

**THE EFFECT OF SODIUM THIOSULPHATE ON THE  
COLIFORM AND *BACTERIUM COLI* COUNTS OF  
NON-CHLORINATED WATER SAMPLES**

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For the sampling of chlorinated water for bacteriological examination it has long been established practice to use sterile bottles containing sufficient sodium thiosulphate to neutralize any residual chlorine in the sample. The thiosulphate must be placed in the bottle before sterilization. Some bacteriological laboratories prepare two sets of water sampling bottles, with thiosulphate for chlorinated water and without thiosulphate for non-chlorinated water. Other laboratories add thiosulphate to all sampling bottles on the assumption that where there is no need for thiosulphate its presence would have no effect on the bacteriological results. The present investigation was undertaken to determine whether or not the presence of thiosulphate in samples of non-chlorinated water would exert any noticeable effect upon the coliform and faecal *coli* counts in the time which normally elapses between collection and examination.

The requisite amount of sodium thiosulphate to be added has been variously recommended as 'one or two small crystals' (Report, 1939), 'a minute crystal' (Thresh, Beale & Suckling, 1943), 'a minute crystal or a drop of a saturated solution' (Taylor, 1949), and 'from 0.02 to 0.05 gramme' (Report, 1946). These amounts are, in fact, much greater than is necessary to neutralize all the residual chlorine to be expected in a water sampling bottle of the usual size. Titration experiments have shown that 0.1 ml. of a 2.5% solution of sodium thiosulphate ( $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ ) will neutralize the chlorine in about 170 ml. of filtered water containing up to 5 p.p.m. of residual chlorine. For the ordinary sampling bottle of capacity up to 6 oz. (170 ml.), therefore, 0.1 ml. of a 3% solution of crystalline sodium thiosulphate should be sufficient to neutralize the chlorine in all samples, including those from most swimming baths. This dosage was chosen for the experiments to be described, 0.4 ml. of the 3% solution being added to a 24 oz. bottle before sterilization.

Previous investigations by means of a 70-tube method have shown that changes may occur in the coliform and faecal *coli* counts of water samples stored without sodium thiosulphate at room or refrigerator temperature for 24 hr. (Report, 1952). Even when the period of storage is limited to 6 hr., similar changes may still occur, though less frequently; fewer changes occur in samples stored at refrigerator

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temperature than in those stored at room temperature (Report, 1953). In the present investigation, therefore, it was decided to store samples both with and without sodium thiosulphate, in order to determine whether the presence of this substance modified in any way the changes in coliform and faecal *coli* content to be expected in its absence. The conditions of storage chosen were 6 hr. in the refrigerator.

#### METHODS

Samples of unchlorinated water were collected from rivers, reservoirs, lakes, springs and wells in the following counties: Berkshire, Caernarvonshire, Cheshire, Denbighshire, Lancashire, Monmouthshire and Oxfordshire. The samples were examined at five laboratories—Birkenhead, Conway, Manchester, Newport (Mon.) and Oxford.

The methods of collecting and examining the samples were substantially those already described (Report, 1952). Each sample was collected in a sterile Winchester bottle which contained no sodium thiosulphate and which was conveyed to the laboratory without delay.

On arrival at the laboratory, within 1 hr. of sampling, the Winchester was inverted several times and a portion of the sample, approximately one-tenth, discarded. From the Winchester, after restoppering and vigorous shaking, three similar sterile 24 oz. bottles, labelled A, B and C, were filled completely. Bottle C differed from the others in that it contained 0.4 ml. of a 3% solution of Analar quality sodium thiosulphate,  $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ , added before sterilization of the bottle. Bottle A was examined immediately; bottles B and C were stored in the refrigerator (2–5° C.) for 6 hr. and then examined.

#### *Technique of examination*

Ten tubes were inoculated with each of seven twofold diminishing volumes of the water sample. In accordance with the technical procedure described in Report No. 71 (1939), volumes of 32, 16, 8 and 4 ml. of the samples were pipetted into tubes containing the same volumes of double-strength MacConkey broth; volumes of 2 ml. and less into tubes containing 5 ml. single-strength MacConkey broth. Sample volumes of less than 1 ml. were obtained by dilution of the water samples with sterile quarter-strength Ringer solution.

Inoculated tubes were incubated at 37° C. and were examined after 24 hr. Those showing acid and gas production were subcultured and incubated at 44° C. to test for the presence of faecal *coli*. Tubes not producing acid and gas at 37° C. in 24 hr. were incubated for a further period of 24 hr. and if at the end of that period acid and gas were produced these tubes were then subcultured and incubated at 44° C.

#### *Methods of statistical analysis*

The total number of tubes with a positive reaction, out of the seventy inoculated, was recorded for each examination of a sample. From this number the density of organisms could be estimated quite simply (Report, 1952, Appendix 1). For a number of reasons, statistical and practical, it is more convenient, in studying the

*Storage with thiosulphate*

After 6 hr. storage in the refrigerator 18·8% of the samples held in bottles containing thiosulphate showed a significant change in coliform content—9·4% showing an increase and 9·4% a decrease.

Table 2. *Effect of thiosulphate upon the presumptive coliform content of water samples stored for 6 hr. at refrigerator temperature*

	No. of samples	Changes of nine positive tubes or more						
		No. showing			Percentage showing			
		Increase	No change	Decrease	Increase	No change	Decrease	Increase or decrease
Without thiosulphate	64	6	48	10	9·4	75·0	15·6	25·0
With thiosulphate	64	6	52	6	9·4	81·2	9·4	18·8
		Theoretical percentage			2·9	94·2	2·9	5·8

*Comparison between storage with and without thiosulphate*

In both series the percentage of increases was the same but decreases were less frequent in the bottles containing thiosulphate. The total changes were thus rather less frequent in the samples with thiosulphate, but the difference is not statistically significant.

The figures, however, permit a much more precise examination of the effect of thiosulphate. For each sample, bottles B and C were treated identically, except that bottle C contained thiosulphate. If thiosulphate encouraged the development of coliform organisms, the total of positive tubes from the examination of each bottle C would tend to be greater than the total for the corresponding bottle B, and conversely. The average difference between the totals of tubes positive for coliform organisms after storage with and without thiosulphate was  $-0.72$  tubes with a standard error of  $0.74$ , and so is of no statistical significance. Thus the behaviour of the coliform count of samples after 6 hr. storage in the refrigerator appears to be quite unaffected by the presence of thiosulphate.

*Faecal coli content*

The number of samples tested was forty-seven. The figures in Table 3 present the findings in terms of a difference of nine or more positive tubes between the sample examined within 1 hr. of collection and after 6 hr. storage at refrigerator temperature with and without thiosulphate.

*Storage without thiosulphate*

After 6 hr. storage in the refrigerator 17·0% of samples without thiosulphate showed a significant change in faecal *coli* content—4·2% showing an increase and 12·8% a decrease. These results show rather more variation than those reported elsewhere, where of 135 samples stored without thiosulphate in the refrigerator for

6 hr. 10.4 % showed a significant change in faecal *coli* content—1.5 % showing an increase and 8.9 % a decrease (Report, 1953). The difference, however, could well be due to chance.

Table 3. *Effect of thiosulphate upon the faecal coli content of water samples stored for 6 hr. at refrigerator temperature*

		Changes of nine positive tubes or more						
		No. showing			Percentage showing			
	No. of samples	Increase	No change	Decrease	Increase	No change	Decrease	Increase or decrease
Without thiosulphate	47	2	39	6	4.2	83.0	12.8	17.0
With thiosulphate	47	1	40	6	2.1	85.1	12.8	14.9
		Theoretical percentage			2.9	94.2	2.9	5.8

#### *Storage with thiosulphate*

After 6 hr. storage in the refrigerator 14.9 % of the samples with thiosulphate showed a significant change in faecal *coli* content—2.1 % showing an increase and 12.8 % a decrease.

#### *Comparison between storage with and without thiosulphate*

In both series the percentage of decreases was the same but increases were less frequent in samples with thiosulphate. The total changes were thus slightly less frequent in the samples with thiosulphate. More detailed statistical analysis shows that the average difference between the totals of tubes positive for faecal *coli* after storage with and without thiosulphate was +0.87 tubes, with a standard error of 0.52. This difference is therefore no more than a chance fluctuation. Thus the behaviour of the faecal *coli* count of samples after 6 hr. storage in the refrigerator also appears to be quite unaffected by the presence of thiosulphate.

#### SUMMARY

A quantity of 0.1 ml. of a 3 % solution of crystalline sodium thiosulphate in a 6 oz. bottle is sufficient to neutralize up to 5 p.p.m. of residual chlorine; the use of large quantities of thiosulphate is unnecessary, except possibly for some swimming-bath samples. The investigation has shown that this amount of thiosulphate has no significant effect on the coliform or faecal *coli* content of non-chlorinated water samples during 6 hr. storage at refrigerator temperature. There therefore appears to be no disadvantage in adding this amount of thiosulphate to all water sampling bottles.

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