A CHEAP AND EFFICIENT MEDIUM FOR THE PLATE COUNT OF MILK

By H. BARKWORTH
South-Eastern Agricultural College, Wye, Kent
and J. G. DAVIS
National Institute for Research in Dairying, Shinfield, Berks

The plate count is no longer considered the only test for the bacterial count of milk. There has recently been a tendency for the methylene-blue, or even the recently developed resazurin test, to displace the colony count as a measure of hygienic quality. Many workers, however, after experience with the dye reduction tests, have returned to the plate count, especially for advisory work, and consider that the new simple tests give inadequate information. Moreover, the plate count is still considered the best test for pasteurized milk. The questions of cost and availability of medium constituents, therefore, assume considerable importance in wartime.

Two media (Min. Health, 1937; Min. Agric. 1934) have received official recognition in England for routine plate counts of milk. Both media (yeastrel agar and milk agar) contain 0·5% peptone, so that a considerable amount can be used if a large number of samples is tested. A shortage of peptone has already been experienced and the price has increased by over 100%. Experiments were therefore undertaken to find an alternative satisfactory medium containing less peptone.

Plan of the experiment

Twenty different formulae were tried, in batches of four or seven media, and 30–58 samples were tested with each batch of media. B.D.H. (bacteriological) peptone was used. Except where stated, all sampling and laboratory work was done in one laboratory (H.B.). Sampling usually extended over 3 days per batch of media, and the same farm might be resampled, but in any one batch about 75% of the samples would be from different farms. In taking the 274 samples at least one sample was taken from each of the 160 farms sending milk to the creamery.

The samples were taken from churns on arrival at a local creamery. A single set of dilution tubes, 1/10th, 1/100th and 1/1000th, was prepared for each milk sample, using 0·9% saline as a diluent. From the 1/100th and 1/1000th dilutions sufficient plates were prepared to enable a single plate at each dilution, 1/100th and 1/1000th, to be inoculated with each of the media used. The dilution technique of the Ministry of Agriculture was used (Min. Agric. 1934). Plate-count results were based on the highest countable plate, i.e. the 1/100th dilution if countable.

CRITERIA OF SUITABILITY

It was considered that the suitability of any new medium should be assessed on three factors: the significance of the difference between plate counts on the new and the old media, the magnitude of the difference, and ease of counting.

(*a*) *Significance of the difference* was established by a '*t*' test on the differences between logarithms of the plate counts on the two media.

(*b*) *Magnitude of the difference.* If the mean difference were too small to be of practical import and if the variability of the difference were not excessive,[1] then a medium might prove an acceptable substitute even if the difference (over thirty or more samples) was significant.

Barkworth (1936) has given the following figures for mean increase in the logarithm of the plate count due to the addition of 1% skim milk:

	No. of samples	Mean increase (log of count)	S.D. of increase
Thomas (raw milk)	264	0·174	0·299
Barkworth (raw milk)	134	0·067	0·335
Provan (pasteurized milk)	124	0·249	0·299

Barkworth, Irwin & Mattick (1941) have established that at $P=0{\cdot}01$ the just significant difference between two samples (one plate per sample) is 0·2980 log, approximately 2·0 times the actual value. This means that if the true values of the two results compared did not differ, as great a difference or greater than that observed would arise by chance less often than one in 100 times. Mattick, McClemont & Irwin (1935) found that two samples were significantly different, $P=0{\cdot}05$, when the ratio of their geometric mean colony numbers was greater than 1·6.

An attempt was made to find a medium in which the mean difference was not more than 0·05 log (1·12 times actual value), but it was held that a medium could not be considered a suitable substitute if the mean difference exceeded 0·1 log (1·3 times actual value). Thus differences due to media would be less important than differences between samples.

(*c*) *Ease of counting.* Although size of colony is probably the most important factor, texture of the colony and contrast with the medium also play a part. Many workers prefer the slightly opaque background of the Min. Agric. medium produced by adding 1% skim milk *after* final filtration, and most of our media were made in this manner.

For routine practice it is desirable that all colonies should be easily appreciated by the eye. The presence of a high proportion of subvisible colonies necessitates frequent resource to the hand lens when counting, thus increasing labour. Even with the hand lens it is not possible to be sure of all 'colonies' and accuracy of counting is certainly less when there are many very small colonies.

[1] Small mean differences are unlikely to be significant if accompanied by high variability of the differences.

Results

The results are given in Table 1 and discussed in detail below.

First batch. Media containing yeastrel, or yeastrel and only 0·1% peptone, were tried using the medium of the Min. Agric. (1934) as a standard (medium no. 1). The difference between the standard and even the best of the new media was excessive being 0·14 log. It is notable that no. 4 (0·3% yeastrel, no peptone) gave significantly and markedly lower counts than no. 3, which contained only 0·1% yeastrel and no peptone.

Table 1. *Composition of media and significant differences between media*

				Percentage composition								
Batch no.	No. of samples	Medium no.	Mean* difference (log)	Whole milk (filtered)	Separated milk (added after final filtration)	Lab-lemco	Yeastrel	Peptone	Agar fibre	Dextrose	Significance of difference between successive media	
1	58	1	0·00	—	1·0	0·3	—	0·5	1·5	—	$P=0·01$ in all cases	
		2	−0·14	—	1·0	—	0·1	0·1	1·5	—		
		3	−0·21	—	1·0	—	0·1	—	1·5	—		
		4	−0·35	—	1·0	—	0·3	—	1·5	—		
2	58	1	0·00	—	1·0	0·3	—	0·5	1·5	—	1–5	N.S.†
		5	−0·02	—	1·0	0·1	—	0·2	1·5	—	5–6	$P=0·02$
		6	−0·05	1·0	—	—	0·3	0·5	1·5	—	6–7	$P=0·01$
		7	−0·09	—	1·0	—	0·1	0·2	1·5	—	1–6	$P=0·01$
3	50	6	0·00	1·0	—	—	0·3	0·5	1·5	—	6–8	$P=0·05$
		8	−0·04	—	1·0‡	0·1	0·1	0·1	1·5	—	8–9	$P=0·05$
		9	−0·07	—	1·0	0·1	0·1	0·1	1·5	—	9–5	$P=0·01$
		5	−0·14	—	1·0	0·1	—	0·2	1·5	—		
4	41 (past. milk)	1	0·00	—	1·0	0·3	—	0·5	1·5	—	1–6	$P=0·01$
		6	−0·06	1·0	—	—	0·3	0·5	1·5	—	6–5	N.S.
		5	−0·06	—	1·0	0·1	—	0·2	1·5	—	6–10	$P=0·01$
		10	−0·22	1·0§	—	0·2	0·2	0·1	1·5	0·1		
6	36	6	0·00	1·0	—	—	0·3	0·5	1·5	—	6–5	$P=0·05$
		1	−0·02	—	1·0	0·3	—	—	1·5	—	6–12	$P=0·05$
		11	−0·04	—	1·0	0·1	0·1	0·2	1·5	—		
		5	−0·06	—	1·0	0·1	—	0·2	1·5	—		
		12	−0·07	—	1·0	—	0·1	0·2	1·5	—		
		13	−0·16	—	1·0	—	—	0·2	1·5	—		
		14	−0·24	—	1·0	0·3	—	—	1·5	—		
7	31	1	0·00	—	1·0	0·3	—	0·5	1·5	—	1–15	$P=0·01$
		15	−0·12	—	2·0	0·6	—	—	1·5	—	15–18	N.S.
		16	−0·12	—	2·0	1·0	—	—	1·5	—		
		17	−0·14	—	1·0	1·0	—	—	1·5	—		
		18	−0·15	—	1·0	0·6	—	—	1·5	—		
		19	−0·26	—	2·0	0·3	—	—	1·5	—		
		20	−0·34	—	1·0	0·3	—	—	1·5	—		

* The first medium in each batch is taken as zero level.
† N.S. = not significant.
‡ Filtered.
§ Unfiltered.

Second batch. The previous results suggested that 0·2% peptone and 0·1% lab-lemco might be a useful formula and this was tried out (no. 5). As a check, a medium was made up to the same formula, using yeastrel instead of lab-lemco, and both the existing standard media were used, Min. Health no. 6 and Min. Agric. no. 1. The differences were much less than before but the two lab-lemco media gave the highest counts, the new formula, no. 5, taking second place to the Min. Agric. medium.

Third batch. A further attempt was made to devise a satisfactory medium containing only 0·1% peptone. Both lab-lemco and yeastrel were incorporated and the medium was made and divided into two portions—one, no. 9, being made as usual, whereas for the other portion, no. 8, the separated milk was added previous to final filtration.

The new media, no. 5, did not do as well as before but the colonies were larger than those on the 0·1% peptone media, nos. 8 and 9, which were very difficult to count. Comparing nos. 8 and 9 there was in this trial a slight significance, $P=0{\cdot}05$, in favour of the filtered separated milk, but the mean difference is small, 0·03 log, and the effect may be due to background and illumination effects as much as to any true increase in colony number.

Fourth batch. Media 5, 6 and 7 were again tried, this time using samples of pasteurized milk, and a final attempt made to use 0·1% peptone, adding 0·1% dextrose. In this medium, no. 11, the whole milk was not filtered and visibility was very poor. There was very little to choose between the other three media.

Fifth batch. This time seven media were used. The dilution tube holds only 9 ml. (1/100th dilution) or 10 ml. (1/1000th dilution) of milk and diluent. Bias might be created if as many as seven plates were prepared from each tube and the media added in constant order. To avoid this the media were inoculated in random order, a separate order being prepared for each sample from Tippetts random numbers (Fisher & Yates, 1938).

This batch (thirty-one samples) contained an excessive proportion of samples with colony counts so high that only estimates could be made, consequently the results were not acceptable for statistical analysis. The order of superiority of the media agreed closely with that in batch 6.

Sixth batch. The same media were tried again, namely, 1, 5 and 6 and sundry variants of medium 6, using 0·2% peptone but different combinations of lab-lemco and yeastrel. There was practically no difference between the first five media. The mean difference between media 6 and 12 is only 0·07 log and the t values were 2·24 for media 6 and 5 and 2·11 for media 6 and 12. These values are significant at $P=0{\cdot}05$.

Seventh batch. Finally an attempt was made to see if a medium with no peptone could be used provided the skim milk and lab-lemco content were increased. This does not appear to be workable.

FURTHER RESULTS

Another laboratory (J. G. D.) also made tests on fourteen samples of pasteurized milk with the media used in batch 6. The order of the media was 14, 13, 6, 1, 5, 12, 11. The mean difference between media 14 and 11 was 0·11 log, $t=2{\cdot}66$, significant at $P=0{\cdot}02$.

Discussion

All workers agreed that ease of counting could not be obtained with a medium containing less than 0·2% peptone. The new medium no. 5, 0·2% peptone and 0·1% lab-lemco, compares favourably with established media from the point of view of mean count and also colony size.

Table 2. *Comparative position of media*

Batch	Order of media	Difference (highest to lowest)	Difference between media 1 and 6	
2	1, 5, 6	0·05 log	0·05	$P=0·01$
3	6, 5	0·14 log	—	—
4	1, 6, 5	0·06 log	0·06	$P=0·01$
6	6, 1, 5	0·06 log	−0·02	N.S.
J. G. D.	6, 1, 5	0·04 log	−0·01	N.S.

Medium 1 Min. Agric.
„ 6 Min. Health.
„ 5 0·2% peptone, 0·1% lab-lemco.

There might be an advantage in using 0·1% lab-lemco and 0·1% yeastrel (see media 8 and 9, batch 3 and media 11, batch 6). However, simplicity is a valuable factor in a routine media and it is doubtful if any practical superiority would accrue.

Comparison of lab-lemco and yeastrel

In two trials, medium 1, with lab-lemco, gave significantly higher counts than medium 6 with yeastrel, and in two other trials there was no significant difference. Trials with medium 5 and its variants suggest, except for batch 3, that lemco is better than yeastrel.

Wilson (1935) investigated a number of extracts (at 0·3%) and found that yeastrel gave the highest counts. He then added whole milk to the medium and found that with 0·3% yeastrel the optimum concentration of milk was 1%. Our results suggest that when milk is an ingredient there is an interaction and that yeastrel does not occupy the position it does when milk is absent. With 1% milk, lab-lemco appears to be a more suitable extract.

A possible explanation is that yeastrel and milk supply much the same constituents, as far as growth of milk bacteria is concerned, but neither supplies those present in lemco. Alternatively yeastrel may have an inhibiting effect on bacteria stimulated by milk. Thus it has been observed that yeastrel considerably increases the size of colonies of *Staphylococcus aureus* and that when large colonies are formed the surrounding medium has fewer colonies than the rest of the plate, i.e. *Staphylococcus* exerts an inhibiting effect on other bacteria.

CONCLUSIONS

1. Ease of counting cannot be obtained in a medium containing less than 0·2% peptone.

2. The following medium appears to form a useful substitute for the present standard English media of the Min. Health (1937) and Min. Agric. (1934):

1·0% skim milk.	0·2% peptone.
0·1% lab-lemco.	1·5% agar fibre.

The skim milk is added after final filtration.

3. When milk is a constituent lab-lemco appears to be preferable to yeastrel as a supplement to peptone.

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