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Membrane filtration media for the enumeration of coliform organisms and *Escherichia coli* in water: comparison of Tergitol 7 and lauryl sulphate with Teepol 610

By a Joint Committee* of the Public Health Laboratory Service and the Standing Committee of Analysts†

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SUMMARY

In a multi-laboratory trial with the membrane filtration technique, three surfactants – Teepol 610 (T610), Tergitol 7 (T7) and sodium lauryl sulphate (LS) – were compared in media for the enumeration of coliform organisms and *Escherichia coli* in water. A total of 179 samples of water (87 raw and 92 marginally chlorinated) were examined for colony counts of coliform organisms, and 185 water samples (94 raw and 91 marginally chlorinated) for *E. coli*. Slight differences in the confirmed colony counts between the three media were noted, but few of these were observed consistently in every laboratory. In most laboratories, T7 gave slightly higher counts of *E. coli* than LS with chlorinated waters; a higher incidence of false-positive results for *E. coli* at 44 °C was also noted with T7. As there were no outstanding differences in the trial, sodium lauryl sulphate, which is chemically defined, cheap and readily available, is therefore recommended for use at a concentration of 0·1 % instead of Teepol 610 in the standard medium for the enumeration of coliform organisms and *E. coli* in water by the membrane filtration technique.

INTRODUCTION

The membrane filtration technique is now used as a routine in many laboratories in the United Kingdom for the enumeration of *Escherichia coli* and other coliform organisms in water. The medium currently recommended for this purpose in Report No. 71 on 'The Bacteriological Examination of Water Supplies' is membrane enriched teepol broth (Report, 1969). However, production of Teepol 610,

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the particular blend of surfactant used in this medium, ceased in 1976 and supplies are now limited. A suitable alternative surfactant is therefore essential. Following preliminary work with a number of such agents, Tergitol 7 and lauryl sulphate – both widely used elsewhere for water examination – were chosen for comparison with Teepol 610 in a multi-laboratory trial. The results of this work are described in this paper.

MATERIALS AND METHODS

Media

Comparative media trials were designed in which Tergitol 7 or sodium lauryl sulphate replaced Teepol 610 in the standard medium – 0·4 % membrane enriched teepol broth (Oxoid MM360) – described in Report No. 71 (1969). The three media, T7, LS and T610 were based on the dehydrated portion of membrane enriched teepol broth (Oxoid MM360). The LS medium was specially prepared by Oxoid Ltd, with 0·1 % of pure lauryl sulphate already included. The other two media were made by adding either Teepol 610 or Tergitol 7 during reconstitution, according to the method recommended by the manufacturers for MM360. Each of these surfactants was distributed from a single batch to 10 participating laboratories. Teepol 610 contains 34 % of the active constituents – secondary alkyl sulphates – and Tergitol 25 %; these two media were therefore treated similarly to give a concentration of 0·4 % of these two surfactants in the final medium. Sodium lauryl sulphate however normally contains 95–99 % of active constituent. For this trial, a specially pure form of sodium lauryl sulphate (BDH No. 44244) containing 99 % active constituents was incorporated in the bulk dehydrated medium to give a final concentration of 0·1 %. The participating laboratories made up 250 ml of each medium and dispensed them in aliquots of 50 ml in screw-capped bottles; these were sterilized by steaming for 30 min on 3 consecutive days and then incubated at 37°C for 24 h and checked visually to exclude contamination. They were then kept at room temperature in the dark. Bottles opened for use were discarded after 1 month.

Membrane filters and support pads

Cellulose acetate membranes (Oxoid) 47 mm in diameter with a pore size of 0·45 μm were obtained in two large batches. Each membrane was pre-sterilized and individually packed. Supplies of these membrane filters, together with sterilized absorbent pads (Whatman No. 17), were distributed to each participating laboratory.

Water samples

Samples of raw or partly treated water, mostly from surface sources, were examined by all laboratories; when necessary, these were diluted after preliminary screening so as to yield a count of approximately 25 yellow colonies on the membranes. Samples of water chlorinated in the laboratory were also used; these were prepared experimentally from raw waters by the marginal chlorination method

used in previous comparative media trials (PHLS, 1968) and more recently in a comparison between minerals-modified glutamate medium and lauryl tryptose lactose broth for the enumeration of coliform organisms by the multiple tube method (PHLS/SCA, 1980). This was based on chlorination in the presence of excess ammonia at 4°C for a time sufficient to allow the survival of some coliform organisms including *E. coli*. Organisms stressed in this way are considered to be useful for accentuating differences between media.

Incubation

All membranes were incubated for 4 h at 30°C followed either by 14 h at 35 or 37°C for coliform organisms, or by 14 h at 44°C for *E. coli*.

Colony counts and confirmatory tests

Where practicable, all yellow colonies on membranes at 35 or 37°C were counted, and then subcultured into tubes of lactose peptone water for incubation at 37°C. The tubes were examined for the presence of acid and gas at 24 h, and if necessary after 48 h. In addition, they were subcultured for colonial morphology and purity after about 6 h onto plates of MacConkey and nutrient agars for incubation overnight at 37°C. Since some species of *Aeromonas* also produce acid from lactose, thus giving yellow colonies on membranes with Teepol broth and red colonies on MacConkey agar, the oxidase test was essential for excluding them; it was performed on colonies from nutrient agar. Yellow colonies on membranes were confirmed as coliform organisms if acid and gas were produced in lactose peptone water (LPW), the oxidase test was negative and typical red colonies were formed on MacConkey agar.

Yellow colonies on membranes at 44°C were also counted and subcultured into lactose peptone water for acid and gas production at 44°C, and into tryptone water for indole production at 44°C. When the work load permitted, subcultures were also made from LPW onto nutrient agar for the oxidase test, and to MacConkey agar for colonial morphology and purity. Yellow colonies on these membranes were regarded as *E. coli* if acid and gas were produced in LPW at 44°C, if the indole reaction was positive in TW after the addition of Kovac's reagent (Cowan, 1974) and the oxidase reaction was negative.

If yellow colonies on membranes were numerous, subcultures were made from a half, quarter or other specific sector of the membrane. All yellow colonies from such sectors were counted, thus eliminating possible bias due to colony selection. Membranes overcrowded with colonies were rejected. The approximate numbers of pink, non-acid producing colonies on membranes were also recorded, since they can interfere with the growth and development of yellow coliform colonies.

RESULTS

The membrane counts of confirmed coliform organisms and *E. coli* obtained by all the laboratories with the three media under review – Tergitol 7 broth (T7), lauryl sulphate broth (LS) and Teepol broth (T610) – have been analysed

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statistically. These results represent coliform counts from 179 water samples (87 raw and 92 chlorinated) and *E. coli* counts from 185 water samples (94 raw and 91 chlorinated). For the results to be acceptable, the numbers of yellow colonies on the membranes ranged from 0 to approximately 100.

Comparison of T7 and LS with T610

Tables 1 and 2 summarize the findings from different laboratories in terms of which media gave the higher confirmed colony count. Some of the laboratories examined only small numbers of samples and their results have therefore been combined. In these tables, the results between T610 and T7 are compared in Section I, and between T610 and LS in Section II.

The combined results for all laboratories (Table 1) indicate that, for coliform organisms in raw water, T610 tended to give higher counts than T7 or LS, but in chlorinated waters, T7 and LS gave higher counts more often than T610. The results from individual laboratories however did not reveal a consistent trend.

Table 2 shows the colony counts for confirmed *E. coli* on membranes. The overall results show no significant difference between pairs of media, except that T610 gave higher counts than LS with chlorinated waters more often than it gave lower counts. This finding however was mainly influenced by the results from one laboratory. The same laboratory also found that T610 gave higher counts than T7 with chlorinated waters, but other laboratories found the reverse – higher counts with T7 – and the differences thus cancelled each other out in the combined results.

Comparison between T7 and LS

The confirmed coliform results are summarized in Section III of Table 1. These show that the number of samples in which T7 gave higher colony counts on membranes than LS is very nearly the same as for those in which LS gave higher counts than T7 (36:38). No individual laboratory found a significant difference between the two media; with chlorinated waters especially, the results were uniform.

The confirmed *E. coli* results are summarized in Section III of Table 2. There is some evidence that T7 gave higher colony counts than LS more often than it gave lower counts, especially with chlorinated waters. But this difference between the media was not apparent in every participating laboratory.

The actual results of confirmed coliform and *E. coli* colony counts are shown in Figs. 1 and 2. For clarity, the counts are plotted on a logarithmic scale with zero readings on the axes. The diagonal lines on the graphs represent equal counts with the two media shown. There is some scatter about these lines throughout the range of counts, indicating that comparison between the two media is the same regardless of how many organisms were present. The scatter of results is somewhat greater for chlorinated than for raw water samples.

Although Table 2, Section III shows that T7 gave more *E. coli* counts which were higher than those given by LS than vice versa, Figs. 1 and 2 show that the concentration of points below the diagonal lines (i.e. samples where the T7 count exceeded the LS count) are not strikingly different from those above the lines.

Table 1. *Comparison of membrane filtration media: counts of confirmed coliform organisms*

Type of water sample	Laboratory	I T610 compared with T7				II T610 compared with LS				III T7 compared with LS			
		T610 >T7	T610 =T7	T610 <T7	Total no. of samples tested	T610 >LS	T610 =LS	T610 <LS	Total no. of samples tested	T7 >LS	T7= LS	T7 <LS	Total no. of samples tested
Raw	Thames	15	4	12	31	15	5	11	31	15	6	10	31
	Truro	5	—	5	10	4	—	6	10	3	—	7	10
	Gloucester	8	2	2	12	9	—	3	12	2	2	8	12
	Medmenham	4	1	9	14	3	5	6	14	8	3	3	14
	Others	11	2	7	20	11	2	7	20	8	2	10	20
	Total	43	9	35	87	42	12	33	87	36	13	38	87
Chlorinated	Thames	14	4	13	31	16	1	14	31	13	5	13	31
	Truro	8	2	17	27	7	4	16	27	12	2	13	27
	Medmenham	5	3	8	16	6	4	6	16	7	2	7	16
	Others	6	4	8	18	4	3	11	18	6	5	7	18
	Total	33	13	46	92	33	12	47	92	38	14	40	92

T610, membrane broth with Teepol 610; T7, membrane broth with Tergitol 7; LS, membrane broth with sodium lauryl sulphate.

Table 2. *Comparison of membrane filtration media: counts of confirmed* E. coli

Type of water sample	Laboratory	I T610 compared with T7				II T610 compared with LS				III T7 compared with LS			
		T610 >T7	T610 =T7	T610 <T7	Total no. of samples tested	T610 >LS	T610 =LS	T610 <LS	Total no. of samples tested	T7 >LS	T7 =LS	T7 <LS	Total no. of samples tested
Raw	Thames	14	7	18	39	21	1	17	39	23	2	14	39
	Truro	5	2	7	14	7	—	7	14	5	—	9	14
	Gloucester	7	2	5	14	7	2	5	14	7	3	4	14
	Medmenham	4	2	8	14	5	1	8	14	7	—	7	14
	Others	8	—	5	13	3	5	5	13	6	—	7	13
	Total	38	13	43	94	43	9	42	94	48	5	41	94
Chlorinated	Thames	21	2	10	33	21	3	9	33	21	4	8	33
	Truro	6	3	14	23	9	5	9	23	13	2	8	23
	Medmenham	7	1	9	17	9	1	7	17	8	1	8	17
	Others	7	1	10	18	10	2	6	18	8	6	4	18
	Total	41	7	43	91	49	11	31	91	50	13	28	91

T610, membrane broth with Teepol 610; T7, membrane broth with Tergitol 7; LS, membrane broth with sodium lauryl sulphate.

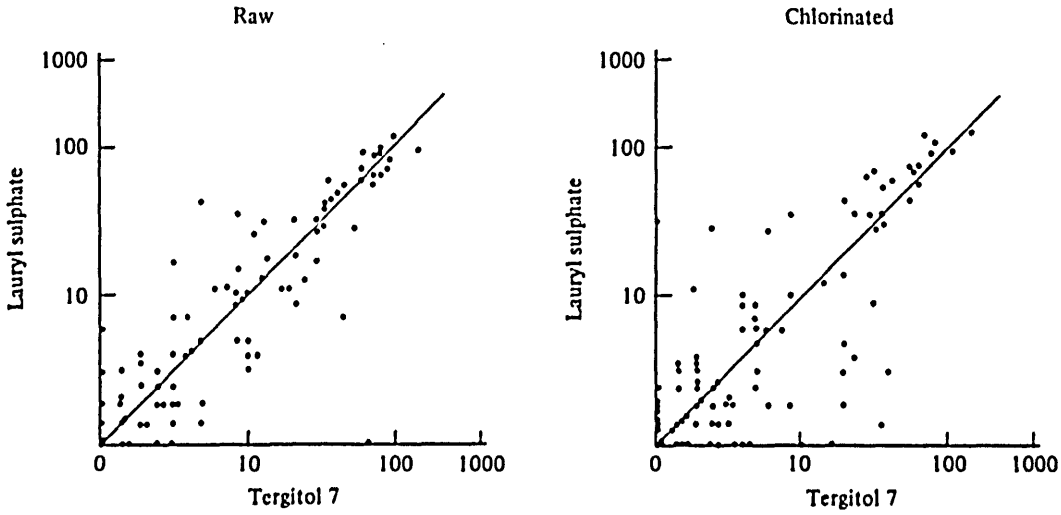

Fig. 1. Confirmed coliform results for T7 and LS membrane filtration media with raw and chlorinated waters.

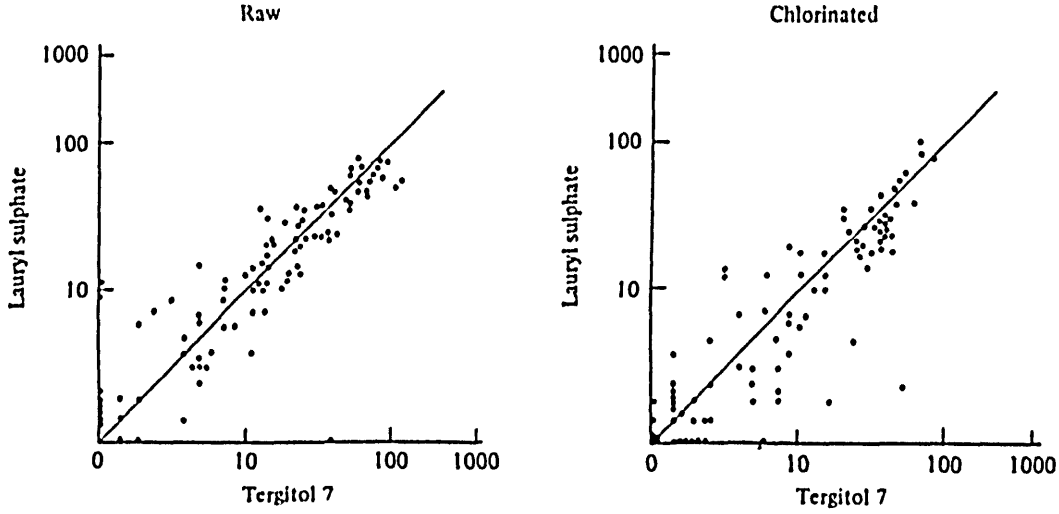

Fig. 2. Confirmed *E. coli* results for T7 and LS membrane filtration media with raw and chlorinated waters.

Coliform counts at 35 and 37 °C

Seven laboratories incubated membranes for coliform organisms at 37 °C and three laboratories at 35 °C. There was no difference between these two sets of laboratories in their conclusions about the comparison between T7 and LS. One laboratory examined duplicate sets of samples at both temperatures. Again, there was no significant difference between the membrane counts at either temperature; however, only 10 raw and 10 chlorinated samples were used and these all came from one source.

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Table 3. *Water samples yielding false-negative results* with* T610, T7 *and* LS

Temperature of incubation	Type of water sample	Total no. of samples tested	False negative results		
			T610	T7	LS
35/37°C	Raw	87	0	6	7
	Chlorinated	92	19	11	13
44°C	Raw	94	3	8	3
	Chlorinated	91	0	0	14

* False-negative water results are defined as those in which a water sample gave either (i) no yellow colonies, or (ii) only yellow colonies which did not confirm as coliform organisms or *E. coli* when one or both of the other media gave yellow colonies which did confirm.

T610, membrane broth with Teepol 610; T7, membrane broth with Tergitol 7; LS, membrane broth with sodium lauryl sulphate.

Water samples giving false-negative results

Table 3 shows the false-negative results given by the three media. A false-negative result for a water sample with one medium means that it gave a nil count when one or both of the other media yielded confirmed coliform organisms. A nil count was recorded either when no yellow colonies developed on the membrane, or when there were only yellow colonies which could not be confirmed as coliform organisms. No significant differences in false-negative rates were found between the three media, although the incidence of false-negative results tended to be higher with chlorinated than with raw water samples.

False-positive membrane colony results

These are defined as yellow colonies on membranes which failed to confirm as coliform organisms at 35° or 37° or as *E. coli* at 44°C. The rate of such false-positive membrane results varied considerably between laboratories. For example, at one laboratory the incidence of false-positive results among coliform counts averaged 85% whereas in another laboratory it was only 6%. The results for all laboratories for each medium are shown in Table 4. For raw waters, no laboratory found any difference between the rate of false-positive results both for coliform organisms and *E. coli* with any medium. With chlorinated waters, however, every laboratory obtained a slightly higher rate of false-positive results at 44°C with T7 than with LS, thus making the combined results significant with Cochran's statistical test. For coliform organisms, the overall rate of false-positive results was higher with T7 – but this was due mainly to results from one laboratory; all other laboratories found little or no difference so that, applying Cochran's test to the combined results, there was no significant difference between these two media. False-positive rates with T610 were usually intermediate between T7 and LS.

Table 4. *False-positive results with T610, T7 and LS*

Organisms sought	Type of water sample	Teepol 610		Tergitol 7		Lauryl sulphate	
		Total colonies	No. not confirmed (false-positive)	Total colonies	No. not confirmed (false-positive)	Total colonies	No. not confirmed (false-positive)
Coliform organisms	Raw	3223	1230 (38·2%)	3113	1194 (38·4%)	2922	1130 (38·7%)
	Chlorinated	2367	1118 (47·2%)	2733	1312 (48·0%)	2580	972 (37·7%)
E. coli	Raw	2367	313 (13·2%)	2461	332 (13·5%)	2227	288 (12·9%)
	Chlorinated	1562	259 (16·6%)	1697	318 (18·7%)	1459	189 (13·0%)

T610, membrane broth with Teepol 610; T7, membrane broth with Tergitol 7; LS, membrane broth with sodium lauryl sulphate.

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DISCUSSION

Almost from the start of the trial, it seemed that there were no outstanding differences in the performance of the three media for the enumeration of coliform organisms or *E. coli* on membranes. With regard to coliform organisms, T610 tended to give higher membrane colony counts than T7 or LS with raw waters, whilst with marginally chlorinated waters, T7 and LS tended to give higher counts than T610. There was excellent agreement between the counts obtained on T7 and LS with chlorinated waters. For *E. coli* in raw waters, there was little to choose between the three media, although T7 tended to give slightly higher counts. With chlorinated waters, T7 again tended to give the higher counts, although one laboratory found considerably higher counts with T610; in all laboratories, LS consistently gave the lowest counts. Statistical analysis of the observed differences between the three media did not, however, reveal any clear-cut conclusion. The choice between LS and T7 as a replacement for T610 in membrane enriched teepol broth (Report, 1969) therefore depended on other factors.

The rate of false-negative results – either (i) no yellow colonies or (ii) yellow colonies none of which could be confirmed as either coliform organisms or *E. coli* when these were present on one or both of the other two media – tended to be higher with chlorinated than with raw water samples, but there was no significant difference between the three media. The incidence of false-positive results – that is the proportion of yellow colonies on membranes which did not confirm as coliform organisms at 35/37°C or as *E. coli* at 44°C – varied greatly between laboratories, probably reflecting the different bacterial content of the various water samples examined. However, when the combined results for raw water samples from all laboratories were analysed, there was no significant difference in the incidence of yellow colonies which failed to confirm from the three media. But with chlorinated water samples at 44°C, the incidence of colonies which failed to confirm as *E. coli* was significantly higher with T7 than with LS.

Both T610 and T7 are mixtures of surfactants controlled by specified manufacturing processes; the commercial production of T610 ceased in 1976, and if T7 were to be recommended as an alternative to T610, a similar problem might arise if this too were discontinued. On the other hand, specially pure LS is chemically defined, cheap and can be obtained as a powder which contains 99% of active constituents. It can thus be incorporated in a dehydrated medium. In contrast, both T610 and T7 have to be added separately to the media during reconstitution. Moreover T7 broth is usually turbid which could lead to batches of medium being discarded in error because they appeared contaminated.

In some laboratories colonial differentiation, especially the acid reaction, was considered to be much better with LS than with T7. Two laboratories also noted that, on incubation for coliform organisms, spreading of the colonies on membranes occurred less with LS than with T7. Although there were no outstanding differences between the numbers of pink, non-acid-producing colonies which grew on membranes with the three media, some laboratories noted fewer of such interfering colonies with LS.

Although the medium containing LS tended to give slightly lower counts of *E. coli* in chlorinated waters, this is not regarded as a great disadvantage, as there is in practice simultaneous examination for coliform organisms, and LS was found to be as successful as T7 for the isolation of these organisms in chlorinated waters. For all these reasons, and especially since it is cheap, readily available, chemically defined and can be incorporated directly in media before dehydration, lauryl sulphate (0·1 %) is recommended as a substitute for T610 in membrane enriched broth for the enumeration of coliform organisms and *E. coli* in water by the membrane filtration technique. However, T7 (0·4 %) also gives satisfactory results, and if necessary, it may be used as an alternative.

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