from thirty day old cultures. They found that Witte's peptone in agar gave higher counts from water samples.

Cook (1916) plated twenty soils on four agars and incubated the plates up to five days. He found that all soils do not behave in the same manner toward the different media.

In the last decade a tremendous amount of work was done on evaluation before tryptone-glucose-skimmilk agar was introduced as standard agar for the examination of milk and diary products. (STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS) (1941) Typical of the work was that done by Foltz and Martin (1938) on the comparison of the old standard and new standard agar as media for determining the bacterial count in ice cream. Two hundred and seventy-nine samples of vanilla ice cream were plated on

the two agars. The authors calculated the geometric average, arithmetic average, and ratio of new standard agar counts to old standard agar counts. The results clearly show the superiority of tryptone-glucose-milk agar to the old standard agar.

Abele (1939) as referee on the Committee on standard methods for the examination of milk and dairy products of the A.P.H.A. presented a detailed history of the work done before tryptone-glucose-milk agar was accepted as standard agar for dairy products, with twenty-two references on all aspects of the milk plate count including the effect of variation in temperature of incubators, the effect of variation in pH and composition of media, the effect of different plating techniques, etc. He discussed the complexities of using the plating method in evaluating solid nutrient media.

Mallmann and Breed (1941) compared the standard agar for water analysis with the new standard milk agar for determining bacterial counts in water. A total of six hundred and fifty-four water samples from various sources were plated on the two agars. The results indicate that the new milk standard agar gives counts comparable with the agar in use for water analysis.

Leifson (1943), in a study on the preparation and properties of bacteriological peptones, prepared three casein peptones and compared them with various commercial

peptones. Growth tests were made by preparing a 1 per cent peptone agar with 0.5 per cent NaCl at pH 7.1-7.3. The agar was poured into petri dishes and the bottom ofthe plate divided into six sections by means of a wax pencil. Twenty-four hour cultures of the bacteria were diluted one loopful to 5 ml. water and one loopful streaked on a section of the plate. Observations were made after 24-48 hours of incubation. Both the size and relative numbers of colonies were recorded. Twenty-four different organisms were tested on the various agars. The findings indicate that with most bacteria the usual 1 per cent concentration of peptone is far from optimum as regards the amount of growth obtained. Several experiments using the casein peptones in concentrations of 0.5 per cent to 10 per cent showed the optimum concentration of all three of these peptones (casein) to be somewhere in the neighborhood of 8 per cent. However, the optimum peptone concentration is lower in infusion media than in the media without infusion.

Hook and Fabian (1943) studied the influence of the type of peptone on the bacterial plate count of raw milk. They prepared various peptones from both animal and vegetable sources and substituted these peptones for the tryptone in Standard Milk Agar. Raw milk samples were plated on the modified agars using Standard Milk Agar as a control. They observed that some of the peptones from vegetable sources gave higher plate counts than Bacto-tryp-

tone but were inferior to Bacto-tryptose. Peptones prepared from spleen, heart, and pork were found to be superior to Bacto-tryptone in their ability to grow organisms from raw milk.

In the following work a study was made on some of the existing methods in use for evaluating solid nutrient media. The work was done in two phases. Part I was devoted to studies on Standard Agar for water analysis. Part II to studies on Standard Agar for diary products. In both phases the standard agars now in use were subjected to various modifications and these modified agars were used as a basis for the evaluation studies and also as a means for improving the standard agars now in use.

An attempt was made in this work to adapt the Frost "little plate" as a means of evaluating plating media. Frost (1915) (1916) described a method of counting viable organisms in milk which on the surface had some advantages over the standard plate count. The method consisted of mixing 0.5 ml. of milk with 0.5 ml. of the nutrient agar which had previously been melted and cooled to 50°C. One tenth of a milliliter of this mixture was spread over a 4 sq. cm. area on a clean, sterile slide. The plate was allowed to harden and then incubated in a moist chamber at 37° for 4-8 hours. The plate was then dried in an oven under 100°C. and stained with alcoholic methylene blue. A count was made of the microscopic colonies and with the

appropriate factors the number of organisms in the milk sample could be determined. Frost and other researchers claimed that the "little plate" gave comparable results with the standard plate count. The chief advantage of this method was the savings in laboratory equipment and media. Another advantage was the saving in time as a milk count could be made in 4-8 hours.

It was envisioned that the Frost method could be used in evaluation studies as it is relatively simple to measure colony size on the "little plate". Theoretically a medium which is "nutritionally" better than another medium would produce larger colonies at any point of the development of the colony. An experiment was set up in an attempt to utilize this method.

Part I Studies on Standard Agar for Water Analysis

Experiment I:

The first experimental work was done using standard agar for water analysis as a base. (STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE) (1936). This medium contains peptone in a concentration of 0.5 per cent.

Darby and Mallmann (1939) in a study on media for coliform organisms observed that when they varied the Bacto-peptone concentration in a liquid medium, a 2 per cent concentration of the peptone gave the best growth with Escherichia coli and a 3 per cent concentration showed a slight inhibitory effect. A comparison of Bacto-peptone and Bacto-tryptose was made and much more rapid growth occurred with the Bacto-tryptose. When the concentration of tryptose was altered the highest growth rates were obtained with a 2 per cent and 3 per cent concentration. To see if these same relationships would hold true in a solid medium the following experiments were set up:

To study the effect of the concentration of peptone in the plating media three modified agars were prepared using standard agar as a base and altering the concentration of peptone. The concentrations used were 1 per cent peptone, 1.5 per cent peptone, and 2.0 per cent peptone. Armour Peptonum siccum was used.

Over a time interval of about two weeks 50 samples of river water were plated on standard agar and the three

modified peptone agars.

The results are tabulated in Table A. The samples were grouped according to the number of colonies found on standard agar. The arithmetic mean for each group and for the total of 50 samples is shown. Using the efficiency of the standard agar as 100 per cent the relative efficiency of the three modified agars was calculated and shown for each group and for the total.

In the following report the unmodified agar will be referred to as "standard agar" and the modified agars will be called by the concentration and type of protein nutrient used, i.e., agar where 1 per cent peptone has been substituted for 0.5 per cent peptone in the standard formula will be called "1 per cent peptone agar", etc.

The data for Experiment I indicate that a 1 per cent concentration of peptone in the plating medium is the optimum concentration. The 1 per cent peptone agar proved to be 26 per cent more efficient than standard agar, 10 per cent more efficient than 1.5 per cent peptone agar, and 65 per cent more efficient than 2 per cent peptone agar on the total of 50 samples. Of the three modified peptone agars only the 2 per cent concentration of peptone gives an agar which is not more efficient than standard agar. The 1 per cent peptone agar was most efficient when the colony count in standard agar was between 0 and 299. The same is true of the other two modified agars. Only in this range did the

Table A The Comparative Bacterial Counts of 80 River Water Samples Plated on Standard Agar and Three Modified Agars

Range of Plate Counts	No. of Samples	% of Samples	Arithmetic Average of Plate Counts				Efficiency of Plating Media (Standard Agar = 100%)			
			Standard Agar	1% Peptone Agar	1.5% Peptone Agar	2.% Peptone Agar	Standard Agar	1% Peptone Agar	1.5% Peptone Agar	2% Peptone Agar
0-299	7	14%	238	594	378	314	100%	249%	178%	132%
300-599	6	12%	444	668	460	417	100%	151%	104%	93%
600-999	15	30%	765	1145	930	544	100%	154%	122%	71%
1000-2999	12	24%	1417	1830	1602	810	100%	129%	113%	57%
3000 above	9	18%	4696	5182	5286	2680	100%	110%	114%	56%
TOTAL	80	100%	1528	1915	1760	982	100%	126%	116%	61%

2 per cent peptone agar show greater counts than standard agar.

Although it is not shown in Table A, there were 5 samples in the higher plate count ranges where standard agar gave higher counts than any of the three modified agar. This can be attributed to either plating error or to the difference in the flora of these 5 samples.

Experiment II:

To study the effect of using a different protein hydrolysate nutrient in the plating media, four agars were prepared using Bacto-tryptose as a substitute for Armour peptone. The concentrations used were 0.5 per cent to compare with standard agar and 1, 1.5, and 2.0 per cent. Forty-nine river water samples were plated on the four agars. The results are tabulated in Table B. The samples were grouped according to colony count on 0.5 per cent agar and the arithmetic mean and per cent efficiency were calculated and shown as in Table B. Here the counts on 0.5 per cent tryptose agar were used as 100 per cent.

It can be seen that the data would indicate that the most efficient of the four modified tryptose agars is the 1 per cent tryptose agar. This agar was 19 per cent more efficient than 0.5 per cent tryptose agar, 51 per cent more efficient than 1.5 per cent tryptose agar, and 62 per cent more efficient than 2 per cent tryptose agar. However in the case of the tryptose agar the 1 per cent concentration

Table B The Comparative Bacterial Counts of 49 River Water Samples Plated on Four Modified Agars *

Range of Plate Counts	No. of Samples	% of Samples	Arithmetic Average of Plate Counts				Efficiency of Plating Media (0.5% Tryptose agar=100%)			
			0.5% Tryptose Agar	1% Tryptose Agar	1.5% Tryptose Agar	2.0% Tryptose Agar	0.5% Tryptose Agar	1.0% Tryptose Agar	1.5% Tryptose Agar	2.0% Tryptose Agar
0 - 199	11	22%	150	157	127	89	100%	108%	86%	59%
200 - 399	28	57%	290	326	220	167	100%	112%	76%	58%
400 & over	10	20%	480	561	284	300	100%	117%	58%	63%
TOTAL	49	100%	308	360	207	173	100%	119%	68%	57%

* (Tryptose substituted for peptone in standard agar)

is most efficient when the colony count is four hundred or over. The efficiency decreases as the colony count gets lower. The efficiency of the 1.5 per cent tryptose, which is in all cases lower than that of 0.5 per cent tryptose agar, decreases as the count increases. Two per cent tryptose agar acts like the 1 per cent agar in the respect that its efficiency increases as the colony count increases.

It is interesting to note that the increase of the concentration of tryptose in a tryptose agar in no way produces the same magnitude of effect as when the peptone concentration was increased in a peptone agar. This is especially apparent in the case of the 1.5 per cent agars. The 1.5 per cent peptone agar produced higher plate counts than standard 0.5 per cent agar. In the case of the 1.5 per cent tryptose agar the counts were lower than the 0.5 per cent tryptose medium.

Experiment III:

To compare 1 per cent peptone agar with 1 per cent tryptose agar, 28 river samples were plated on both agars and also on standard agar. The results are tabulated in Table C. The efficiency of the two modified agars was calculated on the basis of 100 per cent for the arithmetic mean of the standard agar.

Table C shows the counts obtained when 28 river water samples were plated on standard agar and the two most

Table C The Bacterial Count of 28 Samples of River Water as Determined by Plating on Standard Agar and Two Modified Agars

Sample Number	Standard Agar Plate Count	1% Peptone Agar Plate Count	1% Tryptone Agar Plate Count
99	520	980	1,150
100	430	1,180	1,090
101	580	1,050	1,120
102	710	1,050	1,260
103	460	1,270	1,020
104	390	680	690
105	400	660	670
106	370	630	640
107	490	750	810
108	430	600	670
109	350	480	370
110	280	530	470
111	280	560	420
112	270	340	320
113	300	500	420
114	340	1,020	960
115	320	900	710
116	310	340	250
117	310	390	360
118	380	480	260
119	440	620	480
120	150	310	200
121	370	560	430
122	270	540	360
123	260	370	490
124	470	500	480
125	430	420	270
126	330	600	520
Average	383	672	627
% Efficiency	100%	175%	164%

efficient modified agars. The arithmetic average on the counts on the total numbers of samples gives the 1 per cent peptone agar an efficiency of 175 per cent compared with 100 per cent of standard agar and 164 per cent of 1 per cent tryptose agar. All the samples showed higher counts on 1 per cent peptone agar than on standard agar, however, 3 samples showed higher counts on the standard agar than on 1 per cent tryptose agar and 9 samples had higher colony counts on 1 per cent tryptose agar than on 1 per cent peptone agar. This very clearly shows the necessity for plating large numbers of samples when the plating method is used to evaluate solid media.

Experiment IV:

To determine the effect of the time of incubation on the plate counts of river water using standard agar, the 1 per cent peptone, and 1 per cent tryptose agar, five samples were plated and colonies counted at the end of 7, 18, 24, and 48 hours.

Table D presents the data showing the effect of time of incubation on the colony counts using standard agar and the two best modified agars. Standard agar gave higher counts after 7 hours incubation on all 5 samples. On all samples but one, 1 per cent peptone agar proved its superiority at the end of 18, 24, and 48 hours incubation. Sample 124 gave the highest counts on 1 per cent tryptose agar at the end of 24 and 48 hours of incubation.

Table D The Effect of Time of Incubation on the Colony Count of River Water Plated on Standard Agar and Two Modified Agars

Sample Number	Time of Incubation (hours)	Standard Agar Plate Count	1.% Peptone Agar Plate Count	1.% Tryptose Agar Plate Count
#120	7	8	2	2
	18	330	450	410
	24	440	620	480
	48	800	970	900
#121	7	9	5	1
	18	90	270	180
	24	150	310	200
	48	230	500	400
#122	7	5	2	4
	18	260	460	310
	24	370	560	430
	48	710	1,030	910
#123	7	7	3	2
	18	270	430	330
	24	270	540	360
	48	750	1,160	590
#124	7	13	2	7
	18	160	340	320
	24	260	370	490
	48	810	730	930

Experiment V:

A study was made of the relative efficiency of standard agar, 1 per cent tryptose agar, and 1 per cent peptone agar in demonstrating the growth curve of a pure culture of E. coli. A flask of peptone broth was seeded with a 24 hour culture of E. coli and the initial population determined by plating on standard agar and the two modified agars. The broth was incubated at 37° and at the end of 8, 24, and 48 hours the bacterial population was again determined by plating on the three agars.

In Table E is tabulated the data obtained when standard agar and the two best modified agars were used to determine the number of organisms in a flask of peptone broth which has been seeded with a pure culture of E. coli. Figure I is a graphical presentation of the comparative growth curves obtained by plating on standard agar and 1 per cent peptone agar. It is interesting to note that on standard agar the count remains the same at the end of 24 and 48 hours. The counts on 1 per cent peptone agar would indicate that the organisms have entered the death phase sometime after 24 hours. The counts on 1 per cent tryptose agar would indicate that the growth phase is still in existence between 24 and 48 hours.

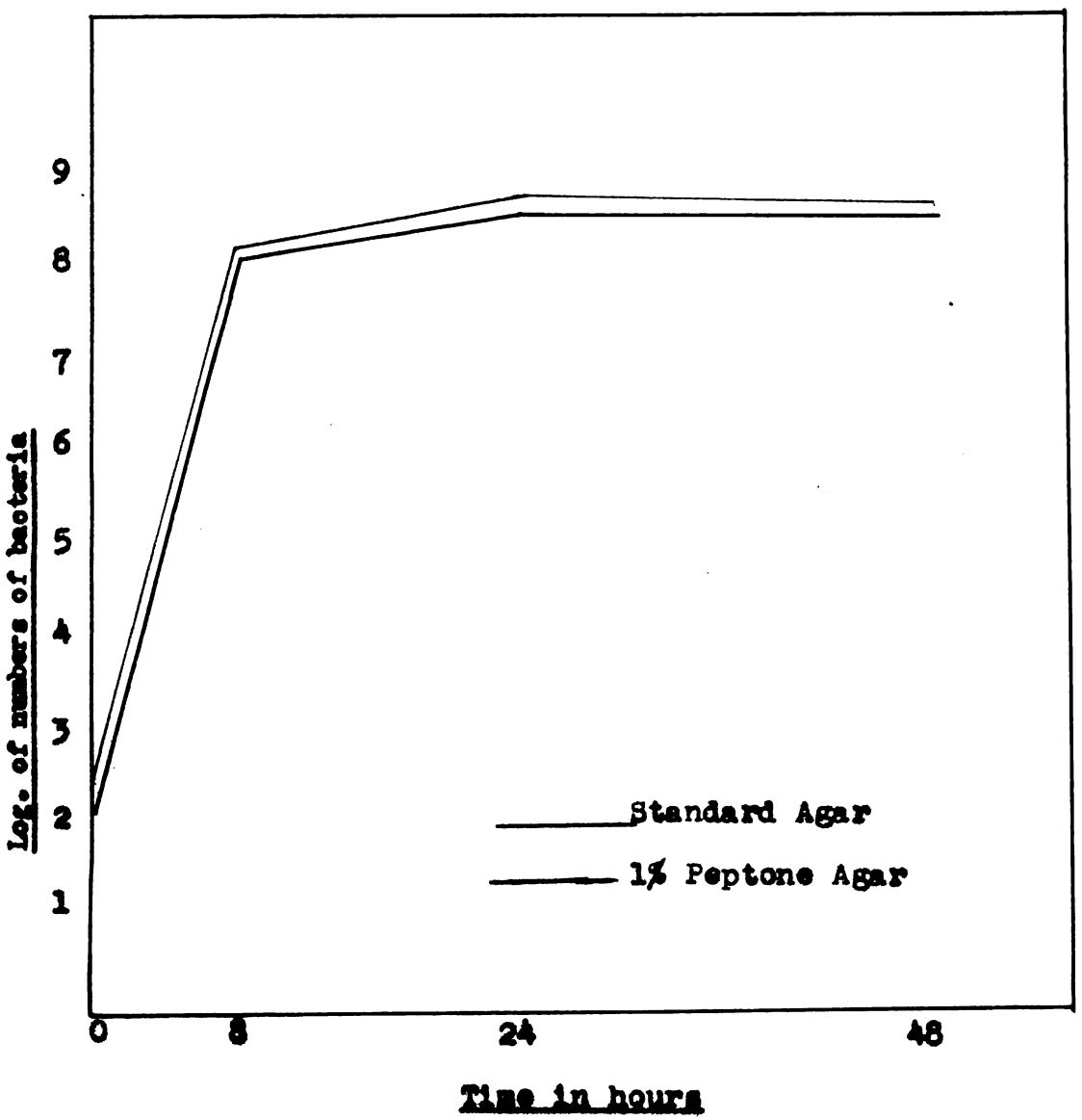
Experiment VI:

Using the Frost little plate technique a comparison was made of Standard Agar and five modified agars. The

Table E The Growth Rate of E. coli in Broth as Determined by Plating on Standard Agar and Two Modified Agars

Time in Hours	Standard Agar	1% Peptone Agar	1% Tryptose Agar
0	440	486	447
8	118,000,000	140,000,000	139,000,000
24	370,000,000	450,000,000	360,000,000
48	370,000,000	410,000,000	380,000,000

Fig. I Comparative Growth Curves of E. coli in Peptone Broth as Determined by Plating on Standard Agar and 1% Peptone Agar



method used was as follows:

A suspension was made in saline of a 24 hour agar slant culture of E. coli. The suspension was diluted to a concentration which had previously been determined to give a colony count which was in the proper range for counting. One-half milliliter amounts of this suspension were added to equal amounts of the six agars which had previously been melted and cooled to 50°C. One-tenth milliliter amounts to the agar-suspension mixture were spread on four square centimeter areas on clean sterile slides. Five sets were made for each agar. The "plates" were allowed to harden and then incubated in a moist chamber at 37°C. At intervals of 2, 4, 6, 8, and 24 hours one set each of the different agars were removed from the moist chamber and dried in an oven at 80°C. When dry the "plates" were stained with alcholic methylene blue, washed with water, and dried.

The microscope used was calibrated for use with three objectives of the microscope. The "little plates" were examined and the colonies counted in 25 to 50 fields. The size of 25 to 50 colonies was measured with the ocular micrometer. The results are tabulated in Table F with the average colony size in millimeters and the colony count per plate given for the six different agars at the various incubation times.

Table F The Comparison of Standard Agar and Five Modified Agars by the Frost "Little Plate"

Colony Size (millimeters)

Incubation Time	.5% Peptone Agar	1% Peptone Agar	1.5% Peptone Agar	.5% Tryptose Agar	1% Tryptose Agar	1.5% Tryptose Agar
2 Hours	.012	.015	.008	.005	.007	.007
4 Hours	.017	.038	.009	.014	.025	.008
6 Hours	.096	.056	.011	.047	.082	.016
8 Hours	.10	.110	.015	.088	.099	.043
24 Hours	.162	.181	.028	.102	.172	.098

Colony Count

Incubation Time	.5% Peptone Agar	1% Peptone Agar	1.5% Peptone Agar	.5% Tryptose Agar	1% Tryptose Agar	1.5% Tryptose Agar
2 Hours	21,500	28,800	18,200	18,000	20,300	10,000
4 Hours	21,700	35,000	18,200	19,500	21,000	12,800
6 Hours	28,500	42,900	22,600	19,700	22,800	14,500
8 Hours	28,500	42,900	26,000	19,800	22,900	15,100
24 Hours	30,500	43,200	41,200	26,000	22,900	18,700

Of the six agars tested with the Frost "little plate" method the greatest colony size was produced by the 1 per cent peptone agar. This same agar also gave the largest colony count of the agars tested. In this respect the results of this experiment agree with the results obtained when the standard plate count was used in the evaluation studies. However, not all the data obtained with the "little plates" are in agreement with the previous data. The colony counts on the 0.5 per cent peptone agar were at all periods of incubation higher than that on the 1 per cent tryptose agar. This is in direct contrast to the results obtained in the previous experiments.

The difference in substrates might very well account for the lack of agreement of some of the results obtained. In one case a pure culture of an organism was used, in the other case the flora of the substrate was quite variable. All the inherent errors present in the standard plate count technique are magnified in the "little plate" method as the quantity of inoculum used is much smaller. The Frost "little plates" seem to be of value when colonies are to be measured, but any quantitative work based on colony counts is open to the same criticisms that are applicable to the standard plate count.

Part II Studies on Standard Agar for Dairy Products

Experiment VII:

In a survey of the literature concerning the evaluation of Tryptone-Glucose-Extract agar no mention was found of any attempt to increase the concentration of the protein in the accepted formula. The concentration of tryptone in the TGE formula is 0.5 per cent. If the same relationship holds true for the TGE agar as does for the standard agar for water analysis, and increase in the concentration of the tryptone would increase the efficiency of this agar. This was tested in the following experiment:

Two batches of Difco TGE agar were made up and to one was added Bacto-tryptone to produce a final concentration of 1 per cent. Fifty samples of milk, including both raw and pasteurized samples, were plated on both agars. Table G presents the data obtained. The plate counts were tabulated both on a total basis of the 50 samples and on the basis of raw or pasteurized samples.

Increasing the concentration of the tryptone in the TGE formula from 0.5 per cent to 1.0 per cent resulted in a agar which gave higher colony counts from both raw and pasteurized milk samples. If the average count on TGE is considered 100 per cent then the efficiency of the modified TGE agar for the 50 milk samples was 123 per cent. For the 27 raw milk samples the efficiency of the modified agar was 276 per cent and for the 23 pasteurized milk samples the

Table G The Comparative Plate Counts Obtained by Plating 50 Milk Samples on T.G.E. Agar and a Modified T.G.E. Agar

Number of samples	T.G.E. Agar		T.G.E. Agar plus .5% tryptone	
	Arithmetic mean	Geometric mean	Arithmetic mean	Geometric mean
50	376,000	24,000	464,000	29,000

Analysis of Above Data on the Basis of Type of Milk Samples

	T.G.E. Agar	T.G.E. Agar Plus .5% tryptone
Number of samples	Arith. mean	Arith. mean
27 (raw milk)	645,000	1,790,000
23 (pasteurized milk)	59,000	77,000

efficiency was 130 per cent.

Experiment VIII:

Another modification of TGE agar was tested with 23 milk samples. This modification consisted of increasing the tryptone concentration to 1 per cent as was previously done, and also increasing the concentration of beef extract from 0.3 per cent to 0.6 per cent. The milk samples were plated on standard TGE, the modified agar used in Experiment VII, and the new modified agar. The results are shown in Table H.

The results obtained when the concentration of both tryptone and beef extract were increased indicate that thismodification is a more efficient plating medium than the standard TGE agar and the first modified agar. The ratio of the counts on the double modified agar to the counts on standard TGE agar was 1.42 and the ratio of the counts on the double modified agar to the counts on agar where just the concentration of tryptone was increased was 1.14. The addition of an extra 0.3 per cent of beef extract probably introduces small amounts of growth stimulating substances which would account for the higher colony counts on the modified agar.

Experiment IX:

The third and final modification consisted of adding buffer salts to TGE. The concentration of salts

Table H The Comparative Plate Counts Obtained by Plating 23 Milk Samples on T.G.E. Agar and Two Modified T.G.E. Agars

	T.G.E. Agar		T.G.E. Agar plus .5% tryptone		T.G.E. Agar plus .5% tryptone plus .3% beef extract	
Number of samples	Arith. mean	Log mean	Arith. mean	Log mean	Arith mean	Log. mean
23	109,000	8,690	136,000	11,000	155,000	12,700

Table I The Comparative Plate Counts Obtained by Plating 18 Milk Samples on T.G.E. Agar and Buffered T.G.E. Agar

	T.G.E. Agar		Buffered T.G.E. Agar	
Number of samples	Arithmetic mean	Geometric mean	Arithmetic mean	Geometric mean
18	617,000	152,000	402,000	119,000

added was as follows:

0.4 per cent K_2HPO_4

0.15 per cent KH_2PO_4

This medium was used to plate out 18 milk samples using standard TGE agar as a control. The results are shown in Table I.

All of the 18 samples plated on the buffered TGE agar produced lower plate counts than when the samples were plated on the standard agar. The efficiency of the medium was lowered when the buffer salts were added. A possible explanation of this may be made on the basis that the organisms normally found in milk are favored by a pH on the acidic side and the buffer salts would to some extent keep the hydrogen ion concentration near its initial value. The relationship of buffer salts to plating media efficiency should be checked further before any definite conclusion can be drawn.

The use of the Frost "little plate" was fairly successful in the evaluation of the modified agars in the first part of this work. The use of the "little plates" to evaluate the modifications made on TGE agar met with no success. Repeated attempts failed to produce results that were comparable to those obtained by the plating method and even failed to produce results that were consistent in themselves.

SUMMARY

A 1 per cent concentration of peptone substituted for the 0.5 per cent concentration of peptone in standard agar for water analysis produces a plating medium which is superior to any of the other modifications tried.

The 1 per cent peptone agar exhibits greatest efficiency when the colony count on standard agar from river water samples is between 0 and 299.

In the experiment where the effect of time of incubation was studied, 1 per cent peptone gave higher counts at 18, 24, and 48 hours. Standard agar gave the highest counts at the end of 7 hours.

The 1 per cent peptone agar gave higher colony counts from samplings in all growth phases of E. coli.

Measuring the colony size of E. coli by the Frost "little plates" further showed the superiority of a 1 per cent concentration of peptone.

In the modifications on TGE agar a superior plating medium was achieved when the concentration of tryptone in the formula was increased to 1 per cent. Increasing the concentration of beef extract to 0.6 per cent again improved the medium.

The addition of buffer salts to the formula of TGE agar is a detriment to its efficiency.

The plating method of evaluating nutrient solid

media is a laborious process which only gives good results when a very large number of samples are tested. It is also quite important to utilize samples which contain varied flora. A more correct evaluation of the medium is obtained when the samples used most closely resemble the type of flora for which the medium is to be used.

If by increasing the nutrients in a plating medium higher colony counts are obtained it may be assumed that either more of the same organisms are developing in the medium or that different organisms are growing where they would not grow before. Either of these developments is important since the ultimate aim in a plating medium which is used for quantitative work is the ability to grow all the viable organisms in the sample.

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