A STATISTICAL EXAMINATION OF THE SIGNIFICANCE AND COMPARATIVE VALUE OF MILK AGAR FOR RAW AND PASTEURISED MILK

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Hiscox *et al.* (1932) have shown that the addition of 1 per cent. fresh unheated skim milk[1] to the official medium (Ministry of Health Memo. 139/Foods) recommended for making routine plate counts of milk increases the number of colonies on the plates. Hiscox and her co-workers tested samples of both raw and pasteurised milk but made no statistical test of the significance of the increase in count. Thomas (1934) also published results for 264 samples of raw milk tested on both standard and milk agar and states: "The addition of sterile milk to the standard agar has a favourable influence on the number and size of the colonies that will develop in the examination of raw milk samples." The value and importance of milk agar would be further enhanced if it could be shown that not only was growth freer but also that the increase in colony numbers was statistically significant. Barkworth (1935) made a statistical examination of Thomas's figures and also of 134 samples of raw milk of his own testing. He agrees with Thomas on the better size of colony and claims that the increase in colony numbers is statistically significant. Later Provan (1935) presented results for pasteurised milk, but based his conclusions on the arithmetic values. He remarks strongly on the improved growth. In the present paper a statistical analysis is made of Provan's results, on the same basis as previous work (Barkworth, 1935), *i.e.* the logarithm of the plate count, and the figures for pasteurised milk are compared with those for raw milk.

In considering any possible increase in recorded count three questions arise: the amount and variability of the increase, the significance of the increase, and the possible correlation of the increase with the count on standard agar. After taking the logarithm of the plate counts the figures were treated by the method of analysis of variance using the sample totals, the treatment totals and the interaction between these two. The interaction could have been calculated direct from the differences, but the method employed gives information on the variation between samples. Significance between variances was tested by the z test of Fisher (1930, p. 194), and the t test (*loc. cit.* p. 99)

[1] The milk is added to the agar broth after this has been adjusted and filtered, and the medium is then ready for tubing and final filtration.

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was used to test significance between means. Variability of increase was expressed as standard deviation, using the formula

$$\sqrt{\frac{S(x-\bar{x})^2}{(n-1)}}.$$

In examining any effect due to the addition of 1 per cent. of milk to the medium we must remember that the 1/10 dilution plate of a routine milk count with standard agar is in itself a 1 per cent. milk medium, being made of 1 ml. saline containing 0·1 ml. milk and 10 ml. of agar medium. The milk ratio falls off rapidly on further dilution and in the 1/100 and 1/1000 plates is 1 : 1100 and 1 : 11,000. On the other hand, corresponding plates poured with 1 per cent. milk agar give final milk ratios of 1 : 55, 1 : 100 and 1 : 108·9, using 10 c.c. agar and 1 c.c. of inoculum. It must also be borne in mind that results are based on the limits of 30–300 colonies per plate, so that the 1/10 plate only covers results up to 3000 colonies per ml. and so on. Having regard to the final milk ratio it is obvious that the 1/10 plate must receive separate treatment. The results for the analysis of variance for this and the remaining dilutions are seen in Table I, and in Table II are given the other statistical values.

Pasteurised milk

From Table II it will be seen that the increase is significant at all levels of count and it should be particularly noted that while the increase is only 0·06 for the 1/10th plate, for the 1/100th and 1/1000th plates the figures are 0·31 and 0·48. This suggests that high counts in pasteurised milk are largely due to just those organisms which will not grow on standard agar. Good processing will give results of five to ten thousand per ml. and these counts requiring milk agar start at 30,000 per ml. and are just the stage where faulty operating is indicated. Hence the importance of using milk agar for pasteurised milk.

It will be noticed that taken over all plates, that is *between* dilutions, the correlation between the count on standard agar and the increase is significant. The correlation *within* dilutions is not significant until the 1/1000th plate is reached.

Raw milk

Table II shows that there is no correlation between the increase and the count on standard agar, but the increase is practically always significant, even with the 1/10 plate.

A 1/10 plate of standard agar has a milk ratio of 1 : 100 and with milk agar the ratio is 1 : 55, so that the figures suggest that at least at this level 2 per cent. milk agar might be even better than 1 per cent.

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Table I. *Analysis of variance*

Dilution	Factors	Thomas (raw milk) No. of samples	Sum of squares	D.F.	Variance	Barkworth (raw milk) No. of samples	Sum of squares	D.F.	Variance	Provan (pasteurised milk) No. of samples	Sum of squares	D.F.	Variance
1/10 plate	Samples	52	16·598	52	0·325	28	5·9750	27	0·2213	46	16·0402	45	0·3564
	Agars		0·479	1	0·479		0·5092	1	0·5092		0·0959	1	0·0959
	Interaction		3·186	51	0·062		2·0615	27	0·0764		0·8674	45	0·0193
	Total		20·263				8·5457				17·0035		
1/100 plate	Samples	—	—	—	—	—	—	—	—	58	13·1436	57	0·2306
	Agars		—	—	—		—	—	—		2·8587	1	2·8587
	Interaction		—	—	—		—	—	—		2·2558	57	0·0396
	Total		—				—				18·2581		
1/1000 plate	Samples	—	—	—	—	—	—	—	—	20	3·2752	19	0·1724
	Agars		—	—	—		—	—	—		2·3377	1	2·3377
	Interaction		—	—	—		—	—	—		0·9270	19	0·0488
	Total		—				—				6·5399		
1/100 and 1/1000 plates	Samples	212	283·312	211	1·343	106	111·0205	105	1·0573	78	44·5095	77	0·5780
	Agars		3·579	1	3·579		0·0632	1	0·0632		4·9823	1	4·9823
	Interaction		8·478	211	0·0402		5·1581	105	0·0491		3·3970	77	0·04412
	Total		295·369				116·2418				52·8888		
All plates	Samples	264	596·42	263	2·27	134	207·626	133	1·5611	124	178·7724	123	1·4534
	Agars		4·01	1	4·01		0·3022	1	0·3022		3·8376	1	3·8376
	Interaction		11·72	263	0·0446		7·4898	133	0·0563		5·5050	123	0·0448
	Total		612·15				215·4180				188·1150		

Table II. *Statistical values*

	Correlation (plain agar count with increase), *r*			Significance of difference between means and no. of samples*			Mean increase (log. of count)			S.D. of increase		
	Thomas (raw)	Barkworth (raw)	Provan (past.)	Thomas (raw)	Barkworth (raw)	Provan (past.)	Thomas (raw)	Barkworth (raw)	Provan (past.)	Thomas (raw)	Barkworth (raw)	Provan (past.)
1/10 plate	−0·045	0·0031	0·2347	Sig. 52	Sig. 28	Sig. 46	0·1427	0·1907	0·0646	0·3507	0·3913	0·1963
1/100 plate	—	—	0·1843	—	—	Sig. 58	—	—	0·3140	—	—	0·2813
1/1000 plate	—	—	−0·0641	—	—	Sig. 20	—	—	0·4835	—	—	0·3124
1/100 and 1/1000 plates	−0·1138	0·1286	Sig. 0·2724	Sig. 212	Non-sig. 106	Sig. 78	0·182	0·0346	0·3574	0·2854	0·3123	0·2970
All plates	−0·0372	−0·0581	Sig. 0·5206	Sig. 264	Sig. 134	Sig. 124	0·174	0·0673	0·2488	0·299	0·335	0·2992

* Values of t at $P = 0·05$.

SUMMARY

The use of 1 per cent. milk agar gives a significant increase in colony numbers for both raw milk and pasteurised milk at all levels of count. There is a marked increase with pasteurised milk on the 1/100 and 1/1000 plates. Milk agar also gives better colony growth, tending to more rapid and accurate counting. With raw milk there is no correlation between the increase and the count on standard agar.

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