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Comparison of membrane filtration and multiple tube methods for the enumeration of coliform organisms in water

BY THE PUBLIC HEALTH LABORATORY SERVICE STANDING COMMITTEE ON THE BACTERIOLOGICAL EXAMINATION OF WATER SUPPLIES*

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SUMMARY

The membrane methods described in Report 71 on the bacteriological examination of water supplies (Report, 1969) for the enumeration of coliform organisms and *Escherichia coli* in waters, together with a glutamate membrane method, were compared with the glutamate multiple tube method recommended in Report 71 and an incubation procedure similar to that used for membranes with the first 4 hr. at 30° C., and with MacConkey broth in multiple tubes. Although there were some differences between individual laboratories, the combined results from all participating laboratories showed that standard and extended membrane methods gave significantly higher results than the glutamate tube method for coliform organisms in both chlorinated and unchlorinated waters, but significantly lower results for *Esch. coli* with chlorinated waters and equivocal results with unchlorinated waters. Extended membranes gave higher results than glutamate tubes in larger proportions of samples than did standard membranes. Although transport membranes did not do so well as standard membrane methods, the results were usually in agreement with glutamate tubes except for *Esch. coli* in chlorinated waters. The glutamate membranes were unsatisfactory. Preliminary incubation of glutamate at 30° C. made little difference to the results.

INTRODUCTION

In the fourth edition of Report 71 on 'The bacteriological examination of water supplies' (Report, 1969), membrane filtration methods are recommended as alternatives to multiple tube methods for the enumeration of coliform organisms and

* The P.H.L.S. Standing Committee on the Bacteriological Examination of Water Supplies is composed of the following members of the P.H.L.S. Staff: Dr W. H. H. Jebb (Oxford), Chairman (until 30 September 1971); Dr B. Moore (Exeter), Chairman (from 1 October 1971); Dr L. A. Little (Wakefield), Secretary; Dr G. I. Barrow (Truro); Dr R. D. Gray (Newport); Dr J. E. Jameson (Brighton); Dr J. H. McCoy (Hull); Dr R. Pilsworth (Chelmsford); Dr J. A. Rycroft (Southend); Dr A. J. Kingsley Smith (Conway); Mrs H. E. Tillett (Colindale), Statistician (from 17 July 1971); Miss J. M. Watkinson (Manchester); together with Dr R. G. Allen, Water Research Association, for whom Mr R. W. Collingwood acted; Dr F. W. Bunting, Society of Medical Officers of Health; Dr N. P. Burman, Metropolitan Water Board; Dr G. U. Houghton, South Essex Waterworks company (until 26 November 1971); Dr A. E. Martin, Ministry of Health; Dr E. Windle Taylor, Metropolitan Water Board.

Requests for reprints should be addressed to Dr L. A. Little, Public Health Laboratory, Wood Street, Wakefield.

Escherichia coli in water. The recommendations were based on extensive work from 1953 to 1969 carried out at the Metropolitan Water Board (MWB) together with the experience of other laboratories where these methods have been adopted (Windle Taylor, 1953–70; Burman, 1960; Windle Taylor & Burman, 1964; Burman, 1967). The Public Health Laboratory Service Standing Committee on the Bacteriological Examination of Water Supplies therefore conducted multi-laboratory trials to compare these membrane methods with the recommended multiple tube techniques. As a result of previous multi-laboratory trials (PHLS, 1968, 1969) a glutamate medium for the multiple tube technique was recommended in the fourth edition of Report 71 in preference to MacConkey broth. The assessment of membrane culture techniques by the MWB was however carried out mainly in comparison with MacConkey broth and only later were membrane results compared with some of the earlier modifications of glutamate medium. For the purpose of the present trials therefore, the membrane techniques were compared with both MacConkey broth and glutamate medium using multiple tube methods.

The membrane filtration technique involves a preliminary incubation of two membranes for 4 hr. at 30° C. followed by 14 hr. at 35° C. for the total coliform count and 44° C. for the *Esch. coli* count. This had been shown to give higher counts, particularly of coliform organisms or attenuated organisms (Burman, 1967). Preliminary trials at the MWB (Windle Taylor, 1965–6) applying a similar incubation procedure to glutamate medium in the multiple tube method also yielded increased coliform counts without any reduction in those of *Esch. coli*. This modified multiple tube incubation technique was therefore also included in the present series of trials. Repeated attempts were made to incorporate the advantages of glutamate into the membrane filtration technique, although considerable difficulty was experienced. The best modification of glutamate media suitable for membranes devised at the MWB was included in these trials. The standard membrane technique and the two modifications of it described in Report 71 were therefore used, together with the glutamate membrane method, and compared with the three multiple tube methods for enumerating coliform organisms and *Esch. coli* in water samples. Seven laboratories in different parts of the country participated in these trials but not all of them used every method or both chlorinated and unchlorinated water.

MATERIAL AND METHODS

Membrane methods

The three basic membrane filtration techniques as described in Report 71 were used for this investigation.

These were:

(1) The standard technique with incubation of both membranes at 30° C. on 0·4 % enriched Teepol medium (0·4 ET) for 4 hr. followed by 14 hr. at 35 and 44° C. respectively.

(2) The extended technique with incubation of both membranes at 25° C. on 0·4 ET for 6 hr. followed by 18 hr. at 35 and 44° C. respectively.

(3) The transport technique with incubation of both membranes on transport medium (TM) for 24 hr. at 25° C. followed by their transfer to 0·4 ET and incubation at 35 and 44° C. respectively for a further 18 hr. In addition, a membrane glutamate medium (MG) developed at the MWB was used (Windle Taylor, 1967–8). This contained L(+)-glutamic acid sodium salt, 6·5 g.; lactose, 30 g.; L(+)-arginine monohydrochloride, 0·02 g.; L(−)-aspartic acid, 0·024 g.; L(−)-cystine, 0·02 g.; K_2HPO_4, 1 g.; NH_4Cl, 2·5 g.; $MgSO_4.7H_2O$, 0·1 g.; $CaCl_2.2H_2O$, 0·02 g.; ferric ammonium citrate, 0·05 g.; thiamin (aneurin hydrochloride), 0·001 g.; bromocresol purple (1 % ethanolic solution), 12 ml.; distilled water to 1000 ml.; pH after sterilization, 6·7; penicillin 100 μg./ml. was added immediately before use. The general method of preparation and sterilization of this medium was as described for glutamate medium in Report 71. Membranes were incubated on this medium for 4 hr. at 30° C. followed by 20 hr. at 35 and 44° C. respectively, and then a further 18 hr. at 35 and 44° C. Counts of yellow colonies were made after 24 and 42 hr. incubation.

As some of the laboratories participating in the trial did not have the equipment required for automatic temperature changes after the resuscitation periods, the temperature changes were made by hand and the 14 hr. incubation periods were extended to 18 hr. to avoid inconvenient incubation times. This was unlikely to affect the counts unless large colonies coalesced and obscured smaller colonies.

Multiple tube method

The multiple tube technique with MacConkey broth and improved formate lactose glutamate medium (IFLG) was used exactly as described in Report 71. All presumptive positive results at 18 hr. were recorded as coliform organisms but those at 24 and 48 hr. were confirmed as coliform organisms by subculture to lactose ricinoleate broth (LRB) incubated at 37° C. The presence of *Esch. coli* was confirmed by subculture of all presumptive positive tubes to LRB for gas formation and to peptone water for indole production, both incubated at 44 ± 0·25° C. Some laboratories examined an additional series in IFLG incubated at 30° C. for the first 4 hr. (IFLG (4 hr., 30°)).

Sources of media

The 0·4 ET, the IFLG and the MacConkey broth used were from single batches of Oxoid dehydrated media purchased in bulk and distributed to each laboratory where they were made up and sterilized. The membrane glutamate medium was made in one batch at the MWB for distribution. The membrane transport medium and the LRB were prepared by the methods normally in use at each laboratory.

Water samples

Unchlorinated water samples were used as available and, if necessary, were diluted so as to give some tubes with negative results among one 50 ml., five 10 ml., and five 1 ml. portions. Samples of chlorinated water were prepared from raw waters by the marginal chlorination method (PHLS, 1968). This was based on chlorination in the presence of excess ammonia at very low temperatures

Table 1. *Relation between degrees of bacterial content and bacterial counts*

Degree of bacterial content	Most probable numbers by multiple tube methods	Counts by membrane methods
1	0	0
2	1	1
3	2	2
4	3	3
5	4	4
6	5	5
7	6	6
8	7	7
9	8	8
10	9	9
11	10	10
12	11	11
13	12	12
14	13	13
15	14	14, 15
16	17	16, 17
17	18	18, 19
18	20	20–22
19	25	23–27
20	30	28–32
21	35	33–37
22	40	38–45
23	50	46–70
24	90	71–125
25	160	126–180
26	> 180	> 180

and for times sufficient to allow the survival of at least some coliform organisms detectable by one or more of the methods under investigation.

Randomization of order of examination

In order to avoid the possibility of the results being influenced by the order of setting up the samples by the various methods, each laboratory was supplied with a series of randomized letters representing the sequence in which the methods were to be used for each sample. This order was then recorded on each result sheet.

RESULTS

The most probable number of organisms (MPN) was obtained by the use of McCrady's tables as printed in appendix C, table II, of Report 71. For the 11 tubes inoculated the MPN gives one or other of 26 possible results which, for the purpose of this analysis, have been expressed as degrees of bacterial content (DBC). The counts on membranes were also transformed into the corresponding 26 degrees of bacterial content (Table 1). Most of the comparisons between the methods have been based on and expressed in these 26 degrees of bacterial content. Rough interpolation was used for the two combinations of positive tubes which are not included in the set of tables used. Degrees of bacterial content are convenient for

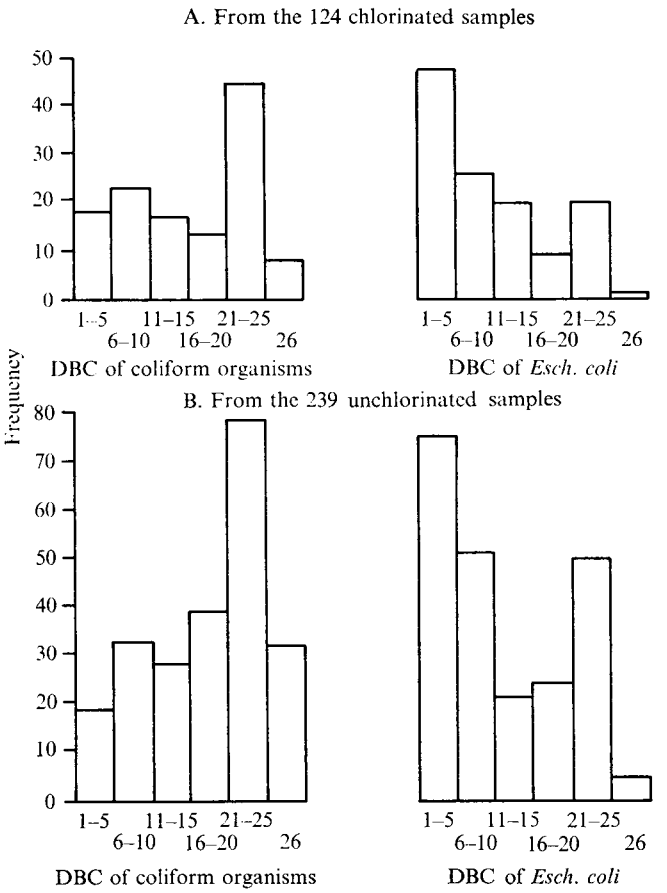

Fig. 1. Distribution of DBC of coliform organisms and *Esch. coli* as found by IFLG method.

statistical analysis and, like the actual numbers of tubes yielding acid and gas which were used in a previous trial (Report, 1969), avoid giving undue weight to high counts.

It became clear after preliminary analysis that the difference between DBC given by tube and membrane techniques depended on whether samples were of chlorinated or unchlorinated water and whether coliform organisms or *Esch. coli* were sought. The type of sample and bacterium sought were therefore analysed separately.

As was to be expected the DBC of samples from widely varying sources did not follow any regular distribution (Fig. 1), nor were DBC results from any single laboratory regular in their distribution. Comparisons were therefore based on the more normally distributed differences between DBC of paired results from two methods applied to the same sample. For each water sample the difference between the DBC given by IFLG and the other method under comparison was calculated and these differences were averaged for all samples. If this average difference is significantly greater or less than zero, the two methods can be said to give different results.

Table 2. *Chlorinated water samples; differences of DBC for paired multiple tube methods*

Methods of testing	Laboratory	Coliform organisms: No. of samples	Coliform organisms: Mean difference of DBC	Coliform organisms: Standard error of mean	*Esch. coli*: No. of samples	*Esch. coli*: Mean difference of DBC	*Esch. coli*: Standard error of mean
IFLG, MacConkey	Manchester	12	4·0*	1·6	12	4·1*	1·1
	MWB	14	−0·1	1·2	14	4·4*	1·5
	Newport	—	—	—	—	—	—
	Oxford	9	−2·6	2·1	9	1·4	1·2
	Wakefield	19	0·1	0·8	18	2·4*	1·0
	All laboratories	54	0·4	0·69	53	3·1*	0·62
IFLG, IFLG (4 hr., 30°)	Manchester	13	−0·4	0·9	13	1·5	1·1
	MWB	14	−2·3*	1·0	14	−0·6	0·7
	Newport	—	—	—	—	—	—
	Oxford	3	4·0	—	3	0·0	—
	Wakefield	15	−0·9	0·8	15	1·7	0·9
	All laboratories	45	−0·9	0·56	45	0·8	0·50

* Mean difference significantly greater than zero ($P < 0·05$).

Chlorinated waters

Between November 1969 and June 1971 five laboratories reported results from a total of 124 chlorinated water samples, in each of which coliform organisms and *Esch. coli* had been enumerated by at least two methods. The possible methods were MacConkey, IFLG and IFLG (4 hr., 30°) multiple tube methods and standard, extended, transport and glutamate membrane filtration methods. The last was little used.

Table 2 shows no overall significant difference between the DBC of coliform organisms obtained with IFLG and MacConkey media or IFLG and IFLG (4 hr., 30°), although on average IFLG gave slightly higher DBC than MacConkey and slightly lower than IFLG (4 hr., 30°). The DBC of *Esch. coli* with IFLG were on average very significantly higher than with MacConkey and slightly, but not significantly, higher than with IFLG (4 hr., 30°).

A comparison was made between the IFLG method and the four membrane methods in turn. Table 3 shows that for all laboratories combined, the average DBC of coliform organisms was significantly lower for IFLG than standard and extended membranes, but the difference between IFLG and transport membranes was not significant. Glutamate membrane gave significantly lower average DBC than IFLG. In contrast, the average DBC of *Esch. coli* with IFLG was significantly higher than with any of the four membrane filtration methods.

It should be noted that, although there were definite differences in average DBC between methods, the actual variation between individual samples was considerable. Fig. 2 demonstrates this variability for three pairs of methods for coliform organisms and *Esch. coli*. The proportions are shown of paired results from samples where both methods gave the same DBC; where the first method

Table 3. *Chlorinated water samples; differences of DBC for paired IFLG and membrane methods*

Methods of testing	Laboratory	Coliform organisms			*Esch. coli*		
		No. of samples	Mean difference of DBC	Standard error of mean	No. of samples	Mean difference of DBC	Standard error of mean
IFLG, standard membrane	Manchester	29	−3·8*	0·9	29	0·6	1·2
	MWB	33	−1·9	1·2	33	5·9*	1·1
	Newport	10	−0·3	1·8	10	3·5	1·6
	Oxford	20	−2·0	1·3	20	1·1	0·9
	Wakefield	32	−2·8*	0·9	31	4·5*	0·9
	All laboratories	124	−2·5*	0·52	123	3·3*	0·49
IFLG, extended membrane	Manchester	27	−3·8*	1·0	27	−0·1	1·2
	MWB	33	−4·1*	1·4	33	5·8*	1·1
	Newport	—	—	—	—	—	—
	Oxford	12	−1·5	1·8	12	1·6	1·4
	Wakefield	32	−3·3*	1·0	31	4·7*	0·9
	All laboratories	104	−3·5*	0·61	103	3·4*	0·57
IFLG, transport membrane	Manchester	29	−3·7*	1·6	27	2·0	1·6
	MWB	33	1·2	1·5	33	7·2*	1·2
	Newport	—	—	—	—	—	—
	Oxford	3	5·0	—	3	7·3	—
	Wakefield	24	−0·2	1·2	24	6·8*	1·4
	All laboratories	89	−0·7	0·85	87	5·5*	0·80
IFLG, glutamate membrane	All laboratories	27	5·4*	1·57	26	6·0*	1·31

* Mean difference significantly greater than zero ($P < 0·05$).

gave greater or smaller DBC of up to five degrees; and where the difference was six or more. These diagrams show that even where one method gave a significantly higher average DBC than a second method, the difference for some samples was in favour of the second method. For example, figure 2A (i) shows that, although IFLG gave significantly lower average DBC of coliform organisms than standard membrane, IFLG gave higher DBC in 26% of the samples. The proportion of samples where the membrane method gave a higher coliform or *Esch. coli* DBC than IFLG was slightly greater for extended membrane than for standard membrane. Transport membrane gave the lowest proportion.

As the five laboratories sampled different water supplies, their results were dissimilar in many ways. However, in the comparison of methods none of the combined findings for chlorinated water samples were significantly contradicted by the results from any individual laboratory.

One method sometimes showed the presence of coliform organisms which were not found in the same sample by a different method. This could mean that the actual method used for detecting the presence of coliform organisms and *Esch. coli* could influence any action to be taken. Taking all the chlorinated water samples examined, IFLG and most of the other methods detected coliform organisms in a similar number of samples, although there was not complete agreement about

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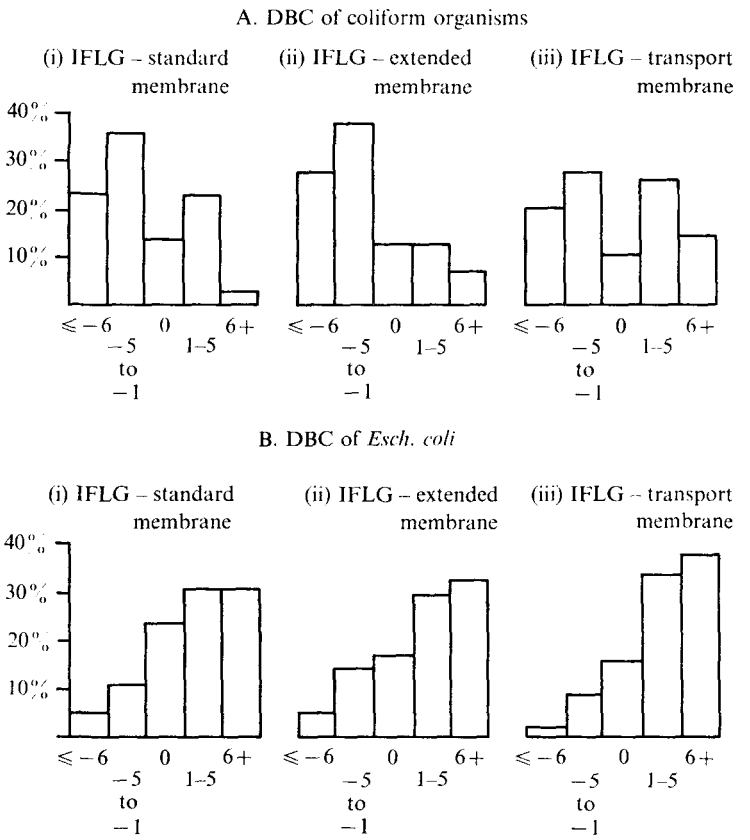

Fig. 2. Distribution of difference in paired results from chlorinated water samples.

Table 4. *Chlorinated water samples; number of samples in which organisms were detected or not detected by IFLG and other methods*

	Coliform organisms				*Esch. coli*			
	Found by IFLG		Not found by IFLG		Found by IFLG		Not found by IFLG	
Other method	Found by other method	Not found by other method	Found by other method	Not found by other method	Found by other method	Not found by other method	Found by other method	Not found by other method
MacConkey	51	1	0	2	40	5	1	7
IFLG (4 hr., 30°)	42	0	2	1	37	2	1	5
Standard membrane	114	5	2	3	80	24	3	16
Extended membrane	97	2	2	3	66	21	4	12
Transport membrane	77	7	2	3	46	32	0	9
Glutamate membrane	15	9	1	2	18	8	0	0

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which samples contained the organisms (Table 4). It was uncommon for the other two multiple tube methods to yield organisms where IFLG did not and vice versa. Coliform organisms, detected in samples by IFLG, were not found in five (4 %) of 119 samples by standard, in two (2 %) of 99 samples by extended, in seven (8 %) of 84 samples by transport and in nine (38 %) of 24 samples by glutamate membrane. However, IFLG detected *Esch. coli* in larger numbers of samples than did the other methods. Where *Esch. coli* was found by IFLG, they were not detected in five (11 %) of 45 samples by MacConkey, in two (5 %) of 39 samples by IFLG (4 hr., 30°), in 24 (23 %) of 104 samples by standard, in 21 (24 %) of 87 samples by extended, in 32 (41 %) of 78 samples by transport and in eight (31 %) of 26 samples by glutamate membrane. There were few chlorinated water samples in which no coliform organisms were detected by IFLG, but in which they were detected by other methods. The number of such samples was too small to evaluate relative frequencies.

Analysis of repeatability

Sixty of the chlorinated waters were tested in duplicate in an attempt to assess the repeatability of four methods. Each of the 60 samples was examined twice by IFLG and twice by standard membrane; most of them were also examined twice by extended and transport membrane. Only the first result of each pair was used for the main analysis already described. The results of the analysis of repeatability showed that none of the methods invariably gave the same DBC twice. Order of examination and magnitude of DBC were not associated with the size of the difference. The differences of DBC of coliform organisms and of *Esch. coli* were similar and were therefore combined. The variability was significantly less in membrane filtration methods than in multiple tubes with IFLG. In 120 duplicate multiple tube tests for coliform organisms or *Esch. coli* using IFLG, there was no difference between the two results in 31 (26 %) samples; in 52 (43 %) there was no difference or a difference of one DBC, and in 100 (83 %) the difference was not more than five DBC. The corresponding proportions for the 292 duplicate tests with membrane filtration methods were 40, 70 and 93 %. It is claimed as an advantage of membrane filtration that the results are likely to lie within narrower limits of variation than with a multiple tube method which is dependent on a MPN. These variations are discussed in Report 71. The present experimental results detected less variation of DBC with membranes in duplicate tests on the same samples.

Unchlorinated waters

Between November 1969 and November 1970 seven laboratories reported results from a total of 239 unchlorinated water samples. Each sample had been tested by at least two of the methods already mentioned for coliform organisms and *Esch. coli*.

Table 5 shows that the overall DBC of coliform organisms was significantly higher for IFLG than for MacConkey. There was no overall significant difference between IFLG and IFLG (4 hr., 30°). IFLG gave a significantly higher average DBC of *Esch. coli* than MacConkey and a slightly but not significantly lower average than IFLG (4 hr., 30°).

Table 5. *Unchlorinated water samples; difference of DBC for paired multiple tube methods*

Methods of testing	Laboratory	Coliform organisms: No. of samples	Coliform organisms: Mean difference of DBC	Coliform organisms: Standard error of mean	*Esch. coli*: No. of samples	*Esch. coli*: Mean difference of DBC	*Esch. coli*: Standard error of mean
IFLG, MacConkey	Conway	9	7·0*	2·3	9	6·2*	2·3
	Manchester	84	1·2*	0·5	85	1·1	0·6
	MWB	24	1·3*	0·5	24	2·5*	1·0
	Newport	—	—	—	—	—	—
	Oxford	11	0·2	1·6	11	0·3	0·6
	Southend	39	3·6*	1·1	39	3·8*	1·0
	Wakefield	29	1·2	0·9	29	0·4	0·9
	All laboratories	196	1·9*	0·37	197	1·9*	0·38
IFLG, IFLG (4 hr., 30°)	Conway	—	—	—	—	—	—
	Manchester	85	−0·7	0·6	85	−0·6	0·5
	MWB	24	−0·1	0·6	24	−1·0	1·1
	Newport	—	—	—	—	—	—
	Oxford	5	−1·2	—	5	0·8	—
	Southend	13	2·6*	1·0	13	1·7	1·4
	Wakefield	23	−0·8	1·1	23	−1·5	1·1
	All laboratories	150	−0·3	0·39	150	−0·6	0·35

* Mean difference significantly greater than zero ($P < 0·05$).

Table 6 compares the results from IFLG and membrane filtration methods for unchlorinated water samples; with these, laboratories did not always agree on which method gave the highest average DBC. With chlorinated waters, however, each laboratory gave results which were either significantly in favour of the same method or else inconclusive.

IFLG gave significantly lower average DBC of coliform organisms than either standard or extended membranes. The overall average differences in DBC of coliform organisms between IFLG and transport membrane were not significant, although two laboratories gave significantly lower and one laboratory significantly higher results with IFLG. The differences in average DBC of *Esch. coli* between IFLG and the membrane methods were less conclusive. Overall, IFLG gave higher results than standard membrane but in one laboratory it gave significantly lower results. There was no significant overall difference between IFLG and extended membrane, although one laboratory favoured IFLG and one extended membrane. The comparison with transport membrane was similar to that with standard membrane – overall results in favour of IFLG but one laboratory in favour of the membrane method. It has been suggested by one participating laboratory that the laboratory differences with transport membranes could be due to the lack of buffering and consequent difficulty of stabilizing the pH. IFLG gave a very significantly higher average DBC than glutamate membrane for both coliform organisms and *Esch. coli*.

The distribution of differences between results from IFLG and three of the

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Table 6. *Unchlorinated water samples; differences of DBC for paired IFLG and membrane methods*

Methods of testing	Laboratory	Coliform organisms: No. of samples	Coliform organisms: Mean difference of DBC	Coliform organisms: Standard error of mean	*Esch. coli*: No. of samples	*Esch. coli*: Mean difference of DBC	*Esch. coli*: Standard error of mean
IFLG, standard membrane	Conway	14	1·2	1·9	14	2·1	2·2
	Manchester	83	−3·8*	0·7	81	−2·0*	0·8
	MWB	24	−0·5	0·4	24	2·5*	1·0
	Newport	27	1·5	0·8	27	5·7*	0·9
	Oxford	11	−6·3*	1·3	11	−1·6	0·9
	Southend	39	−2·4*	0·9	39	1·3	0·9
	Wakefield	39	−5·2*	1·0	36	3·9*	1·5
	All laboratories	237	−2·7*	0·35	232	1·1*	0·44
IFLG, extended membrane	Conway	—	—	—	—	—	—
	Manchester	83	−4·1*	0·7	83	−1·9*	0·9
	MWB	24	−0·7	0·6	24	3·2*	1·1
	Newport	—	—	—	—	—	—
	Oxford	9	−6·8*	1·3	9	−1·1	1·1
	Southend	—	—	—	—	—	—
	Wakefield	7	−5·6*	1·5	6	6·0	2·7
	All laboratories	123	−3·7*	0·47	122	−0·4	0·63
IFLG, transport membrane	Conway	—	—	—	—	—	—
	Manchester	80	−3·8*	0·6	81	−2·4*	0·8
	MWB	24	2·8*	1·1	24	8·4*	1·4
	Newport	—	—	—	—	—	—
	Oxford	4	1·0	—	4	0·0	—
	Southend	35	0·9	1·3	35	4·2*	0·9
	Wakefield	24	−5·3*	1·5	26	5·3*	1·7
	All laboratories	167	−0·3	0·39	170	1·7*	0·54
IFLG, glutamate membrane	All laboratories	91	6·9*	0·85	84	5·2*	0·86

* Mean difference significantly greater than zero ($P < 0·05$).

membrane methods are shown in figure 3. Standard and extended membranes gave significantly higher average DBC of coliform organisms than IFLG but in 23 and 13 % of samples respectively their results were lower. Standard and transport membranes gave significantly lower average DBC of *Esch. coli* than IFLG, but gave higher results in 35 and 30 % of samples respectively. As with the chlorinated water samples (see figure 2) extended membrane gave higher results than IFLG in a slightly greater proportion of samples than did standard membrane, whereas transport membrane gave the smallest proportion. In the comparison of methods illustrated in figures 2 and 3 there are larger proportions to the left of the diagrams – where membrane methods gave higher results than IFLG – for unchlorinated water than for chlorinated water samples.

Table 7 shows the presence or absence of these bacteria in the unchlorinated water samples as found by IFLG and the other methods. With the exception of glutamate membrane, each method detected coliform organisms in nearly all of

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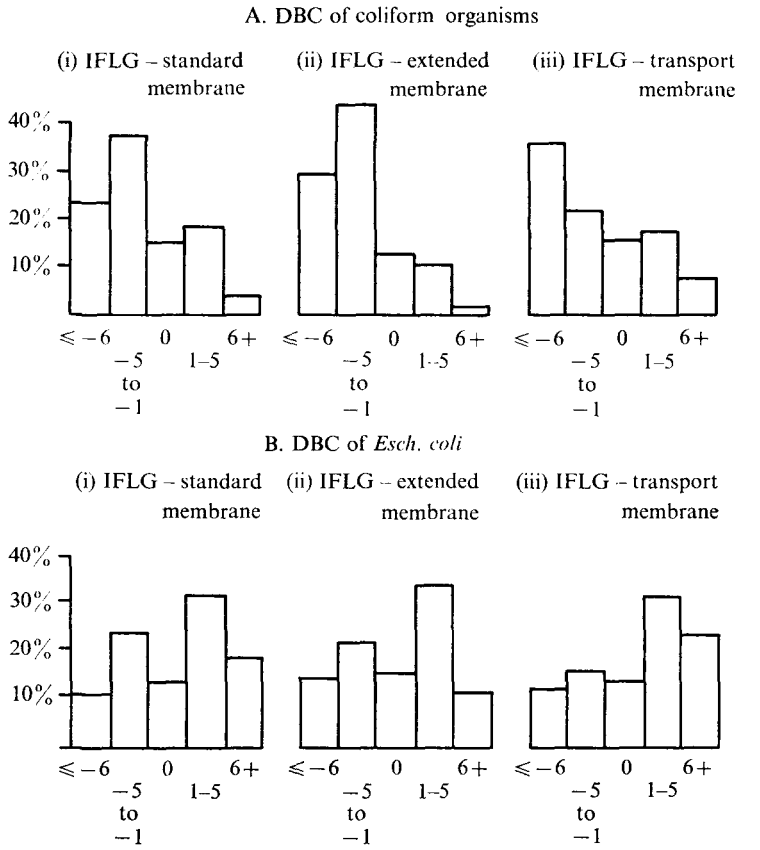

Fig. 3. Distribution of differences in paired results from unchlorinated water samples.

Table 7. ***Unchlorinated water samples; number of samples in which organisms were detected or not detected by IFLG and other methods***

	Coliform organisms				*Esch. coli*			
	Found by IFLG		Not found by IFLG		Found by IFLG		Not found by IFLG	
Other method	Found by other method	Not found by other method	Found by other method	Not found by other method	Found by other method	Not found by other method	Found by other method	Not found by other method
MacConkey	190	2	1	3	144	21	11	21
IFLG (4 hr., 30 °)	148	0	2	0	116	9	12	13
Standard membrane	233	0	4	0	175	23	22	12
Extended membrane	121	0	2	0	91	7	16	8
Transport membrane	162	2	1	2	118	29	11	12
Glutamate membrane	57	22	2	1	32	38	3	11

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these water samples. Agreement between methods on the presence or absence of *Esch. coli*, however, was not so close. Altogether, *Esch. coli* was detected in more of the samples by IFLG than by other methods, except for standard membrane, where the numbers were similar, and IFLG (4 hr., 30°) and extended membrane which both detected *Esch. coli* in more samples than IFLG. *Esch. coli*, found in samples by IFLG, were not detected in 21 (13 %) of 165 samples by MacConkey, in 9 (7 %) of 125 samples by IFLG (4 hr., 30°), in 23 (12 %) of 198 samples by standard, in 7 (7 %) of 98 samples by extended, in 29 (20 %) of 147 samples by transport or in 38 (54 %) of 70 samples by glutamate membrane. Where *Esch. coli* was not found by IFLG they were more often than not detected by standard or extended membrane.

DISCUSSION

A comparison has been made between the results from four membrane methods and those from IFLG with the same water samples. At the same time some comparisons have been made between IFLG, MacConkey and IFLG (4 hr., 30°) multiple tube methods. Seven laboratories took part, although they did not all carry out every test. Laboratories sometimes differed significantly in the comparisons obtained between membranes and multiple tube methods. The reasons for such differences between laboratories cannot be explained by any one factor, but they confirm the recommendation in Report 71 that 'It is essential that before adopting membrane filtration as a routine procedure in any laboratory or with any particular water supply, an adequate parallel series of tests should be run comparing membranes with multiple tubes, in order to establish their equivalence or the superiority of one or the other.' Despite the differences between individual laboratories, the combined results from all laboratories showed some significant differences in the results obtained with different media.

The three multiple tube methods gave average results of comparable magnitude for coliform organisms except that IFLG detected in general more organisms more frequently than MacConkey in unchlorinated waters. IFLG was better in that it gave significantly higher average DBC of *Esch. coli* than MacConkey with both unchlorinated and chlorinated waters, whereas it was only slightly better than IFLG (4 hr., 30°) with chlorinated waters and slightly worse with unchlorinated waters. IFLG (4 hr., 30°) was slightly better than MacConkey at detecting organisms not found by IFLG and in not failing to detect them when found by IFLG.

The findings of the four membrane methods compared with IFLG show that two of them – standard and extended membrane – achieved better results in some respects than IFLG. Transport membrane was never significantly better than IFLG on the combined results for all laboratories and the results from glutamate membrane were consistently poor. Standard and extended membranes were significantly better than IFLG for coliform organisms for both unchlorinated and chlorinated waters, but were significantly poorer for *Esch. coli* in chlorinated waters; they gave equivocal results with unchlorinated waters. Throughout the trial, extended membrane gave better results than IFLG in larger proportions of

samples than did standard membrane. These two membrane methods seldom failed to detect the presence of coliform organisms found by IFLG, but failures did occur with *Esch. coli*, especially in chlorinated waters where the failure rates for both methods were nearly 25 %; however, they did sometimes detect *Esch. coli* where IFLG had failed. In unchlorinated waters both these membrane methods yielded *Esch. coli* in two thirds of the samples.

As the recommendations on membrane filtration in the fourth edition of Report 71 were based mainly on work done at the Metropolitan Water Board, the results of the present trials were examined to see if they supported those recommendations. As stated in the introduction, the original MWB work used MacConkey broth in multiple tubes as the standard reference method. The current work has used IFLG as the standard method and all the other methods, including MacConkey, have been compared with it.

Considering overall results with unchlorinated waters for coliform organisms, standard and extended membranes gave significantly higher results than MacConkey; for *Esch. coli*, standard membranes gave lower results than IFLG, and there was no significant difference between extended membranes and IFLG. The standard membrane results, however, were not significantly lower than those with MacConkey in any laboratory. These results were, therefore, in accord with the earlier work at the MWB that with unchlorinated waters standard and extended membranes gave results as high as or higher than MacConkey for coliform organisms and *Esch. coli*.

With chlorinated waters for coliform organisms, standard and extended membranes gave higher results than IFLG, and there was no significant difference between IFLG and MacConkey; these two membrane methods thus gave higher results than MacConkey. This result is more favourable for membranes with coliform organisms in chlorinated waters than previously found at the MWB. With chlorinated waters for *Esch. coli*, on the other hand, IFLG gave significantly higher results than any membrane method or MacConkey. In this trial, there was no overall significant difference in results for *Esch. coli* in comparison between extended membranes and MacConkey with chlorinated waters.

It may be inferred from these results that IFLG would be better than the present membrane methods for detecting *Esch. coli* in chlorinated waters. But these results have also shown that standard and extended membranes gave higher results than IFLG tubes for coliform organisms in chlorinated waters. Coliform organisms are not, however, a separate group but include *Esch. coli*. This would suggest therefore that higher numbers of *Esch. coli* could be obtained by confirming the identity of the colonies on the coliform membranes. This would take no longer and involve no more work than the equivalent IFLG tube method. The failure of the membrane methods for *Esch. coli* in chlorinated waters, appears therefore to be related to incubation at 44° C. as it does not occur at 37° C. Chlorinated waters should not contain coliform organisms or *Esch. coli* and as any such organisms found would normally be subcultured for confirmation, reliance on the coliform membranes for detecting *Esch. coli* would not involve any additional work. Furthermore, such results occur so infrequently in normal quality testing at waterworks that local

comparisons of membrane and multiple tube methods on routine samples would be unlikely to show any significant differences in actual practice. Indeed, the differences found in this paper were only demonstrated by deliberately producing inadequately chlorinated samples of water in which there were some surviving organisms.

Certain assumptions have however been made in reaching these conclusions. Although *Esch. coli* is included in coliform counts, it does not necessarily follow that the higher coliform counts obtained in the present work with membranes did in fact include *Esch. coli* because confirmation was not carried out. Other possibilities are that the membrane coliform counts consisted largely of false positive results due to yellow colonies other than coliform organisms or of coliform organisms other than *Esch. coli*, which would imply that IFLG is detecting *Esch. coli* but not some other coliform organisms. This is less likely but can only be resolved by indentification of the coliform organisms on the membranes from chlorinated samples. Meanwhile, it is recommended that membranes may continue to be used for chlorinated samples provided that any coliform organisms isolated at 35 or 37° C. are subcultured for confirmation as *Esch. coli*. In practice, whatever laboratory methods are used, any chlorinated waters which yield coliform organisms but not *Esch. coli* should be resampled as a routine.

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