THE CLEANING AND STERILIZATION OF MILK BOTTLES

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(Based on a report to the Ministry of Health)

Introduction

Under Article 21 of the Milk and Dairies Order, 1926, it is laid down that all vessels and appliances used or intended to be used for containing, measuring, or stirring milk, or for any other purpose for which they may be brought into contact with milk, shall be thoroughly washed as soon as may be after use, and shall be cleansed and scalded with boiling water or steam before they are used again.

These regulations have caused trouble in practice. Small dairies, which usually sterilize their bottles the day before they are filled, find no great difficulty in conforming to the Order, though they often disregard it. The larger dairies, however, which fill their bottles immediately after they are cleansed, are faced with the alternatives of either employing steam for sterilization and filling the milk into hot bottles, or relying for sterilization on caustic detergents and thus contravening the terms of the Order. For this type of treatment mechanical washing machines are commonly employed in which, after being cleansed by a suitable hot detergent solution, the bottles are rinsed, cooled and delivered directly to the filling machine. A third alternative, namely that of cooling the bottles after steaming, might be employed, but so far as we are aware this has not been provided for by any of the makers of large machines.

Three years before the war the Ministry of Health asked us if we would undertake a survey of bottle-washing plants to find out whether the results obtained by treatment with caustic detergents were as good bacteriologically as those following steam sterilization. The survey, which was carried out in Greater London, lasted from October 1937 to July 1939. Altogether 105 plants belonging to 26 different types were examined, 204 visits were paid, and 2406 bottles were taken for sampling. In addition a preliminary investigation was found necessary to determine the best technique for the bacteriological examination of washed milk bottles, and a long and careful series of observations was made on the disinfectant action of caustic soda. The results were embodied in a full report to the Ministry containing 95 tables and 9 figures. The paper shortage forbids the publication of such a report in war-time. Since, however, our findings and recommendations may be of interest to milk dealers, the Ministry has kindly agreed to the publication of the report in the greatly abridged form presented here. In this version tabular matter and references to the work of previous observers have had to be restricted to a minimum, and many of the less important findings have had to be described without statistical support. Readers who are anxious for the evidence on which any particular conclusion is based are at liberty to consult the full report, a copy of which has been deposited in the library of the London School of Hygiene and Tropical Medicine.

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Section A. Technique of examining milk bottles bacteriologically

Comparison of different methods

Three methods are available for the bacteriological examination of milk bottles, namely the swab method, the rinse-bottle method, and the roll-bottle method. The swab method, though advocated by Speck & Black (1937), presents obvious difficulties in its technical standardization, and has been reported by Mattick & Hoy (1937) to give lower counts than those obtained by the rinse-bottle technique. Our observations were therefore confined to a comparison of the rinse-bottle and the roll-bottle methods.

A preliminary study was made of the *distribution of organisms in washed bottles.* The free drainage water, which measured about 0·5 ml. in pint bottles, was withdrawn, suitably diluted, and plated out in milk agar (*Report,* 1937). Twenty ml. of ¼ Ringer solution were then added to each pint bottle, which was closed with a sterile rubber bung. The bottle was rolled round till the sides were thoroughly wetted, left in the horizontal position for 15–30 min., and again rolled round; portions of the rinsings were then withdrawn and plated out. 10 ml. of milk agar, melted and cooled to 50° C., were then added to the bottle, and rolled round so as to form a thin film on the inner wall. The plates and bottles were incubated at 37° C. and counted after 3 days. The results are given in Table 1.

Table 1. *Showing the distribution of organisms in washed bottles. Counts expressed as per 1 pint bottle*

		Mean count at 37° C.		
Experiment	No. of bottles	Drainage water	Rinse water	Bottle wall
1	6	259	65	43
2	6	19,400	10,800	1,260
3	6	11,700	9,100	5,000
4	6	55	59	147
5	6	110	270	67
6	6	13,400	5,600	2,800
7	6	4,800	2,400	1,800
8	6	100	19	17
9	6	54	20	13
Total	54	49,878	28,333	11,147
A.M.	6	5,542	3,148	1,239
Percentage	—	56	32	12

About half the number of reproducible organisms in the bottles appeared to be contained in the free drainage water; of the remainder roughly two-thirds could be detached by simple rinsing and soaking, while the other third remained adherent to the bottle wall.

Direct comparison showed that the rinse-bottle technique yielded a count about 15 % higher than the roll-bottle technique, probably because some of the clumps of organisms were disintegrated during the rinsing process. It appears, therefore, that if a suitable rinse-bottle method is used about 85 % of the organisms in the bottle will be counted, whereas if the roll-bottle method is used, this figure will be nearer 70 %.

A study of the various ways in which the rinse-bottle method may be carried out showed that, on the whole, vigorous shaking of the bottle detached rather more organisms than gentle rolling, and that leaving the rinse water in contact with the bottle for 15–30 min. led to a count about 30 % higher than one made without soaking. In the interests of uniformity it was concluded that simple rolling of the bottle, so as to wet the internal surface thoroughly, on two occasions at 15–30 min. interval, was the most suitable rinsing technique to recommend for practical use.

Length of incubation of plates

Comparative observations on plates made from 55 bottles showed that the number of countable colonies both at 22 and at 37° C. was about 50 % higher after 66 than after 42 hours' incubation.

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Ratio of 22° C. to 37° C. bottle counts

Various sets of observations were made, and the combined figures are summarized in Table 2. Though there was considerable variation in the 22° C./37° C. ratio in bottles from different plants, the average figure for all plants was not far from unity.

Suggested standard technique for routine examination of milk bottles

The same apparatus is recommended for the plate count as is described in the Ministry of Health's report on 'The Bacteriological Examination of Water Supplies' (*Report*, 1939*b*), and the same yeastrel milk agar as that laid down in the Ministry of Health's memorandum on 'Bacteriological Tests for Graded Milk' (*Report*, 1937). The only exception is the necessity for a 5 ml. pipette for measuring the quantity of rinse water to be delivered into Petri dishes in addition to the 10 ml. pipette used for delivering 20 and 30 ml. quantities of Ringer solution into milk bottles. The same pipette can be used for both purposes provided it bears a 5 and a 10 ml. mark.

Method of carrying out the test

One dozen bottles should be collected, either after they emerge from the washing plant if they are to be filled at once, or immediately before filling if they are allowed to stand in crates for some time after washing is completed. If they are taken from a large plant, a complete row of bottles should be picked out, so as to detect jet blockage or other type of inefficiency in any of the columns. The mouth of each bottle should be covered with a dry paper cap that has been sterilized in the hot-air oven for 2 hr. at 160–170° C. Sterile forceps are required for this operation. On no account should the fingers be allowed to come into contact with the inside of the cap or with the mouth of the bottle. The cap should be held firmly in position with an elastic band.

Table 2. *Ratio of 22–37° C. bottle counts expressed as pints, after 66 hr. incubation*

Type of plant examined	No. of plants examined	No. of bottles examined	22° C. A.M.	22° C. S.E.	37° C. A.M.	37° C. S.E.	Ratio 22° C./37° C.
Large (series *A*)	5	83	361	77	420	75	1/1·16
Large (series *B*)	23	265	2946	469	2811	423	1·05/1
Small rotary	19	215	5918	1471	6289	1627	1/1·06
Hand-washing with steam sterilization	42	480	4277	633	4368	661	1/1·02

The bottles should be brought back to the laboratory in the upright position and examined as soon as possible. If more than 2 hr. are likely to elapse between the collection of the bottles and their examination, they should be placed in a special container at a temperature of 34–40° F. No bottle should be examined that has been collected more than 24 hr. previously.

In the laboratory the cap should be removed, and 20 ml. of ¼ Ringer solution should be added to ⅓, ½, and 1 pint bottles, and 30 ml. to 1 quart bottles. A rubber bung, sterilized by boiling for at least 5 min., should be inserted into the mouth of each bottle.

The bottle should be held horizontally in the hands, and rolled gently till the whole of the internal surface is thoroughly wetted. It should then be laid horizontally on the bench. 15–30 min. later the bottle should again be rolled gently in the hands so that the whole of the internal surface is wetted. The rubber bung should then be removed, and 5 ml. of the rinse water should be delivered into each of two sterile Petri dishes.

Into each dish 10 ml. of milk agar, melted and cooled down to 48° C., should be poured. Mixing should be carried out by a combination of rapid to-and-fro shaking and circular movements lasting 5–10 sec., the plate being kept flat on the bench throughout. The exact procedure consists in five to-and-fro movements followed by five circular movements in a clockwise direction, succeeded by five to-and-fro movements at right angles to the first set followed by five circular movements in a contra-clockwise direction.

When the agar is thoroughly set, the plates should be inverted and incubated bottom upwards, one at 22° C. and the other at 37° C.

Both plates should be counted after 64–72 hr., using a suitably illuminated counting box of the type referred to in paragraph 45, p. 12, of Memo 139/Foods (*Report*, 1937), and a magnifying glass of 4 in. focal length magnifying 2½ diameters.

In plates containing 500 colonies or less every colony should be counted. In plates containing more than 500 colonies a sector containing at least 300 colonies should be counted, and the approximate number of colonies in the whole plate estimated by use of the appropriate multiplication factor.

The *plate counts* on ⅓, ½ and 1 pint bottles should be converted into *bottle counts* by multiplying by 4, and on quart bottles by multiplying by 6, and all bottle counts should be expressed as per pint bottle. Thus *bottle counts* on ⅓ pint bottles should be multiplied by 3, on ½ pint bottles by 2, and on 1 quart bottles by ½. Bottle counts should be recorded to the nearest integer under 10, to the nearest 5 under 100, to the nearest 10 under 1000, and to the nearest 100 over 1000. All bottle counts estimated from plates containing more than 500 colonies should be reported as 'approximate only'.

In making the report mention should be made of the time of collection of the bottles, the length of the interval elapsing between their collection and their examination in the laboratory, and the temperature at which they have been held meanwhile.

Table 3. *Example of method of working out and expressing the bottle count*

Half-pint bottles	Plate count on 5 ml. rinsings		Bottle count expressed as pints		Mean bottle count
Bottle no.	22° C.	37° C.	22° C.	37° C.	
1	7	23	60	180	120
2	37	60	300	480	390
3	21	18	170	140	155
4	90	78	720	620	670
5	60	48	480	380	430
6	19	30	150	240	195
7	8,000 *ca.*	12,000 *ca.*	64,000	96,000	[80,000]
8	53	63	420	500	460
9	10	9	80	70	75
10	24	20	190	160	175
11	33	48	260	380	320
12	85	68	680	540	610
Total 11	—	—	—	—	3,600
A.M.	—	—	—	—	330

Note. The count on bottle 7, being more than twenty-five times the next highest count in the series, is omitted in calculating the mean bottle count, and a note made to this effect on the report.

The count on each bottle, expressed as the number of colonies, *not* of bacteria, developing per bottle, or preferably as the bottle count, should be calculated for each bottle separately at 22 and 37° C., and the arithmetic mean of the two counts on each bottle worked out. In calculating the mean, any count more than twenty-five times the next highest count in the series should be omitted, and a note made to this effect. The average of the twelve mean bottle counts should then be taken. The final figure thus obtained is therefore based on twenty-four plate counts from twelve bottles, each made with a 5 ml. quantity of rinsings. An example will make this clear (Table 3). In making the report each of the mean bottle counts in the last column of the table and the final average should be given.

The arithmetic mean on a series of twenty-four counts from twelve bottles, made strictly in accordance with the technique just described, may be regarded as subject to the following standard errors:

Mean bottle count	Standard error %	Percentage limits of bottle count
10–50	±12·7	±38
50–200	± 9·0	±27
200–500	± 4·7	±14
500–3000	± 3·7	±11

Thus, if the usual allowance is made of three times the standard error, it is probable that the observed mean bottle count will fall between the limits of ±11 % and ±38 % of the real mean bottle count. For example, if the real mean bottle count is 570, then the observed mean bottle count will be within the rang of 570 ± 63, or approximately between 510 and 630.

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Section B. Survey of 105 bottle-washing plants

The survey comprised a study of 105 bottle-washing plants. Of these, sixty-seven were large plants belonging to sixteen different types, twenty-three were small rotary plants belonging to seven different types, twelve employed steam sterilization after washing, and three were hand-washing plants only. The distinction between large and small plants was somewhat arbitrary.

The main features of the large plants were as follows: all were of the 'straight-through' or 'come-back' type; all had a pre-rinse section; in the detergent section some relied on soaking only, some on jets only, and some on both; only three plants used brushes; the amount of post-detergent rinsing varied considerably; the length of drainage of the bottle in the inverted position varied from 15 sec. to 2½ min.; the time taken for each bottle to pass through the machine varied from 3 to 40 min.; and the number of bottles washed per hour varied from 1200 to 12,000.

The small plants, on the other hand, were of the rotary type, and relied exclusively on jets; all had a pre-rinse, a detergent, and a rinse section; the length of drainage in the inverted position varied from 5 to 15 sec.; the time taken for each bottle to pass through the machine varied from 1 to 4 min.; and the number of bottles washed per hour varied from 600 to 3600.

In the steam sterilization plants the bottles were washed either by hand or in a small rotary machine, and were then exposed in a steam chest to a temperature of 190–210° F. for 7–30 min.

In the hand-washing plants the bottles received no pre-rinse; they were soaked in a weak detergent solution for 1–10 min., rapidly brushed, and inadequately rinsed in a water tank.

As a rule two visits were paid to each plant at an interval of a month or more. The arrangements for the treatment of the bottles were carefully studied, and samples were taken of twelve bottles after cleansing. At the first visit, but not always subsequently, samples were also taken of the detergent solution, the rinse waters, the main water supply, any softened water in use, and occasionally of special materials such as drippings in the discharge section. Observations were also made on the temperature of the detergent solution and of the rinse waters. At small plants, where the custom was to leave the washed bottles overnight before filling them with milk, our usual practice was to collect six bottles immediately after cleansing and six preparatory to being filled.

All samples were brought back to the laboratory and examined at once. Bottle counts were made by the rinse technique described on pp. 98–99. Main water, softened water, rinse water, and drippings from the discharge section were examined by the plate count method using the same yeastrel milk agar medium as for the bottle counts. The detergent solution was titrated for total alkalinity, and for its sodium hydroxide and sodium carbonate content.

Results

Tables 4 and 5 have been prepared to show the arithmetic mean counts, and the mean minimal and maximal counts, with different types of plant. Great variations are seen both in the 22° C. and 37° C. bottle counts between different types of large plant. The number of some types of plant examined was small, but it is nevertheless apparent from Table 4, and from a study of the frequency distribution of the counts on groups of twelve bottles, which space does not permit of reproduction, that (1) certain types, like A, D, E_2, H_2 and J, gave almost uniformly good results; (2) other types, like K_1, gave mostly bad results; and (3) some types, like F and K_2, gave good results at times and bad results at others. Inspection of the individual protocols showed that considerable variations occurred between plants of the same type at different times and in the same plant from time to time, showing how important is the correct management of the plant.

A further study of Table 4 shows that with some plants, such as F and K_1, even the mean minimal counts were quite high. The ratio of the mean maximal to the mean minimal counts affords some idea of the degree of variation that was met with between the counts on individual bottles. The highest ratios were seen in plants giving the worst results, such as C, E_3 and G, but in some plants giving bad results, like K_1 and K_2, the ratios were no higher than in plants giving good results, like A and D. The occurrence of individual high bottle counts in a good plant suggests an unusually severe initial contamination, the failure of one or more jets to work satisfactorily, or contamination from drippings in the discharge section.

From Table 5 it will be noted that the mean bottle counts at 22 and 37° C. were three to four times as high in the small rotary plants, four to twelve times as high in the steam sterilization plants, and sixteen to forty-eight times as high in the hand-washing plants as in the large plants. With the small rotary plants

Table 4. *Bottle counts on large plants*

Identification letter	Type	No. of plants examined	No. of examinations	22° C. A.M	22° C. S.D.	37° C. A.M.	37° C. S.D.	22° C. Mean maximal	22° C. Mean minimal	37° C. Mean maximal	37° C. Mean minimal	Ratio of mean maximal to mean minimal 22° C.	Ratio of mean maximal to mean minimal 37° C.
A	Straight-through. Detergent soak	2	5	107	61	93	71	576	10	574	9	58	64
B	Come-back. Detergent soak	1	2	1,314	1,197	6,522	6,394	3,308	272	9,788	2,642	12	4
C	Straight-through. Detergent jets	1	2	5,562	5,542	11,930	11,759	50,054	212	40,086	1,700	236	24
D	Straight-through. Detergent jets	15	27	201	337	237	284	1,121	30	1,075	38	37	28
E_1	Straight-through. Detergent jets	4	8	1,437	1,442	1,307	1,604	5,006	130	4,790	116	38	41
E_2	Come-back. Detergent soak	6	13	267	358	734	1,133	1,342	27	2,362	172	50	14
E_3	Straight-through, tunnel type. Detergent jets	1	2	5,511	3,538	4,865	4,732	34,017	96	25,252	28	354	902
F	Straight-through. Detergent soak and jets	11	22	2,754	5,398	2,924	4,527	12,499	653	10,839	685	19	16
G	Come-back. Detergent soak. Brushes	1	2	10,303	1,183	10,097	2,250	91,744	160	86,880	238	573	365
H_1	Semi-rotary, tunnel type. Detergent brushes between jets	1	2	93	60	71	13	408	8	332	8	51	42
H_2	Semi-rotary, tunnel type. Detergent jets	5	10	531	516	246	245	1,360	246	754	84	6	9
I	Straight-through. Detergent soak and jets	2	4	1,534	1,547	1,246	1,009	5,568	235	5,484	159	24	34
J	Straight-through. Detergent soak	2	4	72	63	182	187	172	24	691	43	7	16
K_1	Come-back. Detergent soak and jets	4	11	7,362	12,357	8,123	11,720	21,870	877	29,130	1,037	25	28
K_2	Straight-through. Detergent soak and jets	10	21	2,030	2,524	2,698	2,993	16,282	265	16,031	289	61	55
L	Straight-through. Brushes between detergent soak and jets	1	2	6,299	6,194	3,003	2,783	24,120	864	8,750	296	28	30
Total		67	137	—	—	—	—	—	—	—	—	—	—
A.M. and S.E.		—	—	2,008 ±426	4,984	2,324 ±439	5,139	10,160 ±2,184	281 ±51	10,006 ±855	352 ±68	36	28

A.M. = arithmetic mean. S.D. = standard deviation. S.E. = standard error of mean.

One high bottle count excluded from arithmetic mean and standard deviation in machines *D*, E_2, H_2, *J* and K_2, and two high bottle counts in machine *F*.

Table 5. *Bottle counts on small rotary (E_4–P), steam sterilization (Q–R), and hand-washing (S) plants*

Type of plant	No. of plants examined	No. of examinations	22° C.		37° C.		22° C.		37° C.		Ratio of mean maximal to mean minimal	
			A.M.	S.D.	A.M.	S.D.	Mean maximal	Mean minimal	Mean maximal	Mean minimal	22° C.	37° C.
E_4	3	7	2,572	3,847	587	797	10,754	598	2,555	59	18	43
H_3	4	8	1,593	1,781	257	218	5,910	382	556	93	15	6
K_3	2	4	36,823	40,944	53,627	73,620	82,044	9,665	92,444	28,892	8	3
M	3	5	6,885	6,737	1,958	2,725	30,700	1,141	14,655	192	27	76
N	3	7	11,362	19,845	5,666	8,839	41,993	53	14,745	922	792	16
O	7	13	5,867	15,805	5,473	13,624	10,537	815	10,934	894	13	12
P	1	3	2,292	1,055	2,008	510	13,195	27	10,432	51	489	205
Total	23	47										
A.M. and S.E.			7,982 ±2,788	19,113	7,389 ±3,939	27,002	22,867 ±6,444	1,333 ±621	15,788 ±2,648	2,892 ±2,193	17	6
Q	2	2	7,909	7,744	7,417	7,215	16,800	2,880	17,648	2,898	6	6
R	10	15	25,773	44,275	10,009	17,188	51,259	3,099	30,771	386	17	80
Total	12	17										
A.M. and S.E.	—	—	23,671 ±10,738	42,025	9,704 ±3,967	16,355	47,205 ±17,368	3,073 ±1,516	29,227 ±12,706	681 ±357	15	43
S	3	3	96,065	138,602	37,818	20,197	323,683	11,899	127,498	1,481	27	86

A.M. = arithmetic mean. S.D. = standard deviation. S.E. = standard error of mean.

One high bottle count excluded from arithmetic mean and standard deviation in machine R.

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individual protocols showed that good results were often obtained with plants E_4, H_3 and O, but on the whole the counts were distributed over a wide range. The inference is that the nature of the results depends largely on the care and efficiency with which the machine is worked. Again, in some of the steam sterilization plants, very low bottle counts were obtained when the sterilization was carried out carefully, whereas in others in which little care was exercised steam sterilization appeared to be without effect. The very high counts in plants using hand-washing only are sufficient to show that this method should be condemned.

Large plants. Comparison of soak and spray types

In Table 6 the mean bottle counts in plants using a detergent soak, whether followed or not by spray from jets, are compared with those in plants relying on spray treatment alone. Both at 22 and at 37° C. the mean bottle counts were considerably higher in plants relying on soaking than in those relying exclusively on spraying. With either of these counts the odds against such a difference between the two

Table 6. *Large plants. Comparison of soak and spray types*

Soak or spray	Identification letter of plant	No. of examinations	Mean bottle count and standard error of mean 22° C.	37° C.
Soak	A	5	107	93
	B	2	1,314	6,522
	E_2	13	267	734
	F*	22	2,754	2,924
	G	2	10,303	10,097
	I*	4	1,534	1,246
	J	4	72	183
	K_1*	11	7,362	8,123
	K_2*	21	2,030	2,698
	L*	2	6,299	3,003
		86	2,680 ± 646	3,085 ± 620
Spray	C	2	5,562	11,930
	D	27	201	237
	E_1	8	1,437	1,307
	E_3	2	5,511	4,865
	H_1	2	93	71
	H_2	10	531	246
		51	874 ± 286	1,040 ± 495

* Rely mainly on soaking, but contain a few jets for spray treatment as well.

Observed difference between soak and spray 22° C. = 1806 ± 707
Observed difference divided by standard error = 2·6
Observed difference between soak and spray 37° C. = 2045 ± 793
Observed difference divided by standard error = 2·6

groups of plants being due to chance alone are nearly 100 to 1. So far, therefore, as this method of comparison is justifiable, it may be concluded that the spray type of plant yields on the average better results than the soak type.

Large plants. Value of brushes

Only three types of plant, G, H_1 and L, used brushes. Reference to Table 4 shows that with G and L the bottle counts were very high. Since the best results were obtained in plants not making use of brushes, and since several disadvantages attend their use, it would seem desirable to omit them from future plants.

The flora of washed bottles

Our studies were limited to about 250 strains of organisms isolated from twenty-five plants of the large type. Roughly speaking about one-half of the strains examined belonged to the *Chromobacterium* or *Achromobacterium* group of saprophytic bacilli, one-quarter to the cocci, while the remainder were made up of aerobic spore-bearers, thermophilic organisms, and yeasts or moulds. The absence of coliform bacilli, which must have been present in large numbers in the unwashed bottles, suggests that these had been killed by the detergent treatment. Since this treatment would also kill most of the other organisms just mentioned, the conclusion is drawn that the flora of washed milk bottles in large plants is derived mainly from the post-detergent sections. Evidence will be advanced later (pp. 107–8) to show that the rinse waters are responsible for this contamination.

Summary

The results just described for Greater London compare very favourably with those of similar surveys carried out in the United States by Hopkins & Kelly (1919), Smith (1923), and Layson, Huffer & Brannon (1936) (see also *Report*, 1934). The figures recorded in Table 7 show that approximately 50 % of bottles from the large, the small rotary, and the steam sterilization plants conformed to a standard of less than 600 colonies per pint bottle, or approximately 1 colony per ml. capacity.

The figures for the small rotary plants were not so good as for the large plants, there being a greater proportion of high counts among these. The same was even truer of the steam sterilization plants, in which about a quarter of the bottles were grossly contaminated. The results with the plants using hand-washing only were bad, none of them falling below the 2500 mark.

Table 7. *Summary of bottle counts obtained in the survey of Greater London. Bottle counts are reckoned as pints. Each figure represents the mean count of twelve bottles. The* 22° *and* 37° C. *results are treated as separate counts*

	Large plants		Small rotary plants		Steam sterilization plants		Hand-washing only plants	
Bottle count	No.	%	No.	%	No.	%	No.	%
Less than 600	152	55·5	46	49·0	17	50·0	0	0
600–2,500	67	24·4	23	24·4	6	17·7	0	0
2,500–25,000	52	19·0	19	20·2	3	8·8	1	17
Over 25,000	3	1·1	6	6·4	8	23·5	5	83
Total	274	100·0	94	100·0	34	100·0	6	100·0

The general conclusions to be drawn from this survey are that equally good results may be obtained in large or in small rotary plants relying on treatment with hot caustic detergent as in plants employing steam sterilization, and that the uniformly best results are obtained in properly designed and operated plants of the spray type.

Section C. Treatment with detergents

Analysis of the survey data

An analysis was made of the data obtained in the plant survey to determine the relation between the total alkalinity, the temperature, the type, and the frequency of change of the detergent solution to the final bottle count. Briefly, it was found that good results could be obtained by using either a high alkalinity with a low temperature, or a low alkalinity with a high temperature. A high alkalinity was taken as one of 10 or over, reckoning this in terms of the number of millilitres of normal HCl required to neutralize 100 ml. of detergent solution, and a high temperature as one of 145° F. (62·8° C.) or over.

So many different types of proprietary detergents were in use that insufficient observations were available on most of them to enable a trustworthy comparison to be made. So far as they went, however, the results suggested that the addition of trisodium phosphate or sodium metasilicate improved the detergent and bactericidal properties of caustic soda, and that one particular brand of proprietary detergent was superior to all others.

The greatest diversity was observed between different plants in the frequency with which the detergent solution was changed. The figures given in Table 8 show that equally good results were obtained in plants in which the detergent solution was changed infrequently as in those in which it was changed every day or every two or three days. These results confirm Anderson's (1939) previous findings. Provided (1) the solution is brought up to strength by addition of fresh detergent once or twice a day, (2) an efficient filter is installed so as to remove foreign matter and other materials likely to clog the jets, and (3) the tanks are thoroughly cleaned out every few days to remove all deposit, there seems no reason why the detergent solution should be replaced more than occasionally.

Special observations on the detergent section

The results obtained from analysing the figures in the survey were not as clear-cut as might be wished, mainly owing to the disturbing effect of the treatment the bottles received in the rinse section of the plant. For this reason a special investigation was carried out in which the bottles were examined immediately after leaving the detergent section. Again it was found that good results could be obtained with either a high alkalinity and a low temperature or a low alkalinity and a high temperature. The combination, of course, of a high alkalinity and a high temperature would probably have given the best results, but this was rarely used in practice.

Table 8. *Relation between frequency of change of detergent solution and final bottle counts*

Frequency of change	No. of observations	Arithmetic mean bottle counts	
		22° C.	37° C.
1 day	72	4100	4500
2–4 days	33	2200	2600
5–7 days	22	4400	4300
1–2 weeks	23	2340	1870
2–4 weeks	13	1000	2880
4–16 weeks	20	1590	1960

Table 9. *Summary of mean values of reaction velocity constant k, concentration coefficient n, and temperature coefficient θ, for destruction of* Bact. coli *and of* B. subtilis *spores by* NaOH

Bact. coli: Concentration of NaOH and temperature	Mean k	*B. subtilis* spores: Concentration of NaOH and temperature	Mean k
0·05 % at 20° C.	0·054	5 % at 30° C.	0·019
0·05 % at 30° C.	0·116	10 % at 30° C.	0·062
0·025 % at 40° C.	0·236	2 % at 50° C.	0·076
0·1 % at 20° C.	0·367	1 % at 70° C.	0·289
0·05 % at 40° C.	0·389	5 % at 50° C.	0·393
0·1 % at 30° C.	0·701	2 % at 70° C.	0·634
Mean n 2·69		1·75	
Mean $\theta^{10°\,C.}$ 2·03		1·5 *ca.*	

The counts on bottles taken immediately after leaving the detergent section were much lower than on those taken from the discharge section. Of the organisms growing at 37° C. about one-half were aerobic spore-bearers and one-quarter cocci. Counts at 55° C., however, showed that considerable numbers of thermophilic organisms were also present.

Observations in the laboratory on the disinfectant activity of caustic soda

In order to obtain more accurate information on the germicidal activity of caustic soda, a careful series of quantitative observations was carried out by the roll-tube method on *Bact. coli* and *B. subtilis* spores suspended in 1/1000 milk, using different concentrations of caustic soda at different temperatures. For a description of these the reader is referred to a paper by Hobbs & Wilson (1942). The main findings, however, are summarized in Table 9. The reaction velocity k was about 3,000,000 times greater with *Bact. coli* than with *B. subtilis* spores.

Taking the concentration coefficient n for *Bact. coli.* as 2·7, it follows that doubling the concentration of caustic soda increases the reaction velocity about 6·5 times. If, for example, with a given concentration of caustic soda, sterility was reached in 13 min., then doubling the concentration would reduce the time to 2 min., and halving the concentration would increase it to about 85 min.

Taking the temperature coefficient 10° C. for *Bact. coli.* as 2·0, it follows that a rise of 10° C. in the temperature doubles the rate of the reaction velocity. Thus, if sterility was reached at a given temperature in 8 min., then raising the temperature 10° C. (18° F.) would reduce the time to about 4 min., and lowering the temperature 10° C. would increase it to about 16 min. With temperatures, however, of over 40° C. (104° F.), the rate of destruction of vegetative organisms, and with temperatures of over 70° C. (158° F.), the rate of destruction of sporing organisms in the presence of caustic soda was found to be greatly accelerated by the effect of the heat itself.

Table 10. *Percentage concentration of* NaOH *required to destroy approximately* 25 % *of* B. subtilis *spores at different temperatures in different times*

	Minutes				
Temp. ° F.	1	2	4	8	16
120	2·44	1·64	1·10	0·74	0·50
130	2·15	1·44	0·97	0·65	0·44
140	1·90	1·28	0·86	0·58	0·39
150	1·66	1·12	0·75	0·51	0·34
160	1·46	0·98	0·66	0·45	0·30

Note. Doubling these values will result in a destruction of about 60 %, and halving them of about 7 %, of *B. subtilis* spores.

These values are worked out on the basis of the following figures: $k = 0{\cdot}00735$ for all 16 values. $n = 1{\cdot}75$, i.e. rate of destruction is increased 3·4 times when concentration is doubled. $\theta^{10^\circ\,\mathrm{F.}} = 1{\cdot}25$. $\theta^{10^\circ\,\mathrm{C.}} = 1{\cdot}5$.

Recommended concentrations of caustic soda to be used at different temperatures

As numerous workers have pointed out, caustic soda is not an ideal detergent. Its wetting power is low, it does not by itself soften water and is therefore a poor emulsifying agent for oils and grease, and its ability to keep loosened dirt in suspension is poor. In practice these defects may be remedied by the addition of such substances as sodium metasilicate, trisodium phosphate, sodium carbonate, or sodium hexametaphosphate. If these substances are used, they should be regarded as supplements to caustic soda, not as substitutes, since their germicidal activity is very much less than that of caustic soda.

American workers, in particular, have recommended high concentrations of caustic soda for dairy detergents. Minimum figures of 1·5 % and maximum figures of 3·5 % are often quoted. It seems to us very doubtful whether, on public health grounds, such concentrations are either necessary or desirable. Based on our experimental observations we have constructed Table 10 to show the time taken by different concentrations of caustic soda at different temperatures to destroy approximately 25 % of *B. subtilis* spores. This percentage has been chosen because it should afford a fairly wide margin of safety for the destruction of non-sporing pathogenic bacteria. It will be seen that a 0·51 % solution at 150° F. will destroy 25 % of *B. subtilis* spores in 8 min., and a 1·12 % solution in 2 min. Provided that (*a*) large particles of milk clot and other gross material are removed in the pre-rinse section, (*b*) the detergent solution is properly filtered to remove suspended matter, (*c*) the solution is kept up to strength by frequent addition of the necessary amount of detergent, (*d*) the tanks are cleaned out every few days, and (*e*) due allowance is made for the intermittent exposure of bottles to the detergent solution in spray machines, there seems no bacteriological reason why higher concentrations of caustic soda than those given in the table should be required.

SECTION D. RINSING

In the large plants that we examined the rinsing section usually consisted of a series of jets fed from one, two or three tanks of water at different temperatures, the water being recirculated and passed from one tank to the next in the reverse direction to the movement of the bottles. Generally, but not always, the bottles received a final rinse from one or more jets fed with water directly from the main. The water draining from the bottles scoured by the final jets was used to feed the last of the tanks of recirculated rinse water.

In small rotary plants there was usually only one tank from which rinse water was recirculated to a series of jets. One or two jets fed with main water supplied the final rinse.

Table 11. *Relation between the plate count of the recirculated rinse water and the discharged bottle*

22° C.				37° C.			
	Plate count of				Plate count of		
No. of observations	Rinse water per ml. Group	Rinse water per ml. Mean	Bottle	No. of observations	Rinse water per ml. Group	Rinse water per ml. Mean	Bottle
31	0–1,000	310	300	16	0–1,000	360	520
27	1,001–10,000	4,700	1,100	25	1,001–10,000	4,300	1,200
23	10,001–100,000	38,000	2,800	38	10,001–100,000	33,000	1,800
4	>100,000	1,200,000	8,300	6	>100,000	550,000	5,900
85	Weighted mean	68,400	1,610	85	Weighted mean	54,900	1,670

Table 12. *Relation of temperature to bacterial count of recirculated rinse waters (Plants in which chlorine was added to the rinse water excluded)*

No. of observations	Temp. ° F. Group	Temp. ° F. Mean	Plate count at 22° C.	Plate count at 37° C.
30	60– 79	71	102,000	77,700
25	80– 99	87	17,100	27,300
17	100–119	106	12,400	35,500
8	120–139	131	1,000	8,300
18	140 and over	160	780	4,300

Relation of recirculated rinse water to final bottle counts

In order to exclude the effect of imperfect treatment with detergent or of treatment with very hot rinse waters, such as were common in the small rotary machines, we have restricted our analysis to large plants of the hydro-soaker or all-jet type. The summarized results are set out in Table 11.

The close, though not linear, association between the count on the recirculated rinse water and that on the final bottle shows that the greater the degree of bacterial contamination of the rinse water, the higher was the count on the discharged bottle. Further observations showed that the count on the recirculated rinse water was about twenty to thirty times as high as that on the final rinse water.

Relation between temperature and plate count of recirculated rinse waters

In compiling Table 12 the data collected in the survey from both large plants and small rotary plants were used, excluding only those in which chlorine was added to the rinse water.

An inverse relationship existed between the temperature and the plate count of the rinse water. In the lower range of temperatures, 60–79° F., the water was heavily contaminated, whereas in the higher ranges, 120° F. and over, it was very much less so. It will also be noted that in the lower ranges of temperature the 22° C. count exceeded that at 37° C., but that in the higher ranges the converse was true.

Bacterial growth in rinse waters

Plant observations strongly suggested that the contamination of the recirculated rinse waters was due to actual growth of bacteria in the water. To prove this, four experiments were carried out in which rinse water taken from a plant was incubated at 37° C. and examined at intervals by the plate count method. Table 13 presents a typical result. Under these conditions bacterial multiplication occurred freely. There is therefore no reason to doubt that recirculated rinse water constitutes a favourable medium for bacterial growth, and that the degree to which it is contaminated profoundly influences the final bottle count.

Bacterial flora of rinse waters

A study of the flora of recirculated rinse waters showed that cocci and members of the *Chromobacterium* and *Achromobacterium* groups together constituted about 80 % of the organisms present. Aerobic spore-bearers, presumably carried over from the detergent section, and yeasts and moulds constituted most of the remainder. Coliform bacilli, mainly of the intermediate-aerogenes-cloacae group, were found in less than a quarter of the samples examined, and then in only very small numbers.

Table 13. *Bacterial growth in rinse water incubated at* 42° C.

	Plate count per ml. at	
Time in hr.	37° C.	55° C.
0	7,500	3,500
3	9,600	9,300
6	75,000	92,000
11	11,000,000	10,000,000
24	35,000,000	9,000,000

Effect of chlorine on bottle counts

In spite of Article 21 of the Milk and Dairies Order, 1926, which prohibits the use of oxidizing and preservative agents, chlorine was being used in many of the large plants surveyed, either for washing out the rinsing section of the plant after the run was over, or for adding to the rinse water in an endeavour to check bacterial growth during the washing process. Analysis of our figures did not suggest that either method had any great effect on the final bottle count. Addition of chlorine to the recirculated rinse water seemed to have some effect in controlling bacterial growth in plants, like F and K_2, that were normally troubled by gross contamination of the rinse water, but to have little effect in plants of the D and E_2 type in which the rinse water count was generally low.

Bacterial contamination of softened and of final rinse waters

Samples of water were taken from thirty-seven softening plants belonging to twelve different types. Though the ingoing water was of good bacterial quality, it issued from some plants, though not from others, with a high plate count, indicating that contamination had occurred in the plant.

When a softening plant was installed, the final rinse water was generally of about the same quality as that issuing from the softening plant, but in some plants a much higher count was observed on the final rinse water than on the softened water or on the water supplied directly from the mains. The reason for this was not definitely established, but, as found by Anderson (1939), there seemed little doubt that in the favourable temperature prevailing in the plant bacterial multiplication was occurring in and on the final jets, as well as in the manifolds to which the jets were attached. In some jets a gelatinous type of scum developed consisting of bacterial zoogloea.

Summary

In this section evidence has been brought to show that the recirculated rinse water used in most large bottle-washing machines is heavily contaminated with bacteria, and that this contamination is chiefly dependent on bacterial multiplication in the water itself. The solution of this major problem of bottle-washing is so closely bound up with the problem of cooling that it will be more profitably discussed in the next section.

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SECTION E. COOLING

Experimental observations in the laboratory showed that if milk at 40·1° F. (4·5° C.) was poured into warm bottles its temperature rose considerably. It was found that if the temperature of the milk was not to rise more than 5° F. the bottles had to be cooled to 68° F. (20° C.) before being filled.

Methods of cooling milk bottles

In plants in which the bottles pass directly from the washing to the filling machines their temperature, as just shown, should not exceed 68° F. It has already been found that, if rinse water is recirculated at a temperature below about 120° F. (48·9° C.), bacterial multiplication occurs in the water, leading to contamination of the bottle. The problem is, therefore, how to rinse the bottle and cool it to 68° F. or below without contaminating it. To avoid contamination during rinsing the water must be above 120° F., and yet to cool it water of 68° F. or less is required. To change directly from a rinse at 120° F. or over to one at 68° F. is impracticable owing to the risk of cracking the bottles. How then is the bottle to be rinsed without contaminating it, and cooled without cracking it?

Practically all workers who have considered this problem recommend for its solution chlorination of the rinse water (Myers, 1930; Slawson, 1932; Paley, 1935; Mattick & Hoy, 1937; Moore, 1937). By this means bacterial growth in the recirculated water can be checked, so that rinses of progressively lower temperature can be used for removing the detergent and for cooling the bottles. There is no doubt that when chlorine is added to water free from all but a trace of organic matter and allowed to act in a suitable concentration for a given time, the resulting product is for all practical purposes sterile. But the sterilization of rinse water, which varies from hour to hour in its content of organic matter and in the number of bacteria to be killed, presents a difficult problem if gross over-dosage is to be avoided. Unless the concentration of chlorine is subject to laboratory control at frequent intervals during the running of the plant, the results are almost bound to be haphazard and unpredictable.

Another objection to the chlorination of rinse waters is that it is merely a substitute for cleanliness. Many observations on large plants of the D and I type, for example, showed that the plate count of the recirculated rinse water was below 1000 per ml. In numerous other plants, which had been designed or were being operated without regard to bacteriological requirements, the contamination of the rinse water was sometimes prodigious. The right way, in our opinion, to deal with this trouble is not to atone for a wanton disregard of cleanliness by the plentiful use of chlorine, but to see that plants are designed and operated in such a way as to preclude the occurrence of heavy bacterial contamination.

To us a more satisfactory solution appears to be to rinse with hot water at 120° F. or over inside and out in order to remove the detergent, and to cool by a series of *external* rinses only, decreasing successively in temperature till the bottle is down to about 70° F. A final internal rinse with main water at a temperature preferably not exceeding 60° F. may then be given. In this way the recirculated rinse water will cool the outside of the bottle without contaminating the inside, and the final bacterial content of the bottle should be little more than on leaving the detergent section.

The value of such a solution can be tried out only under working conditions. Unfortunately, in the absence of a suitable machine, we were unable to test it. We did, however, make a number of observations in the laboratory to find out whether it was likely to be practicable. An internal and an external spray, operated by foot pedals, were constructed, and attached to the main water supply. Each spray was adjusted to deliver 2 l. of water in 25 sec. Narrow-necked pint bottles of known specific heat were selected. They were immersed in a water-bath at 50° C. (122° F.). Each bottle was then subjected to treatment for a given length of time with the internal or external spray, or exposed to air alone as a control, after which it was transferred immediately to a simple form of calorimeter and filled with water at 13–14° C. The change in temperature of the water was noted every minute till equilibrium was reached after about 5 min. The temperature of the bottle after spraying or air-cooling was calculated from the following formula:

$$M_1 \times S_1 \times \underset{(x-T_2)}{\Delta T} = M_2 \times S_2 \times \underset{(T_2-T_1)}{\Delta T}$$

when M_1 = weight of bottle,
M_2 = weight of water added,
S_1 = specific heat of bottle,
S_2 = specific heat of water,
T_1 = temperature of water added,
T_2 = equilibrium temperature,
x = temperature of bottle after treatment.

Numerous combinations of internal and external spraying and air-cooling were tried. These cannot be described in detail for lack of space. Suffice it to say that by use of an external spray at about 66° F. for 30–60 sec., an internal spray at the same temperature for 5–10 sec., followed by air-cooling at 64° F. for 30 sec., it proved possible to reduce the temperature of the bottle from 122° F. to about 72° F. If the bottle had been treated externally with a warm rinse before being subjected to cold water, as it would have been in practice, the final temperature of the bottle would probably have been not far from 68° F., which was the temperature aimed at. By use of an external rinse with cold water at 65·5° F. for 25 sec., an external rinse with artificially cooled water at 49° F. for 12 sec., an internal rinse with cold water at 65·5° F. for 6 sec., followed by air-cooling at 64–66° F. for 30 sec., the temperature of the bottle was reduced to between 66 and 68° F.

It is suggested, therefore, that the method we advocate for rinsing and cooling bottles without contaminating them should be tried in practice.

Section F. Drainage and discharge

The drainage and discharge section of a bottle-washing plant comprises that part between the final rise and the removal of the bottles from the machine.

Observations showed that in large plants the shorter the drainage time in the inverted position, the higher was the final count on the bottle. No plant with a drainage time of 40 sec. or less provided bottles with a low count. The amount of free water in the discharged bottle varied inversely with the length of drainage. The average amount in bottles drained for 40 sec. or less was 0·58 ml., and in those drained for over 40 sec. 0·38 ml. These figures, taken in conjunction with the finding in Section A (p. 97) that about 50 % of the organisms in a washed bottle are contained in the free drainage water, leave little doubt that the final bottle count is partly dependent on the amount of free water remaining in the bottle.

The rate of drainage at different temperatures was measured experimentally with clean ⅓, ½ and 1 pint bottles of the narrow-mouthed and wide-mouthed varieties. It was found to be inversely proportional to the size of the bottle, to be greater in narrow-mouthed than in wide-mouthed bottles, and slightly greater at 72° F. than at 54° F. During the first 5 sec. about 74–96 % of water drained away, depending on the size and shape of the bottle and the temperature of the water. By 30 sec. 84–100 %, and by 30 min. 88–100 % had drained away. In order that as little drainage water as possible should be left behind in washed bottles it is recommended that in practice the following minimum drainage times should be adhered to with water at 50° F. (10° C.):

⅓ pint bottles	narrow-mouthed	30 sec.	½ pint bottles	wide-mouthed	60 sec.
⅓ ,,	wide-mouthed	45 ,,	1 ,,	narrow-mouthed	90 ,,
½ ,,	narrow-mouthed	45 ,,	1 ,,	wide-mouthed	120 ,,

During discharge from large machines bottles may be contaminated in various ways. One of the commonest was found to be by condensation water from the bottle carriers or from the arms of the discharge mechanism. Water from these sources often had a high bacterial content. This type of contamination can readily be avoided by suitable design of the plant. Rubber pistons, which are used in some plants for pushing the bottles from the carriers on to the discharge platform, may also contaminate the mouth of the bottle, and are best avoided.

Section G. Storage of bottles

In small dairies using rotary machines or hand-washing the washed bottles are generally left for some hours, often overnight, before being filled. Observations were, therefore, made both at the plant and in the laboratory to find out what the effect of storage in the upright or in the inverted position was on the bottle count.

The results were very irregular, being probably influenced by a number of factors such as the initial degree of cleanliness of the bottle, the temperature and place of storage, and the type of flora. It was quite impossible to predict the effect of storage on any individual bottle. Sometimes the bottle count rose enormously within 24 hr.; at other times it fell to a level below that of the original washed bottle. On the whole, storage in the upright position favoured bacterial growth more than storage in the inverted position. The difference was often less than would be expected, probably because the drainage water left in bottles taken from small rotary plants is sometimes so alkaline as to constitute a relatively poor medium for bacterial growth.

As previous workers, such as Taylor (1919), Russell & Hoffmann, and Klunker (see Meurer, 1927), and Layson, Huffer & Brannon (1936) have shown, one of the main factors affecting the count during storage is the degree of moisture of the bottle. If bottles are stored inverted in dry, warm weather, the count will usually fall within 24 hr. or less. If, on the other hand, the air is moist and the temperature favourable for bacterial growth, the counts of bottles in the upright, and even in the inverted position, may rise greatly.

SECTION H. THE PRACTICAL IMPORTANCE OF CLEAN BOTTLES

Experimental observations

To gain some idea of the amount of deterioration suffered by milk that is poured into dirty bottles, eleven experiments were carried out.

Six pints of pasteurized milk were mixed in a 5 l. flask, and distributed alternately into (*a*) six bottles autoclaved in the laboratory, and (*b*) six bottles taken at the moment of discharge from large plants of the F and K_2 type. The bottles were incubated for 24 hr. at 22° C. The milk in each bottle was then submitted to the methylene blue reduction test at 37–38° C. (*Report*, 1937), and to a keeping quality test at 17·5° C. similar to, but slightly more elaborate than, that described by Wilson and his colleagues (1935). The degree of cleanliness of the original bottles was gauged by a plate count made on six other bottles taken at the same time from the same washing plants. The results, which need not be tabulated here, may be summarized as follows:

Average bottle count at	22° C.	4830
	37° C.	5760
Average plate count on pasteurized milk at	22° C.	45,750 per ml.
	37° C.	27,690 per ml.
Average reduction time	37–38° C.	10 hr. 35 min.
Average reduction time of milk in	washed bottles	3 hr. 36 min.
	sterilized bottles	3 hr. 55 min.
Average keeping quality time of milk in	washed bottles	28 hr. 3 min.
	sterilized bottles	30 hr. 49 min.

Thus the reduction time was about 20 min. shorter in the non-sterile than in the sterile bottles and the keeping quality about $2\frac{3}{4}$ hr. less.

The degree of shortening of the reduction and keeping quality times was associated not so much with the original bottle counts as with the reduction time of the pasteurized milk filled into the bottles.

The general conclusion appears to be that contaminated bottles may lead to a significant degree of shortening in the life of milk filled into them, but that the exact degree probably depends on the nature and number of the organisms present in the milk. The fewer and less metabolically active these organisms are, the greater is the relative effect of bottle contamination.

Milk-borne epidemics traced to contaminated bottles

Armstrong & Parran (1927) state that of 373 milk-borne outbreaks of typhoid fever in the United States in which the probable mode of contamination was determined, thirty-seven were attributed to the use of bottles from infected homes. In 1929 a total of forty-four outbreaks of milk-borne disease of different types was investigated in the United States; five of these were reported to be due to contaminated and unsterilized bottles that were returned from houses where sickness prevailed (*Report*, 1931). Rawlinson (see Gibbs-Smith & Hobday, 1928) has shown experimentally that bottles washed in water along with other bottles contaminated with typhoid bacilli may themselves become infected.

Bottles may be contaminated in the dairy as well as in the home, particularly if they are filled and capped by hand. How frequent this mode of infection is cannot be stated. Considering, however, that in Armstrong and Parran's review 296 out of 373 outbreaks of typhoid fever were traced to cases or carriers on the farm or in the distributing plant, and that of 44 outbreaks of miscellaneous milk-borne disease in 1929 sick persons or carriers accounted for no fewer than 31 (*Report*, 1931), infection from bottles may be more common than is generally supposed. It should, in fact, always be suspected when sporadic cases of infectious disease occur on a particular milk round, as in the instance described by Smellie (see Harding, 1939).

Measures for preventing the spread of disease by milk containers

To guard against the spread of infection by bottles returned from infected houses, adequate cleansing and sterilization of the bottles are required. Contamination of bottles after sterilization constitutes an entirely different problem with which this report is not directly concerned. Briefly, however, it may be stated that the wide-mouthed glass bottle with the disk cap should be condemned. There are several objections to it, the most notable being (1) the bottle is generally handled by the rim, which becomes grossly contaminated, and (2) it can easily be filled with loose milk by the roundsman and capped with a disk taken from his pocket. If it is used, the presence of a case or carrier in the dairy or on the round may lead to infection, and thus stultify the whole value of pasteurization of the milk and of sterilization of the bottles. It should be permitted only if, in addition to the disk cap, it is fitted with a hooded cap covering the outside of the neck.

The alternatives are (1) a narrow-necked bottle fitted with a press-over aluminium cap; this prevents contamination of the actual rim, but not of the neck immediately below the rim, so that the milk may be infected during pouring; (2) a bottle, preferably in conjunction with a deep press-over aluminium cap, having recessed angles of the type described by Arnold (1938) to minimize the contact of the milk with the outside of the bottle during pouring; this would appear to be the simplest and most satisfactory method, if no practical disadvantages are found to it; (3) a single-service container of the paper board type; provided the paper pulp is of good quality and sanitary conditions are maintained in the preparation, filling and sealing of the package, this type of container constitutes an almost ideal method of handling milk hygienically; it is, however, expensive, and until paper board or some substitute for it can be produced more cheaply than at present, glass bottles with all their attendant disadvantages will continue to be used.

Section I. The bacteriological principles governing the design and operation of bottle-washing plants

It is abundantly clear that many washing plants on the market have been designed by engineers and are operated by technicians without any strict regard for bacteriological principles. Under these conditions it is not surprising that, however clean the bottle may appear to the naked eye, it is often heavily contaminated with bacteria. In discussing the general principles involved it will be convenient for the sake of clarity to deal separately with the large plants, the small plants, and the hand-washing plants followed by steam sterilization.

1. *Large plants*

(1) *Location.* The plant should be placed in a well-lighted room, protected from street dust and smoke, and be accessible all round and on the top. No operative can be expected to obtain good results day after day if these elementary desiderata are neglected.

(2) *General design.* Plants of the straight-through type are to be preferred to those of the come-back type, so as to avoid the danger of contamination of the washed bottles by the operative who is loading on the dirty bottles.

The detergent and rinse tanks should have an opening large enough to enable a man to get inside and scour them without difficulty. A filter, preferably external, should be attached to the detergent tank.

All jets should be constructed of hard metal, resistant to corrosion, to prevent gradual enlargement of the orifice, and, together with the manifolds, they should be readily detachable for cleaning purposes. On the whole we favour the spray type of plant in which the cleansing is exclusively by means of jets. With such a design the bottles can be conveyed in the inverted position through the whole length of the machine, and are therefore much less liable to be chipped, cracked or de-centred than in soaker types in which the bottles are frequently changing their position. Whatever the design of the jet may be, and whether fixed or rising jets are used, some self-centering mechanism should be incorporated in order to make certain that the bottles are uniformly scoured. The conveyor mechanism should be as simple as possible, and easily accessible for cleaning purposes. Intermittent movement is preferable to continuous, since it is possible to estimate more accurately the length of effective treatment that the bottles receive in each section.

Indicator and recording thermometers should be fitted in the detergent and rinse sections, and checked at frequent intervals against standard instruments. Pressure pumps should also be provided.

The whole machine should be enclosed by easily removable panels, so as to protect the detergent and rinse water and the bottles themselves from external contamination.

(3) *Preliminary sorting of the dirty bottles.* This is necessary to exclude (*a*) cracked or badly chipped bottles, and (*b*) all bottles containing substances like clotted milk, dirt, ashes, paraffin, syrup, urine, and moulds. Damaged bottles, and bottles that have been used for paraffin or similar substances, should be destroyed; the others should be soaked for at least an hour in a 1 % solution of caustic soda and, if necessary, treated by a rotary brush, before being put through the washing machine.

(4) *Pre-rinse.* Bottles should be sprayed inside and out with warm water, conveniently taken from the recirculated rinse. The greater the amount of foreign matter that can be removed in this section of the machine, the better, since the work to be done by the detergent in the next section will be diminished and the contamination of the detergent solution itself by milk carried over in the bottles will be lessened. We would suggest using at least three consecutive internal jets for 5–10 sec. each, as well as an external spray, for each bottle. Great care must be taken to see that the drainage water from the pre-rinse section runs directly to waste, and does not contaminate either the post-detergent rinse water or the underside of the conveyor as it approaches the loading platform.

(5) *Detergent.* The bottles may either be soaked in a detergent solution and then sprayed by suitable jets, or they may be treated entirely by jets. Provided particularly dirty bottles are soaked in a caustic solution and hand-washed before being put through the machine, it is very doubtful whether the incorporation of a soak tank in the machine is desirable. Certainly in our survey the best results were recorded in plants of the all-jet type. If, of course, no preliminary hand-sorting and washing are practised, it may be advisable to include a soak tank, but we do not favour this alternative. The passing of all bottles, however dirty, through the machine cannot but lead to gross contamination of the detergent solution and to consequent difficulty in obtaining satisfactory sterilization. The heavier the load on any machine, the more likely is it to break down. The greater the amount of organic matter and the larger the number of bacteria carried over into the detergent solution, the less effective sterilization will be and the longer it will take.

The two main factors concerned in the destruction of bacteria are the concentration and the temperature of the caustic detergent. It is possible to obtain the same result by using either a weak solution at a high temperature or a strong solution at a lower temperature. Table 10 (p. 106) gives the various combinations of concentration and temperature of the caustic soda solution that are required to bring about a given degree of sterilization in a given time. Plants should be designed and operated so as to ensure that each bottle is exposed to detergent solution at the proper strength and temperature for at

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least the time laid down in the table. For this purpose multiple jets will be required, the concentration of detergent must be checked at frequent intervals, and the temperature should be controlled thermostatically and not by the blowing in of steam.

The type of detergent used is of considerable importance. It should contain the concentration of caustic soda laid down in the table so as to be sufficiently germicidal, but it should contain in addition substances such as sodium metasilicate, trisodium phosphate, sodium carbonate, or sodium hexametaphosphate to increase the wetting, softening, emulsifying and deflocculating powers of the caustic soda and so help in the removal of micro-organisms from the surface of the bottle. Various proprietary detergents fulfil these requirements, of which that labelled E in our survey appears to be the most generally effective.

Brushes, except for use in the preliminary hand-washing of very dirty bottles, are unnecessary. Unless kept clean and changed at frequent intervals they may add to the contamination of the bottles, and lead by the detachment of their bristles to blocking of the jets.

Provided that (*a*) the detergent solution is kept up to strength by addition of fresh detergent when required, (*b*) the detergent solution is properly filtered to remove suspended matter, and (*c*) the tanks are cleaned out every few days, there seems to be no advantage in completely changing the detergent solution at frequent intervals.

(6) *Rinsing and cooling.* This is the section that in many modern machines is most open to criticism. Bottles emerging practically sterile from the detergent section are frequently recontaminated by warm recirculated rinse water in which there is prodigious bacterial multiplication. Recontamination of this type constitutes one of the major problems of bottle-washing. The various methods of overcoming it have been discussed on pp. 109–10, and it is not proposed to deal with these again. Briefly, however, if warm recirculated rinse water is to be used, then it must be sterilized. For this purpose Mattick & Hoy (1937) in this country and most American workers recommend some disinfectant process, of which chlorination is the most obvious; but we have given our reasons for believing that chlorination does not constitute a satisfactory practical solution for this problem, and that moreover it tends to encourage dirtiness by masking its effects.

The solution that we offer lies along different lines. Instead of using warm recirculated, and therefore contaminated, water for the internal rinsing of bottles, we would suggest the use of (*a*) an internal and an external *hot* rinse for removing the detergent, followed by (*b*) a number of *external* rinses with recirculated water of progressively decreasing temperatures for cooling the bottle, and (*c*) a final internal and external rinse with cold water from the mains.

If recirculated rinse water is allowed to fall below about 120° F. (48·9° C.), bacterial multiplication will occur. The hot rinse (*a*) to remove the detergent should, therefore, not be allowed to fall below this level. Provided this is attended to, there is no reason why it should not be recirculated to a certain extent, and then used, along with the warm recirculated water (*b*) employed in the external rinse, for the cleansing of the bottles in the pre-rinse section.

The warm recirculated water (*b*) for the external rinse will admittedly be contaminated, but none of this water will be used for rinsing the inside of the bottle. Its chief purpose is to cool the bottle and prepare it for a final internal and external rinse (*c*) with pure water from the mains.

If the temperature of the main water supply is above 60° F., it may be necessary to introduce a rinse, preferably external, with artificially cooled water between the last external rinse (*b*) with warm recirculated water and the final internal and external rinse (*c*) with water from the mains.

In this way we believe that it should be possible to deliver a bottle free from detergent, free from recontamination, and at a temperature of not over 68° F. (20° C.) without adding materially to the cost of operating the machine. Since the bottles emerge hot from the detergent solution, there should be little cooling of the hot recirculated rinse water (*a*), and the cost of maintaining the temperature of this rinse at 120° F. or over should not be very great.

The rinse tanks should be drained every night, scrubbed out with warm 1 % caustic-soda solution, flushed with cold water, and again drained. Jets and manifolds should be removed daily, cleaned, and sterilized either (*a*) in a steam cabinet, (*b*) by a steam hose used till the metal is thoroughly hot, or (*c*) by immersion in a hot 1 % caustic soda solution for 10 min., followed by thorough rinsing with cold water from the main.

(7) *Drainage and discharge:* After the final rinse it is important for the bottles to be kept in the inverted position for a time long enough to allow the majority of the water to drain away. As pointed out in Section F, the rate of drainage varies inversely with the size of the bottle, and is greater with narrow-

mouthed than with wide-mouthed bottles. The requisite times for different sized bottles are laid down on p. 110, but in the construction of a machine that is to be used for all sizes of bottles it would be wise to arrange for a drainage time of not less than 2 min.

At the end of the drainage period the bottles should be gradually transferred to the oblique position and finally discharged upright. Care should be exercised in the design of the machine to avoid any contamination of the inside of the bottles from condensation or other water on the carriers or discharge mechanism. Rubber pistons that come into contact with the mouth of the bottle should also be avoided. The discharge platform should be well lighted so that any imperfectly cleaned bottles can be picked out readily.

The bottles should then be conveyed by an automatic belt to the filling machine, which should preferably be situated in a different room from the washing plant. During this passage the bottles should be protected by a simple form of covering from contamination from dust, as well as from the fingers and the cough-spray of persons in the vicinity.

(8) *Softened water*. The use of soft water is to be recommended in the detergent and rinse sections, except for the final cold rinse, which should be taken straight from the main. Since water is often heavily contaminated in its passage through some types of softening plant, it is advisable to select a plant that is free from this drawback.

(9) *Cleaning*. The drainage and cleaning of detergent and rinse tanks, and the sterilization of the jets in the rinse section, have already been referred to. It remains only to say that the whole machine should be kept thoroughly clean, particular attention being paid to the conveyor mechanism, which is liable to get soiled with milk residues. All such material should be removed manually at the end of each run, and the belt itself should be scrubbed with hot 1 % caustic soda solution.

2. *Small rotary plants*

The general principles of design and operation that have just been described for large plants hold, with a few modifications, for the small rotary type of plant. Their main features may be briefly described.

(1) *Design*. All rotary plants are necessarily of the come-back type. This has the disadvantage that the same operator handles the clean and dirty bottles. Since, however, the clean bottles emerge in the inverted position, this objection is not so serious as in large plants in which the bottles are discharged upright. The machine should be completely enclosed by removable panels, and all tanks and jets should be freely accessible for cleaning purposes.

(2) *Pre-rinse*. It is desirable to include a pre-rinse section so that the majority of the milk remaining in the bottle can be removed and unnecessary contamination of the detergent solution avoided. Warm water should be used and drained away completely.

(3) *Detergent*. A single tank is usually sufficient. Since the time of exposure is generally short, it is desirable to use a strong caustic solution at as high a temperature as possible. A solution of a suitable proprietary detergent containing preferably 1·5 or 2 % caustic soda should be prepared, and maintained at a temperature of not less than 160° F. Multiple jets are of course necessary. A filter mechanism should be incorporated in the detergent tank.

(4) *Rinsing*. The bottles should be sprayed with multiple jets of recirculated water at a temperature of at least 140° F. Since this water soon becomes converted into a weak detergent solution, the bottles should receive a final spray with main water at a temperature of not less than 120° F. This water can then be used for the pre-rinse section.

(5) *Discharge and storage*. The clean bottles should be picked off and stored inverted in suitable drainage racks or crates. If possible a type of crate or drainage rack should be used in which the weight of the inverted bottle is borne by the shoulder. If the mouth of the bottle comes into contact with the bottom of a dirty crate, it will be recontaminated. If crates of this type have to be employed, they should be made of a non-corrodible metal, and should either be sterilized by steam or thoroughly scrubbed over with hot 1 % caustic soda solution and flushed with cold main water before use.

The bottles should be stored inverted in a cool, dry, well-ventilated room, and protected from dust and from human and animal contamination until they are ready for filling. If they are kept moist and warm, bacterial multiplication will almost certainly occur. They should never be stored upright.

3. *Hand-washing plants with steam sterilization*

If the washing is done strictly by hand the following points should be attended to:

(1) The bottles should be rinsed in warm water to remove the grosser milk residues.

(2) They should then be washed in a weak solution of a suitable proprietary detergent maintained at

a temperature of about 110° F. If possible, each bottle should be soaked for 2 or 3 min., and then scoured internally and externally with a rotary brush.

(3) The bottles should be rinsed with cold main water to remove the excess of detergent.

(4) They should then be transferred in the inverted position to freshly washed crates, packed into a chamber, and sterilized by steam. During this process it is important (*a*) to allow the temperature of the bottles to rise to 200–210° F., (*b*) to maintain this temperature for at least 10 min., and (*c*) to permit the bottles to cool down gradually. The bottles should be stored inverted either in the sterilizing chamber itself or if this is impossible in a cool, dry, well-ventilated room protected from dust and human and animal contamination.

Section J. Bacteriological standard to which washed bottles should conform

Various standards have been recommended for judging the cleanliness of washed bottles. Probably the most widely accepted is that laid down in the U.S.A. Public Health Service Milk Ordinance and Code (*Report*, 1935), which states that they shall contribute not more than one bacterial cell or colony per ml. capacity. Mattick & Hoy (1937) regard this standard as unduly lenient, and would prefer that no bottle, reckoned as a pint, should have a bacterial count at either 22 or 37° C. exceeding 200. It is doubtful whether such a standard could be reached at present by more than a minority of plants. The American Public Health Association (*Report*, 1939*a*) recommends a standard of not more than 1000 colonies for quart, 500 for pint, and 250 for half-pint bottles.

We would suggest for practical use a standard essentially similar to that laid down by the Federal Government of the United States. Since not all dairymen are acquainted with the capacity of bottles expressed in the metric system, we would suggest replacing the 'not more than 1 colony per ml. capacity' by 'not more than 600 colonies per pint bottle'. This, of course, infers figures of 200, 300 and 1200 colonies for $\frac{1}{3}$, $\frac{1}{2}$ and 2 pint bottles respectively. The equivalent figure for one bacterial cell per ml. capacity is 567; the margin of 33 would cover a considerable part of the experimental error of counting.

The mode of carrying out the test and of expressing the results has already been described in Section A (pp. 97–8). It may be stated that approximately 50 % of the bottles taken from large and from small rotary plants in our survey would have conformed to this standard.

In large plants it would probably be well to specify that the bottles should be discharged at a temperature not exceeding 68° F. (20° C.); otherwise the milk that is filled into them would be raised unduly in temperature.

Section K. General summary and conclusions

1. A routine survey of milk bottle-washing plants was carried out in Greater London between October 1937 and July 1939. Altogether 105 plants belonging to 26 different types were examined, 204 visits were paid, and 2406 bottles were taken for sampling.

2. For convenience the plants are grouped into (*a*) large plants of the straight-through and come-back type; (*b*) small rotary plants; (*c*) plants employing steam sterilization in a cabinet after washing by hand or mechanical means and (*d*) plants using hand-washing alone.

3. The mean counts at 22 and 37° C. respectively of all bottles, reckoned as pints, washed in large plants were 2008 and 2324; in small rotary plants 7982 and 7389; in plants

using steam sterilization 23,671 and 9,704; and in plants employing hand-washing only 96,065 and 37,818.

4. Taking all large plants together 55·5 % of the bottles had less than 600 colonies at 22 or 37° C., and only 1·1 % had more than 25,000 colonies. The corresponding figures for the small rotary plants were 49 and 6·4 %; for the steam sterilization plants 50 and 23·5 %; and for plants employing hand-washing only 0 and 83 %.

5. A comparison of plants relying wholly or mainly on soaking in the detergent section with those relying exclusively on spray from jets showed that, though good results might be obtained by either method, the spray types of plant in common use in London gave on the average very much better results than the soaker types of plant.

6. The general conclusion is reached that equally good results may be obtained in large or in small rotary plants relying on sterilization by a caustic detergent as in plants employing steam sterilization, and that the uniformly best results are obtained in properly designed and operated plants of the spray type.

7. From a study of the process of disinfection by caustic soda it is concluded that the two main factors responsible for the destruction of bacteria are the concentration and the temperature of the detergent solution.

8. Virtual sterility can be achieved either by the use of relatively strong caustic soda solutions at a low temperature or relatively weak caustic soda solutions at a high temperature.

9. Based on careful experimental observations in the laboratory on the reaction velocity, the concentration coefficient, and the temperature coefficient of caustic soda, Table 10 (p. 106) is presented setting out the concentrations of this substance necessary at different temperatures to bring about a given degree of sterilization within given times. Such concentrations operating at the temperatures stated should afford a fairly wide margin of safety for the destruction of all vegetative and pathogenic organisms.

10. The use of substances such as sodium metasilicate, trisodium phosphate, sodium carbonate and sodium hexametaphosphate to increase the wetting, softening, emulsifying and deflocculating powers of the caustic soda and so help in the removal of micro-organisms from the bottle is to be strongly recommended, but these substances must be used in addition to the basic concentration of caustic soda, and not as substitutes for it.

11. Bottles emerging from the detergent section of properly operated plants are virtually sterile. In nearly all types of plant, however, they become recontaminated during the subsequent process of rinsing. This constitutes one of the major problems of bottle-washing.

12. Contamination is due mainly to the use of warm recirculated rinse water, which constitutes a favourable medium for bacterial growth.

13. There seem to be two methods by which this contamination may be avoided. (*a*) Bacterial growth in the rinse water may be checked by the addition of chlorine or one of its compounds. This solution of the problem, which is widely favoured in the United States and has advocates in this country, is carefully discussed, and the balance of evidence in our opinion is against it. To be effective it has to be carried out with a degree of care and supervision that is unusual in bottle-washing plants, and its uncontrolled use is likely to degenerate into an excuse for dirtiness. (*b*) Warm recirculated rinse water for the internal spraying of bottles may be replaced by a *hot* spray, at a temperature too high for bacterial growth, in order to remove the detergent. The bottle can then be cooled

down by *external* rinses of recirculated water progressively decreasing in temperature, and finally rinsed and cooled internally and externally by a cold spray directly from the main supply. In this way none of the recirculated and therefore contaminated rinse water should enter the bottle, and the final bottle should emerge as sterile from the rinse as from the detergent section.

14. A minor, but sometimes not unimportant, source of contamination is due to the growth of bacteria in the jets and manifolds incorporated in the rinse section. Our observations on this point could not be carried as far as we would have wished, but there seems to be no doubt that under favourable conditions, which have still to be defined, gelatinous masses of bacterial zoogloea may become built up on the jets and be discharged intermittently into the bottles. Frequent cleaning and sterilization of the jets and manifolds are required to prevent this occurrence.

15. It is pointed out that many types of softening plant serve to contaminate the water, and thus prevent a satisfactory bottle from being delivered.

16. An adequate drainage period after the last rinse is desirable to allow the greater part of the water remaining in the bottle to flow away.

17. Contamination of the bottle from carriers and from the discharge mechanism during the process of delivery must be guarded against.

18. The temperature of the bottle on discharge should not exceed 68° F., if the bottles are to be filled at once; otherwise the temperature of the milk is raised to an undesirable extent.

19. If the bottles are not to be filled immediately they should be stored inverted in clean crates or drainage racks, in a cool, dry, well-ventilated room. Storage in a warm, moist atmosphere leads to considerable bacterial multiplication. On no account should the bottles be stored upright.

20. Experimental observations showed that imperfectly cleansed bottles led to deterioration of the milk filled into them, and that the life of the milk was significantly shortened.

21. A review of the literature has disclosed the occurrence of numerous outbreaks of infectious disease, particularly enteric fever, attributed to bottles coming from infected houses and distributed again without adequate sterilization.

22. It is pointed out that milk may also be contaminated from human cases and carriers of infectious disease in the dairy who handle the bottles during the filling, capping, or distributing processes.

23. To guard against the danger of bottles coming from infected houses proper cleaning and sterilization are necessary in the dairy.

24. To guard against infection of bottles from cases or carriers in the dairy, apart from control of the human personnel, much can be done by the use of strictly automatic filling and capping machinery and of suitably designed containers. The ordinary wide-mouthed bottle with a press-in disk cap is peculiarly susceptible to contamination from human and animal sources, and should not be allowed unless fitted with a hooded cap. In its place there may be used either (*a*) a narrow-necked bottle so designed as to minimize the contact of milk with the outside of the neck during the operation of pouring, and closed with a deep press-over aluminium cap covering the rim, or (*b*) a single-service paper board container.

25. Many washing machines at present in use have been designed by engineers and are operated by technicians without any strict regard for bacteriological principles. The

main bacteriological principles governing the design and operation of bottle-washing plants are therefore laid down and discussed in some detail (pp. 113–116).

26. Considerable attention was paid at the beginning of this investigation to the most satisfactory method of ascertaining the degree of bacterial cleanliness of washed bottles. As a result a method was elaborated, which is recommended for use in practice. Briefly it consists in sampling twelve bottles, adding 20 or 30 ml. of diluent solution to each bottle according to the size, rinsing thoroughly, plating two 5 ml. quantities of the rinse water, incubating one plate at 22° C. and the other at 37° C., counting the colonies after 3 days, and calculating the bottle count by multiplying the plate count by four or six as the case may be.

27. In reporting the bottle count it is suggested that the 22 and 37° C. counts on each bottle should be averaged, and the mean counts for each of the twelve bottles recorded separately. The average of the twelve bottle counts should then be taken. If this final mean, which is based on twenty-four plate counts, does not exceed 600 per bottle, reckoned as pints, the bottles shall be regarded as conforming to a satisfactory standard of cleanliness.

In conclusion we should like to express our thanks to all those who have helped us in this investigation. We would particularly mention Dr J. M. Hamill, late of the Ministry of Health, at whose request the inquiry was begun; Dr W. A. Lethem, of the Ministry of Health, whose previous confidential report to the Ministry proved of great value; Dr A. T. R. Mattick of the National Institute for Research in Dairying, Reading, and Mr E. B. Anderson of the Central Laboratory, United Dairies, Ltd., with whose general co-operation the work was undertaken and who kindly provided us with certain data for analysis; Dr A. Bradford Hill, Mr Russell, Mr Cheeseman and Mr Martin of the Statistical Department, London School of Hygiene and Tropical Medicine, all of whom assisted us in various ways in the treatment and computation of the extensive data collected; Dr E. B. Hendry for help and advice in the titration of detergent solutions; and Miss Irene Maier, whose technical assistance in certain parts of the investigation was greatly appreciated. To the Medical Officers of Health, Sanitary Inspectors and Dairy Managers, too numerous to mention by name, who went often to very considerable trouble to enable us to gain the information we wanted, we tender our sincere thanks.

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