

# A COMPARATIVE STUDY OF SELECTIVE MEDIA USED TO DETECT AND CONFIRM COLIFORMS AND *ESCHERICHIA COLI* IN WATER SAMPLES USING MEMBRANE FILTRATION

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(excerpts),

## ABSTRACT

New membrane filter media were developed to make the detection and confirmation of *E.coli* and coliforms more rapid. Two of those media, Tryptone bile agar plus MUG and Membrane lauryl sulphate broth plus MUG, included the substrate 4-methylumbelliferyl- $\beta$ -D-glucuronide, which was cleaved by the *E.coli* enzyme  $\beta$ -glucuronidase to form 4-methylumbelliferone (which fluoresces under longwave UV light at 366nm). Another two media, **CHROMagar ECC** and **CHROMagar ECC liquid**, utilised chromogenic substrates which were cleaved to give a pink-coloured colony for coliforms, and a blue-coloured colony for *E.coli*. The new media coliform and *E.coli* recoveries were compared with those of standard membrane filtration using Membrane lauryl sulphate broth. With the exception of Membrane lauryl sulphate broth plus MUG, all of the new media compared favourably with standard Membrane lauryl sulphate broth, in many instances giving higher counts. The **CHROMagar ECC liquid** particularly, gave increased sensitivity for both coliforms and *E.coli* at low and high counts over standard Membrane lauryl sulphate broth. The **CHROMagar ECC liquid**: 1) was at least as sensitive as standard membrane filtration methods; 2) did not require confirmatory tests; and 3) was very easy to interpret. Thus, where coliforms or *E.coli* are likely to be isolated and will need to be confirmed, it should be recommended that the **CHROMagar ECC liquid** be used in the place of traditional membrane filtration media.

TABLE 1

**Comparison of the Numbers of *E.coli* Detected from Water Samples Growing on a Variety of Media**

Colonies detected*	<300	300-500	>500
Media used			
MLSF	0% (0)	67% (2)	33% (1)
TBM	0% (0)	67% (2)	33% (1)
MLSFB	100% (3)	0% (0)	0% (0)
CHROMagar ECC liquid	0% (0)	33% (1)	67% (2)
CHROMagar ECC	0% (0)	33% (1)	67% (2)

\*The percent % (total number of plates in brackets) of coliforms detected using a variety of media colony banding of 300+, in a sample size of 3.

**TABLE 2****A Comparison of the Numbers of *E.coli* Detected from Water Samples Growing on a Variety of Media.**

Colonies detected*	0	1	2	>2
Media used				
MLSF	63% (32)	24% (12)	14% (7)	0% (0)
TBM	57% (29)	16% (8)	16% (8)	12% (6)
MLSFB	63% (32)	14% (7)	12% (6)	12% (6)
CHROMagar ECC liquid	51% (26)	22% (11)	12% (6)	16% (8)
CHROMagar ECC	65% (33)	12% (6)	12% (6)	12% (6)

\*the percent % (total number of plates in brackets) of *E.coli* detected using a variety of media with a colony banding of 0-2 cfu / 100ml, in a sample size of 5 liters.

**TABLE 3****The Cost (per sample) of Testing Water Samples using a Variety of Media**

Media used:	MLSF	TBM	MLSFB	CHR.ECC liquid	CHR.ECC
Cost (per sample)*	£ 2.35	£ 2.20	£ 2.20	£ 1.60	£ 2.00

\* Costs are based on 1 MLSO grade 1 testing and analysing each sample.

Although the basic media for the new methods listed above are all more expensive than standard Membrane lauryl sulphate broth (sometimes much more expensive), the biggest overall cost to the laboratory is staffing. This is reduced in all of the new methods due to the lack of time-consuming confirmation techniques. The **CHROMagar** media, although much more expensive than other media in itself, works out to be the cheapest option, due to the need for only one plate per sample and the non-requirement for any confirmation tests.

**CONCLUSION**

Of the methods tested, TBM agar (tryptone bile agar plus MUG), **CHROMagar ECC** and **CHROMagar ECC liquid** all compared very favourably against standard membrane filtration using MLSF (membrane lauryl sulphate), and in many cases gave higher counts.

Only the Membrane lauryl sulphate broth containing MUG was shown to be less sensitive than the standard MF method using Membrane lauryl sulphate broth alone. It is unknown as to why this was the case, but further work should be carried out regarding the effects of substances in Membrane lauryl sulphate broth on the MUG substrate and on varying the concentrations of MUG in the broth.

The most successful of the media used was the **CHROMagar ECC liquid**. Due to the very favourable results obtained by this media, the ease of use, the large savings on staffing time and laboratory materials, and, not least of all, the shorter turn-around-time (24 hours), where it is likely that coliforms or *E.coli* are likely to be isolated and will need to be confirmed it should be recommended that this media be used in the place of standard membrane filtration.