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A STATISTICAL COMPARISON OF TWO METHODS FOR DETERMINING THE BACTERIAL COUNT OF MILK

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The Standard method as stated in "Standard Methods of Milk Analysis" and a modified method (Bowers-Hucker medium incubated at 32° C.) were compared. Average colony counts were obtained by both methods of a variable number of replicate plates (20-100) from 18 representative samples, each plate being counted three to six times. This procedure was followed because it was felt that, for this particular study, a large number of counts (4,709) by several technicians from a relatively small number of samples offered a more dependable basis for comparison than would single or duplicate counts from a larger number.

The modified method produced a greater number of more luxuriant colonies, and comparisons evidenced a marked tendency toward increased counting accuracy.

The augmented visibility of the colonies, probably an important factor in counting accuracy, was no doubt due in the main to the inherent opaqueness of the Bowers-Hucker medium.

For these reasons it was concluded that the modified method is significantly better than the Standard for the purposes for which it is intended.

BULLETIN 423: A STATISTICAL COMPARISON OF TWO METHODS FOR DETERMINING THE BACTERIAL COUNT OF MILK

J. M. FRAYER[1]

INTRODUCTION

For some time there has been a growing notion that the procedure (medium and incubation temperature) recommended by the American Public Health Association in "Standard Methods of Milk Analysis" is inadequate. It has been increasingly recognized that it accurately estimates neither the actual number of viable organisms present nor a constant proportion of them. Insofar as this is true, it does not correctly reflect the sanitary quality of the milk under examination and thereby falls short of its primary objective as a method for the quantitative bacterial analysis of milk.

It is generally admitted that only an approximately true estimate of the number of bacteria present can be expected by the use of any plate method. This is due, among other things, to bacterial clumping; to their varying nutritive demands which can not all be satisfied by any one medium; to diverse optimum growth temperatures which can not all be met by any one fixed incubation temperature; to the degree of reliability of the original sample; to the accuracy of the apparatus used; to the greater or less strictness of adherence to prescribed technique; and, finally, to the personal equation of the operator. The relative importance and significance of many of these factors have been rather extensively explored. However, because of the distrust now felt touching the adequacy of the present Standard method, much interest has been aroused in the development of a medium and the discovery of an incubation temperature which will induce the vigorous growth of a larger proportion of the viable organisms which are present. The general production of larger colonies which are more easily seen would tend to make counting easier and increase its accuracy.

Since the Standard method is imbedded firmly in regulatory laws and is used extensively as a yardstick for premium payment, proposals looking toward change should be made only after exhaustive studies have clearly indicated the benefits which might be expected to accrue.

Assuming that all other factors are under control, an attempt has been made to determine whether there is a marked tendency, attribu-

[1] The writer is indebted to Mr. Alec Bradfield, formerly assistant animal and dairy husbandman, and to Charles Livak, student assistant, for aid in the conduct of these trials and in summarizing data.

table to method, which will not only enhance growth but make it easier for technicians more closely to duplicate their own results and those of others. It may be argued that duplicability is not the desired end per se. However, it is held that, if one or the other method shows a consistent and marked central tendency towards supporting greater growth and towards greater counting accuracy, such a tendency is significant.

PROCEDURE

The question at once arises as to what criteria will best measure the advantages or disadvantages of any proposed modification. Biological studies are usually so complex and the development of a simplifying index or formula without serious impairment is so difficult that the adequacy of such indices is always a moot point.

The study of the relative merits of the two methods hereinafter described involved making a large number of replicate plates from each sample. The raw milk samples used were produced under varying sanitary conditions and deemed to be representative of a fairly wide range of bacterial flora. Three technicians counted each plate of each series and in four of the 18 series each technician counted each plate twice.

There were three chief objectives in mind from which it was hoped to draw conclusions.

1. By making from 20 to 100 plates from each sample and counting each plate from three to six times, it was hoped to provide figures which would be much more dependable, for purposes of comparing the two methods, than would those obtained from single or duplicate plates counted by one person.

2. By making simple statistical studies involving determination of coefficients of variability, high counts, low counts, averages, ranges, relations of averages to ranges and frequency distributions, it was hoped to determine which method showed a closer tendency toward consistency.

3. By making simple statistical studies from figures provided by the counting of each plate by three technicians, it was hoped to determine something of the effects of the two methods on the results secured by several workers. By the recounting of each plate by each of three technicians, it was hoped to determine something of the effect of method on individual counting accuracy.

These comparisons should throw light on the relative usefulness of the two methods from this important point of view.

Two methods were selected for comparison:

1. Standard agar incubated at 37° C. for 48 hours, the procedure recommended in the 1934 edition of "Standard Methods of Milk Analysis" (1) and generally used in this country for this purpose. It will hereafter be designated as the Standard method.

2. Tryptone-glucose-skimmilk agar as described by Bowers and Hucker (2),[1] incubated at 32° C. for 48 hours as recommended by Pederson et al. (3), selected because of the excellent results obtained with it by several investigators, including Bradfield (4) of this Station. It will hereafter be referred to as the modified method.

All samples were handled and plated in strict accordance with the Standard methods procedure except that the Bowers-Hucker medium and the 32° C. incubation temperature were used on one-half of the plates. All media were standardized to a pH of 6.6 to 6.8. Particular attention was given to uniformity of the details of plating technique used by the three operators. The incubators were of the water-jacketed type. Temperatures were checked several times daily. The "Lumi-Lens" counting device was used. Every effort was made to control relevant details so that adequate comparisons could be made from the resultant data.

In any study involving statistical analysis one must choose a measure of central tendency. Without discussing the theory of averages, suffice it to say that the arithmetic average was selected for use in this case. This decision was made after all computations had been made, using both the arithmetic and geometric means and, in some instances, the median. Although the absolute figures differed somewhat, averages, etc., based on colony counts by the Standard and modified methods were substantially in the same ratio and, consequently, comparisons led to the same conclusions. Throughout the study, colony counts per plate were used rather than total bacterial counts because the number, size, and visibility of the colonies were the factors of paramount importance.

DISCUSSION

Objectives 1 and 2 are partially fulfilled and presented in the summary tables on pages 6-8, 10-13, and 16-18.

Table 1 shows a frequency distribution of all counts for each series with the percentage of total for each class. This involves 4,709 colony counts. It is believed that the frequency distribution is a valuable supplement to an array or average of a large group of individual values

[1] Agar (A. H. Thomas & Co., No. 40), 1.5 percent; tryptone (Difco), 0.5 percent; glucose Pfanstiehl, 0.1 percent); fresh skimmilk, 0.5 percent; distilled water.

TABLE 1.—DISTRIBUTION OF COLONY COUNTS

Class intervals	Series number and number of colonies per plate											
	I. 81-100 to 261-280				II. 1-20 to 161-180				III. 11-20 to 121-130			
Method	Standard		Modified		Standard		Modified		Standard		Modified	
	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%
	8	2.7	...	...	14	5.1	...	...	26	19.3	...	...
	29	9.8	3	1.0	33	12.1	...	...	45	33.3	...	...
	45	15.2	18	6.2	81	29.7	...	...	25	18.5	...	...
	66	22.2	44	15.1	94	34.4	14	5.2	22	16.3	8	5.7
	63	21.2	74	25.4	39	14.3	74	27.7	15	11.1	21	14.9
	61	20.5	101	34.8	11	4.0	110	41.2	2	1.5	24	17.0
	24	8.1	38	13.1	1	0.4	55	20.6	...	...	16	11.3
	1	0.3	11	3.8	...	...	13	4.9	...	...	33	23.4
	...	...	1	0.3	...	...	1	0.4	...	...	21	14.9
	...	...	1	0.3	...	...	...	...	...	...	16	11.4
									...	...	1	0.7
									...	...	1	0.7
Totals	297		291		273		267		135		141	
Average number colonies per plate	160		180		62		109		33		78	
	VII. 1-3 to 25-27				VIII. 11-20 to 101-110				IX. 6-10 to 66-70			
	8	5.7	...	...	1	0.7	...	...	1	0.7	...	...
	25	17.7	...	...	8	5.5	...	...	1	0.7	...	...
	36	25.5	5	3.6	26	18.0	...	...	9	6.4	6	4.3
	29	20.6	20	14.2	24	16.7	...	...	36	25.5	16	11.4
	20	14.2	36	25.5	25	17.4	...	...	24	17.0	15	10.6
	14	9.9	48	34.0	24	16.7	21	15.6	21	14.9	26	18.4
	5	3.6	20	14.2	23	16.0	58	43.0	29	20.6	30	21.3
	4	2.8	11	7.8	10	6.9	43	31.8	11	7.8	14	9.9
	...	...	1	0.7	2	1.4	12	8.9	6	4.3	8	5.7
			...	...	1	0.7	1	0.7	3	2.1	13	9.2
											6	4.3
											3	2.1
											4	2.8
Totals	141		141		144		135		141		141	
Average number colonies per plate	10		16		56		79		31		38	
	XIII. 1-2 to 15-16				XIV. 101-120 to 261-280				XV. 6-8 to 30-32			
	2	3.2	2	4.2	2	3.3	...	...	1	1.7	...	...
	6	10.0	5	10.4	14	23.3	...	...	1	1.7	...	...
	18	30.0	10	20.8	21	35.0	2	3.3	11	18.3	4	7.4
	16	26.7	11	22.9	10	16.7	21	35.0	10	16.7	19	35.2
	12	20.0	10	20.8	12	20.0	26	43.3	14	23.3	10	18.5
	4	6.7	4	8.3	1	1.7	6	10.0	13	21.7	6	11.1
	1	1.7	5	10.5	...	...	3	5.0	8	13.3	11	20.4
	1	1.7	1	2.1	...	...	1	1.7	2	3.3	2	3.7
							1	1.7	...	...	2	3.7
Totals	60		48		60		60		60		54	
Average number colonies per plate	7		8		158		188		19		20	

Series number and number of colonies per plate												
IV. 6-10 to 46-50				V. 81-100 to 361-380				VI. 121-140 to 421-440				
Standard		Modified		Standard		Modified		Standard		Modified		
Cases	%	Cases	%	Cases	%	Cases	%	Cases	%	Cases	%	
6	4.4	...	...	2	1.5	...	...	3	2.1	...	...	
45	33.3	1	0.7	5	3.6	...	...	...	...	...	...	
37	27.4	5	3.5	4	2.9	...	...	5	3.5	...	...	
25	18.5	21	14.9	11	8.0	...	...	7	4.9	...	...	
14	10.4	36	25.5	32	23.2	...	...	27	18.8	3	2.1	
4	3.0	49	34.8	33	23.9	...	...	25	17.3	7	5.0	
2	1.5	17	12.1	25	18.1	3	2.5	33	22.9	23	16.3	
2	1.5	10	7.1	16	11.6	8	6.5	20	13.9	27	19.2	
...	...	2	1.4	9	6.5	9	7.3	17	11.8	40	28.4	
				1	0.7	11	8.9	7	4.8	24	17.0	
				...	...	16	13.0	...	...	13	9.2	
				...	...	30	24.4	...	...	1	0.7	
				...	...	29	23.6	...	...	...	...	
				...	...	13	10.6	...	...	2	1.4	
				...	...	4	3.2	...	...	1	0.7	
135		141		138		123		144		141		
	19		31		189		302		242		287	

X. 21-40 to 201-220				XI. 11-13 to 44-46				XII. 121-140 to 421-440			
13	9.2	...	...	3	5.0	...	...	2	5.0	...	...
32	22.7	...	...	3	5.0	...	...	...	...	...	...
48	34.0	...	...	7	11.7	3	5.0	...	...	...	...
26	18.4	...	...	13	21.7	8	13.4	...	...	...	...
17	12.2	3	2.1	11	18.3	2	3.3	2	5.0	...	...
5	3.5	29	20.1	11	18.3	11	18.3	4	10.0	...	...
...	...	52	36.1	3	5.0	16	26.7	5	12.5	...	...
...	...	36	25.0	3	5.0	8	13.3	6	15.0	1	2.5
...	...	17	11.8	2	3.3	6	10.0	10	25.0	...	...
...	...	7	4.9	1	1.7	5	8.3	10	25.0	2	5.0
				2	3.3	1	1.7	...	...	7	17.5
				1	1.7	...	...	1	2.5	9	22.5
								...	...	5	12.5
								...	...	8	20.0
								...	...	5	12.5
								...	...	3	7.5
141		144		60		60		40		40	
	73		159		25		30		290		367

XVI. 11-20 to 91-100				XVII. 91-95 to 166-170				XVIII. 21-30 to 101-110			
1	1.7	...	...	3	5.3	...	...	8	14.0	...	...
5	8.3	...	...	6	10.4	...	...	18	31.6	...	...
20	33.3	...	...	7	12.3	...	...	11	19.3	...	...
13	21.7	2	3.3	4	7.0	...	...	11	19.3	...	...
10	16.7	21	35.0	7	12.3	1	1.8	6	10.5	1	1.7
4	6.7	29	48.4	7	12.3	2	3.5	3	5.3	8	13.3
3	5.0	8	13.3	7	12.3	4	7.0	...	...	31	51.7
4	6.6	...	...	10	17.5	2	3.4	...	...	13	21.7
				2	3.4	6	10.5	...	...	7	11.6
				1	1.8	12	21.0				
				1	1.8	8	14.1				
				1	1.8	13	22.8				
				...	...	5	8.8				
				...	...	1	1.8				
				1	1.8	3	5.3				
60		60		57		57		57		60	
	57		73		116		141		45		88

and is almost indispensable to a study of this kind. In series 15 to 18, inclusive, each technician counted each plate twice. The counts used in the frequency distribution are the average of the two recounts. Class intervals were adjusted to the number and size of counts. It is a common statistical practice to use not less than 10 nor more than 25 classes. This rule was adhered to as closely as possible.

In most cases the modal clumping is more pronounced in counts obtained by the modified than in those secured by the Standard method. The exceptions occur in series where the averages are lower or higher than those usually counted. This observation sustains the recommendation in "Standard Methods of Milk Analysis" (1) that wherever possible plating should be so planned that resultant colonies will fall between 30 and 300 colonies per plate. A complete picture of the story which these figures have to tell can not be obtained by looking at them from a single angle or by judging from a single series of indices.

The next group of three tables throws some light on the comparative ranges and range relatives.

Comparisons involving all counts arranged according to magnitude of average colony counts obtained by the Standard method are shown

TABLE 2.—COMPARISONS INVOLVING ALL COUNTS

Arranged according to magnitude of average colony counts by Standard method

Series number	(1) High colony counts		(2) Low colony counts		(3) Average colony counts		(4) Ranges		(5) Relation average to range Average = 100	
	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*
13	16	15	2	2	7	8	14	13	200	162
7	23	26	1	7	10	16	22	19	220	119
15	28	32	9	14	19	20	19	18	100	90
4	42	50	6	15	19	31	36	35	189	113
11	44	43	12	18	25	30	32	25	128	83
9	53	67	9	16	31	38	44	51	142	134
3	62	130	12	44	33	78	50	86	152	110
18	76	108	21	70	45	88	55	38	122	43
8	101	106	20	62	56	79	81	44	145	56
16	99	90	36	60	57	73	63	30	111	41
2	126	167	6	68	62	109	120	99	194	91
10	135	211	30	108	73	159	105	103	144	65
17	166	164	91	114	116	141	75	50	65	35
14	201	262	112	150	158	188	89	112	56	60
1	221	264	84	104	160	180	137	160	86	89
5	266	373	88	213	189	302	178	160	94	53
6	320	424	129	204	242	287	191	220	79	77
12	361	427	124	279	290	367	237	148	82	40
Average	130	164	44	86	88	122	86	78	128	81

S* = Standard method.
M* = Modified method.

in table 2. The highest counts obtained by any one of the technicians by both methods on all series are found in column 1. The modified method yields a significantly greater average high count than does the Standard. With three exceptions, the serial high averages by the former exceed those by the latter. The lowest counts obtained by any individual for all the series are shown in column 2. The average obtained by the modified is about twice that secured by the Standard method. The lowest count for each individual series noted when the former method is used exceeds that secured by the latter.

The colony averages for each series obtained by both methods are shown in column 3. These figures resulted from averaging the mean counts obtained by three people counting the same plate. These averages are more than usually significant because they are the results of 40 to 297 counts per series for each method. Here again both the serial average and the grand average of counts by the modified method exceed those by the Standard—the grand averages significantly so.

The ranges between the highest and lowest counts for each by both methods are shown in column 4. They do not take into account the size of the averages involved. Notwithstanding, the average range for all series is less by the modified than by the Standard method and, with the exception of five series, the individual ranges are less.

For purposes of more adequate comparison a relative was developed by dividing each range by its corresponding average colony count and multiplying by 100. The resultant relatives are shown in column 5. The average relative for counts obtained by the modified is significantly less than that for those from the Standard method. This indicates that from this angle the former gives the more consistent results. This statement is based on the assumption that, all other factors being equal, the smaller the relative range, the more dependable is any one of the counts from a series of replicate plates from the same sample of milk.

The picture is developed somewhat further in table 3, in which are displayed comparisons involving the colony counts for each series by each technician, which are analyzed in the same manner as in table 2. This group of figures indicates a rather close agreement in the counting by the three technicians.

Table 4 carries this type of analysis a step further, and compares plate averages. These figures were obtained by taking the mean of the counts by three people on the same plate. As might have been expected, this process smooths out some of the inequalities inherent in

TABLE 3.—COMPARISONS INVOLVING COLONY COUNTS OF TECHNICIANS 1, 2, 3

Series number	High colony counts						Low colony counts						Average colony counts						Range						Relation average to range. Average = 100					
	Tech. 1		Tech. 2		Tech. 3		Tech. 1		Tech. 2		Tech. 3		Tech. 1		Tech. 2		Tech. 3		Tech. 1		Tech. 2		Tech. 3		Tech. 1		Tech. 2		Tech. 3	
	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*
13	10	14	16	14	11	15	2	2	2	4	6	4	6	7	8	8	8	9	8	12	14	10	5	11	133	171	175	125	62	122
7	21	26	23	24	23	22	1	8	2	7	5	10	9	16	10	16	12	16	20	18	21	17	18	12	222	112	210	106	190	75
15	26	28	27	32	28	31	8	14	13	15	12	14	19	19	18	20	20	20	18	14	14	17	16	17	95	74	78	85	80	85
4	40	46	42	44	42	50	6	18	9	19	10	15	19	31	18	31	19	31	34	28	33	25	32	35	179	90	183	81	168	113
11	42	39	44	43	37	40	13	18	13	19	12	18	26	30	25	29	24	30	29	21	31	24	25	22	112	70	124	83	104	73
9	53	66	48	66	53	67	9	20	16	16	16	21	30	39	30	37	33	39	44	46	32	50	37	46	147	118	107	135	112	118
3	59	110	62	104	59	130	12	44	17	45	14	54	33	78	36	73	29	84	47	66	45	59	45	76	142	85	125	81	155	90
18	75	108	76	108	64	106	21	72	34	70	31	74	39	88	51	87	46	89	54	36	52	38	33	32	138	41	102	44	72	36
8	86	95	101	94	90	106	20	64	32	62	22	64	54	79	68	77	46	81	66	31	69	32	68	42	122	39	101	42	148	52
16	94	88	92	84	99	90	26	66	43	60	43	63	49	75	64	70	60	74	68	22	49	24	56	27	139	29	77	34	93	36
2	126	167	116	157	108	139	6	71	10	68	8	67	65	116	63	105	58	107	120	96	106	89	100	72	185	83	168	85	172	67
10	135	211	118	201	118	205	30	125	30	108	30	127	82	164	71	149	67	163	105	86	88	93	88	78	128	52	124	62	131	48
17	166	162	132	163	144	164	100	117	94	114	91	120	125	141	112	139	112	142	66	45	38	49	53	44	53	32	34	35	47	31
14	201	259	200	240	190	262	123	154	118	164	112	150	154	188	167	188	152	189	78	105	82	76	78	112	51	56	49	40	51	59
1	213	264	221	234	220	236	84	127	91	128	90	104	158	183	162	183	160	174	129	137	130	106	130	132	82	75	80	58	81	76
5	266	369	254	347	240	373	88	213	93	214	104	220	194	309	188	288	186	310	178	156	161	133	136	153	92	50	86	46	73	49
6	320	424	304	396	320	389	131	230	129	219	132	204	244	287	238	283	243	289	189	194	175	177	188	185	77	68	74	63	77	64
12	...	...	339	406	361	427	...	...	124	279	140	320	...	...	283	354	297	380	...	...	215	127	221	107	...	...	76	36	74	28
Average	114	146	123	153	123	158	40	80	48	90	49	92	77	109	90	119	87	124	74	65	75	64	74	67	123	73	110	69	105	68

S* = Standard method.
M* = Modified method.

TABLE 4.—COMPARISONS INVOLVING PLATE AVERAGES
Arranged according to magnitude of grand average colony counts by Standard method

Series number	(1) High average colony counts		(2) Low average colony counts		(3) Grand average colony counts		(4) Ranges		(5) Relation average to range Average = 100	
	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*
13	12	14	4	4	7	8	8	10	114	125
7	22	23	3	8	10	16	19	15	190	94
15	28	30	11	15	19	20	17	15	89	75
4	41	46	9	18	19	31	32	28	168	90
11	40	38	13	19	25	30	27	19	108	63
9	50	66	14	20	31	38	36	46	116	121
3	58	107	15	50	33	78	43	57	130	73
18	70	107	29	72	45	88	41	35	91	40
8	92	95	26	65	56	79	66	30	118	30
16	91	86	40	64	57	73	51	22	89	30
2	114	140	9	71	62	109	105	69	169	63
10	118	193	30	120	73	159	88	73	121	46
17	146	163	98	117	116	141	48	46	41	33
14	195	254	112	157	158	188	76	97	48	52
1	215	224	88	126	160	180	127	98	79	54
5	244	352	98	216	189	302	146	136	77	45
6	310	403	131	229	242	287	179	174	74	61
12	345	416	132	300	290	367	213	116	73	32
Averages	122	153	48	93	88	122	73	60	105	63

such work. The relatives are smaller but, nevertheless, lead to the same conclusion in regard to the comparative merits of the two methods.

A significant fact brought to light in preparing the preceding summary tables was the amazing range in counts obtained from an identical sample of milk by either method. This, of course, is nothing new, as it has been set forth many times as a weakness inherent in any plate count method. It was to be expected that this trend would persist whether all counts, the counts of several persons, or the mean plate counts were being considered. It is felt that herein lies an inherent and vital defect in the practice of comparing two methods of securing counts by making single or duplicate plates of the same sample instead of a large number of replicates. Opinions differ as to whether it is better to make a few plates of many samples or many plates of few samples. Nevertheless, it is felt that a large number of replicate plates yields values upon which comparisons may be confidently based.

Another way to compare the respective merits of two or more methods is to obtain an expression of the relative amount of variation from the mean counts. This coefficient of variability or per-

S* = Standard method.
M* = Modified method.

centage variation is obtained by working out the average deviations from the mean counts, dividing by the mean count, and multiplying by 100. This is a much simpler computation than to obtain it from the Standard deviations and it appears that the same relative differences are observed whichever way is used to compare the efficiency of the two methods.

Coefficients of variability due to all causes, obtained separately for the counts of each technician and each series, are shown in table 5. It

TABLE 5.—COEFFICIENTS OF VARIABILITY—ALL CAUSES
Arranged according to magnitude of average colony counts by Standard method

Series number	Standard method			Modified method		
	Technician 1	Technician 2	Technician 3	Technician 1	Technician 2	Technician 3
13	30.00	30.00	17.50	38.43	26.50	35.44
7	44.67	39.10	29.50	22.00	18.75	14.69
15	21.05	18.61	20.00	17.26	21.40	21.95
4	32.42	26.06	28.53	16.32	15.45	17.64
11	22.12	24.00	19.53	15.17	15.00	15.17
9	27.80	24.47	21.33	24.18	25.70	23.08
3	36.03	33.22	28.03	20.10	19.10	17.61
18	31.31	20.84	19.91	8.18	7.76	6.85
8	24.81	20.22	25.71	9.25	9.00	8.04
16	19.81	19.76	20.58	7.67	7.93	6.42
2	30.98	27.79	28.60	13.73	12.96	11.92
10	27.71	27.35	24.30	9.97	10.46	10.21
17	8.38	9.63	7.99	5.67	6.55	6.22
14	10.52	12.84	11.38	8.99	6.57	10.19
1	16.19	15.68	15.51	9.57	10.42	11.78
5	15.24	15.73	12.20	8.64	9.70	9.95
6	12.15	11.57	13.50	8.86	8.86	7.88
12	....	11.55	12.00	....	7.18	6.96
Averages	24.19	21.58	19.78	14.35	13.29	13.44

appears that there is a trend towards a decrease in the magnitude of the coefficients of variability as the size of the average colony counts increases; the average coefficients for the counts of each technician are surprisingly uniform, being significantly smaller by the modified than by the Standard method; and the individual serial coefficients from counts obtained by the modified are almost always materially smaller than those derived from counts obtained by the Standard method.

Additional summary comparisons which may throw more light on the problem are shown in table 6. The number of separate counts made in each series with both methods appears in column 1 and allows the reader to judge as to the relative dependability of the average counts. The serial average bacterial counts appear in column 2 and the grand average colony counts in column 3. They are again presented here because they constitute an essential step in the development of the

TABLE 6.—FURTHER COMPARISONS INVOLVING ALL COUNTS

Arranged according to magnitude of average colony counts by Standard method

Series number	(1) Number of counts involved in each series		(2) Average bacterial counts		(3) Average colonies per plate		(4) Coefficients of variability all causes		(5) Coefficients of variability due to counting		(6) Percentage increase M* over S*
	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	
13	60	48	70,000	80,000	7	8	21.43	28.88	20.00	13.25	+ 14.29
7	141	138	10,000	16,000	10	16	32.30	17.37	20.20	6.38	+ 60.00
15†	120	108	38,000	40,000	19	20	18.68	18.90	7.47	6.00	+ 5.25
4	135	141	3,800	6,200	19	31	27.00	15.39	7.84	5.61	+ 63.16
11	60	60	250,000	300,000	25	30	20.40	13.50	8.20	5.00	+ 20.00
9	141	141	62,000	76,000	31	38	23.33	23.97	7.22	3.82	+ 22.58
3	135	141	330,000	780,000	33	78	31.30	18.63	12.00	6.63	+136.36
18†	114	120	45,000	88,000	45	88	21.22	7.39	13.33	2.22	+ 95.56
8	144	135	560,000	790,000	56	79	21.39	8.56	16.32	3.06	+ 41.07
16†	120	120	285,000	365,000	57	73	18.77	6.30	13.16	3.42	+ 28.00
2	273	267	620,000	1,090,000	62	109	27.35	12.37	10.51	5.91	+ 75.81
10	141	144	7,300	15,900	73	159	26.11	9.94	8.60	5.02	+117.81
17†	114	114	232,000	282,000	116	141	7.53	5.82	6.31	1.86	+ 21.55
14	60	60	1,580,000	1,880,000	158	188	10.98	8.00	4.97	2.71	+ 18.92
1	297	291	16,000	18,000	160	180	13.50	8.63	6.43	6.16	+ 12.50
5	138	123	378,000	604,000	189	302	13.19	8.75	5.53	4.33	+ 59.78
6	144	141	24,200	28,700	242	287	10.54	6.55	2.89	4.38	+ 18.60
12	40	40	29,000	36,700	290	367	10.80	6.21	2.53	3.87	+ 26.53
Total	2,377	2,332									
Averages			252,000	361,000	88	122	19.77	12.51	9.64	4.98	+ 46.54

S* = Standard method.
M* = Modified method.
† = Each of three technicians counted same plate twice.

values which follow. The coefficients of variability due to all causes are shown in column 5. Unlike those shown in table 5, these serial coefficients are obtained by finding the mean of three counts for each plate, determining the deviations from the averages of these means, the coefficients of variability being calculated, as usual, from the means of these deviations for each series. Comparisons of the averages of these coefficients as well as of the serial coefficients (with two exceptions) indicate a noticeable and, sometimes; a marked trend towards consistency of replicate counts made from the same sample of milk and, conversely, a greater tendency towards a smaller relative divergence of the individual counts from the mean of all the counts of a series. It is believed that this difference in favor of the modified method may be fairly attributed to the method as a whole.

The coefficients of variability due to the mechanics of counting were isolated and are shown in column 5. The results seem significant. The difference between the averages of coefficients is nearly two to one in favor of those obtained from counts by the modified method.

The individual counts of a series tend to clump about the mean which is nearly twice as great with the modified as with the Standard method. There are many individual cases of greater relative accuracy than is indicated by the average. However, in the last two series (6 and 12), wherein the largest number of colonies per plate was found, there is a slight difference in favor of the Standard method. Although both coefficients are small, this variation might well be due to crowded plates which would tend to vitiate any advantages due to method.

The percentage increases of average serial counts secured by the modified over those by the Standard method are shown in column 6. An increase occurred in every case, being in some instances small and never very large. This seems due in the main to the character of the colony count averages which more nearly represent optimum growth numbers and can be assumed to result from the relatively large number of counts

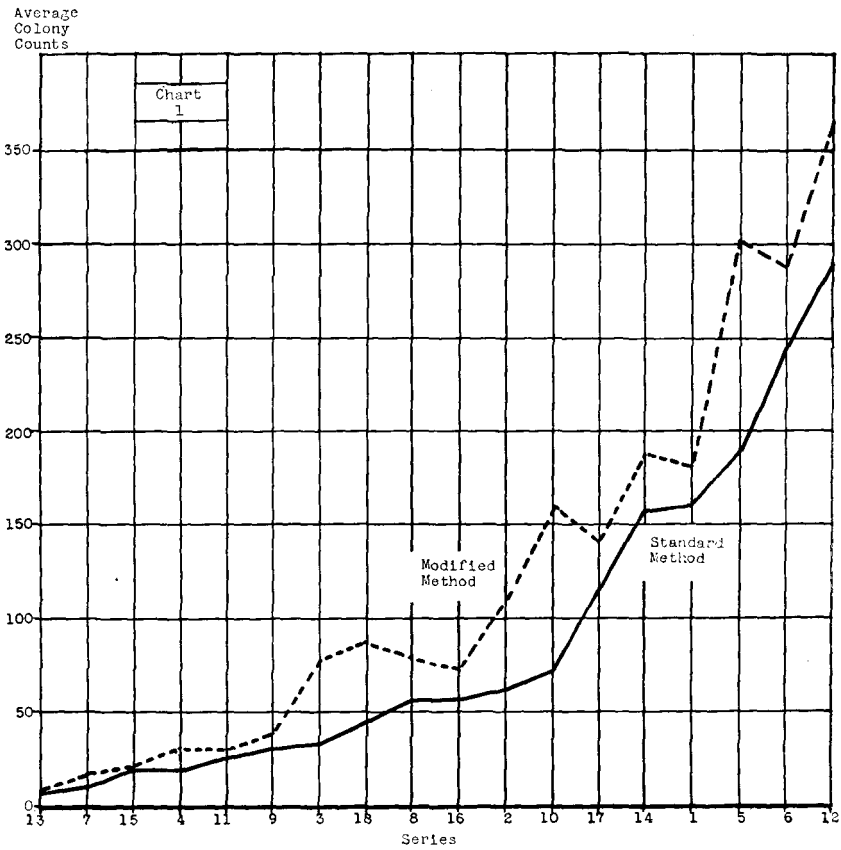

CHART 1.—Comparison of average colony counts arranged according to magnitude of counts by the Standard method.

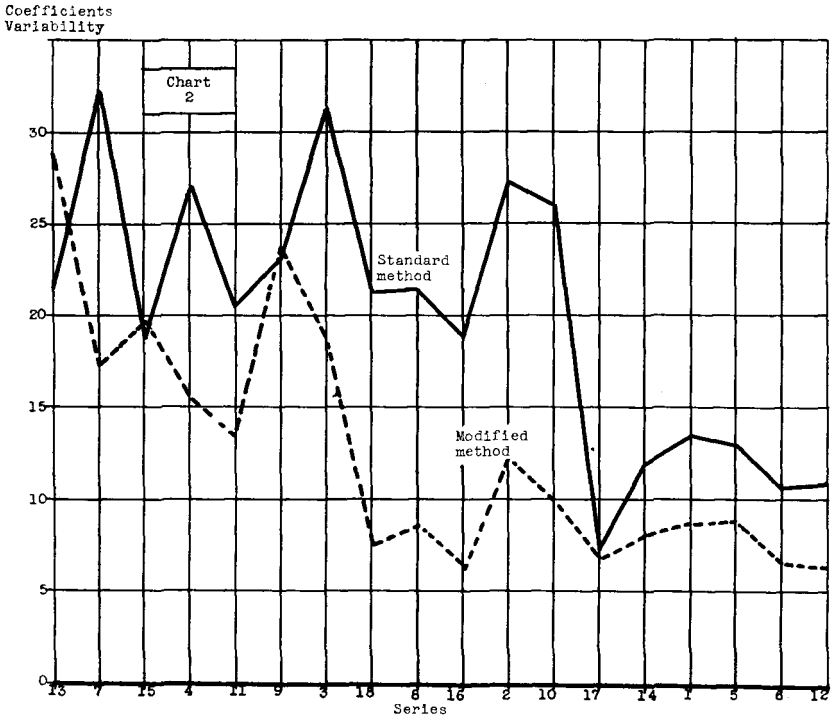

CHART 2.—Coefficients of variability—all causes. Arranged according to magnitude of counts by Standard method.

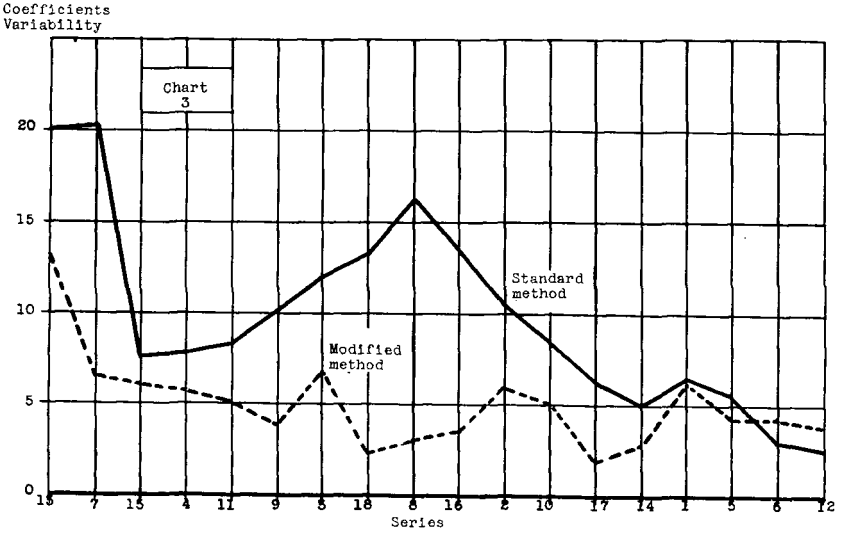

CHART 3.—Coefficients of variability—due to counting. Arranged according to magnitude of counts by Standard method.

involved in each series. Higher counts were obtained in every series by the Standard than some by the modified method, as appears in the tables showing high and low counts for each series by both methods.

The various comparisons, from different angles, of the effect of the method used on the results obtained, due to causes thought to be inherent therein, decidedly favor the modified method. Possible reasons for this are disussed later.

Graphs 1 to 3 compare the grand average colony counts for each series, the coefficients of variability due to all causes, and the coefficients of variability due to counting, these values being arranged according to the magnitudes of the colony counts obtained by the Standard method.

In fulfillment of objective 3 (p. 4) it is hoped to learn something about the effect of method on collective and individual counting accuracy. In order to bring out the counting variations in each series, table 7 was prepared to indicate the relation of the mean average colony counts to the average ranges between the high and low counts of the same plate handled by three technicians. The figures in column 2, as might be expected, show that low absolute ranges occur in series with low average counts and are sometimes larger when the modified rather

TABLE 7.—RELATION OF MEAN AVERAGE TO AVERAGE RANGE BETWEEN HIGH AND LOW COUNT OF SAME PLATE COUNTED BY THREE TECHNICIANS

	(1)		(2)		(3)	
Series number	Average colony count		Average plate range		Relation of average range to mean average Average = 100	
	S*	M*	S*	M*	S*	M*
13	7	8	3.5	2.8	50.0	35.0
7	10	16	5.1	2.6	51.0	16.2
15	19	20	3.6	3.3	18.9	16.5
4	19	31	3.9	4.5	20.5	14.5
11	25	30	5.2	3.8	20.8	12.7
9	31	38	5.7	3.8	18.4	10.0
3	33	78	10.1	13.4	30.6	17.2
18	45	88	15.5	4.9	34.4	5.7
8	56	79	23.9	6.3	42.7	8.0
16	57	73	19.0	6.2	33.3	8.5
2	62	109	17.0	16.1	27.4	14.8
10	73	159	16.9	21.1	23.6	13.3
17	116	141	19.0	6.8	16.3	4.8
14	158	188	20.5	13.0	13.0	6.9
1	160	180	26.6	29.0	16.6	16.1
5	189	302	28.3	34.2	15.0	11.3
6	242	287	17.0	30.5	7.0	10.0
12	290	367	15.0	28.0	5.2	7.6
Averages	88	122	14.2	12.8	24.7	12.7

S* = Standard method.
M* = Modified method.

than the Standard method is used. Nevertheless the average of the serial absolute ranges is smaller with the former than with the latter. However, the relative ranges as recorded in column 3 show that individually (with two exceptions) the differences are much in favor of the modified method, some running as high as nearly seven to one, the average being nearly two to one. This would indicate a decided tendency towards agreement of colony counts within a series of replicate plates when the modified method is used.

Comparisons of duplicate counts, involving 930 individual colony counts by the same technician, are shown in table 8. Averages of the means of duplicate counts from the same plate are shown in column 1. Good agreement was secured in the grand average by both methods by all technicians. Averages of ranges between duplicate counts of same plates for all technicians appear in column 2, grand averages of absolute ranges significantly favoring the modified method. These differences are smaller between two counts by the same technician than they are between the high and low counts of three technicians, a tendency which has been often noted.

The average relation between means and ranges of duplicate counts on same plate is significant, both as regards differences between values obtained by the Standard and by the modified methods and the differences between the work of the several technicians. Relatives from the modified agree much more closely between technicians than do those by the Standard method. This would seem to indicate that some factor, or factors, inherent in the former method tends to assist individuals not only in duplicating their own counts more closely but also those of

TABLE 8.—COMPARISONS OF DUPLICATE COUNTS BY SAME TECHNICIAN

Series number	(1) Averages of the means, duplicate counts, same plates						(2) Averages of ranges between duplicate counts of same plates						(3) Average relation between ranges and means of duplicate counts on same plate Average = 100					
	Tech. 1		Tech. 2		Tech. 3		Tech. 1		Tech. 2		Tech. 3		Tech. 1		Tech. 2		Tech. 3	
	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*
15	18	19	18	20	20	20	2.6	1.7	2.2	1.5	1.9	1.9	14.3	9.4	12.2	7.5	9.6	10.0
16	49	75	64	70	60	74	16.4	5.9	10.0	5.0	6.8	3.4	32.7	7.6	15.3	7.2	11.3	4.6
17	125	141	112	138	112	142	10.2	5.3	14.5	6.5	6.0	4.2	8.3	3.8	12.9	4.6	5.4	3.0
18	37	88	50	87	45	89	6.9	4.2	19.2	4.4	5.3	3.0	18.7	4.9	38.6	5.2	12.4	3.4
Averages	57	81	61	79	59	81	9.0	4.3	11.5	4.4	5.0	3.1	16.25	6.4	19.8	6.1	9.7	5.2

S* = Standard method.
M* = Modified method.

TABLE 9.—COEFFICIENTS OF VARIABILITY AS AN INDEX OF THE EFFECT OF MEDIA AND INCUBATI TEMPERATURE ON DUPLICABILITY OF INDIVIDUAL COUNTING

Series number	Coefficients of variability											
	Technician 1				Technician 2				Technician 3			
	(1) From grand average		(2) From technician's average		(3) From grand average		(4) From technician's average		(5) From grand average		(6) From technician' average	
	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*	S*	M*
15	6.84	4.15	7.22	4.37	5.58	4.02	6.11	3.75	5.00	4.85	4.75	4.8
16	14.38	4.04	16.73	3.93	5.68	3.45	7.89	3.06	5.93	2.30	5.63	2.2
17	4.40	1.89	4.08	1.89	6.26	2.30	6.48	2.34	2.58	1.50	2.68	1.4
18	7.08	2.41	8.90	2.41	18.89	2.52	18.51	2.55	5.89	1.73	6.39	1.7
Averages	8.18	3.12	9.23	3.15	9.10	3.07	9.75	2.92	4.85	2.59	4.86	2.5

S* = Standard method.
M* = Modified method.

others. In other words, it helps to minimize the so-called personal equation in counting.

The coefficients of variability as an index of the effect of media and incubation temperature on duplicability of individual counts are shown in table 9. These were determined in two ways for the counts of each technician. In column 1 they are shown from the grand serial averages for the counts of technician 1, while in column 2 they are derived from the average of his counts for each series. The results secured by technicians 2 and 3 are similarly analyzed and presented in columns 3 to 6.

Close agreement was secured between coefficients arrived at in either way for the same series and method. A significant difference is again seen in favor of the modified method. Some differences occurred in the results of the work of the three technicians when operating the Standard method but they were much less significant when the modified method was used. This shows from another angle the greater dependability of the results secured by the modified method and the greater probability of close agreement in recounts of the same plate by the same or several individuals.

SUMMARY AND CONCLUSIONS

Two outstanding methods of obtaining the bacterial counts of milk were compared by making a variable number of replicate plates (20 to 100) of 18 representative milk samples by both methods and counting each plate from three to six times, making 4,709 counts in all. The resultant figures were then analyzed by simple statistical methods which were calculated to show from different angles something of the relative value of each method for the purpose intended.

No method has as yet been devised whereby with mathematical precision all the bacteria present in a given sample of milk can be counted. The range between counts from the same sample of milk is still far too great, even when the modified method (p. 5) is used, although the range is significantly less than when the Standard method (p. 5) is used.

The data herein presented seem to justify the following conclusions.

1. The modified method, involving the use of a special medium and incubation temperature, produced a greater number of more luxuriant colonies from the same sample of milk than did the Standard method.

2. When the Bowers-Hucker medium was used, owing to its inherent opaqueness, the visibility, especially of the smaller colonies, was increased, a larger proportion of the organisms present were grown, and counting accuracy was augmented.

3. The modified method seems to be much better adapted to the purpose than is the present Standard method.

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