A STUDY OF THE BACTERIOLOGICAL EXAMINATION OF GRADE "A" (CERT.) MILK[1].

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The following paper contains a study of the results of bacteriological examinations of samples of Grade "A" (Certified) milk, some of which were 24 hours old and others 30 hours old at the time of examination. This work was started in 1916 and is still being carried on. Some of the results have already been published[2]. For purposes of comparison, these results, together with further series, are included in the present paper.

Conditions of Milk Production at the Farm and Carriage by Rail.

The samples of milk were derived from Farms I, II, III and IV.

All these farms are equipped for clean milk production since they possess water, light, steam, covered milking pails and the other necessary dairy appliances.

Farms I, II and III have special sheds for milking, but Farm IV is without special provision in this respect.

The milk was cooled to temperatures which varied between 40° F. and 66° F. on Farm I, between 50° F. and 66° F. on Farm II, 30° F. and 68° F. on Farm III, and 43° F. and 61° F. on Farm IV.

It was then bottled and sample (pint or quart) bottles were taken at random from the bulk and sent each week to the laboratory, in ventilated boxes, by rail. No attempt was made to keep them cool on the journey with the result that the temperatures on arrival at the laboratory varied between 36° F. and 76° F.

In one series the age of the milk was 24 hours, and in a second series 30 hours, on arrival at the laboratory.

The fact that the bulk was so small, allowed the milk to be readily affected by temperature changes and at certain periods of the year the temperatures of cooling and during transit were very unfavourable.

[1] The expenses of this work were in part defrayed by the Nestle and Anglo-Swiss Milk Co.

[2] Freear, Buckley and Stenhouse Williams (1919), *A Study of Two Types of Commercial Milk.* Cambridge Univ. Press.

Bacteriological Examinations.

All the samples were examined by plating dilutions from 1/10 c.c. to 1/10,000 c.c. of the well mixed milk on neutral whey agar plates, which were incubated for five days at 22° C. and then counted. Lactose fermentation tests were also carried out by inoculating quantities of milk varying from 1 c.c. to 1/10,000 c.c. into litmus lactose peptone water and incubating at 37° C.

The presence or absence of gas in the Durham's tubes was noted on the fifth day.

Standards of Comparison.

The results of the bacteriological examinations have been considered in relation to the American standard of 10,000 bacteria per 1 c.c. of milk and also in relation to the 30,000 standard which has been adopted in this country. The lactose fermentation tests have been included as they yield useful information, a fact which will appear later.

Results of the Bacteriological Examinations of 201 Weekly Samples from Farms I, II and IV at the end of 24 hours.

Table I shows that 201 samples have been examined, 82 of these came from Farm I between November, 1916 and September, 1918, 77 from Farm II between April, 1919 and December, 1920, and 42 from Farm IV between February, 1920 and January, 1921.

Results in comparison with the 10,000 *standard.*

When the results of the bacteriological examinations are considered it is seen that two or 2·4 per cent. of the samples from Farm I showed counts which were above 10,000 and that seven or 8·5 per cent. produced acid and gas when inoculated into litmus lactose peptone water in quantities of 1 c.c. or less.

Three or 3·7 per cent. of the samples from Farm II gave counts of more than 10,000 and 20 or 26 per cent. gave rise to the production of acid and gas in lactose peptone water.

Seven or 16·6 per cent. of the samples from Farm IV were above the 10,000 standard and 14 or 33·3 per cent. showed acid and gas.

When the results from Farms I, II and IV are combined it is seen that 201 samples have been examined, and that 12 or 6 per cent. of them have failed to maintain the 10,000 standard and that 41 or 20·6 per cent. have contained organisms capable of fermenting lactose with the production of acid and gas.

Results in comparison with the 30,000 *standard.*

In this country a maximum of 30,000 bacteria per 1 c.c. has been adopted as the standard. It was thought advisable, therefore, to consider the above results in relation to that standard.

In Table I the results are tabulated in this form. The table shows that the

percentages of occasions when the bacterial counts from Farms I, II and IV rose above 30,000 were 2·4, 1·3 and 9·5.

Table I. *Milk 24 hours old.*

10,000 standard.

Farm	No. of samples	No. of counts above 10,000	Percentage of counts above 10,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	82	2	2·44	7	8·5
II	77	3	3·7	20	26·0
IV	42	7	16·6	14	33·3
I, II and IV	201	12	6	41	20·6

30,000 standard.

Farm	No. of samples	No. of counts above 30,000	Percentage of counts above 30,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	82	2	2·44	7	8·5
II	77	1	1·3	20	26
IV	42	4	9·5	14	33·3
I, II and IV	201	7	3·5	41	20·6

When the results were combined the percentage of failure to maintain the standard was 3·5.

COMPARISON OF RESULTS AT THE END OF 24 HOURS.

When the combined results are considered in relation to this standard and are compared with the results on the 10,000 basis it is seen that the lower standard gives an advantage of 2·5 per cent.

It is of interest to note that the lower standard is of no advantage to Farm I but that Farms II and IV benefit to the extent of 2·4 per cent. and 7·1 per cent. The explanation lies in the fact that the bacterial counts from Farm I have always been well below the 10,000 limit except upon the two occasions recorded, when they were above 30,000, whilst Farms II and IV did not exhibit so uniform a standard of purity.

Clear evidence of this is furnished by the fact that 8·5 per cent. of the samples from Farm I contained lactose fermenting organisms as compared with 26 per cent. and 33 per cent. in the cases of Farms II and IV.

The value of the lactose fermentation test as a control of the count is demonstrated, since Farm II possessed all the mechanical appliances found on Farm I and the bacterial counts lay very close together.

The lactose fermentation tests, however, showed that there was a difference in the quality of the labour. Indeed, it would appear that when a certain skill has been attained this test is a very valuable aid in judging the quality of that skill.

The absence of a milking shed on Farm IV makes it difficult to decide the exact influence of labour in this case, but from the work which we have carried

out in a cowshed which is no better than that on Farm IV we are inclined to the opinion that better results could be obtained with improved labour.

Bacteriological Examinations at the end of 30 hours.

In view of the fact that the official regulations relating to the sale of Grade "A" (Certified) milk require that it shall reach the consumer within 48 hours of production and that the maximum number of bacteria present at any time during that period shall not be more than 30,000, it became important to determine the bacteriological condition of the milk at ages greater than 24 hours.

A series of experiments was, therefore, set up for the study of such milk at 30 hours old. 219 samples have been examined from Farms I and III.

117 of these were received from Farm I between August 8th, 1918 and December 9th, 1920. Farm III provided two sets of samples ("A" and "B"). In the first instance, from October 23rd, 1919 to January 20th, 1921, 62 samples (called "A" in the Table) were taken from the first milk coming over the cooler. Later, it was decided to examine samples of milk taken at a later stage in the same milking. These are called "B" in the Table and the period of examination extended from March 4th, 1920 to January 20th, 1921.

Results in comparison with the 10,000 *standard.*

Table II shows the results obtained with these samples when compared with a standard of 10,000 bacteria per 1 c.c.

Table II. *Milk* 30 *hours old.*

10,000 standard.

Farm	No. of samples	No. of counts above 10,000	Percentage of counts above 10,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	117	16	13·6	23	19·6
III "A"	62	15	24·6 (24·2)	25	40·3
III "B"	40	10	24·6 (25·0)	22	55·0
I, III "A" and "B"	219	41	18·7	70	32

30,000 standard.

Farm	No. of samples	No. of counts above 30,000	Percentage of counts above 30,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	117	8	6·8	23	19·6
III "A"	62	6	9·7	25	40·3
III "B"	40	7	17·5	22	55·0
I, III "A" and "B"	219	21	9·6	70	32

Out of 117 samples examined from Farm I, 16 or 13·6 per cent. showed counts above 10,000 and 23 or 19·6 per cent. contained lactose fermenting organisms.

15 or 24·2 per cent. of the 62 "A" samples from Farm III contained more than 10,000 bacteria per 1 c.c. and 25 or 40 per cent. contained organisms which

produced acid and gas in lactose peptone water. 40 "B" samples from Farm III are recorded of which ten or 25 per cent. gave bacterial counts above 10,000 and 22 or 55 per cent. gave acid and gas in litmus lactose peptone water.

When the results from Farms I, III "A" and III "B" are combined, the average error is 18·7 per cent. and one-third of the samples showed the presence of lactose fermenting organisms.

Results in comparison with the 30,000 *standard.*

Table II shows the results of the examination of milk from these farms, when 30,000 bacteria per 1 c.c. was used as a standard of comparison. From this Table it appears that the liability to failure to maintain the standard when compared with the liability to failure to maintain the 10,000 standard is reduced from 13·6 per cent. to 6·8 per cent. on Farm I, from 24·2 per cent. to 9·7 per cent. on Farm III "A" and from 25 per cent. to 17·5 per cent. on Farm III "B."

COMPARISON OF THE RESULTS AT 24 AND 30 HOURS.

These results are brought out in Table III in which the bacterial contents of the samples, at 24 and 30 hours old, are compared.

Table III. *Comparison of Counts at the end of* 24 *and* 30 *hours.*

10,000 standard.

Farm	Age of milk	No. of samples	No. of counts above 10,000	Percentage of counts above 10,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	24	82	2	2·4	7	8·5
I	30	117	16	13·6	23	19·6
		199	18	9·0		
I, II and IV	24	201	12	6·0	41	20·6
I, III "A" and "B"	30	219	41	18·7	70	32·0

30,000 standard.

Farm	Age of milk	No. of samples	No. of counts above 30,000	Percentage of counts above 30,000	No. of samples giving A. and G. in 1 c.c. or less	Percentage of samples showing A. and G. in 1 c.c. or less
I	24	82	2	2·4	7	8·5
I	30	117	8	6·8	23	19·6
		199	10	5		
I, II and IV	24	201	7	3·5	41	20·6
I, III "A" and "B"	30	219	21	9·6	70	32

In order to construct this table it was necessary to adopt a commercial standard of cleanliness. The general excellence of the samples from Farm I over a period of more than four years justifies their use as a basis for comparison. During this time 199 samples have been examined, 82 at 24 hours and 117 at 30 hours old. 9 per cent. of these have been found to be above the 10,000 standard and 5 per cent. above the 30,000 standard.

If the results of the examination of milk from Farm I at the end of 24 and 30 hours be considered in relation to the 10,000 standard it is seen that the liability to failure to maintain the standard is increased from 2·4 per cent. at the end of 24 hours to 13·6 per cent. at the end of 30 hours and the percentage of samples showing acid and gas in litmus peptone water has risen from 8·5 per cent. to 19·6 per cent.

On the 30,000 basis the number of samples which showed counts above the standard increased from 2·4 per cent. at the end of 24 hours to 6·8 per cent. at the end of 30 hours.

It follows, therefore, that, if 30,000 bacteria per 1 c.c. be accepted as the standard, Farm I is liable to an increased error of 4·4 per cent. when the examinations take place at the end of 30 hours.

If, now, the combined figures from Farms I, II, III "A," III "B" and IV be considered, it is seen that 201 samples from Farms I, II and IV were examined when 24 hours old and that 6 per cent. failed to maintain the 10,000 standard.

Further, 219 samples from Farms I, III "A" and III "B" were examined when 30 hours old, and the percentage of failures amounted to 18·7, an increase of 12·7.

When, however, the comparison was made on the 30,000 basis it was found that the percentage of failures to maintain the standard had risen from 3·5 per cent. at the end of 24 hours to 9·6 per cent. at the end of 30 hours, an increase of only 6·1 per cent.

The work which has been carried out demonstrates that the milk from Farm I when 30 hours old is liable to exceed the standard on 25 occasions in the course of a year. If the figures for the combined farms be taken this number rises to 35. These variations would be materially increased if the 10,000 standard were adopted since the rise would amount to 11·2 per cent. in the case of Farm I and 12·7 per cent. in the case of the combined farms.

The adoption of such a standard for official purposes would involve the combined farms in an increased liability to error of nearly 100 per cent.

Table III further shows that the percentages of occasions when lactose fermenting organisms were found rose from 8·5 per cent. to 19·6 per cent. in the case of Farm I and from 20·6 per cent. to 32 per cent. in that of the combined farms, when the time of examination was increased from 24 to 30 hours.

It is clear that as the age of the milk increases, it becomes more difficult to maintain the standard, and the liability to error at the end of 30 hours is already sufficiently serious to raise the question of the reconsideration of the present conditions relating to the handling of the milk. The considered opinion of the Milk Industry is required upon this point.

If the industry is in a position to supply this milk to the consumer within 24 hours of milking, then the present methods of production and carriage, with no attempt at keeping the milk cool on the journey, may perhaps be continued.

If that is impossible, and no guarantee can be given that the milk will reach

the consumer within a period of less than 30 hours, then the whole question of methods for chilling the milk both at the farm and during the journey requires consideration. The figures in Table IV demonstrate that, if the milk were sold within 30 hours, then chilling would be necessary during the months May to September. These are technical problems to which answers are required if our future work is to be of maximum advantage to the industry.

Table IV. *Seasonal Variation in Counts of Certified Milk.*

Farms I and II.

Milk 24 *hours old.*

Period	Counts under 30,000	Counts over 30,000	Period	Counts under 30,000	Counts over 30,000
Oct.–April	87	1	May–Sept.	72	2

Farms I, III "A" and III "B."

Milk 30 *hours old.*

Period	Counts under 30,000	Counts over 30,000	Period	Counts under 30,000	Counts over 30,000
Oct.–April	131	4	May–Sept.	88	17

The facts recorded show that milk produced under the best conditions can travel for 24 hours and still maintain a very high standard of bacterial purity. When the time is prolonged beyond this, the danger of error steadily increases.

They further show that it is inadvisable to raise the standard at the present juncture. This is the more important since the successful production of Grade "A" (Certified) milk involves the employment of persons possessing certain technical knowledge, a form of labour which is very limited in this country at the present time.

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