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A comparison of the International Standards Organisation reference method for the detection of coliforms and *Escherichia coli* in water with a defined substrate procedure

S.I. Niemela[1], J.V. Lee[2] and C.R. Fricker[3]

[1]Pikkukoskentie, Helsinki, Finland, [2]WEMRU, Central Public Health Laboratory, London, UK, and [3]CRF Consulting, Child's Acre, Reading, UK

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ABSTRACT

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Aims: This study investigated the use of the International Standards Organisation (ISO) procedure for the comparison of microbiological methods. Using this procedure the ISO reference procedure for the detection of coliforms and *Escherichia coli* in water was compared with a defined substrate method (Colilert^{TM}).

Methods and Results: A total of 20 laboratories from 13 European countries compared the use of Colilert/Quanti-Tray^{TM}, a quantitative defined substrate test (DST) for the presence of coliforms and *E. coli* with the ISO reference procedure, which utilizes tergitol-TTC medium. Results of the study showed that DST detected significantly more coliforms and *E. coli* than did the reference procedure. In the case of *E. coli* the recoveries were also higher when using DST and the difference seen was statistically significant. The confirmation rate obtained when using the DST product suggested that no confirmation of wells positive for *E. coli* was necessary during routine use.

Conclusions: Colilert is a suitable alternative to the ISO reference procedure for the detection of coliforms and *E. coli* in water. The methods used during the comparison study indicated that confirmation of all colonies/positive wells led to the most accurate information and it is recommended that for future comparison studies this should become standard practice. Confirmation of a small proportion of colonies led to misleading conclusions and should be avoided when comparing microbiological methods.

Significance and Impact of Study: It has been demonstrated that the ISO reference procedure fails to detect a significant proportion of coliforms and *E. coli* in drinking water. Colilert/QuantiTray^{TM} is a more suitable alternative.

Keywords: coliforms, comparing microbiological methods, defined substrate test, *Escherichia coli*, membrane filtration, water.

INTRODUCTION

The microbiological quality of drinking water is safeguarded through a programme of frequent analyses of water samples leaving the treatment works, in the distribution system and at customers' taps. These frequent analyses for the indicator organisms, coliforms, *Escherichia coli*, faecal streptococci and sulphite-reducing clostridia are prescribed in the European Union Drinking Water Directive (EUDWD) and will be incorporated into the national law of member states. The EUDWD specifies the International Standards Organisation (ISO) 9308.1 method to be used for the detection of coliforms and *E. coli* that involves membrane filtration and incubation on a tergitol-TTC agar medium (Chapman 1951). In Article 7, Part 5b, the European

Correspondence to: Dr C.R. Fricker, Child's Acre, Church Lane, Three Mile Cross, Reading, RG7 1HD, UK (e-mail: cfricker@compuserve.com).

Directive states: 'Methods other than those specified in Annex III, Part 1, may be used, providing it can be demonstrated that the results obtained are at least as "reliable" as those produced by the methods specified. Member States which have recourse to alternative methods shall provide the Commission with all relevant information concerning such methods and their equivalence'. 'Reliable' in this context was not defined. It is generally accepted however, that alternative methods should have a performance which is equivalent or better. A method is normally considered to be 'equivalent', if the (confirmed) recovery of target organisms is not significantly different from the reference method. For the purposes of this study, and in accordance with the recently developed ISO methods comparison protocol (ISO CD 17994 2001) the test method (Colilert-18®) was considered to be equivalent to the reference method if the mean difference in recovery of target organisms was not significantly different from zero, significance being tied with the approximate 95% confidence limits defined by the expanded uncertainty with coverage factor $k = 2$ (Anon 1995).

In recent years, methods based on the detection of β-D-galactosidase and β-D-glucuronidase have been widely adopted for the detection of coliforms and *E. coli*, respectively (Edberg *et al.* 1988; Sartory and Howard 1992; Palmer *et al.* 1993). One such method, the Colilert-18®/Quanti-Tray® system (IDEXX Laboratories, Portland, ME, USA) has been used extensively for the detection of these organisms in water (Edberg *et al.* 1990; Fricker and Fricker 1996; Fricker *et al.* 1997).

This study was performed to compare the performance of defined substrate technology (DST) with the ISO reference procedure for the detection of coliforms and *E. coli* in water in a wide variety of water sources from 13 European countries. Whilst other studies have been performed recently to compare these two procedures (PHLS, 2000), because of the wide range of laboratories participating in this study the types of samples and target organisms may be wider, giving a more useful comparison. Initially all laboratories were asked to perform tests for both coliforms and *E. coli* and to confirm the identity of one 'presumptive' target organism from each membrane and DST. During this phase of the study, one laboratory confirmed all isolates from a proportion of samples tested. During a second phase of the study, five laboratories were selected to perform tests for *E. coli* only and all isolates were confirmed.

MATERIALS AND METHODS

The laboratories

A total of 20 laboratories were involved in the study from the following European states: Austria, Denmark, Finland, France, Germany, Ireland, Italy, The Netherlands, Norway, Portugal, Spain, Sweden and the UK. A protocol for performing the tests was distributed to all laboratories in an attempt to ensure consistency between the analysts.

Samples

Each laboratory chose the type of water sample to be used in this study based on the water types normally analysed. Thus laboratories which usually analyse water which has been treated with chlorine used samples which had been disinfected, whilst those laboratories which normally analyse nondisinfected water used contaminated ground water or good quality surface water. Laboratories were asked to try and use samples which contained between 5 and 15 CFU 100 ml^{-1} whenever possible.

Samples used for comparisons were taken from the same bottle in every case. For disinfected samples, a procedure based upon that of Cowburn *et al.* (1994) was used. The procedure used was as follows. A 10 l container was filled with tap water and warmed to 37°C for 2 h followed by an overnight incubation at 4°C. The following day, 500 ml of good quality sewage effluent was added to the container and thoroughly mixed by shaking and the use of a magnetic stirrer. A solution of 10–15 mg of free chlorine was then added to the diluted sewage effluent and mixed by stirring for 5 min. Samples (1 l) were then removed into sterile 1 l bottles containing 5 ml of 18% sodium thiosulphate solution at 1 min intervals. These samples (100 ml) were then analysed for the presence of coliforms and *E. coli* using Colilert-18® medium and Quanti-Tray 2000 trays and the remainder of the sample was stored at 4°C overnight. After incubation, the 2000 Quanti-Tray trays were examined to determine appropriate samples for use in the comparison study. Samples which contained 15–70 target organisms were used directly for comparison of the two procedures and those containing 150–700 were diluted 1 : 10 prior to use.

Media comparison

Selected samples were used to compare the performance of tergitol-TTC medium incubated at 37 and 44°C and DST/Quanti-Tray® incubated at 37°C for detecting coliforms and *E. coli*. Samples (100 ml) were filtered through 0·45 micron membrane filters and placed onto tergitol medium before incubation. Colilert-18®/Quanti-Tray® was used according to the manufacturer's instructions.

After incubation the number of 'presumptive' colonies was counted on the membranes and the number of yellow (positive for total coliforms) and yellow and fluorescing (positive for *E. coli*) wells was counted from the Quanti-Trays.

Confirmed counts

During the first phase of the study where all 20 laboratories participated, one colony or well was selected from each membrane or Quanti-Tray® to determine the general 'true positive rate' for each laboratory. For membranes, the 'presumptive' colony nearest to the centre of the membrane was selected for confirmation. Colonies were plated onto nutrient agar and examined for production of cytochrome oxidase, ability to ferment lactose at 37 and 44°C and the ability to produce indole from tryptophan using tryptone water and Kovacs reagent. For Quanti-Trays, the well nearest to the bottom left-hand corner of the tray which showed yellow colouration and/or fluorescence was tested. If both yellow and yellow/fluorescent wells were present, one of each type was selected for confirmation. A small volume of liquid was removed through the back of the tray using a sterile hypodermic needle and syringe and plated onto MacConkey agar. After incubation a well-isolated lactose-fermenting colony was selected and identified as described above. If no lactose-fermenting colonies were present, a nonlactose-fermenting colony was selected.

To determine the 'true positive rate' for each laboratory, the percentage of colonies and wells examined which 'confirmed' as being coliforms or *E. coli* was determined and each number of 'presumptive' organisms was multiplied by this percentage to give the 'confirmed count'. During the second phase of the trial, where only five laboratories participated, all colonies were confirmed. One laboratory (no. 19) confirmed all colonies from part of their samples during the first phase of the trial. These results were analysed together with the second-phase results.

Mathematical treatment

The data were analysed according to the draft proposal ISO CD 17994 (2001) for the establishment of equivalence between microbiological methods which prescribes calculation of 100 times the logarithmic (ln) difference ('relative difference percentage') between the confirmed counts for each sample. The assessment of equivalence is based on the mean and the expanded uncertainty range (Anon 1995) computed on the basis of the mean and standard deviation of the relative difference and the number of samples.

RESULTS

The number of samples analysed by different laboratories varied considerably and is included in Tables 4 and 5. In total over 2500 samples were used to compare the ISO reference method and DST/Quanti-Tray®. Laboratory no. 19 analysed most of the samples and all colonies from membranes and wells from DST from samples yielding <10 colonies or positive wells were confirmed in this laboratory. The numbers quoted do not include samples where the result for at least one method was zero or too numerous to count.

Table 1 shows the number of presumptive colonies (and corresponding DST counts) observed using the different methods.

It can be seen that DST gave higher presumptive coliform counts than did the ISO reference method whereas the reference method gave higher presumptive counts for *E. coli* than DST. The differences as such are not meaningful because they become modified by the confirmation coefficients shown in Table 2.

It is clear that there were considerable differences between the confirmation coefficients obtained in different laboratories and between methods. In most cases, DST had higher confirmation coefficients for both coliforms and *E. coli*, although it was noticeable that in some laboratories the confirmation coefficient for coliforms was lower with DST than for the reference method. The ranges, mean values and standard deviations of the confirmation coefficients are shown in Table 3.

Table 4 shows a summary of the results used for the assessment of equivalence of the two methods for total coliforms according to the latest version of the ISO draft

Table 1 Numbers of presumptive colonies (and corresponding DST counts) observed

Laboratory	Presumptive DST TC	Presumptive tergitol TC	Presumptive DST EC	Presumptive tergitol EC
1	8378	6966	192	335
2	22 340	13 597	3566	5997
3	1219	1073	75	232
4	620	378	232	272
5	1753	1524	588	889
6	1383	1471	449	894
7	2277	1320	140	373
8	1521	1047	512	466
9	1715	2610	655	949
10	165	209	566	176
11	383	259	24	39
12	1447	611	176	251
13	5068	5137	524	723
14	2935	1359	351	464
15	939	209	264	262
16	3131	751	1517	1342
17	4321	4235	588	1140
18	2507	1590	468	1196
19	11 944	11 327	765	1028
20	909	974	211	726
Total	74 991	56 647	11 863	17 754

TC, total coliforms; EC, *Escherichia coli*; DST, defined substrate technology.

Table 2 The numbers of cultures tested in each laboratory to determine an average confirmation coefficient (in parenthesis) for each of the four methods

Laboratory	DST TC	Tergitol TC	DST EC	Tergitol EC
1	148 (0·53)	109 (0·21)	97 (0·56)	125 (0·18)
2	356 (0·77)	310 (0·48)	323 (0·86)	308 (0·59)
3	99 (0·91)	109 (0·83)	57 (0·93)	60 (0·78)
4	52 (0·90)	22 (0·82)	22 (0·91)	25 (0·64)
5	50 (0·90)	50 (0·86)	50 (0·92)	50 (0·70)
6	28 (1·0)	30 (1·0)	28 (0·96)	28 (1·0)
7	51 (0·75)	46 (0·74)	29 (0·90)	41 (0·46)
8	39 (0·95)	133 (0·90)	41 (0·88)	129 (0·76)
9	20 (0·75)	18 (0·67)	18 (0·83)	19 (0·63)
10	38 (1·0)	32 (0·66)	12 (0·92)	24 (0·46)
11	30 (0·90)	28 (0·93)	7 (1·0)	14 (0·50)
12	34 (0·62)	34 (0·91)	24 (0·92)	31 (0·94)
13	113 (0·88)	107 (0·65)	114 (0·97)	118 (0·85)
14	101 (0·83)	96 (0·77)	68 (0·75)	82 (0·30)
15	89 (0·57)	52 (0·60)	48 (1·0)	48 (0·56)
16	70 (0·60)	55 (0·82)	58 (0·97)	59 (0·83)
17	100 (0·91)	55 (0·67)	100 (0·97)	42 (0·26)
18	26 (0·92)	27 (0·96)	25 (0·88)	27 (0·41)
19	1894 (0·97)	2265 (0·74)	1766 (0·99)	3806 (0·42)
20	25 (1·0)	25 (0·92)	25 (1·0)	25 (0·44)
Total	3363	3603	2912	5061

TC, total coliforms; EC, *Escherichia coli*; DST, defined substrate technology.

Table 3 Range, mean and standard deviation of the confirmation coefficients of all participating laboratories

Method	Range of confirmation coefficient	Mean confirmation coefficient	S.D.	S.E.M.
DST total coliforms	0·53–1·0	0·83	0·15	0·034
Tergitol total coliforms	0·21–1·0	0·76	0·19	0·043
DST *E. coli*	0·56–1·0	0·91	0·10	0·022
Tergitol *E. coli*	0·18–1·0	0·59	0·23	0·051

The mean values were calculated by summing the confirmation coefficient for each laboratory and dividing by 20 (the number of laboratories which submitted confirmation data).

protocol for comparison of microbiological methods (ISO CD 17994 2001). The corresponding results for *E. coli* are shown in Table 5. Samples which yielded a zero result for either of the two methods were excluded because of the use of confirmation coefficients. When confirmation coefficients are used, samples which have small numbers of 'presumptive' organisms which in fact are not the target organism, will still yield a positive result. For example, if with a particular sample one method (the more specific) yields a result of zero, whilst the other method (the less specific) gives a presumptive count of two (with both organisms not genuinely being the target organism) and the confirmation coefficient is 0·5, then the less specific method will appear to be more sensitive which may not be the case.

The grand mean of the mean relative differences for total coliforms was 52·0 with a standard deviation of 47·8. With 20 laboratories, the standard deviation of the grand mean ('standard error') was 10·7. As a consequence, the mean (52·0) can be termed significant with considerable confidence.

The grand mean of the mean relative differences for *E. coli* was 1·4, with a standard deviation 62·0. The standard deviation of the grand mean ('standard error') was 13·9. The expanded uncertainty range contains the origin and extends beyond the limits –10% and +10%. According to the ISO CD 17994 (2001) principles the comparison is 'inconclusive'. More data are required to reach a conclusion.

It was decided to improve the design by confirming all presumptive observations during the second phase of the study.

The inconclusive results in some laboratories occurred because the expanded uncertainty range contained the value 0% and the lower end of the range of the expanded uncertainty was below −10% or the upper end above +10%. Overall DST gave significantly higher recovery of total coliforms. Only one of 20 laboratories found DST significantly less productive than the tergitol medium (Table 4). The results for *E. coli* (Table 5) were more ambiguous. The evaluations were almost evenly divided between positive, negative, and inconclusive.

Table 6 shows the data generated during the second phase of the study, where all isolates were confirmed in six laboratories (data from phase 1 of the study where laboratory no. 19 confirmed all colonies/wells from samples with <10 organisms detected is included).

With the exception of laboratory no. 17, the results were far more uniform during phase 2, the main reason being the more reliable confirmation when all presumptive colonies and wells were tested. Table 7 gives a summary of the confirmation tests.

Compared with phase 1 results (Table 2) the confirmation rates were considerably more homogenous and higher when the random variation associated with fractional confirmation was avoided by confirming all presumptive results.

Whilst three laboratories during phase 2 would not individually have been able to conclude whether the two methods were equivalent, when all data are looked at together it was apparent that DST detected significantly more *E. coli* than the reference method. This is one of the benefits of multicentre studies, where the large number of samples analysed allows a conclusion to be reached. If the number of samples is small, the data are inconclusive.

Table 4 Evaluation of the equivalence of the two methods for total coliforms according to ISO CD 17994 (2001)

Laboratory	N	n_0	n	Mean relative difference	S.D.	Expanded uncertainty interval		Evaluation
						LO	HI	
1	218	2	216	93·5	76·2	83·1	103·9	+
2	650	60	590	70·8	127·6	60·3	81·3	+
3	112	28	84	42·6	63·1	28·8	56·4	+
4	33	17	16	93·3	141·7	22·5	164·1	+
5	44	0	44	46·5	72·7	24·6	68·4	+
6	60	0	60	−5·1	63·8	−21·6	11·4	?
7	80	14	66	31·0	171·8	−11·3	73·3	?
8	38	1	37	52·9	76·1	27·9	77·9	+
9	61	2	59	−47·6	46·9	−59·8	−35·4	−
10	29	14	15	21·2	112·4	−36·8	79·2	?
11	24	2	22	43·1	73·8	11·6	74·6	+
12	45	0	45	71·3	96·0	42·7	99·9	+
13	153	0	153	39·5	68·1	28·5	50·5	+
14	138	5	133	80·5	76·2	67·3	93·7	+
15	41	14	27	145·5	85·0	112·8	178·2	+
16	52	5	47	156·2	111·8	123·6	188·8	+
17	200	1	199	27·8	78·1	16·7	38·9	+
18	132	6	126	39·3	62·7	28·1	50·5	+
19	472	0	472	39·6	51·4	34·9	44·3	+
20	50	0	50	−2·2	74·3	−23·2	18·8	?

N, total number of samples; n_0, number of samples excluded because of zero results; n, number of samples retained for analysis.
+, methods not equivalent, alternative method (DST) significantly higher; −, methods not equivalent, alternative method (DST) significantly lower; ?, unable to determine if the methods are equivalent, more samples required.

When the data of the six laboratories were merged, the mean relative difference of all 804 samples was 22·1, with a standard deviation of 72·9. The limits of the expanded uncertainty range were low (LO) = 16·9 and high (HI) = 27·2. Both limits are on the positive side of the origin, indicating a significantly higher confirmed average recovery of *E. coli* by DST in this study.

The results from laboratory no. 17 showed a much larger difference between DST and the tergitol medium. For this reason, a statistical comparison of the data from the remaining five laboratories (excluding laboratory no. 17) was performed. The results of the evaluation showed that the mean relative difference was 16·9 with a standard deviation of 68·5. The limits of the expanded uncertainty were LO = 11·9 and HI = 21·9 demonstrating that DST recovered significantly more *E. coli* than the tergitol medium even excluding the results from laboratory no. 17.

The results from the phase 2 study cannot be disputed as every colony and Quanti-Tray well was confirmed. It was interesting therefore to compare the results for *E. coli* for the six laboratories obtained in both phases of the study. These results are shown in Table 8.

DISCUSSION

There have been many studies describing the potential benefits of DST over more traditional techniques (Edberg *et al.* 1988; Cowburn *et al.* 1994) for the detection of coliforms and *E. coli*. However, the requirement of the European Drinking Water Directive that the ISO reference procedure be used in all member states unless evidence can be given to demonstrate that other methods are suitable prompted the study reported here. In addition to comparing the two methods, this study also investigated the way in which methods might be compared. In the first phase of the study, only a single colony or well was confirmed for each sample, except in one laboratory where samples that yielded <10 CFU/100 ml had all presumptive colonies/wells confirmed. The results of this comparison suggest that when comparing two microbiological methods, it is desirable that all presumptive isolates be confirmed. The use of a small proportion of colonies for confirmations leads to huge variations in the proportion of colonies which confirm. This phenomenon can be seen by comparing the proportion of isolates which confirmed in the two phases of the study as

Table 5 Evaluation of the equivalence of the two methods for *E. coli* according to ISO CD 17994 (2001) during phase 1 of the study

Laboratory	N	n_0	n	Mean relative difference	S.D.	Expanded uncertainty range		Evaluation
						LO	HI	
1	170	85	85	57·8	77·3	41·0	74·6	+
2	625	143	482	−24·9	76·3	−31·6	−17·6	−
3	64	36	28	−85·9	89·5	−119·7	−52·1	−
4	31	18	13	18·7	98·9	−36·1	73·5	?
5	50	0	50	−0·5	77·0	−22·3	21·3	?
6	63	7	56	−75·5	55·1	−90·2	−60·8	−
7	35	15	20	−53·9	75·2	−87·5	−20·3	−
8	31	2	29	−10·7	96·3	−46·4	25·0	?
9	63	13	50	−86·5	83·0	−110·0	−63·0	−
10	49	24	25	164·8	111·0	120·4	209·2	+
11	13	5	8	24·5	58·3	−16·7	65·7	?
12	38	10	28	−37·2	71·7	−64·3	−10·1	−
13	150	19	131	−19·5	78·9	−33·3	−5·7	−
14	133	48	85	61·8	92·8	41·7	81·9	+
15	30	7	23	41·5	84·7	6·2	76·8	+
16	59	6	53	−2·8	75·8	−23·6	18·0	?
17	173	39	134	62·0	85·7	47·2	76·8	+
18	126	13	113	−12·0	76·3	−26·4	2·4	?
19	51	0	51	57·9	36·4	47·7	68·1	+
20	50	4	46	−52·2	73·6	−73·9	−30·5	−

N, total number of samples; n_0, number of samples excluded because of zero results; n, number of samples retained for analysis.
+, methods not equivalent, alternative method (DST) significantly higher; −, methods not equivalent, alternative method (DST) significantly lower; ?, unable to determine if the methods are equivalent, more samples required.

Table 6 Evaluation of the equivalence of the two methods for *E. coli* during phase 2 of the study according to ISO CD 17994 (2001)

Laboratory	N	n_0	Mean relative difference	S.D.	Expanded uncertainty range		Evaluation
					LO	HI	
2	75	12	18·9	70·2	2·6	35·1	+
5	49	0	−5·0	39·1	−16·2	6·2	?
12	60	1	9·8	70·2	−8·3	27·9	?
14	30	0	6·3	50·0	−11·9	24·5	?
17	45	5	109·4	89·0	82·9	135·9	+
19	545	164	19·9	70·7	13·8	26·0	+

N, total number of samples; n_0, number of samples containing a confirmed zero count.
+, methods not equivalent, alternative method (DST) significantly higher; ?, unable to determine if the methods are equivalent, more samples required.

Table 7 The numbers of *E. coli* cultures tested in each laboratory to convert presumptive counts to confirmed counts. The average rate of confirmation is given in parentheses. All presumptive positives were tested

Laboratory	DST *E. coli*	Tergitol *E. coli*
2	475 (0·99)	383 (0·85)
5	593 (0·98)	805 (0·74)
12	515 (0·97)	521 (0·86)
14	512 (0·97)	516 (0·87)
17	518 (0·87)	221 (0·85)
19	1765 (0·99)	3430 (0·46)

shown in Tables 2 and 7. Furthermore, in Table 8 the vast differences in relative mean differences can be seen for the laboratories involved in both phases of the trial. Thus it is clear that to obtain the best quality data, all isolates should be confirmed.

Defined substrate technology gave higher recoveries than tergitol for total coliforms and many reasons may be hypothesized for this. Overgrowth of background organisms on the tergitol medium was a frequent occurrence resulting in difficulty in reading the membranes. In this situation coliform colonies could well be obscured. In addition, heavy growth of nontarget flora could result in the inhibition of growth of coliform organisms, leading to a reduction in the

Table 8 Mean relative differences (RD) of the two media for *E. coli* determined in both phases of the study

Laboratory	Mean RD (phase 1)	Mean RD (phase 2)
2	−24·9	18·9
5	−0·5	−5·0
12	−37·2	9·8
14	57·8	6·3
17	62·0	109·0
19	57·9	19·9

number detected (Toze *et al.* 1990, 1994; Niemi *et al.* 2001). DST detects coliforms on the basis of possession of the enzyme β-D-galactosidase and many organisms which possess this enzyme do not ferment lactose on primary isolation, when inhibitory agents are present. However, when subsequently inoculated in a noninhibitory medium, they often ferment lactose. This would lead to an increase in the number of coliforms being detected by DST and also to a reduced confirmation coefficient. This phenomenon was investigated in one laboratory and it was shown to be due to the presence of coliforms, which were unable to ferment lactose, but were O-nitrophenol galactoside (ONPG) positive. These organisms are coliforms but the procedure used in the trial would not confirm them as confirmation relied on fermentation of lactose. These 'environmental' coliforms form a substantial part of the natural flora in some waters and have been shown to be common in other countries (Fricker *et al.* 1997). Thus the confirmation coefficient for DST was probably underestimated in many laboratories. There are other potential reasons for the increased recovery of organisms when using the DST medium contrary to membrane filtration onto Tergitol agar. It is well recognized that organisms which are damaged, particularly in the outer membrane, become increasingly sensitive to surface active agents. The tergitol medium contains such material whilst DST does not. Furthermore, the actual process of membrane filtration is relatively harsh with a potential to allow organisms to dry on the surface of the membrane. Incubation of the water sample with the Colilert powder prevents this and also reduces nutrient shock as the DST medium contains minimal nutrients.

In phase 1 of the study, by far the majority of the laboratories involved showed that DST recovered significantly higher numbers of coliforms than did the tergitol medium. This was not the case with *E. coli* where there was a fairly even spread of events between (i) those laboratories finding DST detected more *E. coli* than tergitol, (ii) those finding no difference and (iii) those finding that tergitol detected more *E. coli* than DST. Membranes incubated at 44°C were much easier to read although the presumptive counts were much higher than with DST, presumably because of the growth of thermotolerant coliforms. Overall, during phase 1, DST was shown to detect significantly more coliforms than the tergitol medium whilst there was no significant difference between the two methods for *E. coli*. However, using the ISO procedure for determining if two microbiological methods are equivalent, the statistical tests employed showed that more data were required to reach a firm conclusion. For this reason, the second phase of the study was undertaken.

The results from the second phase of the study showed that DST detected significantly more *E. coli* than did the reference method. In one laboratory (no. 17), the difference between the two methods was extremely large and suggested that this data might constitute 'an outlier'. There were no reasons to doubt the data generated but for completeness, the data were analysed twice – once including data from laboratory no. 17 and once excluding it. In both cases statistical analysis showed that DST recovered significantly more *E. coli* than the reference method. There has been a report that Colilert fails to detect some strains of *E. coli* within its 18 h incubation period (Schets *et al.* 2001). It is certain that some strains of *E. coli* fail to cleave sufficient methylumbelliferyl glucuronide (MUG) to produce fluorescence within 18 h but this has not been seen in other studies. Furthermore, notwithstanding any glucuronidase-negative strains in this study, DST still detected more *E. coli* than the reference method. No single method is able to recover all strains of a particular organism or group of organisms and most membrane filtration methods currently in use rely on the ability of *E. coli* to grow at 44°C, ferment lactose and produce indole from tryptophan. The occurrence of strains of *E. coli* which are negative for one or more of these traits is higher than the occurrence of strains which are negative for β-D-glucuronidase. A complicating factor in the work reported by Schets *et al.* (2001) is that to produce the samples used in the study, surface waters were diluted in 0·1% peptone. Colilert is a defined substrate medium and the addition of significant amounts of organic substrates (0·1% peptone is sufficient to support the growth of bacteria to at least 10^7 ml^{-1}) can affect the results. When such large amounts of peptone are added, bacteria are able to utilize it as a substrate rather than MUG. This could have the effect of delaying the time at which bacteria started to utilize MUG as a substrate resulting in a delay in the time taken to be able to detect fluorescence. Thus these results should be treated with caution.

In conclusion this study has demonstrated that DST is more sensitive than the ISO reference procedures for the detection of both coliforms and *E. coli*. DST is a patented technology and the results generated in this study cannot be extrapolated to other media which detect coliforms and *E. coli* on the basis of possession of β-D-galactosidase and β-D-glucuronidase. Furthermore, results from this study

strongly suggest that when comparing two microbiological methods, all presumptive isolates should be confirmed. Selecting 'representative' colonies from membranes is often subjective and can lead to misleading results. The conclusion drawn in the ISO document, that all organisms should be confirmed wherever possible, is supported by this study and results generated from studies where only a proportion of colonies is confirmed should be treated with caution.

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