THE ADVANTAGES OF SKIM-MILK AGAR FOR THE DETERMINATION OF THE SANITARY QUALITY OF MARKET MILK

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Attention was called by Sherman (1) to the advantages in using an agar containing a fermentable carbohydrate in the bacteriological control of market milk. He presented data which showed that the colonies of bacteria normally present in milk were two to ten times larger on lactose agar than on plain agar. He made comparative bacterial counts on plain and lactose agar which showed an average increase, in favor of lactose agar, of 43 per cent on raw milk and 378 per cent on pasteurized milk. Sherman mentioned that "glucose is apparently just as efficient as is lactose, both as to number and size of colonies," and that "since glucose is cheaper it should perhaps be recommended as the standard for routine work." He also suggested that the amount of sugar could be reduced to 0.1 per cent "without impairing the value of the medium." Simmons (2), and others, have confirmed Sherman's findings.

Ayers and Mudge (3) suggested a milk powder agar for use in determining the numbers and kinds of bacteria in market milk and other dairy products. They found that milk powder agar gave from 3 to 75,000 per cent higher counts than those obtained on plain agar. The colonies were larger and consequently could be counted with greater ease and accuracy. The casein agar reported by Ayres (4) was found to be unsatisfactory unless incubation periods longer than two days were employed.

The modification of milk powder agar suggested by Zoller (5) was found by Norton and Seymour (6) to be a definite improvement. This modified milk powder agar gave higher counts than any other medium tested by these workers, but it was criticized because of the complicated procedure involved in its preparation.

EXPERIMENTAL

The results here reported include data obtained on approximately 760 samples of milk taken from the milk supply of 250 dealers located in various parts of the State of New York. These milk samples were taken three times during the year at periods representing winter, spring, and summer conditions. The samples of milk were plated in duplicate, in dilutions of

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1–100 and 1–1,000, on standard nutrient agar and on a milk agar having the following composition:

Fresh raw skim milk	2.0 per cent
Bacto peptone	0.5 per cent
Bacto beef extract	0.1 per cent
Glucose	0.1 per cent
Agar	1.5 per cent

This medium is a modification of one employed with good results by J. M. Sherman (unpublished data) almost twenty years ago, in which he used 0.2 per cent skim milk powder, 1 per cent peptone, and 1.5 per cent agar. In the medium here reported, skim milk was used instead of milk powder with the belief that it would be more uniform in consistency, freer from sediment, and would give uniformly high bacterial counts. Small amounts of beef extract and glucose were added on the assumption that they might improve the nutritive value of the medium, and support the luxuriant growth of certain types of bacteria which, in the absence of these constituents, might grow very poorly or not at all. A medium of the above composition is simple, easily made, and should cost as little or probably less than the present plain agar suggested by Standard Methods.

Ayers and Mudge (3) say "attempts to make a medium by using skimmed milk, with its casein dissolved, and adding peptone and beef extract were not successful." If much larger amounts of skim milk, or phosphate buffers, are added to the medium, precipitation will occur. The required amounts of glucose and skim milk may be added to the adjusted, autoclaved, and filtered nutrient medium just previous to its final sterilization. If the medium previous to the addition of milk is adjusted to pH 7.0, the reaction of the completed medium will be approximately pH 6.5. If thought advisable, the pH could be raised by the addition of sodium hydroxide.

Table 1 shows the general trend of the results obtained from the comparative counts made on the three sets of samples. These data are reported

TABLE 1

Average percentage increase in favor of skim-milk agar between counts made on standard agar and on skim-milk agar

KIND OF MILK	WINTER SAMPLES FEB 6–7		SPRING SAMPLES MARCH 28–29		SUMMER SAMPLES JUNE 6–7	
	No. tested	Per cent increase	No. tested	Per cent increase	No. tested	Per cent increase
Pasteurized	221	127	199	351	198	196
Raw	25	5	56	16	56	18

separately because of the extreme differences in temperature when the tests were made. Many extremely high counts were observed during the warmer weather. Frequently neither set of plates could be accurately counted on the highest dilution used, which probably tended to obscure many of the existing differences in counts. On the average, the skim-milk agar count on pasteurized milk was two to four times as large as the corresponding count on standard agar. A slight increase in favor of skim-milk agar was also observed on the smaller number of samples of raw milk tested.

The presence in pasteurized milk of certain bacteria which are unable to grow on standard agar is the obvious reason for higher counts on skim-milk agar. That varying numbers of such bacteria are often found in pasteurized milk is well known. Probably the presence of these types of bacteria explains the results reported in Table 2.

TABLE 2

Some striking differences in counts from pasteurized milk on standard agar and on skim-milk agar

NUTRIENT AGAR COUNT	SKIM-MILK AGAR COUNT	DIFFERENCE IN FAVOR OF SKIM-MILK AGAR
		Per cent
7,900	1,230,000	15,470
2,400	73,500	2,962
29,900	815,000	2,626
2,100	57,300	2,152
5,600	46,500	730
14,900	117,000	685
19,500	119,000	510
28,500	123,000	332
2,400	9,300	287
3,500	10,300	190

It will be observed from the distribution of percentage differences reported in Table 3 that 60 per cent of the 608 pasteurized milk samples showed a definitely higher count on the skim-milk agar; about 35 per cent

TABLE 3

Relative distribution of percentage differences in counts from pasteurized milk on plain agar and skim-milk agar, using plain agar as standard

RANGE IN PERCENTAGE	NUMBER OF SAMPLES
−26 per cent to −60 per cent	28
−25 per cent to − 1 per cent	70
0 per cent to 25 per cent	145
26 per cent to 200 per cent	274
201 per cent to 500 per cent	50
501 per cent to 2000 per cent	33
2001 per cent to 5000 per cent	5
Over 5000 per cent	3
	Total 608

gave approximately the same count (−25 per cent to +25 per cent); and less than 5 per cent of the samples tested gave a lower count on the skim-milk agar. It is obvious that the difference between the counts on different samples is far from consistent. This very inconsistency brings out most clearly the advantages of the skim-milk medium.

Since logarithms of bacterial counts tend to minimize small differences which might be considered significant and also offer an opportunity to present large groups of data in a small space, the logarithms of the bacterial counts for each period have been plotted in Figures I, II, III, and IV.

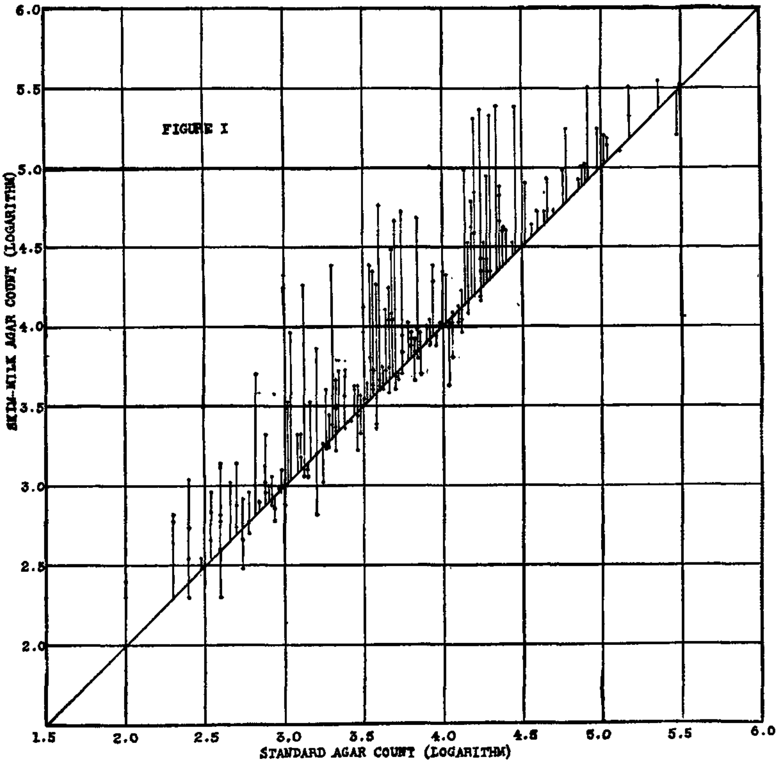

FIG. I. The logarithm of the number of bacteria *in pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken February 6 and 7.

If the skim-milk agar count exceeds the plain agar count, its logarithm appears above the diagonal line; if it is less, it appears below this line. The distance vertically from each dot to the diagonal line represents a single logarithmic difference. In certain cases, several of these differences are plotted on the same vertical line. This means that the standard agar counts on several samples were the same, whereas the skim-milk agar counts varied. Each dot represents one skim-milk agar count. The logarithms of these comparative counts on pasteurized milk appear in Figures I, II, and III. A glance at these graphs shows that the bulk of the skim-milk agar counts are higher than the standard agar counts. The logarithmic differences on certain samples are conspicuous. The general trend of the three charts is similar, although the counts on both media tend to be higher as warmer weather approaches.

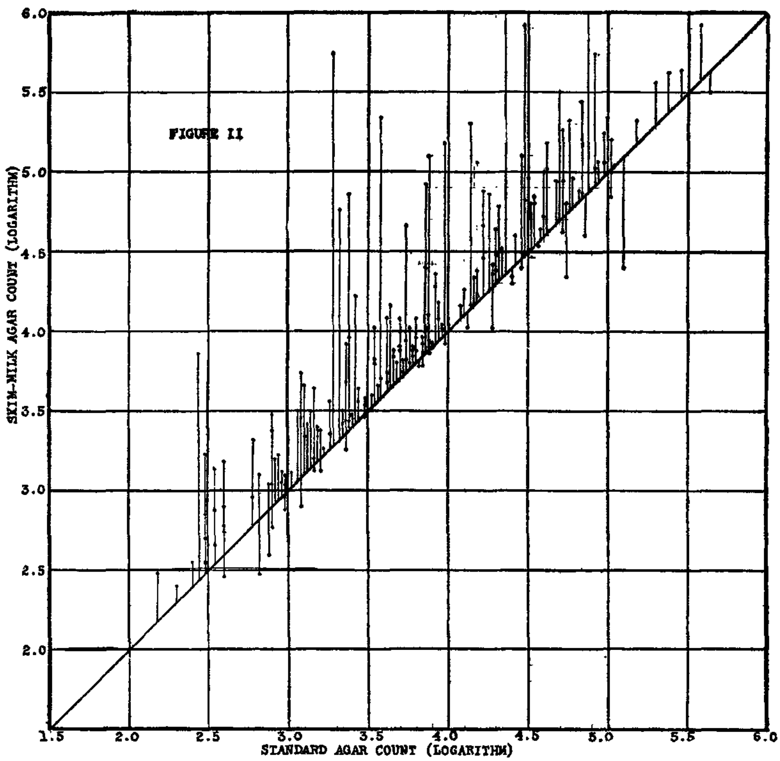

FIG. II. The logarithm of the number of bacteria *in pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken March 28 and 29.

Because of the small numbers of raw milk samples tested, the results for the three periods are presented together in Figure IV. The logarithmic differences in these counts on raw milk are believed to be of but little significance.

These data have been analyzed according to the percentage difference in counts, based on logarithmic averages as employed by Robertson and Frayer (7). The logarithmic averages of the standard and skim-milk agar counts on pasteurized milk and the percentage differences calculated from these averages are presented in Table 4. Similar calculations were made

TABLE 4

Theoretical percentage differences in counts from pasteurized milk based on logarithmic averages

	LOGARITHMIC AVERAGES		DIFFERENCE IN COUNTS: PERCENTAGE
	SKIM-MILK AGAR COUNTS	STANDARD AGAR COUNTS	
			Per cent
Winter samples Feb. 6–7	3.787	3.585	59
Spring samples Mar. 28–29	4.024	3.804	66
Summer samples June 6–7	4.616	4.366	78

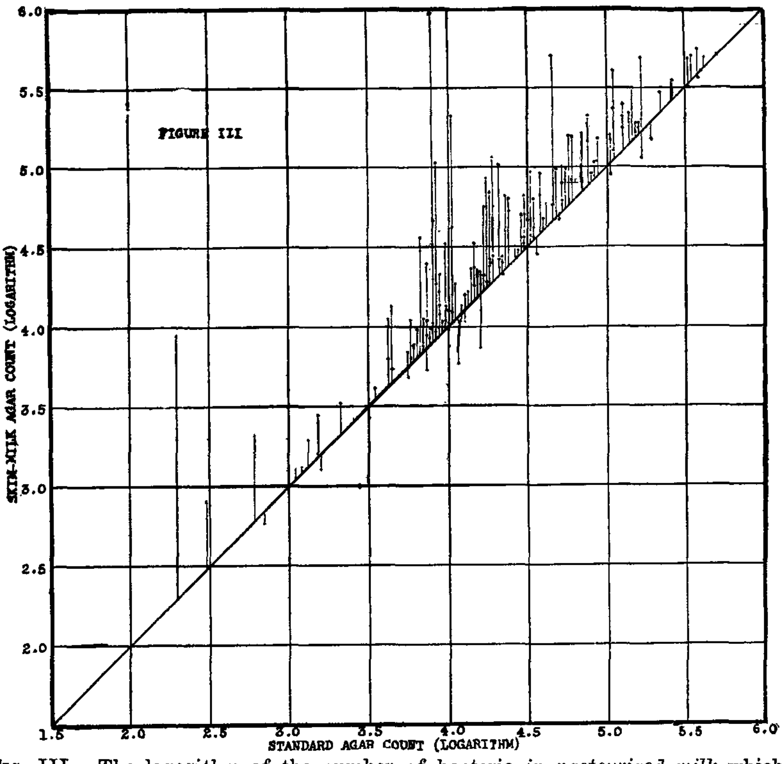

FIG. III. The logarithm of the number of bacteria *in pasteurized milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken June 6 and 7.

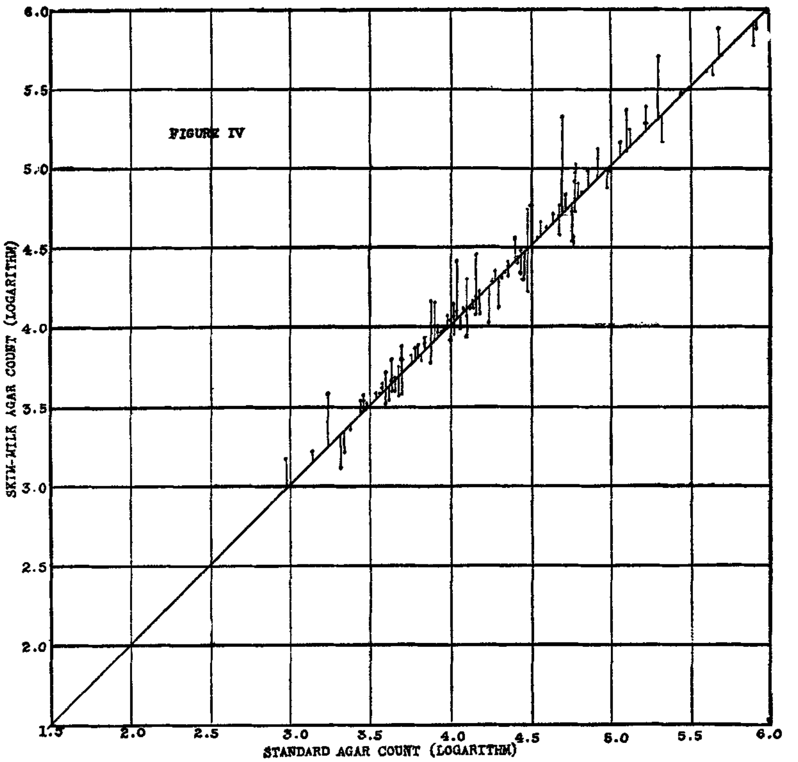

FIG. IV. The logarithm of the number of bacteria *in raw milk* which grew on standard agar and on skim-milk agar.

The standard agar counts are plotted on the diagonal line.

Samples taken February 6 and 7; March 28 and 29; and June 6 and 7.

on the raw milk data, but were found to have but little significance. For each set of logarithmic averages the percentage difference in count was calculated from the theoretical plate counts corresponding to these averages. This is illustrated for the spring samples of pasteurized milk:

Logarithmic average of skim-milk agar count is 4.024, which is equivalent to 10,570 bacteria per cc.

Logarithmic average of standard agar count is 3.804, which is equivalent to 6,370 bacteria per cc.

$$\frac{10{,}570 - 6{,}370}{6{,}370} \times 100 = 66 \text{ per cent theoretical percentage difference in counts.}$$

So great a percentage difference in counts, when based on logarithmic averages, is believed to be truly significant.

SUMMARY

The fermentable carbohydrates and other milk constituents in skim-milk agar make it a desirable medium to use in the routine control of market milk and other dairy products.

The skim-milk agar counts on 618 samples of pasteurized milk were, on the average, two to four times as large as the corresponding counts on standard agar. The counts on 137 samples of raw milk were only slightly higher.

The colonies were much larger and consequently could be counted with greater ease and rapidity.

The slight opacity of the medium prevents the glare often experienced when artificial lighting devices are used. Acid-producing and protein-digesting types of bacteria can be differentiated on this medium.

It supports the growth of bacteria responsible for mastitis in cows.

It is simple and easy to make and no more expensive than the present standard agar.

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