IDEXX Summary 13A

Topic: Colilert vs. LMX broth (Merck's Readycult)

Title : Use of two presence/absence systems for the detection of *E. coli* and coliforms from water.

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Report Highlights:

- Study done by Thames Water Utilities (London, UK) on detection of coliforms and *E. coli* using Colilert and LMX broth.
- LMX has a high false positive rate and must be confirmed

	# of samples	Coliform False Positive Rate	*E. coli* False Positive Rate
Colilert	**174**	**0%**	**0%**
LMX broth	**210**	**15.7%**	**9.6%**

Use of two presence/absence systems for the detection of *E.coli* and coliforms from water.

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The Colilert system presence/absence test and LMX broth were compared for their ability to recover *E.coli* and coliforms from water. Both methods gave similar recoveries for both groups of organisms. The LMX system however, gave a significant number of false positives, largely due to the presence of *Aeromonas* spp. It is concluded that care must be taken in the interpretation of results obtained using some presence/absence methods where no confirmation of the identity of bacteria is performed.

INTRODUCTION

The routine monitoring of the bacteriological quality of drinking water relies on the use of the indicator organisms *E.coli* and coliforms and they are used to indicate faecal contamination or other water quality problems such as failures of disinfection, bacterial regrowth within the distribution system or ingress. The most commonly employed technique for the detection of these organisms in water is membrane filtration. Normally, water (100 ml) is concentrated by membrane filtration and the membranes placed onto a selective and differential medium such as membrane lauryl sulphate broth (Anon, 1994) which inhibits the growth of Gram positive bacteria and contains lactose and a pH indicator for detection of fermentation of lactose. Two membranes are normally used for each sample, one incubated at 37°C (for coliforms) and the other at 44°C for *E.coli*). Membranes are incubated for 18 hours and examined for yellow colonies which are then identified using standard phenotypic tests (Anon, 1994). The World Health Organisation guidelines for these organisms are such that a single *E.coli* or coliform organism in 100 ml of potable water is deemed to be unsatisfactory and these guidelines have been used as the basis for most drinking water regulations throughout the world. For many situations

therefore, it is not necessary to accurately quantify the numbers of indicator bacteria present in a sample. Presence/absence methods have therefore become widely adopted in water laboratories, particularly in Germany, the United States and South America since these methods offer a significant advantage in terms of labour saving and the reduced time required to obtain a confirmed result.

Colilert (IDEXX, Chalfont St Giles, UK) is a product which is widely used in the United States and is becoming more widely used in the United Kingdom. Comparative trials in the U.K. have shown that it gives equivalent results to the U.K. standard membrane filtration method (Cowburn *et al.*, 1994; Fricker *et al*, 1994). In extensive tests, Colilert detected *E.coli* in similar numbers of samples as did membrane filtration and in fact detected coliforms more frequently. The Colilert test uses the principle of defined substrate technology whereas many other products are available which whilst including chromogenic or fluorogenic substrates for specific enzymes (e.g. β-glucuronidase and β-galactosidase), also contain other carbon and energy sources. Defined substrate technology is a patented process where the major carbon and energy sources for bacterial growth are also indicators of growth. Thus in Colilert, the two substrates for the enzymes β-glucuronidase and β-galactosidase are the major sources of carbon and energy and result in the formation of a fluorescent and a coloured product which indicates that growth of *E.coli* or coliforms has occurred. One product which contains substrates for β-glucuronidase and β-galactosidase together with other carbon and energy sources is LMX broth (Merck, Poole, U.K) which has recently become available and is recommended for

use in detecting *E.coli* and coliforms in water and foods. The plethora of newly formulated media which are being developed prompted a study to determine the relative effectiveness of a defined substrate medium and a more traditionally formulated medium containing substrates for the enzymes *B*-galactosidase and *B*-glucuronidase.

MATERIALS AND METHODS

Water samples were obtained from a wide geographical area and consisted of raw waters (both ground and surface supplies), partially treated water, marginally chlorinated water and final distribution water. Raw, partially treated and distribution water were collected into sterile glass bottles, transported in cooled, insulated containers and analysed within six hours. Marginally chlorinated water was prepared according to the methods of Cowburn *et al.* (1994). Briefly these were raw water or sewage effluent diluted 1:20 in tap water. This mixture was then chlorinated to give an initial concentration of 1.5-2 ppm free chlorine and the solution was continually stirred before samples were taken after at one minute intervals between 10-25 minutes after the chlorine had been added. LMX was prepared as a triple strength broth, distributed in 50 ml amount in glass bottles and stored at 2-8°C in the dark until required. Water samples (100 ml) were added to the LMX medium and to sterile glass bottles to which Colilert was then added. All samples were incubated for 24 hours in a waterbath (Fricker et al 1994), Colilert samples at 35°C and LMX at 37°C.

After incubation, all samples showing a colour change (indicating the presence of coliforms) and or having demonstrable fluorescence (indicating the presence of *E.coli)*

were plated on two plates of MacConkey agar, one incubated at 37°C and the other at 44°C. Lactose fermenting colonies on the 37°C plate were treated as presumptive coliforms and those on the 44°C as presumptive *E.coli*. Colonies were then tested for acid production at 37°C and 44°C, production of indole from tryptophane at 44°C and oxidase production. Coliform organisms produce acid from lactose at 37°C and are oxidase negative, *E.coli* also produces acid from lactose at 44°C and produces indole from tryptophane at the same temperature. Organisms which could not easily be shown to be coliforms or *E.coli* were further identified using API 20E (Biomerieux, Basingstoke, UK).

Further experiments were conducted to establish the growth of *Aeromonas* spp. in the two media as these organisms frequently cause false positive reactions in media used for the isolation of coliforms. Briefly, organisms were cultured in nutrient broth and diluted in sterile water to give counts in the range 1-10^4 per ml. The numbers of colony forming units per ml were determined using membrane filtration or pour plate techniques using yeast extract agar. These samples were also used to inoculate LMX broth and Colilert which were incubated according to the manufacturers instructions.

RESULTS

A total of 986 samples were tested. These consisted of 82 raw waters, 75 partially treated waters, 160 marginally disinfected samples and 669 treated waters. The recovery of *E.coli* and coliforms from these waters is shown in Table 1. There was no significant difference in the recovery of *E.coli* or coliforms with the two methods ($p > 0.05$). LMX broth recovered coliforms from more samples than did Colilert (177 vs 174) whereas Colilert recovered *E.coli* from more samples than did LMX (49 vs 47). The

major difference between the two media was the confirmation rate for both coliforms and *E.coli*. No false positive reactions were seen with Colilert and on every occasion when a yellow colour was seen, a coliform was identified and similarly when fluorescence was seen, *E.coli* was identified. In this study we did not encounter any *E.coli* strains which did not produce fluorescence. With LMX the overall confirmation rate for coliforms was 84.3% and that for *E.coli* 90.4%.

Most of the false positive results for coliforms in LMX were due to *Aeromonas* spp. and therefore the growth of some environmental isolates of these organisms in the two media was investigated. Table 3 shows the growth of *Aeromonas* strains in the two media. LMX supported the growth of 17 of 64 strains whereas only one strain gave a positive reaction in Colilert.

DISCUSSION

The use of media containing chromogenic or fluorogenic substrates for the enzymes *B*-galactosidase and *B*-glucuronidase for simultaneous detection of coliforms and *E.coli* is increasing (Sartory and Howard, 1992; Brenner *et al.*, 1993) and whilst these are not always superior to standard methods (Walter *et al.*, 1994), further development of such media is likely to lead to more widespread use, particularly if no confirmation of results is required. The Colilert test is widely used, particularly in the United States and a number of studies have shown that it gives equivalent results to membrane filtration techniques, in that similar numbers of samples are found to be contaminated when using either method (Cowburn *et al.*,1994; Fricker *et al.*, 1994). The benefits of such presence/absence tests are largely their ease of use and the fact that confirmation of positive reactions is not required, leading to a confirmed result at least one day earlier than would otherwise be attainable. This is of considerable importance to the water industry. Because of the time delay normally encountered before a confirmed result is available, water utilities must act on presumptive results. This usually leads to further sampling and may result in interruption of the water supply to customers. If the presumptive results are later shown to be correct then the action and the expense incurred is justified. However, if the presumptive results are not confirmed then the expense has been wasted. Furthermore, the ability to differentiate between *E.coli* and thermotolerant coliforms can alter the decision that water utilities make about the safety of the water supply.

The results of this study have reinforced the observation that confirmation is not required for Colilert, but has demonstrated that false positive reactions do occur in LMX broth, due largely to the growth of *Aeromonas* spp. In addition a small number of apparent false positive *B*-glucuronidase reactions were seen which were due either to growth of *Aeromonas* spp which possessed *β*-glucuronidase or to fluorescent *Pseudomonas* spp. Thus in routine use, LMX broth would not be of substantial benefit since positive reactions would need to be confirmed, leading to a delay in obtaining an accurate result. Furthermore, whilst Colilert is presented as a pre-dispensed, sterile powder which can be added directly to the water sample, LMX is sold as a dehydrated powder which needs to be reconstituted, dispensed and sterilised. Thus Colilert offers a further saving in staff time, in that no preparation of the medium is required, which makes it particularly suitable for small laboratories with limited facilities and resources. Recently a device has become available with Colilert which allows quantitative results to be obtained without the need for multiple tube tests to be performed. This is an additional benefit to the use of Colilert.

The conclusions of this study are that Colilert is a suitable medium for water quality monitoring and that confirmation of positive reactions is not required. However, if other media such as LMX broth are used, care must be taken in the interpretation of the results and confirmation of positive reactions must be performed.

Table 1. Recovery of coliforms from different water types using LMX broth and Colilert.

Water type	Number samples	LMX broth		Colilert	
		Presumptive	Confirmed	Presumptive	Confirmed
Raw	82	73	69	68	68
Partially treated	75	46	35	36	36
Marginally chlorinated	160	77	62	59	59
Disinfected	669	14	11	11	11
TOTAL	986	210	177	174	174

Table 2. Recovery of *E.coli* from different water types using LMX broth and Colilert.

Water type	Number samples	LMX broth		Colilert	
		Presumptive	Confirmed	Presumptive	Confirmed
Raw	82	25	24	23	23
Partially treated	75	11	9	10	10
Marginally chlorinated	160	15	14	16	16
Disinfected	669	1	0	0	0
TOTAL	986	52	47	49	49

Table 3. Positive *B*-galactosidase reactions obtained in LMX and Colilert with *Aeromonas* spp.

Number of cells added	Number of strains used	Positive reaction in LMX	Positive reaction in Colilert
1-10	9	1	0
$10\text{-}10^2$	13	3	0
$10^2\text{-}10^3$	27	8	0
$10^3\text{-}10^4$	15	5	1

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