A ROUTINE METHOD OF BACTERIOLOGICAL ANALYSIS AND GRADING OF ICE-CREAMS: WITH RECORDS OF ELEVEN YEARS' APPLICATION

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Clearly defined standards have been laid down for estimating the bacteriological quality both of water and milk, but no authoritative standards seem to exist for ice-cream. Such text-books and literature as we have been able to consult are strangely silent on the subject. About eleven years ago we were called upon to produce a scheme for testing, as a measure of food control, the qualities of the ice-creams sold by various vendors in the city of Alexandria. We then decided to adopt the existing standards for milk, and established a tentative scheme of examination to permit of grading ice-creams by semi-direct readings as for milk. The time would now appear opportune to put on record our method, since safe ice-cream is a Services' as much as a civilian requirement—of like importance with a safe milk or a safe water. Recently, the British military authorities have approached us concerning our grading of the two kinds of ice-cream on the local market, namely, milk ice-cream ('gelati') and water ice-cream ('granita'), and have asked us to undertake on their behalf the examination of the process of manufacture of two series of Alexandria milk ice-creams before and after supervision on the spot. The present paper deals with the method, the findings obtained over a decade therewith, and the fitting into the picture of the army tests, because of the great importance which the latter attach to the good hygienic control of production.

The scheme being founded fundamentally on a water and on a milk basis, let us consider some of the general ideas which guided us in drawing it up. We may state the rule for a *water* as:

> 10–20 presumptive *B. coli* per 100 c.c. (i.e. readings of +10 and +5 c.c. respectively in the MacConkey tubes, but not in less); and

> 1–2 typical *B. coli* per 100 c.c. (i.e. confirmed typicals in the MacConkey +100 and +50 c.c. tubes respectively),

the lower limits being applicable to *public* supplies, and the higher to small *private* supplies such as moorland and upland surface waters to farms, shallow wells, etc. *Milk* being a good culture medium, the possibility of microbial development between milking and use has to be allowed for, so that here the less exacting higher limits of the water rule become applicable; i.e. > 20 presumptives and > 2 typicals per 100 c.c., equivalent to > 0·01–0·02 presumptives and > 0·001–0·002 typicals per 100 c.c. on a milk basis—assuming a milk to be judged 1000–2000 times more leniently than a water. On the severer 1000 times basis, this means > 100,000 total bacteria per c.c., with positive presumptive *B. coli* readings allowed down to 0·005 but not in less, and positive typical *B. coli* readings down to 0·05 but not in less. Some such ideas as these would appear to have inspired the framers of the Ministry of Health (M. of H.) and of the Milk Marketing Board's (M.M.B.) specifications for milk, judging by the standards laid down in the Milk (Special Designations) Orders of 1923 and 1936, namely, 100,000–200,000 bacteria per c.c. being permitted in a milk, as against the Koch standard of 100 per c.c. for an untreated water, and coliform bacilli to be absent in 0·01 c.c. Under the subsequent heading of 'Method' it will be seen that our practice is to set up quantities of 0·1, 0·05, 0·01 and 0·001 c.c. of the ice-cream, such quantities permitting the grading of the sample directly on the results of the presumptive test for all but one reading, that labelled 'doubtful'. The interpretation of the several readings are:

Readings of *B. coli* test		
+ + + +	Unfit	
+ + + −	Doubtful [representing an 'inferior grade' (M. of H.) milk]	
+ + − −	Just passable	[representing either a 'Grade A' ((M. of H.) or an 'accredited' (M.M.B.) milk]
+ − − −	Passable	
− − − −	Fit	

A presumptive reading of + + + + in an ice-cream (or a milk) means that the sample is to be declared as *unfit*, because such a reading is the equivalent of a

+ 1 c.c. water-reading, which corresponds to at least 100 presumptives per 100 c.c. of a water. And this is not permissible, since > 20 presumptives per 100 c.c. are allowed in a water.

Again, a presumptive reading of + + + − means that the ice-cream is to be declared as *doubtful*, because such a reading is the equivalent of a + 10 c.c. water-reading and corresponds to at least 10 presumptives per 100 c.c. of the water. If the confirmatory test should establish here the presence of typical (faecal) *B. coli*, then the sample is to be condemned and declared as *unfit*, because on a water-basis not more than 2 typicals per 100 c.c. are allowed.

Likewise a reading of + + − − means that the sample of ice-cream is passable on a presumptive basis and *just passable* on a typical basis. Such a reading is the equivalent of a + 50 c.c. water-reading, which corresponds to at least 2 coliforms per 100 c.c.; and, in a water, 2 typicals per 100 c.c. are just permissible, although up to 20 presumptives are allowed.

A reading of + − − − in an ice-cream means that the sample is *passable*, because such a reading is the equivalent of a + 100 c.c. water-reading, which corresponds to at least 1 presumptive per 100 c.c. And, since even 1 typical per 100 c.c. of a water is allowed, much more could 1 presumptive per 100 c.c. be allowed.

Lastly, a reading of − − − − in an ice-cream means that the sample is to be declared as *fit*, because such a reading on a water-basis is equivalent to the absence of *B. coli* in 100 c.c. (even in 160 c.c. —see Table 1) which represents a first class water.

It is well always to bear in mind that typical *B. coli* are usually not of themselves to be considered harmful. They are only indicators of the possible separate presence of harmful germs, like *B. typhosus*, etc. Whenever, therefore, non-lactose fermenter colonies are seen, at the Endo confirmatory stage, to accompany coliform colonies with the characteristic metallic sheen of typicals, particular attention must be paid to the former as possible pathogens.

METHOD

The ice-cream sample, after collection in a wide-necked screw-capped sterile bottle, and cold storage during transit, is liquefied at room temperature on delivery at the laboratory. 0·3 c.c. is pipetted off aseptically into a sterile test-tube and made up to 30 c.c. with sterile water, to make a dilution of 1/100; while 0·5 c.c. of the latter is pipetted off to a second tube and made up to 5 c.c., to make a dilution of 1/1000. For the *B. coli test*, we inoculate with quantities of 10, 5, 1 and 0·1 c.c. respectively of the 1/100 dilution, four tubes of MacConkey's bile salt-lactose-peptone water, containing neutral red and Durham tube; and for the *total count* we inoculate two Petri dishes with quantities of 0·1 and 0·4 c.c. respectively of the 1/1000 dilution, into which are subsequently poured about 15 c.c. of melted agar at 45° C. and the contents mixed. The tubes, and plates (after solidifying), are incubated at 37° C. for 48 hr.

The total count per c.c. is obtained by counting the combined colonies in the two plates and multiplying by 2000, or those in the 0·1 c.c. plate (when the colonies in the 0·4 c.c. plate are too numerous) and multiplying by 10,000. The growths in the four MacConkey tubes are recorded in terms of positive acid and gas production (the presumptive test), and the last positive is plated on Endo, when after 24–48 hr. representative coliform colonies are picked to lactose-peptone-water, peptone-water, and Koser's citrate (the confirmatory test), and any suspicious non-lactose fermenter colonies further examined for pathogens.

At the confirmatory test stage we prefer Endo's medium to all others, because of the ease with which it permits the detection of a variety of typical *B. coli* colonies by their metallic sheen. Further, the use at the next step of this stage of Koser's citrate medium, following its adoption by Harold (1938) in water analysis, has greatly simplified the differentiation between typical (faecal) and non-typical (dust, soil, grain) *B. coli*—compared with a previous reliance on the 'flaginac' method of Houston (1913). A notable weakness of the latter was its failure to recognize the existence of certain indol-positive non-typicals and certain indol-negative typicals, which constitute exceptions to the Houston rule of typicals being indol-positive and non-typicals being indol-negative.*

The confirmatory test thus provides us with four categories of coliforms, and a fifth non-coliform category, as follows:

Typical	$L^+ I^+ C^-$	'Flaginac' typical (Houston), *coli* I faecal (M. of H., 1927, 1939).
	$L^+ I^- C^-$	*coli* II (M. of H.).
Non-typical	$L^+ I^+ C^+$	Intermediate II, *aerogenes* II, *cloacae* I (M. of H.).
	$L^+ I^- C^+$	Intermediate I, *aerogenes* I, *cloacae* II (M. of H.).
	L^-	False presumptive.

Those of the first category are extremely numerous, and include the faecal coliforms of the seven biochemical subgroups defined elsewhere by one of us, with the several species and serological types of each (Compton, 1943).

* Harold (1938) estimated that some 7% of indol-positive coliforms and about 3% of indol-negative, were exceptions to the rule.

Table 1 summarizes the scheme of examination, and sets out the interpretation of the various possible readings of the *B. coli* test on a milk basis and on a water basis. We assume, as already explained, that a milk (or ice-cream) be judged 1000 times more leniently than a water.

RESULTS

Over the 11-year period we examined some 589 samples of milk ice-cream and 241 samples of water ice-cream. For convenience the senior author has the latter. Dealing first with the latter, which cover the period 31 July to 18 December 1944 (mainly autumn months), Table 3 sets out the detailed findings as regards *B. coli* content and total counts.

To appreciate the findings of this table and see them in some sort of perspective it will be helpful to consider them in terms of the grading of Table 2, in the light of civilian sampling over summer months, and in view of army attempts to improve purity of production by on-the-spot supervision of the process of manufacture by Army Inspectors. Table 4 permits of such consideration.

Table 1

B. coli test: Presumptive With following quantities in MacConkey (48 hr. at 37° C.)				Interpretation of foregoing in c.c. on basis of		Confirmatory	Grading in terms of	
0·1 c.c. P allowed	0·05 c.c.	0·01 c.c. T allowed	0·001 c.c.	Ice-cream or milk	Water	Plate on Endo, last tube showing presumptives. Pick coliform colonies to lactose-P.W., P.W. etc. citrate: $L^+ I \pm C^- =$ typical; $L^+ I \pm C^+ =$ non-typical	Absence of typical	Presence of typical
+	+	+	+	+0·001	+1		Unfit	
+	+	+	−	+0·01, −0·001	+ 10, − 1		Doubtful (inferior grade)	Unfit
+	+	−	−	+0·05, −0·01	+ 50, − 10			Doubtful (inferior grade)
+	−	+	−	+0·06, −0·05	+ 60, − 50		Passable	
+	−	−	−	+0·10, −0·06	+100, − 60			Passable
−	+	+	−	+0·11, −0·10	+110, −100			
−	+	−	−	+0·15, −0·11	+150, −110			
−	−	+	−	+0·16, −0·15	+160, −150			
−	−	−	−	−0·16	−160		Fit	Fit

P = presumptive *B. coli*. T = typical *B. coli*.
+ indicates a positive result (acid and gas production, indol formation, growth in citrate) and presence in.
− indicates a negative result (non-fermentation, indol not formed, no growth in citrate) and absence in.
P.W. = peptone water, L = lactose P.W. with neutral red, I = indol, C = Koser's citrate.

classified and summarized them in his several *Annual Reports* from these Laboratories into a threefold category as follows: *fit and passable* ('bons'), *doubtful* ('douteux'), and *unfit* ('mauvais'), in terms of the results of: (*a*) the total number of bacteria met with per c.c., and (*b*) the presumptive *B. coli* test. Since (*a*) and (*b*) parallel each other (compare Table 3), it follows that *B. coli* findings as a rule suffice for grading. Table 2 sets out the distribution of the samples on such grading.

Of the above samples 152 relate to the year 1944, of which fifty-eight represent civilian, and ninety-four military sampling. Municipal Inspectors collected the former while Army Inspectors of a Field Sanitary Section, operating in the city, collected

Analysis of Table 4 shows that prior to 1944 the average degree of bacteriological fitness and passableness of samples over the previous decade was 39·6%. This includes the four war years 1940, 1941, 1942 and 1943, with average respective percentages of 73·5, 62·8, 32·6 and 33·3. The sharp decline in quality which occurred during the years 1942 and 1943 improved in 1944 to a 56·9% degree of passable fitness (column 5). When the army began sampling in 1944 this improvement continued, to become 63·9% (column 7), and with supervised production 100% (column 9). On-the-spot supervision of the process of manufacture took the form of attention to such matters as cleanliness of the hands of workers, scalding beforehand of equipment

Table 2. *Period* 1934–44

Classification	Milk ice-creams	%	Water ice-creams	%
Fit and passable	269	46	67	28
Doubtful	135	23	55	23
Unfit	185	31	119	49
Total	589	—	241	—

and vessels, boiling the milk, adding the ingredients while the milk was near the boiling point, etc. Eight samples thus controlled and collected by Army on the *B. coli* test, random sampling reveals an expectancy of full fitness of only one out of four among ordinary samples; while, after hygienic control of production, a like degree of fitness is attained in four out of four samples.

In our view this finding means that during the El Alamein period of 1942 a certain carelessness crept into production, which automatically went on throughout the following year, until, when the army began being actively interested in 1944, producers began taking heed, with resulting improvement. The climax of improvement came when army supervisors undertook on-the-spot supervision, which showed

Table 3

Findings	*B. coli* test (MacConkey broth: 48 hr. at 37° C.) in 0·1, 0·05, 0·01 and 0·001 c.c. quantities: ++++	+++−	++−−	+−−−	−−−−
Presumptive	19	12	17	18	28
Confirmatory:					
'Typicals' present	4	2	3	4	0
'Typicals' absent	15	9	13	14	28
False presumptive	0	1	1	0	0
	Total counts per c.c. (agar 48 hr. at 37° C.)				
Limit counts	12,000–143,000	10,000–29,300	8,000–28,000	4,600–13,000	2,600–12,000
Average count	38,631	19,043	14,474	7,925	5,890

Table 4

Grading	1934–44 Civilian sampling	%	1944 Civilian sampling (summer)	%	1944 Army sampling (autumn) Non-supervised production	%	Supervised production	%
Fit and passable	173	39·6	33	56·9	55	63·9	8	100
Doubtful	114	26·1	9	15·5	12	13·9	0	0
Unfit	150	34·3	16	27·6	19	22·1	0	0
Totals	437	—	58	—	86	—	8	—

Inspectors between 7 October and 5 December 1944 yielded results nothing short of dramatic, although *a priori* to be expected. Total counts fell to limits of 4000–12,000 (average 6750) per c.c., and the *B. coli* test was negative in the four test quantities (0·1, 0·05, 0·01 and 0·001 c.c.). Judged on total counts this represents an approximate 2½ times (or 60%) improvement; since the average total count on eighty-six army samples before hygienic control was 17,000 per c.c., as against the foregoing 6750 per c.c. on eight samples after control. Again, judged by the *B. coli* test the improvement is approximately 4½ times (about 77%); since before, twenty out of eighty-six samples only were fit (in the sense of a four minus degree of fitness); while after, eight samples out of eight were thus fit. In other words, local producers what they could achieve in the betterment of their ice-creams.

This exposition would be incomplete without calling attention to the equally important question of *water ice-creams*, although it is possible that though they constitute an attractive food, they may not be so popular with the forces as with civilians. Our data relating them (Table 2) indicate a relatively higher percentage of 'unfit' specimens than among milk ice-creams (49 as against 31%). In view of the good results obtained by army supervision of the manufacture of milk ice-cream, there is every reason to suppose that equally good, or even better, results might be obtained by similar supervision of water ice-cream production, so that, eventually the standard might reach that of Alexan-

dria Water Company water itself—a first-class water with absence of *B. coli* in 100 c.c. in 100% of samples.

A final word with reference to *total counts*. It will be noticed (Table 3) that in our series, "unfit" samples of milk ice-cream showed *average* counts of approx. 40,000 viable bacteria per c.c., "doubtful" samples 20,000, "just passable" 15,000, "passable" 8,000, and "fit" samples 6,000. In view of this the recognised total count limit of 100,000 per c.c. appears to us to represent a somewhat too generous limit.

SUMMARY AND GENERAL CONCLUSION

The laboratory method here outlined and utilized by us over the past 11 years, of examining and judging ice-cream on a milk basis, appears to provide a sieve of mesh generous enough to let through the passably 'fit' while stopping the presumably 'doubtful' and 'unfit'.

Eighty-six samples of Alexandria milk ice-creams collected from various vendors in the city by Army Inspectors during the autumn months of 1944 showed, on this method, about 19% 'unfit'—a result in remarkable agreement with fifty-eight samples from Municipal sampling, collected earlier in the year during summer months, which showed 16% 'unfit'.

A small number of samples collected *after* hygienic control of the process of manufacture by Army Inspectors resulted in a total disappearance of unfit samples. The improvement arising from this controlled experiment was estimated at a lowering of 60% in total bacteria per c.c., and a reduction of 77% in coliforms.

The results of the latter experiment show what good results can be achieved in the betterment of milk ice-creams by simple hygienic measures, and should be more widely known in all centres where members of the Services are large consumers of ice-cream.

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REFERENCES

COMPTON, ARTHUR (1943). *C.R. Soc. Med. Hyg. trop. Egypte*, 8, 124.

HAROLD, C. H. H. (1938). *Thirty-Second Annual Report, Metropolitan Water Board*, p. 19.

HOUSTON, SIR ALEXANDER (1913). *Studies in Water Supply*, p. 143. London.

MINISTRY OF HEALTH (1927). *Rep. Publ. Hlth Med. Sub.* no. 41; (1939) no. 71.

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