

COUNTING THE LIVING BACTERIA IN MILK—A PRACTICAL TEST

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The method under test, in the series of analyses reported here, has already been described (1915, 1916a). A comparison of results obtained in a series of milk analyses by this and the standard plate method has also appeared (1916 b). The following advantages have been claimed for the method:

1. It is rapid, requiring only about one-eighth of the time needed for the standard plate method.
2. The technic is simple and requires little glassware or culture medium.
3. It furnishes a means of keeping a permanent record of each analysis.
4. Only the individual bacteria, or groups of the same, that are alive grow into colonies. This makes it possible to use the method for counting the bacteria in pasteurized milk, which is not possible by methods of direct microscopical examination such as those of Slack and Breed.

It seemed desirable to make further tests of the method, and if possible, under practical conditions, such as working in connection with the routine examination of a city milk supply. Through the kindness of Dr. M. J. Rosenau of the Department of Preventive Medicine and Hygiene of the Harvard Medical School, arrangements were made for me to carry on a series of tests in the laboratories of the Boston Board of Health. The work has been made possible further by the courtesy of Dr. F. H. Slack, director of the laboratories, and Dr. W. M. Campbell, who conducts the bacteriological work on milk.

In this laboratory all samples of milk collected by the milk

TABLE 1
The number of bacteria per cubic centimeters of milk obtained by different methods

DATE	NO.	KIND OF MILK	MICROSCOPICAL ESTIMATE (SLACK)	STANDARD PLATES			LITTLE PLATES (FROST)		RATIO
				Routine test		Duplicate (Frost)	Bacteria per cubic centimeter		
				Number of colonies	Bacteria per cubic centimeter				
I. March 15, 1916	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
	(1)	Pasteurized	-	14-10	120,000			52,000	1:0.43
	(2)	Pasteurized	-	7-10	85,000			28,600	1:0.34
	(3)	Raw	+	253-228	2,500,000			1,560,000	1:0.62
	(4)	Pasteurized	-	23-29	200,000			197,600	1:0.98
	(5)	Pasteurized	-	9-17	130,000			31,200	1:0.24
	(6)	Pasteurized	-	13-15	140,000			54,000	1:0.38
	(7)	Pasteurized	-	24-25	245,000			218,400	1:0.88
	(8)	Pasteurized	-	116-155	1,400,000			1,040,000	1:0.74
	(9)	Pasteurized	++	70-31	500,000			1,560,000	1:3.1
	(10)	Pasteurized	+	237-204	2,000,000			1,690,000	1:0.84
	(11)	Pasteurized	+	223-193	2,000,000			854,000	1:0.43
II. March 16, 1916	(12)	Pasteurized	+						
	(1)	Pasteurized	-	164-139	135,000			59,150	1:0.43
	(2)	Pasteurized	+	29-sp.	290,000			96,200	1:0.33
	(3)	Pasteurized	-	63-79	710,000			2,080,000	1:2.90
	(4)	Pasteurized	-	24-22	230,000			187,200	1:0.81
	(5)	Pasteurized	-	20-22	210,000			65,000	1:0.31
	(6)	Pasteurized	-	136-128	132,000			13,000	1:0.10
	(7)	Pasteurized	-	62-76	690,000			1,040,000	1:1.50
	(8)	Pasteurized	-	54-39	460,000			130,000	1:0.28
	(9)	Pasteurized	+	29-26	270,000			640,000	1:2.40
	(10)	Pasteurized	-	98-102	100,000			85,800	1:0.85
	(11)	Pasteurized	+	8-6	7,000,000			1,560,000	1:0.22
(12)	Pasteurized	+	9-sp.	1,900,000			780,000	1:0.41	

NOTE — — = microscopic estimate below 500,000. + = estimate of 500,000 and over. Sp. = spreaders. Column 5 gives counts on duplicate plates.

III. March 17, 1916	(1) 24	Raw	-	Spreaders			182,000	
	(2) 25	Raw	-	Spreaders			132,000	
	(3) 26	Pasteurized	-	12-18	150,000		49,400	1:0.33
	(4) 27	Pasteurized?	+	68-74	700,000		1,040,000	1:1.46
	(5) 28	Pasteurized	-	20-10	150,000		7,800	1:0.05
	(6) 29	Pasteurized	-					
	(8) 30	Pasteurized	-	25-15	200,000		200,000	1:1
	(9) 31	Pasteurized	-	10-4	70,000		78,000	1:1.11
	(10) 32	Pasteurized?	-	4-16	100,000		260,000	1:2.60
	(11) 33	Pasteurized	-	34-48	500,000		936,000	1:1.87
	(12) 34	Pasteurized	-	12-4	80,000		78,000	1:0.97
IV. March 18, 1916	(1) 35	Raw	-	Spreaders			41,600	
	(2) 36	Raw	-	Spreaders			62,400	
	(3) 37	Raw	-	Spreaders			44,200	
	(4) 38	Raw	-	Spreaders			18,200	
	(5) 39	Pasteurized	-	8-0	40,000		3,120,000	1:78
	(6) 40	Pasteurized?	+	178-184	1,800,000		1,716,000	1:0.95
	(7) 41	Pasteurized	-	6-18	120,000		640,000	1:5.33
	(8) 42	Pasteurized	-	7-sp.	70,000		210,000	1:3.
	(9) 43	Pasteurized	-	10-18	140,000		1,040,000	1:7.43
	(10) 44	Pasteurized	+	133-127	1,200,000		13,780,000	1:11.50
	(11) 45	Raw	-	Spreaders			338,000	
			-	Spreaders			200,000	
V. March 20, 1916	(1) 46	Pasteurized	-	Spreaders			10,400	
	(2) 47	Pasteurized	-	Spreaders			18,200	
	(3) 48	Pasteurized	-	Spreaders			117,000	
	(4) 49	Pasteurized	-	Spreaders			832,000	
	(5) 50	Pasteurized	-	Spreaders			182,000	
	(6) 51	Pasteurized	-	Spreaders			8,800	
	(7) 52	Pasteurized	-	Spreaders				
	(8) 53	Pasteurized	-	6-2	40,000		1,157,000	1:29
	(9) 54	Pasteurized	+	64-80	700,000		1,339,000	1:1.91
	(10) 55		+	Spreaders				
	(11) 56		+	338-363	3,500,000		1,300,000	1:0.37
	(12) 57		-	8-6	70,000		43,900	1:0.62

TABLE 1—Continued.

DATE	NO.	KIND OF MILK	MICROSCOPICAL ESTIMATE (SLICK)	STANDARD PLATES			LITTLE PLATES (FROST)		RATIO	
				Routine test		Duplicate (Frost)	Bacteria per cubic centi-meter			
				Number of colonies	Bacteria per cubic centi-meter					
								Number of colonies		Bacteria per cubic centi-meter
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	
	(1) 58	Raw	—	13-14	120,000	3-2	30,000	33,800	1:0.25:0.28	
	(2) 59	Raw	—	0-0	—10,000	1-2	10,000	5,200	1:1:0.52	
	(3) 60	Raw	—	10-6	80,000	11-12	110,000	20,800	1:1.25:0.25	
	(4) 61	Raw	—	7-5	60,000	10-0	50,000	26,000	1:80:0.45	
	(5) 62	Raw	—	11-9	90,000	sp-5	50,000	39,000	1:0.55:0.43	
	(6) 63	Raw	—	0-0	—10,000	6-sp	60,000	7,800	1:6:0.78	
	(7) 64	Raw	—	1-3	20,000	1-2	10,000	7,800	1:0.50:0.39	
	(8) 65	Raw	—	0-0	—10,000	2-sp	20,000	3,250	1:2:0.32	
	(9) 66	Raw	—	0-0	—10,000	1-4	25,000	ap.13,000	1:2.5:1.30	
	(10) 67	Raw	—	5-3	40,000	5-2	35,000	11,830	1:0.9:0.30	
	(11) 68	Raw	—	5-1	30,000	2-2	20,000	27,400	1:0.66:0.90	
	(12) 69	Raw	—	0-0	—10,000	13-3	80,000	22,750	1:8:2.27	
, VI. March 21, 1916	(1) 70	Pasteurized	—	6-2	40,000	sp-5	50,000	26,000	1:1.25:0.65	
	(2) 71	Pasteurized	—	10-16	130,000	15-14	150,000	114,000	1:1.15:0.80	
	(3) 72	Pasteurized	—	37-41	400,000	36-sp	360,000	520,000	1:0.90:1.30	
	(4) 73	Pasteurized	—	10-8	90,000	—5-7	50,000	41,600	1:0.55:0.45	
	(5) 74	Pasteurized	—	3-15	90,000	37-28	320,000	267,000	1:3.55:3.00	
	(6) 75	Pasteurized	—	14-16	150,000	10-13	120,000	197,000	1:0.80:1.31	
	(7) 76	Pasteurized	—	25-3	140,000	22-sp	220,000	44,200	1:1.57:0.31	
	(8) 77	Pasteurized	—	sp-3	30,000	3-3	30,000	15,000	1:1:0.50	
	(9) 78	Pasteurized	—	11-5	80,000	9-10	100,000	35,750	1:1.25:0.44	
	(10) 79	Pasteurized	—	48-53	500,000	160-120	1,400,000	1,222,000	1:2.80:2.44	
		(11) 80	Pasteurized	—	29-31	300,000	24-20	200,000	299,000	1:0.66:1.00
	VII. March 22, 1916									

VIII. March 23, 1916		(1)	81	Raw	+	246-257	2,500,000	sp-340	3,400,000	1,500,000	1:1.36:0.60
		(2)	82	Pasteurized	-	10-12	110,000	1-2	10,000	260,000	1:0.09:2.30
		(3)	83	Pasteurized	-	37-43	400,000	27-sp	270,000	520,000	1:0.67:1.30
		(4)	84	Pasteurized	-	27-24	250,000	30-sp	300,000	20,000	
		(5)	85	Pasteurized	+	49-56	500,000	sp-8-8	80,000	1,000,000	1:0.14:2
		(6)	86	Pasteurized	-	11-sp	110,000	10-sp	100,000	104,000	1:0.90:0.95
		(7)	87	Pasteurized	-	10-2	60,000	11-sp	110,000	20,000	
		(8)	88	Pasteurized	-			10-11	100,000	100,000	1:1
		(9)	89	Pasteurized	-	10-20	150,000	11-25	180,000	30,000	
		(10)	90	Pasteurized	-	19-23	200,000	10-10	100,000	36,800	1:0.50:0.18
		(11)	91	Pasteurized	-	39-sp	400,000	54-45	500,000	653,000	1:1.25:1.63
		(12)	92	Cream	+	0-0		sp-0		no growth	
IX. March 24, 1916		(1)	93	Raw	+	1-0	60,000	1-0	10,000	26,000	1:0.16:0.43
		(2)	94	Raw	+	6-sp	60,000	0-3	15,000	12,000	1:0.25:0.20
		(3)	95	Raw	+	6-sp	60,000	sp-2	10,000	13,000	1:0.16:0.21
		(4)	96	Raw	+	sp-mold		33-36	350,000	91,000	
		(5)	97	Raw	+	0-0	-10,000	4-1	25,000	30,000	1:2.50:3.00
		(6)	98	Raw	+	74	750,000	40-20	300,000	300,000	1:0.40:0.40
		(7)	99	Raw	+	3-4	40,000	6-3	45,000	104,000	1:1.12:2.60
		(8)	100	Raw	+	8-sp	70,000	sp0-sp5	70,000	73,000	1:1.00:1.05
		(9)	101	Raw	+	0-0	-10,000	0-0sp	-10,000	very few	
		(10)	102	Raw	+	400-800	6,000,000	368-268	3,120,000	1,040,000	1:0.48:0.16
X. March 27, 1916		(21)	103	Pasteurized	-	184-sp	1,800,000	sp-sp		+1,000,000	
		(3)	104	Pasteurized	-	146-sp	1,460,000	520-sp	520,000	5,200,000	1:0.35:3.5
		(9)	105	Pasteurized	-	24-sp	250,000	9-4	650,000	26,000	1:2.60:0.1
		(20)	106	Pasteurized	+	24	24,000,000	sp-sp		2,600,000	
		(25)	107	Pasteurized	-	sp	500,000	sp-sp		3,000,000	
		(27)	108	Pasteurized	-	19	190,000	sp-sp			
		(28)	109		-	24	250,000	sp-sp		1,560,000	
		(29)	110		-	67	650,000	sp-sp		1,830,000	
		(30)	111		-	88	900,000	230-sp	2,300,000	3,120,000	1:2.5:3.4

inspector and sent into the laboratory are examined microscopically by the Slack method, and rated as above or below the legal limit of 500,000 bacteria per cubic centimeter. Those samples rated as likely to show more than the permissible number of bacteria are plated out, as also are all samples of pasteurized milk. The presence of streptococci and pus is noted. The microscopical estimate is used further as a guide to the dilution needed in plating. Ordinarily duplicate plates of only one dilution are made. This is usually 1:10,000.

At the time the routine plates were poured I made little plates. From sample 59 to sample 111 (table 1) I also made standard plate cultures.

The medium used for the ordinary plates was made according to the standard methods. It had a reaction of +1.5 and contained one per cent of agar. Ten cubic centimeters were used for each plate. The Petri dishes had clay tops. The temperature of incubation was 37.5°, and the time forty-eight hours. The air of the incubator was saturated with moisture. The counting was done either with the naked eye or with the aid of a reading glass.

The little plates were made in the manner previously described (Frost, 1916 a) and incubated five to seven hours. They were then dried, stained and counted. The same agar was used as that supplied the laboratory. It would have been better, no doubt, to have had a medium containing more agar, since some difficulty was experienced from spreaders, and in highly contaminated milks the little colonies were not so well individualized as would have been the case with a stiffer agar.

The results obtained from these comparative tests are given in table 1. The kind of milk is indicated, i.e., whether raw or pasteurized. The microscopical estimate by the Slack method; the plate counts obtained in the routine analyses, together with the number of colonies actually seen on the plates; the plate counts obtained by the writer; the results obtained from the use of the little plates; and the ratio which the results obtained by the different methods of analysis bear to each other, are indicated in the different columns of the table.

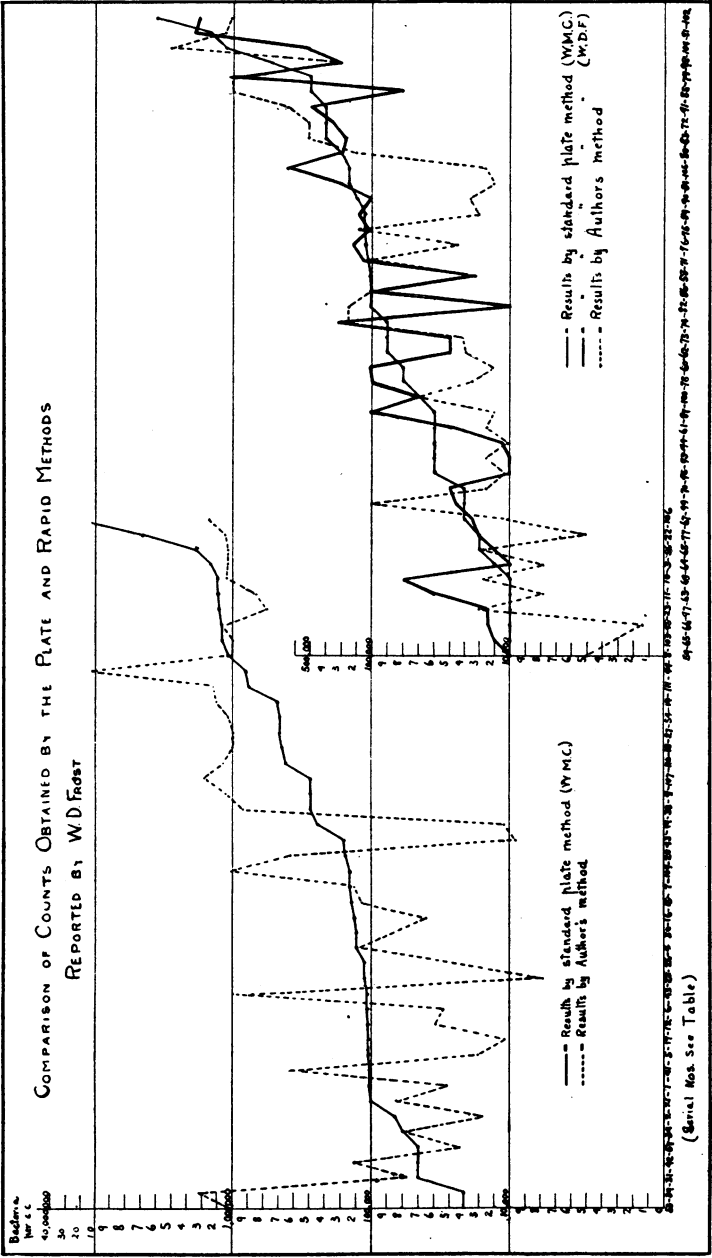


CHART 1

All of the analyses made during the time of the comparative tests are included in the table, although it is apparent that the data are too unsatisfactory in a number of cases to be of much value; yet for the sake of completeness all are given.

The general results are shown in chart 1.

The milks used were both raw and pasteurized, and varied in bacterial content from very good to highly contaminated milks.

One of the first things to note is the fact that because of spreaders on both plates (which unfortunately were unusually prevalent in the laboratory at the time of these tests), no counts could be obtained in 16 out of the 111 tests, and in 16 other samples one of the duplicate plates was spoiled by spreaders.

While spreaders occurred on the little plates under exactly the same conditions, their presence did not materially interfere with a count of the plates, with the possible exception of one. It would seem then that the little plates could be relied upon to give countable colonies with more certainty than the standard plates.

The second thing to notice is that there is considerable variation between the counts on the duplicate plates of the routine series. In ten samples the difference amounted to over 50 per cent (5, 9, 26, 28, 30, 43, 71, 78, 89, and 102) and in eleven other samples it was over 100 per cent (31, 32, 34, 39, 41, 64, 68, 70, 74, 76, and 87).

Again, the number of colonies on the plates in about 40 cases was below 20, which would seem to be the smallest number of colonies that can be relied upon as giving anything like a satisfactory degree of accuracy.

Excluding those samples in which one-half or more of the plates were rendered useless because of spreaders, and also those samples in which both plates had less than twenty colonies, it will be seen that only 28 of the 111 counts can be considered as thoroughly reliable in indicating the exact number of bacteria in the sample when the standard plate method was used.

The criticisms here made of the routine plates would apply with equal force to the duplicate Petri dish cultures made by myself. Of 53 only 10 were entirely satisfactory in that both

plates were free from spreaders and had a sufficient number of colonies present to be thoroughly reliable.

The agreement between the two series of standard plates varies from a ratio of 1:0.09 to 1:8, with an average ratio of 1:1.42. In other words, the plates that I made from one sample contained slightly less than one-tenth as many colonies (9 per cent) as the routine plates, and in another instance my count was 700 per cent higher than the routine, while my average figures were 42 per cent higher than those of Dr. Campbell.

The little plates, on the other hand, were all countable. Some of them were too thickly seeded, and others were overrun to some extent with spreaders, but neither of these things prevented the counting of the plates. It might have been expected, for several reasons, that the counts obtained by this method would not closely approximate the count obtained by the standard plate method, the chief of which is that the medium is somewhat different from the standard in that it is half milk. Again, the short period of incubation might not be sufficient for the development of colonies from some of the slowly-growing bacteria; and finally, crowding might be sufficient to inhibit the growth of some colonies. In spite of these differences and possible handicaps, however, the results obtained in this competitive series show a reasonable agreement.

Compared with the routine standard plates (leaving out of account three of the samples [39, 44 and 53] which are evidently not comparable), the ratio varied from 1:0.05 to 1:7.43, with an average of 1:0.96. This appears to be a better showing than was obtained in duplicate runs by the standard plate method alone where the average variation was 1:1.42. It must be pointed out, however, that this comparison gives a false impression, since the counts obtained by the little plates are on the whole somewhat lower than those obtained by the standard plate method. For example, if we compare the results I obtained by the standard plate method with the routine plates made by Dr. Campbell, we will see that my count fell below the routine count nineteen times, while it was higher in 14 samples. That is, the variation occurred either up or down with about

equal frequency. On the other hand, comparing the results obtained from the little plates with the results from the routine plates, it is found that in 48 samples the count on the little plates fell below the count obtained by the standard plate method, and above in 26 samples. In other words, the count on the little plates was lower than that on the standard plates in two cases, as against one above.

There are marked differences between the results obtained by the two methods in several samples. Some figures on the little plates are very much lower than those obtained by the standard plate method. This might be accounted for by the supposition that colonies did not have time to develop in the short period of incubation, but in some of these cases, at least, a long period of incubation had little effect in raising the count. What seems more likely in these instances is that we are dealing with samples of milks in which the bacteria have a tendency to form groups

Tests comparing the plating of 120 cc. of whole milk with the ordinary dilution methods. (By W. M. Campbell)*

1/20 cc. WHOLE MILK		DILUTION 1:20		DILUTION 1:100	
Number of colonies	Count	Number of colonies	Count	Number of colonies	Count
712	14,240	1,109	22,180	401	40,100
688	13,760	1,042	20,840	365	36,500
181	3,620	257	5,140	98	9,800
173	3,460	229	4,580	87	8,700
660	13,200	870	17,400	220	22,000
720	14,400	680	13,600	200	20,000
185	3,700	415	8,300	141	14,100
616	12,320	1,030	20,600	331	33,100
202	4,040	210	4,200	49	4,900
200	4,000	237	4,740	128	12,800
232	4,640	129	2,580	71	7,100
340	6,800			224	22,400
449	8,980			217	21,700
128	2,560	200	4,000		
110	2,200	240	4,800		
118	2,360	128	2,560		
96	1,920	140	2,800		

* American Journal Public Hygiene, 17, p. 359.

that are not easily shaken apart except as they are diluted with water. This fact has been brought out especially well by a series of tests given in the Reports of the Boston Board of Health (1907).

This grouping of the bacteria no doubt occurs to some extent in all milks, but appears to be more noticeable in the better grades of milk.

The little plates occasionally give much higher counts than the standard plate method; see, for example, nos. 39, 44, and 53 (table 1). In these samples the little plates showed a large number of small colonies of the lactic acid type, and it is quite conceivable that the count obtained by the little plates is more accurate than that obtained by the standard plate method.

TABLE 2
Number of bacteria in plain and lactose agars—from Sherman 1916

SAMPLE NUMBER*	NUMBER OF BACTERIA PER CUBIC CENTIMETER		
	Plain agar	Lactose agar	Ratio
1	817	1,017	1:1.25
2	1,340	2,070	1:1.55
3	1,630	2,000	1:1.23
4	3,230	3,530	1:1.09
(3)	6,000	69,000	1:10.50
(2)	6,500	42,500	1:6.54
5	7,330	7,330	1:1
(1)	7,600	25,300	1:3.33
6	8,000	11,300	1:1.41
7	8,900	12,100	1:1.36
8	9,030	8,970	1:0.99
9	11,800	11,170	1:0.95
(4)	18,100	43,600	1:2.41
(5)	23,800	58,000	1:2.44
(6)	72,000	177,000	1:2.46
10	186,000	610,000	1:3.28
11	192,000	278,000	1:1.45
12	260,000	361,000	1:1.39
13	369,000	463,000	1:1.26
14	53,700,000	109,000,000	1:2.03

* The numbers of the samples are the same as those used by Sherman in his table 1. The numbers in parenthesis are from his table 2. The last four analyses (nos. 15-18) are not given, because of the difficulty of charting such high numbers. See chart 2.

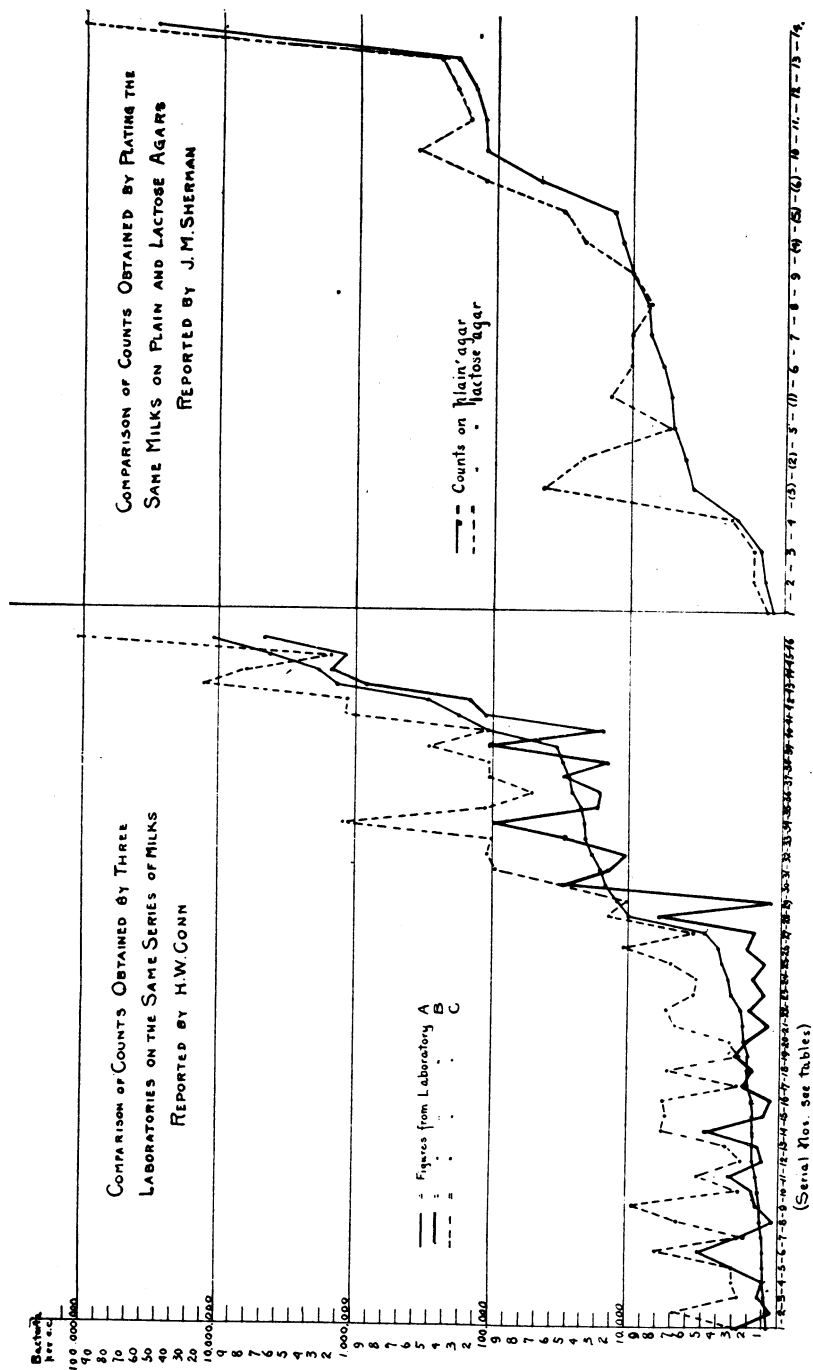


CHART 2

Sherman (1916) has recently shown that sugar agar gives higher counts than plain agar on the same milks. I have combined his tables 1 and 2 in my table 2. These figures are represented graphically in chart 2.

The large amount of milk in the little plates makes this a sugar medium, and it is possible that the discrepancies under discussion can be at least partially explained on this basis.

As far as the accuracy of the little plate method is concerned, when judged by the standard plate method, this study would seem to indicate that on the average the results are in fairly close agreement, with the probability that in about half of the samples the count will be somewhat low, and that other samples will show a much higher count than that obtained in the usual way.

It is interesting, in this connection, to compare the results obtained by different workers using the standard plate method in analyzing milk, and for this purpose I have brought together some of the results obtained by Conn (1915), and incorporated them in table 3. The figures were obtained by taking alternate numbers in Conn's table 2 and arranging them in an ascending scale. The column headed "S. Method and S. Media" was taken, but any other would apparently have given similar variations. The same figures are represented in chart 2.

TABLE 3

Data from "Standards for Determining the Purity of Milk". H. W. Conn. Table 3, p. 2366, Public Health Reports, August 13, 1915. Averages obtained by laboratories A. B. and C. Rearranged in one series using alternate figures.

RESULTS OBTAINED FROM THE SAME MILKS BY			RATIO
Laboratory A	Laboratory B	Laboratory C	
760	2,960	2,725	1:3.9:3.5
800	610	6,900	1:0.7:8.6
950	1,350	2,780	1:1.4:2.9
1,000	1,010	3,000	1:1.0:3.0
1,000	3,100	3,000	1:3.1:3.0
1,010	5,330	8,130	1:5.2:8.1
1,150	2,770	2,250	1:2.4:2.0
1,200	500	6,700	1:0.4:5.6
1,250	1,586	9,650	1:1.3:7.7
1,340	1,840	2,760	1:1.3:2.0

TABLE 3—Continued

RESULTS OBTAINED FROM THE SAME MILKS BY			RATIO
Laboratory A	Laboratory B	Laboratory C	
1,500	3,310	5,381	1:2.2:3.6
1,810	1,160	2,500	1:0.6:1.3
1,830	1,530	3,500	1:0.8:1.9
1,830	5,500	7,800	1:3.0:4.2
1,840	1,100	7,450	1:0.6:4.0
1,850	700	7,710	1:0.3:4.1
2,050	2,560	2,880	1:1.2:1.4
2,100	1,975	7,375	1:0.9:3.5
2,130	2,960	2,725	1:1.3:1.2
2,440	2,320	3,560	1:0.9:1.4
2,660	900	6,930	1:0.3:2.6
2,700	2,175	7,580	1:0.8:2.8
3,325	1,200	5,750	1:0.3:1.7
3,450	2,950	5,510	1:0.7:1.6
3,900	2,100	7,210	1:0.5:1.8
4,100	2,230	14,300	1:0.5:3.5
5,050	1,975	5,750	1:0.4:1.1
11,560	8,000	24,000	1:0.7:2.1
17,200	760	12,300	1:0.04:0.6
25,100	57,000	55,000	1:2.2:2.1
29,000	22,500	99,000	1:0.7:3.4
35,000	13,300	138,000	1:0.3:4.0
39,200	53,000	113,000	1:1.3:3.0
40,000	198,000	1,730,000	1:4.9:43.0
42,300	30,500	145,000	1:0.7:3.4
49,000	29,000	75,000	1:0.5:1.5
52,600	55,000	121,000	1:1.4:2.3
54,600	24,500	124,000	1:0.4:2.2
59,100	135,000	523,000	1:2.2:8.8
130,000	27,400	146,000	1:0.2:1.1
337,000	158,000	1,700,000	1:0.3:5.0
533,000	244,000	1,600,000	1:0.4:3.0
2,350,000	930,000	22,000,000	1:0.4:9.0
3,500,000	2,700,000	8,190,000	1:0.8:2.4
6,720,000	1,600,000	2,700,000	1:0.2:0.4
14,600,000	7,000,000	164,000,000	1:0.5:11.2

The variations, it will be noticed, are similar to those found in comparing my method with the standard plate method, and I believe I am warranted in saying that variations very similar in magnitude to those obtained by using the standard plate method and the little plate method may occur when two or more workers analyze milk by the standard plate method alone.

In considering the accuracy of this method, one of the things that must be determined is the uniformity of the distribution of the colonies on the little plates. Experience in these tests is that the distribution of colonies in the various fields in any little plate is quite uniform. To show this, the counts of twenty fields are given here by the mechanical selection of these fields on ten different plates.

NO. 1 LOW	NO. 5 LOW	NO. 10 OIL IM.	NO. 15 OIL IM.	NO. 20 LOW	NO. 30 LOW	NO. 40 OIL IM.	NO. 50 LOW	NO. 60 LOW	NO. 70 LOW
13	10	8	13	42	62	8	62	0	10
7	9	11	13	53	50	7	62	1	6
6	10	10	12	41	55	11	58	3	12
16	9	6	15	40	57	12	58	2	7
10	8	3	8	34	68	17	62	2	7
10	10	4	8	24	54	19	66	2	8
7	11	3	8	24	47	20	62	0	8
17	13	10	5	26	44	9	46	2	6
16	11	9	5	22	50	9	43	2	8
14	14	11	6	27	58	17	38	3	13
18	13	6	6	25	39	11	43	4	11
11	21	4	6	45	40	13	34	4	7
10	15	3	3	35	50	13	57	1	9
15	9	7	9	29	43	13	53	0	12
16	14	9	5	33	54	13	59	1	11
10	14	7	8	26	60	10	56	4	9
11	8	6	11	32	50	11	55	3	6
6	13	6	12	25	39	14	70	2	4
10	14	4	8	22	53	9	68	2	9
10	19	3	6	35	47	12	68	1	12
20)233	20)245	20)130	20)167	20)640	20)1020	20)248	20)1120	20)39	20)175
12	12	6.5	8	32	51	12	56	2	9

Another matter of importance is counting at different magnifications. In a former communication (1916 b) some emphasis

was laid on the discrepancies obtained with different magnifications used for counting the little plates. The data presented were for counts where the period of incubation was only four or five hours. If the period of incubation is seven or eight hours there seems to be little difference, and that magnification may be used which will give the most satisfactory number to count.

In comparing the two methods other considerations need brief mention.

First, the method is rapid, requiring not over seven or eight hours.

Second, the little plate method is reliable in that it is not likely to fail entirely because of spreaders, too great a dilution, etc.

Third, it permits a study of a relatively large amount of the milk, usually 1/20th of a cubic centimeter.

Fourth, it furnishes a permanent record of the bacterial content of the milk.

Fifth, it requires less material in the way of glassware and media. Microscopical slides, sterilized in the flame just before use, are substituted for Petri dishes, and the amount of medium is not over 1/20th and may be as little as 1/200th part of that used by the standard plate method.

Sixth, it is less time-consuming to make the little plates than it is to make the standard plates, and the only previous preparation necessary is the sterilization of pipettes and test tubes for mixing the samples. The counting of the little plates requires more time if 20 fields are carefully gone over. It seems probable, however, that the extensive counting may not be required in routine work. If the little plate is gone over quickly to gain an idea of the distribution of the colonies, the counting of four or five representative fields will be sufficient. Tests of this kind made during the progress of this work gave very satisfactory results. By varying the magnification, fields with comparatively few colonies in them can always be obtained.

The method may seem complicated to some, but in reality it is not, and if one will take the time to master the principles involved, the method is much simpler than the standard plate method.

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