IDEXX
Literature Cover Sheet

IDEXX #: 2I

Topic: Enterolert™, Colilert® and Colilert-18® vs. UK's MF Method

Title: Quantitative Procedures for the Detection of *E. coli*, coliforms and Enterococci in Water, Using Quanti-Tray® and Enterolert™

Author(s): C R Fricker, E J Fricker, T Goodall, and J Cowburn

Date: November, 1995

Source: Presented at the Water Quality Technology Conference(WQTC), New Orleans, LA

Highlights:

Colilert and Colilert-18
- Based on testing 1,926 samples of disinfected sewerage effluent, the Quanti-Tray system using Colilert or Colilert-18 reagent was found to be comparable to the UK Standard MF procedure.
- For samples where counts greater than 50 cfu per 100ml were observed, Quanti-Tray was "much easier to read than membrane filters."
- Quanti-Tray does not require confirmation and thus "accounts for a considerable time savings."

Enterolert
- Based on testing 984 samples of disinfected sewerage effluent and 184 samples of river water, Enterolert was "shown to give equivalent results to membrane filtration."
- Enterolert provides results in half the time compared to membrane filtration.

Quantitative Procedures for the Detection of *E.coli*, coliforms and Enterococci in Water, Using Quantitray and Enterolert

C R Fricker E J Fricker T Goodall and J Cowburn
Thames Water Utilities
Development Microbiology
Spencer House
Manor Farm Road
Reading
RG2 0JN
United Kingdom

Introduction

Defined substrate technology (DST) has become widely utilised for the detection of *E.coli* and coliforms in water, particularly in the United States (1). However, due to the regulations governing water quality in the United Kingdom, the use of this technology for the monitoring of potable water has been less widely implemented, particularly due to the requirement for quantitative data to be reported to the regulator. This requirement for quantitative data together with the 24 hr incubation period required for the Colilert presence/absence test (the current U.K. standard method is an 18 hr test) has prevented widespread use of Colilert in the U.K. DST has largely been utilised for coliforms and *E.coli* only, although the opportunity exists for the same technique to be applied to other organisms. Possession of *B*-glucosidase by the genus *Enterococcus* offers a further possibility for using DST for water quality monitoring.

Traditional most probable number techniques (MPN) are time consuming and not suitable for use in large laboratories which process hundreds of samples per day. However, the concept of using MPN technology is a good one, since the recovery of damaged organisms is thought to be enhanced with liquid media over that obtained on membranes. Furthermore, if the number of tubes used is large, then the 95% confidence limits which can be achieved are "tight".

This study describes the use of some new DST reagents in both qualitative and quantitative formats. The new reagents described are Colilert 18, which is used for the detection of coliforms and *E.coli* within 18 hr, Enterolert, which detects the presence of enterococci (based n possession of B-glucosidase) within 24 hr and Quantitray, a new procedure for determining quantitative results for *E.coli*, coliforms and enterococci using an MPN procedure.

MATERIALS AND METHODS

Water Samples

Several types of water samples were used during this study, including fully
treated water, water taken after slow sand filtration but before disinfection,
water which had been pre-chlorinated and sampled after coagulation, raw
surface and ground water and water which had been partially disinfected in
the laboratory. For the partially disinfected waters, samples of raw surface
water or sewage effluent which had been diluted 1:20 in tap water were
treated with chlorine at an initial concentration of 2 ppm. After a period
of 10 min, samples were removed every minute for the next 10 min and
the chlorine neutralised with sodium thiosulphate (2).

Reagents and Equipment

All reagents were supplied by the manufacturers (IDEXX, Portland, ME)
and used according to the manufacturers instructions. Experiments for
Colilert were performed at 37°C. Enterolert was incubated at 41°C.
Quantitrays were supplied by the manufacturer, pre-sterilised by gamma
irradiation.

U.K. Standard Methods

All samples were examined using U.K. standard methods (3). For
coliforms and *E.coli* this involved filtration of two 100ml volumes of water
through separate 0.45 *um* membranes and incubation of one of the
membranes at 37°C and the other at 44°C for 18 hr. Yellow or colourless
colonies were then examined for their ability to ferment lactose at 37°C
and 44°C, produce indole from tryptophane at 44°C and to produce
cytochrome oxidase. Colonies which fermented lactose at 37°C and did
not produce cytochrome oxidase were classified as coliforms. Those
which in addition produced indole and fermented lactose at 44°C were
regarded as being *E.coli*.

For enterococcal testing, samples (100 ml) were filtered through 0.45 *um*
membranes and incubated at 37°C for 24 hr followed by a further 24 hr at
44°C. Typical colonies were then further identified using standard
biochemical tests.

Performance of the Colilert test

Samples (100 ml) were placed in sterile plastic bottles and Colilert powder added to the bottle. After mixing to dissolve the powder, samples for presence/absence testing were incubated at 37°C for 24 or 18 hours depending on the formulation being used. For quantitative testing, the samples were added to Quantitrays after the powder had dissolved and the trays sealed in a heat sealer. Where positive reactions were seen with Colilert, the presence of coliforms and/or *E.coli* was confirmed by plating on solid medium and testing of colonies by standard bacterial identification systems as described above. Similarly, all positive enterococcus reactions were confirmed.

RESULTS

The data shown in Table 1 demonstrates that there is no significant difference in the recovery of coliforms when using membrane filtration Colilert 18 or Colilert 24. Table 2 shows the same type of data for *E.coli* and again there was no significant difference between the three methods ($p > 0.05$).

When comparing the recovery of coliforms and *E.coli* using the Quantitray and membrane filtration, 1926 samples of disinfected sewage effluent were used. The range of counts for both coliforms and *E.coli* was 0-200 and the correlation coefficients obtained were 0.88 for coliforms and 0.83 for *E.coli*. Tables 3 and 4 show the recovery of coliforms and *E.coli* respectively using Colilert 18/Quantitray and membrane filtration.

For the studies on enterococci, 984 samples of disinfected sewage effluent and 184 samples of river water were used. Table 5 shows the comparison of recoveries using Enterolert/Quantitray and the U.K. standard membrane filtration procedure. The correlation coefficient obtained for the two procedures was 0.81. A proportion of the isolates of enterococci obtained using each method were speciated and no particular bias was observed (data not shown). The frequency with which the major species were isolated was (most frequent first), *E.faecium*, *E.faecalis*, *E.durans* and *E.casseliflavus*.

DISCUSSION

This study has demonstrated the effectiveness of a new system for enumerating indicator bacteria in water. Using the Quantitray system, coliforms, enterococci and *E.coli* could all be enumerated in the range 0-

200 organisms per 100 ml. The results obtained were comparable to those obtained with membrane filtration methods. However, when high counts of bacteria were observed (>50) the Quantitray method was much easier to read than membrane filters. Growth of competing bacteria on membranes can often cause inhibition of the growth of target organisms or can mask the colour changes associated with carbohydrate fermentation. Use of the Quantitray system overcomes this problem and allows for accurate and easy counting. Furthermore, no confirmation of bacteria is required which accounts for a considerable time saving.

Two new defined substrate media have also been evaluated during this study. Firstly, the Colilert 18 test which gives results within 18 hours has been shown to give equivalent results to both Colilert 24 and membrane filtration. The medium Enterolert has also been shown to give equivalent results to membrane filtration for the detection of enterococci from water. Furthermore, the results are available after 24 hours whereas with the U.K. standard method results are not available for 48 hours. Because of the development of an 18 hr quantitative test (which is the standard in the U.K.), a multicentre study involving 90% of the U.K. water utilities is now underway in anticipation of the more widespread use of this cost-effective technology.

The views expressed here are those of the authors and do not necessarily reflect those of Thames Water Utilities Ltd.

REFERENCES

1. Edberg, S.C., Allen, M.T., Ludwig, T., Smith,D.B. and the National Collaborative Study. 1989. National field evaluation of a defined substrate method for the simultaneous enumeration of total coliforms and *Escherichia coli* from drinking water: comparison with presence-absence techniques. Applied and Environmental Microbiology, 55: 1003-1008.

2. Cowburn, J.K., Goodall, T., Fricker, E.J., Walter, K.S. and Fricker, C.R. 1994 A preliminary study of the use of Colilert for water quality monitoring. Letters in Applied Microbiology, 19: 50-52.

3. Anon 1994 The Microbiology of Water. Part 1 - Drinking Water. HMSO, London, U.K.

Table 1. Recovery of coliforms from different water types, using Colilert 18 and Colilert 24.

Water Type	Total samples	+ve by at least one method	+ve by MF	+ve by Colilert 24	+ve by Colilert 18
Fully treated	1623	13	11	13	12
Post filtration	131	63	52	55	56
Pre-chlorinated	214	179	138	141	143
Raw	65	52	43	43	41
Disinfected effluent	312	223	183	188	190

All positive reactions were confirmed by standard tests.

Table 2. Recovery of *E.coli* from different water types, using Colilert 18 and Colilert 24.

Water Type	Total samples	+ve by at least one method	+ve by MF	+ve by Colilert 24	+ve by Colilert 18
Fully treated	1623	3	2	3	3
Post filtration	131	18	14	14	16
Pre-chlorinated	214	41	34	34	33
Raw	65	19	14	16	15
Disinfected effluent	312	136	116	113	115

All positive reactions were confirmed by standard tests.

Table 3. Use of Quantitray and membrane filtration for the recovery of coliforms from disinfected sewage effluent.
Correlation coefficient = 0.88.

	Quantitray	
Membrane Filtration	+	-
+	467	52
-	67	1340

All positive reactions were confirmed by standard tests.

Table 4. Recovery of Quantitray and membrane filtration for the recovery of *E.coli* from disinfected sewage effluent.
Correlation coefficient = 0.83.

	Quantitray	
Membrane Filtration	+	-
+	195	25
-	24	1682

All positive reactions were confirmed by standard tests.

Table 5. Recovery of enterococci using Enterolert and membrane filtration disinfected sewage effluent and river water.
Correlation coefficient = 0.81

	Quantitray	
Membrane Filtration	+	-
+	173	13
-	10	788

All positive reactions were confirmed by standard tests.