A Comparison of the Roll-Tube and Standard Plate Methods of Making Bacterial Counts of Milk*

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THE use in Europe of a roll-tube technique for the quantitative and qualitative analysis of milk and other products was observed by one † of the authors. Since this technique appeared to possess certain advantages over the standard plate method, a study was undertaken for the purpose of comparing the results from the two methods.

The history of the development of the roll-tube method of making bacterial counts as outlined by Damm (2) dates back to 1886. In that year, Esmarch described a method of making bacterial counts by introducing the test material into gelatin contained in an ordinary test tube, closing the tube with a cotton stopper covered by a rubber cap, and rotating it by hand under a stream of cold water until the gelatin congealed on the walls. This method had the disadvantage that some of the gelatin would adhere to and penetrate the cotton stopper, and it was difficult to produce a uniform layer of gelatin on the walls of the tube. Subsequent improvements were made in the method, such as introducing mechanical rotating devices. However, many of the handicaps of the procedure were not overcome until a motor-driven roll-tube apparatus, as described by Mundinger and Woeckel (6), was developed. Using this apparatus and a Burri 0.001 ml. loop, Damm (2) obtained milk counts which were closely comparable with those obtained by the standard plate method. Gramm (3) reported on the practical application of this method for counting the bacteria in milk and Lerche (4) pointed out the usefulness of the apparatus and tubes for making surface cultures, such as are usually made on agar slants.

PROCEDURE

The equipment used was a six-tube apparatus made by Paul Funke & Company, with counting lens and tubes. The tubes have an over-all length of 15.3 cm. and an inside diameter of 1.9 cm. A constriction in the tube about 2–3 cm. from the top prevents the medium from wetting the cotton plug when the tube is rapidly rotating in a horizontal position. For making counts the tubes are filled with 7 ml. of an agar medium, containing preferably 2.0 percent of agar in order to give the desired consistency to the medium. The tubes are plugged and sterilized, and just before use are tempered in a water bath to 45° C., inoculated with the milk, and after careful mixing of the contents rotated at a speed (about 2,000 r.p.m.) sufficient to deposit the agar in a layer of

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uniform thickness against the inside wall where it congeals after a short time (about 3 minutes). The tubes are then incubated in a nearly horizontal position with the bottom of the tubes slanting slightly downward to carry down any small amount of moisture that may collect and which may cause the development of spreaders over the agar surface. Occasionally when the tubes were incubated in an absolutely horizontal position, free moisture in amounts sufficient to encourage the growth of spreaders was encountered. The use of a 2.0 percent agar in place of a 1.5 percent agar greatly decreased this difficulty. The gelation of the agar can be speeded up in warm weather by playing a fan upon the rolling tubes or by rolling them in a cool room. After incubating, the counting of the colonies may be facilitated by using a lens attached to a metal cylinder which is slipped over the agar roll-tube.

In comparing the bacterial counts on milk by the standard plate and the roll-tube methods, dilutions of 1:100 and 1:1000 were made using the standard procedure (1). The plates and tubes were prepared with a 2.0 percent tryptone-glucose-extract-milk-agar and incubated at 37° C. side by side in the same incubator. To facilitate the counting of colonies in the tubes, the tubes were marked off with lines and all colonies were counted.

Since the actual length of the agar surface in the tube was approximately 12.0 cm. and the inside diameter about 1.9 cm., the agar surface area, taking into consideration the thickness of the agar layer (about 0.11 cm.) was approximately that of a standard petri dish 9.0 cm. in diameter. Some additional tubes prepared locally had a useful length of 11.5 to 12.0 cm. and an inside diameter of 1.8, thus providing an agar surface area slightly less than that of a standard 9.0 cm. petri dish. Figures 1, 2, and 3 show the roll-tube apparatus, the position of incubating cultures, and the developed colonies and counting lens.

From 6 to 20 plate and roll-tube cultures were made from each of 14 samples of milk, making a total of 227 plate and 228 roll-tube cultures. In addition, plate and roll-tube cultures were made in duplicate from 43 different samples of milk. All inoculations for a given sample were made from one and the same dilution. In each comparison the average number of colonies fell within the range of 30 to 300 per roll-tube or plate as recommended by *Standard Methods for the Examination of Dairy Products* (1).

DISCUSSION OF RESULTS

In Table 1 are summarized the results of the 14 comparisons in which 6 to 20 plate and roll-tube cultures were made from each sample of milk. The average roll-tube count was lower for 11 of the milk samples and higher for 3. In 7 of the 14 comparisons the average roll-tube count was within 10 percent of the plate count, in 10 within 15 percent, in 11 within 20 percent, and in only one was the difference greater than 25 percent.

A statistical summary of the 14 comparisons, using the actual colony counts, is presented in Table 2. Considering the data as they actually were obtained, the variances and standard deviations were higher for the plate count in 11 of the comparisons, and the coefficients of variation were higher for the plate method in 9 comparisons. The mean variance, mean standard deviation, and mean coefficient of variation all were higher for the roll-tube method.

The general applicability of the roll-tube as compared to the plate method can be evaluated best by considering the pooled information from the 14 milk samples. First, it is of importance to test the significance of the differences between the general plate

TABLE 1

SUMMARY OF COMPARISONS OF SIX TO TWENTY REPLICATE PLATE AND ROLL-TUBE COLONY COUNTS OF FOURTEEN SAMPLES OF MILK

Milk No.	No. of Cultures		Colony Range		Average No. of Colonies		Ratio
	Plate	Tube	Plate	Tube	Plate	Tube	Tube/Plate
1	20	20	41–81	27–69	55.7	46.3	0.831
2	14	14	32–56	25–36	41.3	31.8	0.770
3	15	15	192–311	146–199	258.7	172.3	0.666
4	13	13	81–114	70–94	93.8	83.8	0.893
5	19	19	152–225	67–268	214.2	212.1	0.990
		(17)[1]		(181–268)[1]		(227.4)[1]	(1.062)[1]
6	6	6	111–156	104–140	129.2	119.5	0.925
7	19	19	30–65	26–54	49.0	41.3	0.853
8	16	16	70–117	72–106	99.3	93.4	0.941
9	19	20	14–59	37–61	37.3	45.6	1.223
	(16)[2]		(29–59)[2]		(41.4)[2]		(1.101)[2]
10	19	19	87–137	76–149	115.1	104.0	0.904
11	20	20	81–118	72–103	95.4	85.0	0.891
12	20	20	109–154	110–133	129.1	122.2	0.946
13	18	18	130–188	149–178	158.4	164.5	1.039
14	9	9	25–40	23–44	32.7	35.6	1.089
Total	227	228					

[1] After omitting two lowest counts.
[2] After omitting three lowest counts.

and roll-tube means; second, the interaction between milks and methods should be tested to determine if all milks react similarly to the two methods; and third, the difference between the plate and roll-tube variances should be tested for significance.

In pooling sums of squares to arrive at a generalized pooled variance, it is necessary that the data fulfill two conditions, namely: (1) the variances of different samples must be homogeneous, and (2) the means must be independent of their variances or standard deviations (7). The variances in Table 2 were highly heterogeneous for both methods as determined by Bartlett's chi-square test for homogeneity of variances (7). Furthermore, correlations between means and standard deviations were found to be positive and highly significant.

On further examinations of the data in Tables 1 and 2, it was noted that unusually large discrepancies in range, variance, and standard deviation between the two methods occurred in several of the milk samples. As shown in Table 1, the wide range in roll-tube counts of 67–268 for sample 5 was due to two extremely low counts which when omitted left a range of 181–268. When the three lowest counts for the plate method in sample 9 were omitted, the range was changed from 14–59 to 29–59. Other large discrepancies, particularly that in sample 3, could not be explained by occasional divergent counts.

In a study of the reliability of the plate method, using numerous parallel plate cultures, Malcolm (5) observed the occurrence of similar variations. As a result, he recommended the use of five parallel plates in the routine examination of milk for bacterial content.

It will be noted in Table 2 that the discrepancies between variances of the two methods in samples 5 and 9 were markedly diminished when the low counts were omitted. However, the mean variances of the two methods for all 14 samples now were reversed in magnitude. When in addition, sample 3 was omitted, the mean variances of

TABLE 2

STATISTICAL SUMMARY OF THE COMPARISON OF THE COLONY COUNTS FROM REPLICATE PLATE AND ROLL-TUBE CULTURES OF FOURTEEN SAMPLES OF MILK

Milk No.	Degrees of freedom (n–1)		Mean ($\bar{x}$)		Sum of squares (Sx^2)		Variance (V)		Standard deviation (s)		Coef. of variation (C.V.)	
	Plate	Tube	Plate	Tube	Plate	Tube	Plate	Tube	Plate	Tube	Plate	Tube
1	19	19	55.7	46.3	2,010.21	1,798.20	105.80	94.64	10.3	9.7	18.5	21.0
2	13	13	41.3	31.8	610.86	168.36	46.99	12.95	6.9	3.6	16.6	11.3
3	14	14	258.7	172.3	12,893.93	3,267.33	921.00	233.38	30.3	15.3	11.7	8.9
4	12	12	93.8	83.8	1,234.31	744.31	102.86	62.03	10.1	7.9	10.8	9.4
5	18	18 (16)[1]	214.2	212.1 (227.4)	9,650.53	46,388.95 (8,131.90)	536.14	2,577.16 (508.24)	23.2	50.8 (22.6)	10.8	23.9 (9.9)
6	5	5	129.2	119.5	1,403.83	891.50	286.17	178.30	16.9	13.4	13.1	11.2
7	18	18	49.0	41.3	1,334.00	940.11	74.11	52.23	8.6	7.2	17.6	17.6
8	15	15	99.3	93.4	2,779.00	1,509.94	185.27	100.66	13.6	10.0	13.7	10.7
9	18 (15)	19	37.3 (41.4)	45.6	2,979.68 (1,211.94)	850.80	165.54 (80.80)	44.78	12.9 (9.0)	6.7	34.5 (21.7)	14.7
10	18	18	115.1	104.0	2,869.79	4,146.79	159.43	230.36	12.6	15.2	11.0	14.5
11	19	19	95.4	85.0	1,976.55	1,073.95	104.03	56.68	10.2	7.5	10.7	8.9
12	19	19	129.1	122.2	2,039.80	987.20	107.36	51.95	10.4	7.2	8.0	5.9
13	17	17	158.4	164.5	3,908.44	1,792.50	229.91	105.44	15.2	10.3	9.6	6.2
14	8	8	32.7	35.6	176.00	374.22	22.00	46.78	4.7	6.8	14.4	19.2
Total or weighted mean	213	214	108.7	98.2	45,893.92	64,933.79	215.46	303.43	14.7	17.4	13.5	17.7
Total or weighted mean [2]	(210)	(212)	(110.0)	(104.8)	(44,126.18)	(26,676.74)	(210.12)	(125.83)	(14.5)	(11.2)	(13.2)	(10.7)
Total or weighted mean [3]	(196)	(198)	(99.3)	(86.9)	(31,233.18)	(23,409.41)	(159.33)	(118.22)	(12.6)	(10.9)	(12.7)	(12.5)

[1] Numbers in parentheses represent revised data.
[2] Revised values substituted for roll-tube count of milk sample 5 and plate counts of milk sample 9.
[3] Milk sample 3 omitted and revised values substituted for roll-tube count of milk sample 5 and plate count of milk sample 9.

the two methods were more nearly alike. Nevertheless, the data still did not fulfill the conditions necessary for arriving at a generalized pooled variance.

Because the variances and standard deviations were roughly proportional to the means, the counts were transformed into logarithms. After the transformation the variances were still highly heterogeneous. Furthermore, the means and their standard deviations were not independent; however, in contrast to the results obtained from actual counts, the means and standard deviations now were negatively and significantly correlated.

When square roots of the counts were used, better results were obtained than by the use of the actual counts or logarithms. In Table 3 are shown the calculations from square roots corresponding to the calculations from actual colony counts in Table 2.

With all counts included, the chi-square values from tests for homogeneity were far beyond the one percent level of significance. Omitting the two lowest counts secured by the roll-tube method in sample 5 and the three lowest counts obtained by the plate method in sample 9, the chi-square values were only slightly beyond the one percent level.

Of greater significance still was the fact that the means were now independent of their standard deviations except for the difficulty presented by sample 3 in the plate method. When this sample was omitted for the plate method, the correlation approached zero and was not significant. Also, when it was omitted in applying the chi-square test for homogeneity, the resulting chi-square value was no longer significant for the plate method. The effect on the pooled variances and other values is shown by the numbers in parentheses in the last line of Table 3.

Three analyses of variance, one including the square roots of all the counts, one including revised roll-tube data for sample 5 and plate data for sample 9, and one using these revised data and in addition omitting the data for sample 3 entirely, are presented in Table 4.

In applying the F test (7) the difference between methods was found to be significant in the first and second analyses and highly significant in the third. In general, therefore, the counts using the roll-tube method were lower than the counts using the plate method.

The interaction between samples and methods was highly significant in the first and second analyses, and significant almost to the one percent level in the third. This indicates that there were differential responses among the different samples to the two methods. It will be pointed out later that the roll-tube counts tend to be lower than the plate counts, particularly when counts are high.

The question as to whether the variances of the two methods were similar or significantly different can be answered either by testing them for homogeneity or by applying the F test to the ratio of the variances. Both tests were applied to the three pairs of mean variances in the last three lines in Table 3. Using all the data, the difference between the variances shown in Table 3 was found to be significant, with a greater variance occurring in the roll-tube counts than in the plate counts. The reverse was true when the revised values for the roll-tube counts of sample 5 and the plate counts of sample 9 were substituted. If, in addition, sample 3 is omitted, the variances are not significantly different and the counts from the two methods may be said to be equally variable. It also should be pointed out that a test of significance of the interaction in Table 4 is not valid unless the plate and roll-tube variances are similar.

TABLE 3

STATISTICAL SUMMARY OF THE COMPARISON OF THE COLONY COUNTS FROM REPLICATE PLATE AND ROLL-TUBE CULTURES OF FOURTEEN SAMPLES OF MILK (COLONY COUNTS TRANSFORMED INTO SQUARE ROOTS)

Milk No.	Degrees of freedom (n–1)		Mean ($\bar{x}$)		Sum of squares (Sx^2)		Variance (V)		Standard deviation (s)		Coef. of variation (C.V.)	
	Plate	Tube	Plate	Tube	Plate	Tube	Plate	Tube	Plate	Tube	Plate	Tube
1	19	19	7.4	6.8	8.42	10.70	.4432	.5632	.67	.75	9.1	11.0
2	13	13	6.4	5.6	3.28	2.59	.2523	.1992	.50	.45	12.8	8.0
3	14	14	16.0	13.1	21.78	2.98	1.5557	.2129	1.25	.46	7.8	3.5
4	12	12	9.7	9.1	5.51	3.35	.4592	.2792	.68	.53	7.0	5.8
5	18	18	14.6	14.4	10.20	74.25	.5667	4.1250	.75	2.03	5.1	14.1
		(16)[1]		(15.1)		(6.93)		(.4331)		(.66)		(4.4)
6	5	5	11.3	10.9	4.33	4.14	.8660	.8280	.93	.91	8.2	8.3
7	18	18	7.0	6.4	5.59	8.04	.3106	.4467	.56	.67	8.0	10.5
8	15	15	9.9	9.6	11.91	6.97	.7940	.4647	.89	.68	9.0	7.1
9	18	19	6.0	6.7	24.00	2.10	1.3333	.1105	1.15	.33	19.2	4.9
	(15)		(6.4)		(7.64)		(.5093)		(.71)		(11.1)	
10	18	18	10.7	10.2	11.69	14.39	.6494	.7994	.81	.89	7.6	8.7
11	19	19	9.8	9.2	3.80	4.36	.2000	.2295	.45	.48	4.6	5.2
12	19	19	11.3	11.1	7.82	1.95	.4116	.1026	.64	.32	5.7	2.9
13	17	17	12.6	12.8	4.39	6.76	.2582	.3976	.51	.63	4.0	4.9
14	8	8	5.7	5.9	1.59	4.35	.1988	.4833	.45	.70	7.9	11.9
Total or mean	213	214	9.9	9.5	124.31	147.43	.5836	.6889	.76	.83	7.6	8.8
Total or mean [2]	(210)	(212)	(10.0)	(9.5)	(107.95)	(79.61)	(.5140)	(.3755)	(.72)	(.61)	(7.2)	(6.4)
Total or mean [3]	(196)	(198)	(9.6)	(9.2)	(86.17)	(76.63)	(.4396)	(.3870)	(.65)	(.62)	(6.8)	(6.7)

[1] Numbers in parentheses represent revised data.
[2] Revised values substituted for roll-tube count of milk sample 5 and plate count of milk sample 9.
[3] Milk sample 3 omitted and revised values substituted for roll-tube count of milk sample 5 and plate count of milk sample 9.

TABLE 4

ANALYSES OF VARIANCE OF THE SQUARE ROOTS OF THE MEAN BACTERIAL COLONY COUNTS FROM PLATE AND ROLL-TUBE CULTURES OF FOURTEEN SAMPLES OF MILK

Source of variation	*Before revision*			*After revision of roll-tube data for milk No. 5 and plate data for milk No. 9*			*After revision of roll-tube data for milk No. 5 and plate data for milk No. 9, and omission of data for milk No. 3*		
	Degrees of freedom	*Sum of squares*	*Mean square*	*Degrees of freedom*	*Sum of squares*	*Mean square*	*Degrees of freedom*	*Sum of squares*	*Mean square*
Between milks	13	3,756.73	288.98 **	13	3,737.17	287.47 **	13	2,989.93	249.16 **
Between methods	1	24.80	24.80 *	1	33.30	33.30 *	1	14.46	14.46 **
Interaction	13	67.10	5.16 **	13	55.71	4.29 **	13	10.61	.88 *
Within subclasses	427	271.74	.64	422	187.56	.44	394	162.80	.41
Total	454	4,120.37		449	4,013.74		419	3,177.80	

* Significant.
** Highly significant.

In a similar study involving six samples, with average colony numbers of from 183 to 337 per plate or roll-tube method, Damm (2) reported a coefficient of variation of 14.13 and 16.38 for the plate and roll-tube culture methods respectively.

The results of the 43 comparisons of duplicate counts made by the two methods are shown in Table 5. The roll-tube count was lower than the plate count in 27 comparisons and higher in 16. A statistical analysis of the data, using the square roots of the counts showed the difference between the roll-tube count and the plate count for the 43 samples to be highly significant. The t value of 3.21 surpassed the 1 percent level for 42 degrees of freedom. It appears, therefore, that the roll-tube method gives results commonly lower than the plate method.

Further examination of the data in Table 5 revealed that large discrepancies in the average counts between the two methods might be occurring more frequently in samples having high plate counts. By plotting the plate counts against differences between methods in a contingency table and applying the chi-square test for independence, it was found that such was the case. Since the surface area of the agar was slightly less in the roll-tube cultures than in the plate cultures, it is possible that the slightly lower roll-tube counts might have been due to greater crowding of colonies in the tube.

SUMMARY AND CONCLUSIONS

Two series of comparisons were made, using the standard plate method and the roll-tube culture method for determining the numbers of colonies developing from milk. One series consisted of making 6 to 20 replicate cultures by each method of 14 different samples of milk, whereas the second series consisted of making duplicate cultures by each method of 43 milk samples.

When the actual counts or their logarithms were used in the statistical analysis, the variances of different milk samples were found to be highly heterogeneous for both methods. Furthermore, the means and standard deviations were significantly correlated, positively for actual counts, and negatively for the logarithms.

Square roots of the colony counts yielded more satisfactory results. When analyzed by this technique, a significant difference was found to exist between the colony counts obtained by the two methods. In general the counts obtained by the use of the roll-tube method were lower than by the use of the standard plate method. This appeared to be true, particularly with milks of high colony counts and may possibly be due to the fact that the surface area of the agar of the roll-tube cultures was smaller than that of the plate cultures, thus increasing the crowding of the colonies. A comparison of the two methods using a larger tube, therefore would be of interest.

Variances in the two methods appeared to be about equal. Although the roll-tube method gave a slightly lower count than the plate method, the difference was not of sufficient magnitude to justify the rejection of the method.

The following advantages may be cited for the roll-tube method of making bacterial counts. The tubes are subject to less breakage, require less storage space, and are more easily handled than petri dishes. They may be filled with the correct amount of agar, sterilized, and kept on hand in a relatively small space in the refrigerator to be ready for instant use in the laboratory or carried about easily for use in the field, on receiving platforms, and in the plant. They may even be sent through the mail to inspectors and technicians in the field and to operators

TABLE 5

A COMPARISON OF DUPLICATE COUNTS BY THE PLATE AND ROLL-TUBE METHODS ON FORTY-THREE SAMPLES OF MILK

Sample No.	Average No. of Colonies		Square root		Ratio of counts Tube to plate
	Plate	Tube	Plate	Tube	
1	63.5	51.5	8.0	7.2	.811
2	149.0	116.0	12.0	10.8	.779
3	88.0	51.0	9.4	7.1	.580
4	209.0	171.0	14.5	13.1	.818
5	156.5	204.0	12.5	14.3	1.304
6	121.5	89.0	11.0	9.4	.733
7	62.0	34.5	7.9	5.9	.556
8	33.0	30.5	5.7	5.5	.924
9	140.5	47.5	11.8	6.9	.338
10	80.5	63.5	9.0	8.0	.789
11	36.5	43.0	6.0	6.6	1.178
12	50.5	60.5	7.1	7.8	1.198
13	50.5	46.0	7.1	6.8	.911
14	159.5	151.0	12.6	12.3	.947
15	225.0	230.5	15.0	15.2	1.024
16	41.5	52.5	6.4	7.3	1.265
17	64.0	71.5	8.0	8.4	1.117
18	101.0	120.0	10.0	11.0	1.188
19	63.5	57.0	8.0	7.5	.898
20	64.0	52.2	8.0	7.2	.820
21	100.0	51.5	10.0	7.2	.515
22	210.5	223.5	14.5	14.9	1.062
23	75.0	73.0	8.7	8.5	.973
24	134.0	74.0	11.6	8.6	.552
25	116.5	100.0	10.8	10.0	.858
26	82.5	48.0	9.1	6.9	.582
27	285.5	248.0	16.9	15.7	.869
28	240.0	74.5	15.5	8.6	.310
29	59.0	44.5	7.7	6.8	.754
30	70.5	56.5	8.4	7.5	.801
31	41.0	35.0	6.4	5.9	.854
32	113.0	121.0	10.6	11.0	1.071
33	150.0	145.5	12.2	12.1	.970
34	200.0	134.5	14.1	11.6	.673
35	273.0	273.5	16.5	16.5	1.002
36	112.0	115.5	10.6	10.7	1.031
37	61.5	62.0	7.8	7.9	1.008
38	78.0	81.5	8.8	9.0	1.045
39	41.0	56.5	6.4	7.5	1.378
40	35.0	37.0	5.9	6.1	1.057
41	40.5	48.0	6.4	6.9	1.185
42	36.5	34.5	6.0	5.9	.945
43	143.5	65.5	12.0	8.1	.456
Total	4,658.5	3,946.5	427.1	392.1	

Mean difference = .814
t = 3.21
Highly significant for 42 degrees of freedom.

of small plants and receiving stations who have limited laboratory facilities and who prefer not to make their own media. Further savings in the cost of making bacterial counts are possible by using a calibrated inoculating loop for which the tubes are especially adapted. This procedure eliminates the time, skill, and equipment required for making sterile dilution blanks, for cleaning and sterilizing pipettes, making the dilutions, and plating. Another factor which merits some slight consideration at this time, where agar supplies are limited, is the fact that the roll-tube method requires only about 7 ml. of culture medium compared with 10–15 ml. used in the petri dish. Less incubator space is needed, which may be of importance where large numbers of routine analyses are to be made. Also there is less danger of contamination of the tubes than of the plates, a fact which should tend to increase the dependability of the results. Finally, the incubated tubes may be carried around in a compact package for demonstrational use by the field man and others doing educational work. Aside from their use in making bacterial counts, the roll-tubes and apparatus may be used for making surface cultures by whirling the sterile agar in the tubes and after thorough solidification of the agar inoculating with a bacterial suspension and rewhirling the tubes. The tubes provide a larger surface than ordinary agar slants and also a means of uniform inoculation.

On the other hand, the roll-tube method of making bacterial counts requires a special motor-driven apparatus for rotating the tubes, which should be large enough to accommodate twelve or more tubes, so that no time will be lost in waiting for the agar to congeal in the whirling tubes. (Tubes may be removed or added while the apparatus is running.) Congealing is retarded at warm room temperatures, and under such conditions a fan may be necessary to cool the whirling tubes or the whirling may have to be done in a cool room. Because of the collection of small amounts of free moisture in the bottom portion of the tube, the incidence of spreading colonies is apt to be greater with roll-tube cultures than with petri dish cultures, unless care is taken that the tubes are incubated with their bottom ends slightly sloping downward. In the roll-tube method, it is desirable to use a somewhat firmer agar than is necessary in the plate method. Roll-tube cultures are somewhat more difficult to count than corresponding plate cultures, especially when the number of colonies approaches 300. Surface colonies are not as plainly seen in the roll-tube as in the petri dish.

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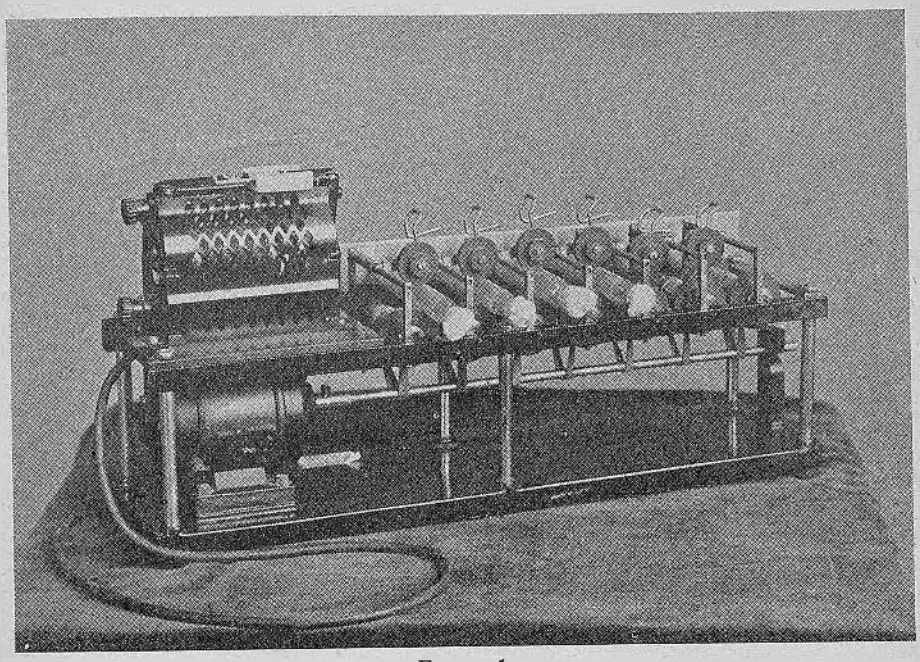

FIGURE 1
A six-tube roll-tube apparatus

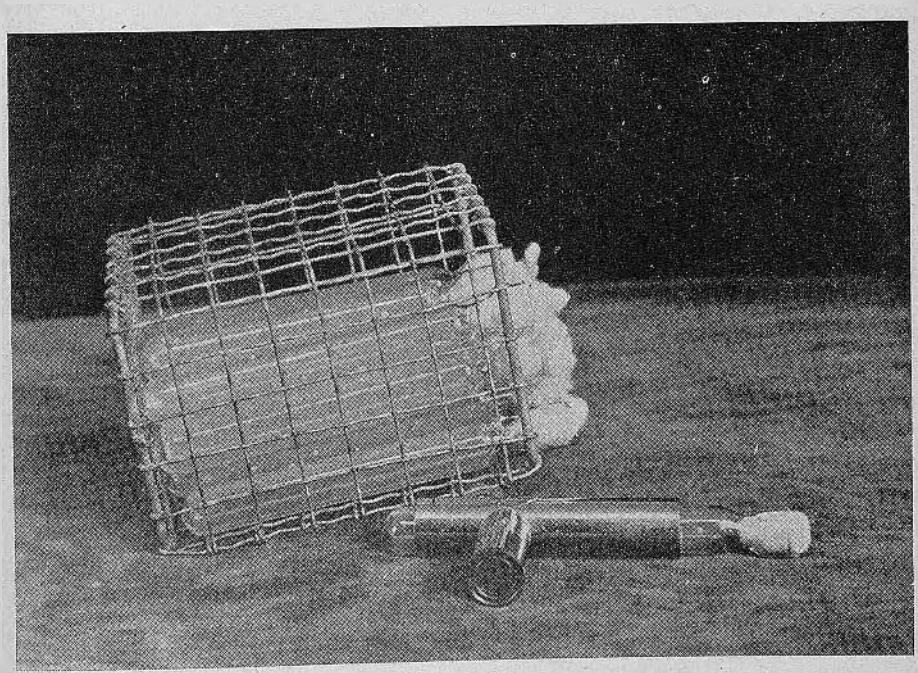

FIGURE 2
Position of incubating roll-tubes and counting lens in position over tube

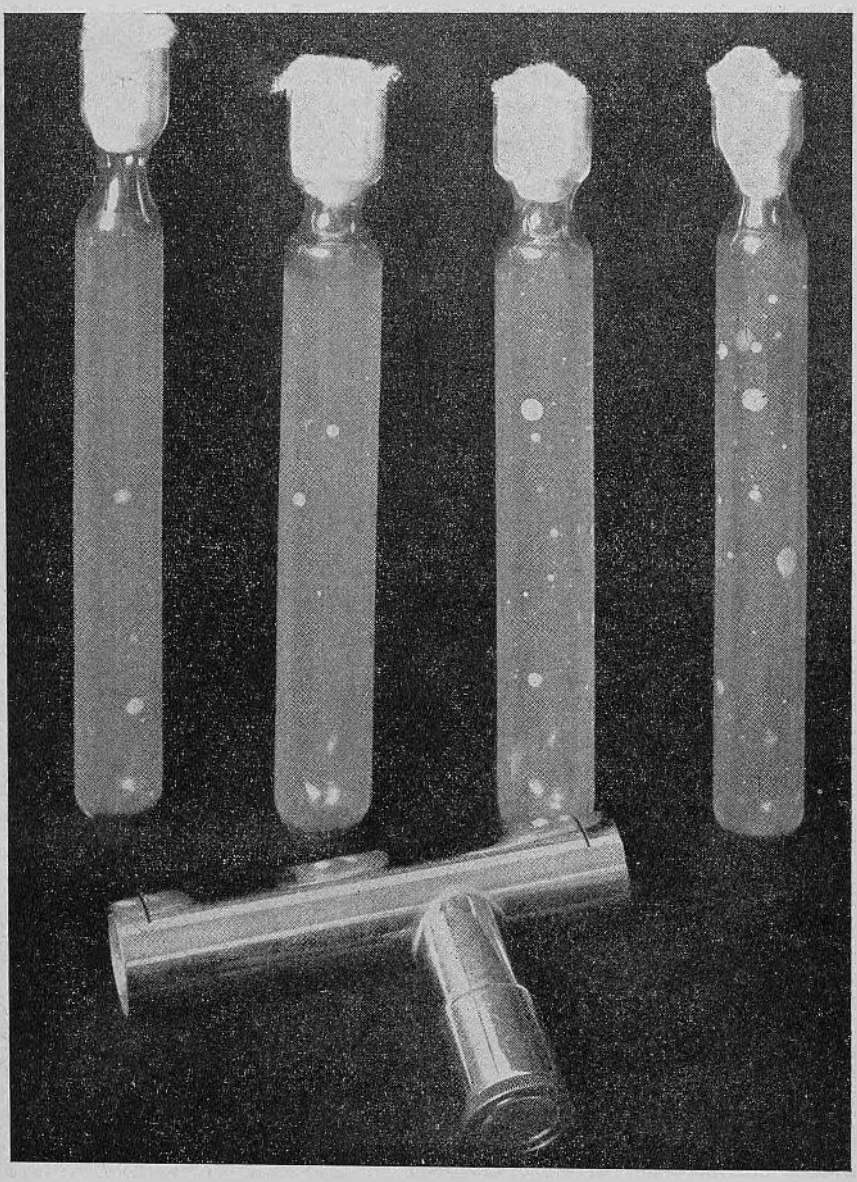

FIGURE 3
Roll-tube cultures showing colonies and counting lens