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USE OF TWO FORMULATIONS OF COLILERT AND QUANTITRAY™ FOR ASSESSMENT OF THE BACTERIOLOGICAL QUALITY OF WATER

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Abstract—The use of standard Colilert (IDEXX) for the detection of coliforms and *E. coli* in potable water has been studied. In a large survey, Colilert recovered *E. coli* from similar numbers of samples to membrane filtration whilst recovery of coliforms was higher with Colilert. Further experiments to study the sensitivity of the two methods using pure cultures, showed that there was no significant difference in their ability to recover *E. coli*. Studies on a newly developed form of Colilert (Colilert 18) which is said to give results within 18 h showed that there was no significant difference between the two forms of the product. Use of the Colilert 18 in a quantitative format using QuantiTray™ showed that the product gave similar quantitative results to membrane filtration, but was easier to read and was less time consuming. Subculture of a proportion of positive samples demonstrated that there was no need to confirm results obtained with the Colilert/QuantiTray™ system. The results of this study suggest that both formulations of Colilert are suitable alternatives to the United Kingdom membrane filtration method for monitoring the bacteriological quality of drinking water. © 1997 Published by Elsevier Science Ltd

Key words—coliforms, *E. coli*, detection, defined substrate technology, quantitation, Colilert

INTRODUCTION

Monitoring the bacteriological quality of drinking water relies on the detection of indicator organisms, namely coliforms and *E. coli*. Membrane filtration is the most widely used method in the United Kingdom, with two membranes being used, one being incubated at 37°C for coliforms and the other at 44°C for *E. coli*. The detection of these organisms is based on their ability to ferment lactose and such fermentation is detected by use of an indicator in the medium (membrane lauryl sulphate broth, MLSB) which is turned yellow by the acid produced during fermentation. All colonies which appear yellow or colourless when growing on this medium are termed "presumptive" coliforms or *E. coli* depending on the temperature at which they were recovered. Subsequent tests including production of acid and gas from lactose at 37 and 44°C, production of indole from tryptophan at 44°C, Gram reaction and production of cytochrome oxidase are used to determine whether the organisms are coliforms or *E. coli*.

Whilst the current method has been extremely useful in aiding the assessment of the bacteriological quality of potable water, it has many shortfalls. The production of gas from lactose has long been thought to be irrelevant and indeed the new Report 71 (Anon, 1994) does not include this reaction in its definition of either *E. coli* or coliforms. The production of indole from tryptophan at 44°C is not exclusive to *E. coli* and some strains of *Klebsiella*, notably *K. oxytoca* are known to produce a positive reaction in this test, whilst some strains of *E. coli* are indole negative. Furthermore, not all strains of *E. coli* have the ability to grow at 44°C, and in particular *E. coli* 0157:H7 which is known to cause haemorrhagic colitis in humans will often not grow or will grow poorly at this elevated temperature. With respect to coliforms, the currently available tests are even more confusing. Whilst a significant proportion of coliforms are anaerogenic (fail to produce gas when fermenting lactose) approximately 10% of coliforms isolated from potable source water within the Thames area do not ferment lactose, probably due to the lack of the enzyme lactose permease (Fricker *et al.*, 1994). The inability to ferment lactose means that under the current definition, these organisms are not classified as coliforms. It seems unreasonable however to exclude organisms on the basis of a single physiological test which requires two enzymes. It defies logic to state that a coliform such as a strain of *Enterobacter cloacae* which possesses the enzyme lactose permease is of any more health or sanitary significance than a strain which does not possess the enzyme.

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There are moves to try and classify organisms as *E. coli* or coliforms on the basis of possession of a single enzyme system, B-glucuronidase for *E. coli* and B-galactosidase for coliforms (Fricker and Fricker, 1994). Whilst this approach does not entirely solve the problem, it simplifies the tests required to recover and identify the desired organisms. Defined substrate technology (Edberg *et al.*, 1991) utilizes this concept and has been used for the detection of *E. coli* and coliforms in water in the United States and become a widely adopted method, using the commercially available product, Colilert. Our initial studies with this product (Cowburn *et al.*, 1994) demonstrated that it gave similar recoveries to the UK standard method for detection of coliforms and *E. coli* in many types of water sample. The current investigation was performed to directly compare Colilert with membrane filtration using membrane lauryl sulphate broth (MLSB) for the recovery of coliforms and *E. coli* in drinking water samples. In addition, since it has been suggested that Colilert may not be as sensitive as membrane filtration for the detection of *E. coli*, experiments were performed to address this issue. Pure cultures of *E. coli* which had been allowed to "starve" in water for 24 h before being diluted to obtain a theoretical level of one organism per 100 ml were used to compare membrane filtration with Colilert.

Further experiments were performed to compare the efficiency of a newly developed product (Colilert 18) which gives results within 18 h with the original Colilert formulation. Furthermore, a system for quantifying results using Colilert (QuantiTray™) was also evaluated by comparison with membrane filtration.

MATERIALS AND METHODS

A total of 7389 drinking water samples taken for various reasons were examined for the presence of coliforms and *E. coli* using membrane filtration and Colilert 24 (IDEXX, Chalfont St Peter, U.K.). Samples (100 ml) were filtered through sterile membranes (0.45 u) and incubated on MLSB as indicated in the U.K. standard method (Anon, 1994). A further 100 ml was poured into a sterile glass bottle and the Colilert reagent added. All Colilert samples were incubated at 37°C and were examined after 24 h incubation. Any samples which were yellow in colour (indicating the presence of coliforms), but not as intense as the comparator supplied by the manufacturers were reincubated for a further 4 h. All samples which were yellow after incubation were examined under long wave ultraviolet light for a characteristic blue fluorescence indicating the presence of *E. coli*. All positive Colilert samples were plated onto two plates of MacConkey agar, one for incubation at 37°C and the other at 44°C. The resultant colonies were identified according to standard methods (Anon, 1994) as were all "presumptive" colonies on MLSB. Any isolate which did not conform exactly to the U.K. water industry definition of coliforms or *E. coli* was identified using the ATB 32E system (Biomerieux, Basingstoke, U.K.).

For the experiments to compare Colilert with membrane filtration using pure cultures of *E. coli*, environmental isolates were cultured in nutrient broth at 37°C for 18 h. Dilutions of the culture were made in sterile distilled water and plated on nutrient agar using a spiral plater (Don Whitley Scientific, Shipley). After incubation for 18–24 h at 37°C, the dilutions were used to seed sterile tap water to give a theoretical yield of one colony forming unit per 100 ml. Experience showed that allowance had to be made for die off during the "starvation" period and a reduction of approximately 50% was predicted. Fifty replicates of each seeded sample were used to inoculate 25 Colilert bottles and to perform 25 membrane filtrations. Colilert samples were incubated at 37°C and the membranes at 30°C for 4 h followed by 14–18 h at 44°C. Only samples which gave less than five colonies on all of the membranes were used for comparative purposes.

A further 1057 samples of water taken after filtration, but before disinfection were used to compare the two forms of Colilert. Samples were prepared in the same way as described above and those using Colilert 18 were incubated at 37°C for 18 h, whilst those using Colilert 24 were incubated for 24 h. All positive results were confirmed by plating the resultant culture on MacConkey agar and identifying the organisms present as described above.

A further 2463 samples of disinfected sewage effluent (Cowburn *et al.*, 1994) were used to determine the relationship between counts of coliforms and *E. coli* obtained by the Colilert 18/QuantiTray™ and those obtained using the membrane filtration method. A total of 1296 wells from the QuantiTrays which turned yellow (indicating the presence of coliforms) and a further 160 wells which fluoresced (indicating the presence of *E. coli*) were subcultured and examined for the presence of coliforms and *E. coli*, to determine the number of false positive reactions and to check if non-fluorescing wells contained *E. coli*.

Qualitative results were compared using MacNemar's test for paired samples. Correlation coefficients were calculated for the numbers of coliforms and *E. coli* by membrane filtration and Colilert 18 in combination with QuantiTray™.

RESULTS

The results presented in Table 1 show that very similar recoveries of *E. coli* were obtained with both the membrane filtration and Colilert methods using pure cultures of *E. coli* containing approximately one colony forming unit per 100 ml. Colilert recovered *E. coli* in more 100 ml replicates than did membrane filtration although the differences were not statistically significant ($p > 0.05$).

Table 2 shows the comparison of membrane filtration and Colilert 24 for the recovery of coliforms and *E. coli* from drinking water samples. Substan-

Table 1. Recovery of *E. coli* from water samples "spiked" to contain approximately one colony forming unit per 100 ml

	Membrane filtration	
Colilert 24	+	−
+	106	35
−	28	181

Table 2. Recovery of coliforms and *E. coli* from treated drinking water using Colilert and membrane filtration

	Colilert 24	Membrane filtration
Presumptive coliforms	296	363
Confirmed coliforms	296	257
Presumptive *E. coli*	70	102
Confirmed *E. coli*	70	71

Table 3. Contingency table showing the numbers of drinking water samples which gave identical and discrepant coliform results using membrane filtration and Colilert 24

	Colilert 24	
Membrane filtration	+	−
+	201	56
−	95	7037

Table 5. Comparison of the number of samples found to contain coliforms using Colilert 24 and Colilert 18 from 1057 post filtration, pre-chlorination samples

	Colilert 24	
Colilert 18	+	−
+	283	46
−	37	691

tially more coliforms were detected and confirmed using Colilert 24, due largely to samples which contained coliforms which did not possess lactose permease. The confirmation rates for both *E. coli* and coliforms were 100% for Colilert. Conversely, confirmation rates for isolates obtained by membrane filtration were 69.6% for *E. coli* and 70.8% for coliforms.

Tables 3 and 4 show the numbers of concordant and discordant results obtained from treated drinking water using Colilert 24 and membrane filtration for the detection of coliforms and *E. coli*, respectively. Tables 5 and 6 show the comparison of Colilert 18 and Colilert 24 for the recovery of coliforms and *E. coli* from post filtration, pre-chlorination samples. No significant difference was seen between the two media ($p > 0.05$) for either group of organisms.

Tables 7 and 8 show the comparison of recoveries of coliforms and *E. coli* from disinfected sewage effluent using the Colilert 18/QuantiTray™ system and membrane filtration. Colilert 18/QuantiTray™ detected coliforms in significantly more samples than did membrane filtration ($p < 0.05$), but there was no significant difference in the numbers of samples found to contain *E. coli* using the two methods. The correlation coefficients for the quantitative results obtained by Colilert 18/QuantiTray™ and membrane filtration were 0.87 and 0.89 for coliforms and *E. coli*, respectively.

Of the 1296 yellow, non-fluorescing wells examined, all were shown to contain coliforms and none contained *E. coli*, although some strains of *Escherichia vulneris* and *Escherichia hermanni* were detected. All of the 160 wells which showed fluorescence contained *E. coli*.

DISCUSSION

From the data presented it can be seen that Colilert performs similarly to the U.K. standard membrane filtration method for detecting *E. coli*, with similar numbers of samples being found to contain *E. coli* by both methods. The differences are not significant when compared by McNemars test for paired samples ($p > 0.05$) and can be accounted for by the uneven distribution of organisms within water samples. Colilert detected non-*E. coli* coliforms in significantly more samples than did membrane filtration ($p < 0.05$). This was due mainly to the occurrence of organisms which did not ferment lactose (probably due to the lack of lactose permease) although they did possess B-galactosidase. These organisms are indeed coliforms and will grow on MLSB but they form pink colonies which are ignored in the U.K. standard method. Comparison of the two formulations of Colilert showed that they both performed with similar efficiency and thus that they are both suitable alternatives to the current membrane filtration technique for monitoring the bacteriological quality of water. However, U.K. regulations require that some drinking water samples must be examined with a procedure which will give quantitative data. The QuantiTray™ system divides 100 ml water samples into 51 discreet "wells" which enable the "most probable number" of organisms to be calculated. This system was compared with membrane filtration both in terms of the number of samples found to contain coliforms and/or *E. coli* and the actual counts of organisms found in individual samples. Comparison of the total number of samples found to contain coliforms using Colilert 18/QuantiTray™ and membrane filtration showed that membrane filtration detected coliforms in significantly fewer samples than did Colilert 18. No significant difference was seen between the numbers of samples found to contain *E. coli*. When the numbers of organisms detected by each method was compared, correlation coefficients of 0.87 and 0.89 for coliforms and *E. coli*, respectively, were obtained, which are significant at the 0.1% level. Both of these figures represent a good correlation between the membrane filtration and QuantiTray™ and thus the two methods give comparable results. In addition, the QuantiTray™ system is very easy to inoculate, requires no confirmations and has a

Table 4. Contingency table showing the numbers of drinking water samples which gave identical and discrepant *E. coli* results using membrane filtration and Colilert 24

	Colilert 24	
Membrane filtration	+	−
+	54	17
−	16	7302

Table 6. Comparison of the number of samples found to contain *E. coli* using Colilert 24 and Colilert 18 from 1057 post filtration, pre-chlorination samples

	Colilert 24	
Colilert 18	+	−
+	74	15
−	19	949

that Colilert, specifically, is suitable for use with the hard waters found in the Thames catchment.

CONCLUSIONS

Two defined substrate media, Colilert 24 and Colilert 18 have been compared with the United Kingdom standard membrane filtration method for the detection of coliforms and *E. coli*. The results indicate that:

(1) Both forms of Colilert® detect coliforms in more samples than does membrane filtration, whilst the detection rate for *E. coli* is similar for all three methods.

(2) A novel system for quantifying bacteria (QuantiTray™), based on the most probable number technique gave similar counts to membrane filtration for both coliforms and *E. coli*.

(3) Colilert® is suitable for the quantification of coliforms and *E. coli* in a wide range of sample types.

(4) Largely due to the fact that there is no need to "confirm" isolates, Colilert® is much simpler to use than membrane filtration and offers a cost-effective alternative.

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Table 7. Comparison of the number of partially disinfected sewage effluent samples found to contain coliforms using Colilert 18/QuantiTray™ and membrane filtration

	Colilert 18/QuantiTray™	
Membrane filtration	+	−
+	598	56
−	49	1760

counting range of up to 200 organisms per 100 ml. Whilst some organizations may report membrane filtration counts of up to 200, realistically, the upper limit of the count which can be made using membrane filtration is approximately 80. The QuantiTray™ system also has the benefit that growth of "background" organisms does not interfere with the ease of counting. Growth of other organisms on membranes can often mask the presence of target organisms and removal of this problem is a major benefit.

It should be noted that the confirmation rates for *E. coli* and coliforms from membranes found during this study were not as high as we would normally expect in our laboratories (Walter *et al.*, 1994). However, the fact that the results obtained with Colilert were confirmed in every case suggests that confirmation is not required with this method. This has two significant benefits for water companies. Firstly, removal of the need for confirmations in the laboratory significantly reduces the workload and hence the costs. Secondly, since confirmation is not required, operational staff can respond, confident in the knowledge that the result reported by the laboratory is correct. In this study, operational responses based on membrane filtration data would have been made unnecessarily in 30% of occasions when "presumptive" results were obtained. This saving in operational response has significant cost implications since resampling and remedial actions would be reduced.

The detection of the enzyme B-glucuronidase has been suggested for the detection of *E. coli* growing on membranes (Sartory and Howard, 1992) but in our hands the medium did not perform as well as described in the original paper (Walter *et al.*, 1994). Other similar media have subsequently been described (Brenner *et al.*, 1993), but we have no experience of their performance. Attempts in this laboratory to develop media which can be used with membrane filtration to simultaneously detect and discriminate between *E. coli* and coliforms using substrates specific for B-glucuronidase and B-galactosidase have not been successful. The benefits of such media would be that a single membrane could be used to detect both groups of organism and that a numerical result would be obtained. This is important in the U.K. in the context of examining samples from distribution zones where quantitative results must be obtained in order to quote maximum and mean levels of contamination for samples which are found to be positive. The use of the QuantiTray™ system allows quantitative results to be obtained and is more efficient than even the use of a single membrane.

Table 8. Comparison of the number of partially disinfected sewage effluent samples found to contain *E. coli* using Colilert 18/QuantiTray™ and membrane filtration

	Colilert 18/QuantiTray™	
Membrane filtration	+	−
+	286	34
−	37	2106

There have been some recent reports which have suggested that Colilert does not perform as well as some other European standard methods (Schets *et al.*, 1993; Gale and Broberg, 1993). The trials reported by Schets and colleagues compared Colilert with Dutch standard methods using a small number of samples and concluded that the Dutch method was more sensitive. The Dutch workers stated that they incubated Colilert for 24 ± 2 h. This is different from what is recommended. The product literature for the Colilert 24 product states categorically that incubation must be continued for a full 24 h period, and that if after that period there is a pale yellow colour lighter than that of the comparator, incubation should be continued for a further 4 h. This discrepancy in procedure may well account for the poor performance of Colilert in the Dutch trial. The trial reported by Gale and Broberg (1993) had no such deviations from procedure but their comparison was between the Colilert most probable number method and the U.K. standard MPN method. It is difficult therefore to compare the results of their trial with those reported here.

Reports that Colilert does not recover *E. coli* as efficiently as membrane filtration are worrying and inconsistent with much of the reported literature. For this reason we specifically undertook to examine the difference in sensitivity between membrane filtration and Colilert presence/absence. In our hands Colilert performed rather better than membrane filtration although the results were not significant ($p > 0.05$). We can offer no credible explanation for the different results obtained in our laboratory and those obtained elsewhere. However, the majority of published reports suggest that Colilert is a sensitive method for the detection of *E. coli* in drinking water (Berger, 1991) and, thus, our results are not surprising.

This study is, we believe, the largest comparison of Colilert with conventional methods which has been reported. The results suggest that Colilert could become a routine method in the water microbiology laboratory. However, further studies are required to determine if different water types will have any effect on Colilert performance. In this study all waters were hard and therefore it would be particularly interesting to determine the performance of Colilert with softer waters. However, we are confident that presence/absence tests and the newly developed QuantiTray™ can have a place in routine water microbiology and