OBSERVATIONS ON THE COLONY PRODUCTIVITY OF SIX MILK PLATING MEDIA

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Colony productivities of 5 media (2 containing skim milk, 2 milk-free, and 1 synthetic) have been compared with that of the official (TGEM) medium, authorized by the American Public Health Association. The chief objective was to discover a suitable milk-free medium to replace the TGEM medium. Work on improving the milk-free media is being continued. The synthetic medium (selected amino acids, vitamins, purines, pyrimidines, mineral salts, glucose and agar) gave about 95 percent productivity of the TGEM medium. A synthetic medium has potential use as a reference productivity standard.

EVER since the American Public Health Association (APHA) accepted the present Tryptone Glucose Beef Extract (Skim Milk) Agar (TGEM) plating medium,[1] effective on July 1, 1939, repeated complaints have been made about the particulate separation (some undissolved portions from skim milk powders, when reconstituted and used, but more often of the progressively precipitating masses of flocculent skim milk solids in the melted medium) and the resulting mistaken colony identification of the particulate matter. Even when fresh skim milk is uniformly distributed, the medium has a slightly cloudy appearance. In addition, one cannot ignore the inconvenience of locating a suitable source of fluid skim milk or of rehydrating skim milk powders and the nuisance of weighing or measuring the quantities needed.

The APHA Committee was responsible for authorizing the addition of skim milk to the plating medium, for reasons which at that time seemed justifiable, the aim being to get with maximal uniformity the highest Standard Plate Counts obtainable by using an improved plating medium and by incubating the poured plates for 48 hours ±3 hours at a preferred temperature of 32°C. Because the Standard Plate Count may not be appreciably reduced in some instances by the failure to include 1 percent of skim milk (average reduction from about 5 to 8 percent), some laboratories have deliberately omitted skim milk from the plating medium. (Although the Eighth Edition of Standard Methods authorized the optional use of 32°C and 37°C for the incubation of plates, it was disclosed before the Ninth Edition of Standard Methods was printed that most laboratories would prefer to incubate their plates at 35°C, if the use of this temperature were authorized. Since some laboratories had unqualifiedly accepted 32°C incubation as official for their jurisdictions, the optional use of either 35° or 32°C incubation temperature is authorized in the Ninth Edition.)

Buchbinder *et al.*[2] directed attention at the 1948 Annual Meeting of the APHA: (1) to the possibilities of one or more milk-free plating media with colony productivities nearly equal to those obtained on the official TGEM medium, and (2) to a realization that a magnitude of colony counts could be adjusted slightly by making small changes in the proportion of the ingredients in the medium.

In 1949, the Laboratory Methods Committee[3] of the *International Association of Milk and Food Sanitarians* (IAMFS) undertook to compare the colony productivity of five candidate plating media with that of the officially recognized

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*The Chairman of the Subcommittee on *Standard Methods for the Examination of Dairy Products* of the American Public Health Association herewith acknowledges the valuable help of C. A. Abele, Luther A. Black, Leon Buchbinder, and Samuel R. Damon in organizing and executing this study, and of both Miss Vivian Pessin, Statistical Consultant to the Subcommittee and formerly Senior Statistician, and Louis Pincus, Senior Statistician, Bureau of Records and Statistics of New York City Department of Health, for guiding the execution and for making the multiple computations needed to establish the relative conformance of the determinations by the candidate media with those on the official APHA medium.

The Subcommittee gratefully acknowledges the unselfish help of each of seventeen participating laboratories: Oak Farms Corporation Laboratories, Dallas Texas; Division of Laboratories, Ministry of Health, Montreal, Canada; Division of Bacteriology and Dairy Research, Department of Agriculture, Ottawa, Canada; H. P. Hood & Sons Laboratory, Boston, Massachusetts; Environmental Health Center Laboratories, Federal Security Agency, Cincinnati, Ohio; City Health Department Laboratories, Denver, Colorado; Division of Laboratories, Department of Health, New Orleans, Louisiana; Caddo Branch Laboratory, Division of Laboratories, State Department of Health, Shreveport, Louisiana; Division of Laboratories, State Department of Public Health, Chicago, Illinois; State Department of Health Laboratories, Austin, Texas; City Department of Health Laboratories, Steubenville, Ohio; Hygienic Laboratory Division, State Department of Health, Charleston, West Virginia; Dairy and Food Control Laboratory, State Department of Agriculture, Madison, Wisconsin; Dean Milk Company Laboratory, Rockford, Illinois; The Borden Company Laboratory, Chicago, Illinois; City Board of Health Laboratory, Chicago, Illinois; and State Board of Health Laboratories, Topeka, Kansas.

Tryptone Glucose Beef Extract (Skim Milk) Agar, Difco (Bacto) dehydrated, Medium No. B2 (TG EM), for plating milk samples using the Agar Plate Method.[1] Directions for their use provided that skim milk was to be added to only one of these candidate media. Among the four skim milk-free media, two had relatively complex formulas.

Based upon the average count obtained on each medium and comparable calculated range distribution about each mean, the data by Pessin and Black[3] disclosed that the colony productivities of the two skim milk-containing media were practically indistinguishable. Two of the skim milk-free media of relatively simple composition possessed many of the desired characteristics of a suitable plating medium, but the colony productivities of each of these exceed the range limits recognized for conformance with the productivity of the official TGEM medium. For obvious reasons, further consideration of the more complex skim milk free media was discontinued.

Following a preliminary report of the results obtained in the IAM FS studies, the Subcommittee on Standard Methods for the Examination of Dairy Products of the AP HA, through the cooperation of two manufacturers of dehydrated plating media, initiated similar tests to determine colony productivities. Their studies included observations on six media, identified as follows:

A. A single batch of Difco agar prepared from the ingredients (an officially recognized procedure), subdivisions of which were shipped to each participating laboratory with directions for adding the necessary amount of rehydrated skim milk prepared from subdivisions of the same lot of powder. In the interests of assuring maximal uniformity, directions, were furnished for rehydration and sterilization and for the addition of the reconstituted milk to known quantities of sterile media in the containers after receipt at each of seventeen laboratories.

B. A candidate TGEM dehydrated medium, prepared according to Formula No. 183-B, by the Baltimore Biological Laboratories, Inc., Baltimore, Md.

C. The officially recognized Difco (Bacto) TGEM dehydrated medium, prepared according to Formula No. B2, by Difco Laboratories, Inc., Detroit, Mich.

D. A candidate dehydrated milk-free medium, by Difco, composed of 0.35% yeast extract, 0.5% tryptone, 0.1% dextrose, and 1.5% agar.

E. A candidate dehydrated milk-free medium, by BBL, composed of 0.9% milk-protein peptone, 0.1% dextrose, and 1.5% agar.

F. A synthetic medium, prepared according to Formula No. S4 by BBL, under the direction of Pelczar and Brown,[4] consisting of 18 amino acids, 8 vitamins, 3 purine bases, 2 pyrimidine bases, potassium acid phosphate, magnesium sulphate, glucose, and agar. (Recent information discloses that a small amount of sodium ethyl oxalacetate added to the original formula for Medium F practically eliminates interfering precipitates and permits reducing the incubation period from 96 hours to 72 hours.)

PROCEDURE

Except for Medium A, all of which was prepared from the identical batch of ingredients in one laboratory for distribution in rehydrated form to each of the seventeen participating laboratories, the remaining five media were furnished by the manufacturer in dehydrated form. Each batch was received in individually-sealed packages without manufacturers identification on the bottle. Each bottle contained about 160 grams, an amount sufficient for at least five liters of prepared medium. The unopened packages were codes with an identifying number for each medium before shipment to each participating laboratory. When each batch of the medium was prepared, each laboratory was directed to determine its pH, using the method normally employed, and to answer a few pertinent questions relating thereto. In the interests of establishing a uniform and reliable electrometric procedure for pH determinations, a separate report[5] on these records will be made at the 1951 annual meeting of the Association.

Directions provided for plating so that from each laboratory 12 usable counts per sample (range limits extended to 20-400 colonies per plate in order to keep the additional work within practical limits) would be available on not less than 25 raw and 25 pasteurized milks. Because of the need for balanced records among the 6 media, no results could be included on any sample with less than 12 usable counts, 2 by each of the 6 different plating media. If any plate among the 12 per sample had spreader growth, an obvious contamination, or a colony count outside of the range, it was necessary to omit from the tabulations the results on the otherwise satisfactory plates on that sample. Regardless of whether the counts per plate conformed to the above 20-400 colony limits, the laboratories were directed to include the counts on all plates poured with the synthetic medium, provided they had no spreaders or obvious contamination. Directions for diluting each type of milk follow.

Dilute each raw milk sample 1:10 by transferring 11 ml of milk to a dilution bottle containing 99 ml of sterile dilution water. From this dilution measure 1 ml to a second dilution bottle containing 99 ml of sterile dilution water. After mixing thoroughly transfer exact 1 ml portions only into each of 12 plates.

Dilute each pasteurized milk sample 1:100 by transferring 1 ml to a dilution bottle containing 99 ml of sterile dilution water. After mixing thoroughly, transfer exact 1 ml portions only into each of 12 plates.

A special order of arranging the plates for each sample, prior to pouring, and also of selecting for successive samples the first pair in the sequence of plates to be poured, appreciably reduced the possibility for high or low count tendencies caused by unequalized sedimentation in and the progressive drying of the 1 ml portions deposited in the plates. Plates for each sample were arranged according to the 6 pairs, as listed below. Test portions were transferred to the

plates in increasing numerical order of plate identity, always starting with Plate 1. Each pair of plates were poured with the respective medium indicated below the plate identities.

Plate Identity	1	2	3	4	5	6
	12	11	10	9	8	7
Medium Used	A	B	C	D	E	F

The order of selecting the pairs of plates first to be poured provided for the pairs of plates for Sample 1 to be poured progressively with Media A, B, C, D, E, and F; for Sample 2, with Media B, C, D, E, F, and A; for Sample 3, with Media C, D, E, F, A, and B; for Sample 4, with Media D, E, F, A, B, and C; for Sample 5, with Media E, F, A, B, C, and D; for Sample 6, with Media F, A, B, C, D, and E; for Sample 7, with Media A, B, C, D, E, and F; for Sample 8, with Media B, C, D, E, F, and A, etc. Despite the need to discard the results on occasional samples because of unsatisfactory plates, the above order was repeated for the pairs of plates to be poured first with the respective media, beginning with each successive seventh sample to be plated. Appropriate sterility controls were used on dilution waters, plates, media, etc.

Because of the slower colony growth response on the synthetic medium, plates poured with it were incubated at 35°C for 96 hours± 3 hours. Plates poured with the other five media were incubated as usual at 35°C for 48 hours ±3 hours. Because of the need for additional incubation of Plates 6 and 7 and the expected smaller size colonies thereon, the analysts were cautioned not to be less diligent than otherwise when counting the colonies on these plates.

ANALYZING THE DATA

Usable counts were reported on 337 raw and 349 pasteurized milks. Data on raw and pasteurized samples were treated separately. International Business Machine equipment, available in the New York City Department of Health, was used for sorting and tabulating the data and for most of the computations. Before sorting the cards, the punch record on each was checked for accuracy.

Two indices were computed to measure the variability among counts on duplicate plates in each laboratory. The counts were converted to logarithms, and the logarithm of the lower count subtracted from that of the higher count. The antilogarithm of the average of these differences is the geometric mean of the ratios of the counts, herein regarded as the first index.

The second index of variability of duplicates was the point below which 95 percent of the ratios are expected to fall. Since the logarithms of bacterial counts have an approximately normal distribution, the distribution of the differences of logarithms of paired counts will also be normally distributed (around zero), provided the order of counts within each pair is random. Fixing the order of counts so that each difference is positive has no effect on the magnitudes of the differences. The effect on the distribution is the removal of the negative half thereof and the addition of its mirror image to the positive half. Thus, the distribution of the absolute values of the differences of logarithms of paired counts is half of a normal distribution. The standard error of the whole normal distribution is computed from the absolute differences, and the interval $(0 + 1.96s)$ contains 95 percent of the area of the distribution of the absolute differences. The antilogarithm of $1.96s$, hereinafter referred to as the "95 percent point," is therefore the level below which 95 percent of the ratios are expected to fall.

Bartlett's test for homogeneity of variances was negative; that is, the laboratories were found to differ very significantly with respect to the variation between the counts of duplicate plates.

Each medium was compared with Medium C, with Medium A, and with Medium F. For the media comparisons, the duplicate counts were added mechanically before conversion of the sum to logarithms. Each logarithm of a sum for the base medium was then subtracted from the corresponding logarithm for the test medium, and the square of the differences determined. For each pair of media, a tabulation was run similar to the tabulations for the laboratories, except that in the case of the media, the directions of the differences were indicated. After correcting for the use of sums instead of averages (by the subtraction of the logarithm of 2), the mean differences and their variances were computed for the various pairs of media.

DIFFERENCES AMONG LABORATORIES

The magnitude of the differences between duplicate counts were studied for each laboratory which reported counts for at least 100 pairs of duplicate plates of the same type of milk. Table 1 lists the geometric means of the ratios of the higher to lower counts, and the levels below which 95 percent of such ratios are expected to fall. Chart I shows the same data graphically, with the laboratories ordered according to their variability.

The geometric means of the ratios ranged from 1.14 to 1.17 for raw milk, and from 1.14 to 1.16 for pasteurized milk. The 95 percent of the ratios ranged from 1.13 to 1.56, and from 1.09 to 1.45 for the raw and pasteurized milk, respectively. From these figures, it appears that duplicates of raw milk vary somewhat more than duplicates of pasteurized milk.

Although there were some exceptions, the magnitude of the differences in the same laboratory generally were similar for raw and pasteurized milk. Laboratories 4 and 16 varied somewhat less for raw milk than for pasteurized milk, while the reverse was true for Laboratory 5.

DIFFERENCES AMONG MEDIA

The productivities of the six media are compared in Table 2. The geometric means of the counts of each medium are listed and are also expressed as a percentage of the geometric means of Medium C, A and F. Asterisks are used to indicate whether the latter percentages differ significantly from 100;

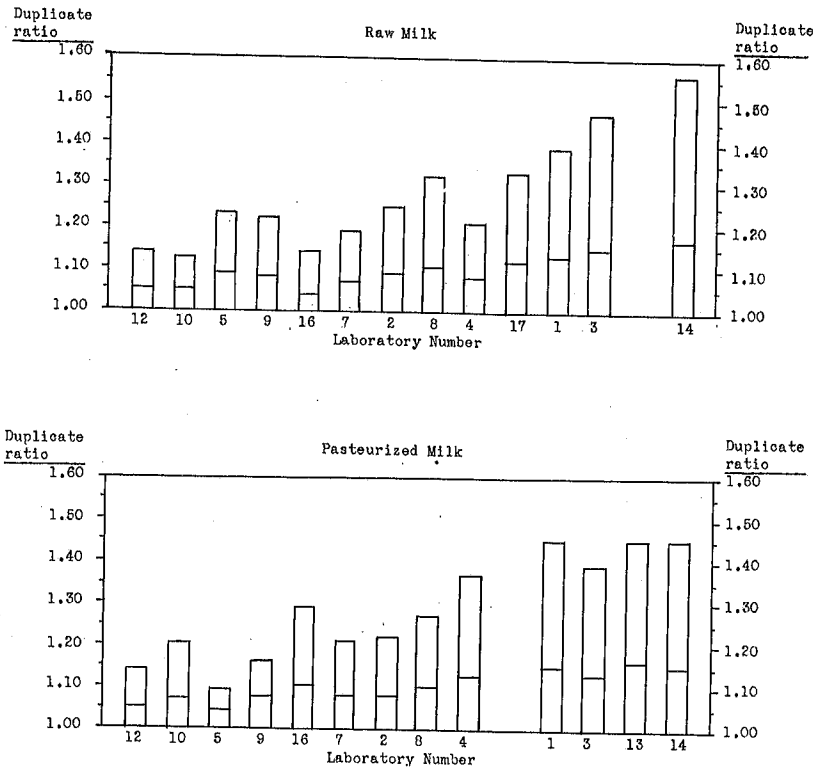

Chart I — Variability of counts as measured by duplicate ratios* by laboratory and type of milk samples.

* A duplicate ratio is defined to be the ratio of the higher to the lower counts on duplicate plates.

The upper limit of each bar is the level below 95% of the duplicate ratios are estimated to fall.

The horizontal line within each bar is the geometric mean of the duplicate ratios.

that is, whether the difference in the productivity of a given medium and the base medium has statistical significance. Chart II gives the relative percentages of the means (the base mean being 100 for Medium C), and the range within which the true precentages are expected to fall 95 percent of the time. When the range does not include 100 percent, the given mean is said to be significantly different from the base mean.

The geometric means of the counts ranged from 101 to 112 for raw milk, and from 93 to 121 for pasteurized milk. The mean counts for raw and pasteurized milk were very close for all except Media A and D. The geometric mean for pasteurized milk on Medium A was much lower than the geometric mean for raw milk, while the reverse was true for counts on Medium D.

Medium A generally had the lowest productivity among the six compared. Its geometric mean on pasteurized samples was significantly lower than that on all other media. Its geometric mean on raw milk was significantly lower than that on Media B, C and D, about the same as on Medium E, and somewhat higher than on Medium F.

The productivity of Medium B approximated that of Medium C and was significantly higher than the productivities on Media A and F.

The productivity of Medium C was significantly higher than that on Media A, E and F, about the same as on Medium B, and significantly lower than that on Medium D.

Medium D had the greatest colony productivity, particularly for pasteurized milk. Its geometric means were 4 and 14 percent higher than the geometric means for Medium C for raw and pasteurized milk, respectively. It was also significantly more productive than Media A and F.

TABLE 1. NUMBER OF DUPLICATE PLATE PAIRS, AND VARIABILITY OF COUNTS AS MEASURED BY DUPLICATE RATIOS*, BY LABORATORY AND TYPE OF MILK SAMPLES

Laboratory	RAW MILK			PASTEURIZED MILK		
	No. of duplicate plate pairs	Geom. mean of duplicate ratios*	95%-point** of duplicate ratios*	No. of duplicate plate pairs	Geom. mean of duplicate ratios*	95%-point** of duplicate ratios*
1	126	1.13	1.39	150	1.15	1.45
2	150	1.09	1.25	150	1.08	1.22
3	150	1.15	1.47	156	1.13	1.39
4	150	1.08	1.21	150	1.13	1.37
5	114	1.09	1.23	114	1.04	1.09
6	....	....	....	84	....	....
7	150	1.07	1.19	180	1.08	1.21
8	132	1.11	1.32	114	1.10	1.27
9	150	1.08	1.21	150	1.07	1.16
10	144	1.05	1.13	144	1.07	1.20
11	42	....	....	54	....	....
12	144	1.05	1.14	156	1.05	1.14
13	84	....	....	150	1.16	1.45
14	168	1.17	1.56	156	1.15	1.45
15	....	....	....	30	....	....
16	150	1.04	1.14	150	1.10	1.29
17	168	1.12	1.33	6	....	....

* A duplicate ratio is defined as the ratio of the higher to the lower count of a duplicate pair of plates.

** The level below which 95% of the duplicate ratios are estimated to fall.

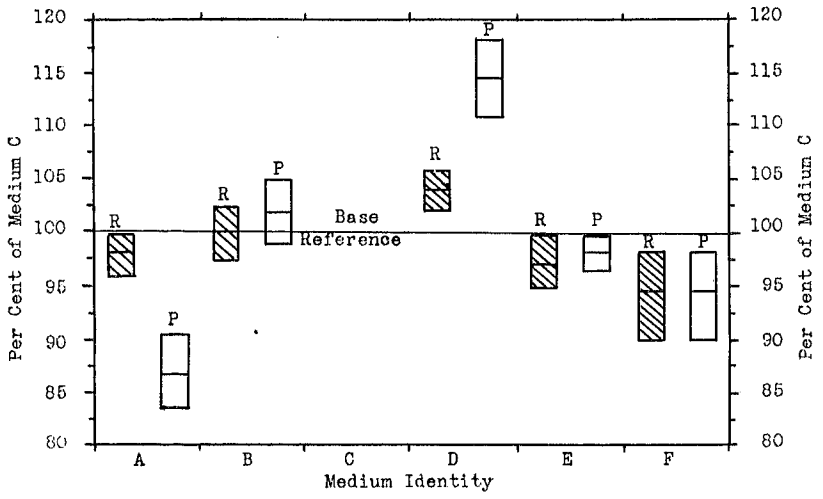

CHART II — COMPARISON OF GEOMETRIC MEAN COUNTS OF SIX PLATING MEDIA, USING MEDIUM C, THE OFFICIAL TGEM MEDIUM, AS A BASE OF REFERENCE

R = Raw milk. P = Pasteurized milk.

The center line of each bar is the geometric mean of Medium X expressed as a percentage of the geometric mean of the medium used as a base of comparison.

The length of each bar represents the range within which the true value of the percentage lies 95% of the time. If this range does not include 100%, Medium X is significantly different from the base medium.

DISCUSSION

Based upon the confirming observations reported herein, namely that the colony productivities on the two dehydrated Media B and C, to which 1 percent of skim milk was to be added after rehydration and before use, the Subcommittee on Standard Methods for the Examination of Dairy Products recommended to the APHA Coordinating Committee on Laboratory Methods, on November 1, 1950, that authorization be granted for the optional use of either Difco's Tryptone Glucose Extract Agar, dehydrated, No. B 2 or BBL's Trypticase Glucose Extract Agar, dehydrated, No. 183-B as official plating media for determining the Standard Plate Count on milk and cream by the Agar Plate Method. The Coordinating Committee on Laboratory Methods acted favorably on the recommendation and referred it to the Committee on Research and Standards. On February 20, 1951, the Committee on Research and Standards voted favorably on the action of the Coordinating Committee on Laboratory Methods.

Thus far, comparative determinations have been made on raw and pasteurized milk only. There is no reason to believe that optional use should not be extended to raw and pasteurized cream. Before use for plating milk and cream, one percent of skim milk (fresh or freshly reconstituted, or sterilized stock thereof) should be added to the prepared medium. No determinations have been made by the Subcommittee to establish independently the equivalent productive value of each BBL ingredient for possible interchangeable use with each Difco ingredient. In the interests of uniformity, it is recommended that a dehydrated form of medium be used.

Formal announcement of the acceptance of the optional use of either of these two dehydrated media for making Standard Plate Counts on milk and cream by the Agar Plate Method was made by Reginald M. Atwater, M. D., Executive Secretary of the American Public Health Association, effective February 20, 1951.[6]

Table 2. COMPARISON OF GEOMETRIC MEAN COUNTS USING SIX DIFFERENT PLATING MEDIA, BY TYPE OF MILK SAMPLE

	MEDIA					
	A	B	C	D	E	F
Geometric mean						
Raw milk	105.5	108.0	108.0	112.3	105.2	101.4
Pasteurized milk	92.5	108.3	106.1	121.1	103.9	99.8
Geometric means as percentages of Medium C						
Raw Milk	98**	100	(100)	104**	97*	94**
Pasteurized milk	87**	102	(100)	114**	98*	94**
Geometric means as percentages of Medium A						
Raw Milk	(100)	102*	102**	106**	100	96
Pasteurized milk	(100)	117**	115**	131**	112**	108**
Geometric means as percentages of Medium F						
Raw milk	104	107**	107**	111**	104	(100)
Pasteurized milk	93**	109**	106**	121**	104	(100)

** P <.01 Significant

* .01 <P <.05 Borderline significance.

Because of the general tendency for increased colony productivity on Medium D and for decreased colony productivity on Medium E than was obtained on Medium C, the Subcommittee, although recognizing the need to replace as soon as possible the milk-containing media with milk-free media for official work, suggests that observations be made on media of similar composition, slightly modified only with respect to the percentages of ingredients used, so as to yield counts essentially in agreement with those obtained on Medium C.

The colony productivity on the original S4 synthetic media yielded an average colony count of essentially 95 percent of that obtained on the official TGEM medium. For nearly a half-century, scientists have talked about a synthetic plating medium, but credit for so nearly a complete achievement, as herein reported, goes to Pelczar and Brown.[4] Although the cost of ingredients for the synthetic medium is much greater than the cost for digest and/or extract type media, there is good reason to believe that the modified S4 medium can be used even now, with due care, as a reference medium for the productivity determination of commercial lots of media.

It is recognized that considerable additional work should be done, first, to confirm the productivities herein reported, and later, to improve the composition of a synthetic medium, the use of which may at the proper time receive official APHA approval for reference productivity determinations. Fortified by the rapidly expanding knowledge on nutrition, including that on single cell type organisms, Earle K. Borman, Connecticut State Department of Health Laboratories, Hartford, Connecticut, as Vice-Chairman of the APHA Subcommittee on Approval of Culture Media, is now organizing studies to improve the synthetic medium.

SUMMARY

The colony productivities of six plating media for the Agar Plate Method, including one of synthetic composition, have been compared.

The comparison confirms an earlier observation that the colony productivity obtained on the officially recognized Difco's Tryptone Glucose Extract Agar, dehydrated, No. B 2 and BBL's Trypticase Glucose Extract Agar, dehydrated, No. 183-B, are essentially identical. Formal authorization for the optional use of either of these media was made by the American Public Health Association on February 20, 1951.

Plans are to make additional comparisons using modified formulas of the two milk-free plating media, the objective being first to obtain on each, essentially identical productivities with that obtained on the currently approved media and then to recommend at the proper time their substitution for the two milk-containing media identified above.

A synthetic plating medium consisting of 18 amino acids, 8 vitamins, 3 purine bases, 2 pyrimidine bases, potassium acid phosphate, magnesium sulphate glucose, and agar has yielded an average colony count of essentially 95 percent of that obtained on the officially recognized Difco's Tryptone Glucose Extract Agar, dehydrated, No. B 2. Steps have been taken to confirm this observation, hoping thereby to establish a basis for recognition at the proper time by the American Public Health Association of a reproducible reference medium for determining the colony productivity of commercial lots of media.

REFERENCE

1. *Standard Methods for the Examination of Dairy Products,* Ninth Ed., American Public Health Association, New York 19, New York.

2. Buchbinder, Leon, Baris, Y., Alff, E., Reynolds, E., Dillon, E., Pessin, V., Pincus, L., and Strauss, A. Studies to Formulate New Media for the Standard Plate Count of Dairy Products, *U. S. Public Health Reports,* **66**, 327 (1951).

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6. Atwater, R. M. Official Acceptance of Plating Media for the Agar Plate Method. *Amer. J. Public Health,* **41**, 609 (1951).

NEW BOOKS & OTHER PUBLICATIONS

The Pharmacological Evaluation of Antioxidants, by A. J. Lehman, O. G. Fitzhugh, A. A. Nelson, and G. Woodward, pages 197-208. Nonpoisonous antioxidants are propylgallate, thiodipropionic acid and its dilauryl and distearyl esters, and gum guaiac. 12 references.

Salmonella Infection as a Food Industry Problem, by W. R. Hinshaw and Ethel McNeil, pages 209 to 240. 103 references. Most of the 150 serological types affect man and animals, with birds and swine the most important animal reservoirs. Eggs have yielded 52 types of *Salmonella.* Infections have come through meat, vegetables, and fruits, (contaminated from animals), milk and cheese (through insects, rodents, etc).

Reactions between Sugars and Nitrogenous Compounds and Relationship to Certain Food Problems, by J. P. Danehy and W. W. Pigman, pages 241 to 290. 199 references. The importance of this subject is in changes caused during processing and handling of various foods, important among which are browning discolorations. The survey deals with experimental and speculative work on the character of the reactions.

Chemical and Microbial Studies on Sliced Canned Bacon, by J. A. Ulrich and H. O. Halvorson, pages 291 to 325. About 100 references. The most desirable cure leaves 2 - 3 percent salt in the product. Smoking is more effective without removing the skin. There is some measurable deterioration during storage but less at 21° – 24°C (70° – 75°F) than at 38°C (100° F).

Certain Aspects of Internal Corrosion in Tin Plate Containers, by R. R. Hartwell, pages 327 to 383. 142 references. Tinned cans now require about 4 percent of the nation's finished steel output. This is a well illustrated discussion of the manufacture of tin plate, corrosion, packaging and storage, variables in canning operations (e.g. oxygen,

(continued to page 129)