

Evaluation Of AquaCHROM[™] ECC, A New Chromogenic Culture Broth For The Detection Of *E.coli* And Other Coliforms

AUTHORS A. Lerner¹, J. Coral¹, L-C. CHAl² ¹ CHROMagar, Paris, FRANCE, ² Faculty of Sci. - Univ. of Malaya, Kuala Lumpur, MALAYSIA.

Revised Abstract

<u>Background</u>

E.coli and coliforms are indicators of drinking water quality, their presence meaning a greater risk of more harmful pathogens. A test allowing for early, simple and reliable detection of fecally contaminated water would enhance public health. There are several commercially available broth media, but all of them are based on the use of a fluorogen, which requires the use of a UV lamp. AquaCHROM[™] ECC is based on the use of two chromogens (no UV), facilitating the water quality control in remote areas where laboratory resources are not available. In this study, we evaluated the performances of AquaCHROM™ ECC. <u>Materials</u>

For the sensitivity/specificity test, 187 well-characterised strains were used: 70 *E.coli*, 29 other coliforms and 87 non coliforms. The inoculum ranged from 100-10,000 CFU/100 ml.

For the detection limit, individual strains of *E.coli*, coliform and mixes, were tested at different inoculum/replicates. In order to determine the performances at room temperature incubation, one E.coli and one Citrobacter were tested at 100CFU/100ml. The colour and temperature were recorded every three hours. In order to determine the performances of AquaCHROM[™] ECC within the MPN method, a total of 50 food samples were purchased from the local markets in Malaya (Malaysia). Each food sample was enumerated for coliforms and *E.coli* with both conventional three-tube-MPN (cMPN) and modified AquaCHROM[™] MPN (mMPN) assays. <u>Results</u>

The sensitivities/specificities of AquaCHROM[™] ECC for *E.coli* (glucuronidase positive) were 100%/100% and for the common coliforms 94%/74%, respectively. The limits of detection for *E.coli* and coliforms were: 1CFU/100ml ; 1 *E.coli*/10⁴ coliform at 18 hours ; 1 E.coli/10⁶ coliform at 24 hours. The AquaCHROMTM ECC incubated at room temperature showed visible colour changes at 24-48 hours.

Concerning MPN method tests, out of 50 food samples tested, 48 (96.0%) were contaminated with coliform. Both MPN assays used in this study generated similar prevalence result for coliform, but varied greatly in the detection of *E.coli*. The cMPN assay detected *E.coli* in only 44.0% of the samples; whilst the mMPN assay had found 64.0% of the samples positive for *E.coli*.

<u>Conclusion</u>

AquaCHROM^M ECC showed excellent detection performances and its use is suitable for water as well as food quality control. Further evaluations could include studies in rural areas and resource constrained settings.

Methods

STUDY No. 1: Medium specificity and sensitivity

186 well-characterised strains were tested: 70 *E.coli*, 29 other coliforms. The inoculum used were about 100 CFU/100ml for *E.coli* and the other coliforms, and 10,000 CFU/ 100 ml for non-coliforms. Each isolate inoculum was added into 100ml of sterile distilled water and then incubated for 24h at 37 °C.

STUDY No. 2: Detection limit

To determine the detection limit, we used two pure cultures: an *E.coli* and one coliform. Each strain was tested at three different levels of contamination, with three replicates per level. Then, we tested a mix of *E.coli*/coliform. 9 different ratios of *E.coli*/coliform were tested, with three replicates for each ratio.

STUDY No. 3: Comparison with other broth media

AquaCHROMTM ECC reading performance, was compared with Colilert (IDEXX) and Readycult (MERCK). 6 different strains of *E. coli* and 5 different strains of other coliforms were added, from the same inoculum, to each one of the three media. Inoculum ranges were 100 CFU / 100ml. The three broth were incubated for 24h at 37°C and interpreted according to the manufacturers indications.

STUDY No. 4: Incubation at room temperature

1 *E.coli* and 1 coliform was tested at 100 CFU/100ml and the medium was incubate at room temperature. The color apparition was recorded every 3 hours.

STUDY No. 5: MPN test

A total of 50 food samples comprise of raw meats, raw chicken parts, prawns, cockles, vegetables and ready-to-eat foods were purchased from the local markets. Each food sample was enumerated for coliform and *E.coli* with both methods, after 24h and 48h of incubation at 35°C: (1) conventional three-tube-MPN (cMPN): 1ml in 3 tubes and 3 consecutive dilutions, (2) modified AquaCHROM[™] ECC MPN (mMPN): 0,1ml in 8 wells and 6 consecutive dilutions

REFERENCES

TOPIC Q02 Fecal Pollution Indicators and Pathogens

Results

STUDY No. 1: Medium specificity and sensitivity

The study results were analysed in according to the prevalence of each specific strain (see Table 1).

For *E.coli*, two classification groups were defined: Glucuronidase positive (Gluc+) and glucuronidase negative (Gluc-).

For the other coliforms, two classification groups were also defined: "common" and "rare".

For the non-coliforms, two classification groups were also defined: "common" and "rare".

• *E.coli* (70):

Result: 60/60 Gluc+ showed a green colour and 10/10 Glucshowed a yellow colour, as expected.

• Other coliforms (29):

-onthecommongroup(Klebsiella, Enterobacter, Citrobacter), 17/18 turned yellow as expected.

-on rare coliforms group (Serratia, Hafnia, E.hermanii, *Yersinia*) 5 / 11 turned yellow as expected.

Non-coliform (86):

-on the most common group (Enterococcus, Streptococcus, Staphylococcus and Salmonella): 51/54 were inhibited as expected.

-on the rare group (Pseudomonas, Aeromonas, Clostridium, Listeria, Shigella, Vibrio, Proteus, Legionella, Acinetobacter), 27/32 were inhibited as expected.

Table 1: AquaCHROM[™] ECC sensitivity for the 3 different groups

	Tested	Green	Yellow	Colorless
	isolates			or inhibited
E.coli	70			
Glucuronidase+	60	60 (100%)		
Glucuronidase-	10		10 (100%)	
Other coliforms	29			
Common ¹	18		17 (94%)	
rare ²	11		5 (45%)	
Non-coliforms	86			
Common ³	54			51 (94%)
rare ⁴	32			27 (84%)

species tested : *Klebsiella, Enterobacter, Citrobacter*

species tested : Serratia, Hafnia, E.hermanii, Yersinia

³ species tested : *Enterococcus, Streptococcus, Staphylococcus and Salmonella*

species tested : Pseudomonas, Aeromonas, Clostridium, Listeria, Shigella, Vibrio, Proteus,

Legionella, Acinetobacter

Conclusions

• AquaCHROM[™] ECC showed excellent specificity and sensitivity (100% for *E.coli* glc + and 94% of common coliforms). • AquaCHROM[™] ECC, owe to the combination of two chromogens instead of one chromogen and one fluorogen, is easier to read

than Colilert-18 and Readycult coliform.

• AquaCHROM™ ECC is suitable for areas where no laboratory facilities are available: no need of UV light and can be incubated at room temperature.

• In this study, we found the performance of AquaCHROM[™] ECC in the detection of coliform was comparable to the conventional assay. However, the sensitivity in *E. coli* detection was at least doubled in mMPN assay compared to cMPN. In general, mMPN assay had a wider range of detection limit (<1.1 to >2.1 x 10^6 MPN/g) compared to a 3 x 3 cMPN format (<0.3 to >110 MPN/g)data not shown.

STUDY No. 2: Detection limit

- In pure strains, *E.coli* were detected from 1 UFC / 100ml and coliforms from 1 UFC / 100ml. • At 18h, *E.coli* were detected in a mixture of 1 *E.coli* to 10⁴ coliforms • At 24h, *E.coli* were detected in a mixture of 1 *E.coli* to 10⁶ coliforms.

STUDY No. 3: Comparison with similar commercially available media



Although for "normal" strains (classical phenotypic behaviour), the three media performed similarly. The fluorescence is difficult to read, because it can be hidden by the colour of the medium. The chromogens are easier to read because the colour difference between green (for *E.coli*) and yellow (for coliforms) is clearer than the difference between a colour with/without fluorescence. For "difficult" strains (*E.coli* with weak β-glucuronidase activity, for instance) there is a clear advantage of the chromogenic over the fluorogen. This late is barely detectable (see picture) while the green chromogen on the AquaCHROM[™] ECC, although weak, is clearly detectable. Note: The packaging of Readycult was found not practical because some capsules do not open properly.

STUDY No. 5: Results of MPN Test

Out of 50 food samples tested, 48 (96.0%) were contaminated with coliform. Both MPN assays used in this study generated similar prevalence result for coliform, but varied greatly in the detection of *E.coli*. The cMPN assay detected *E.coli* in only 44.0% of the samples; whilst the mMPN assay had found 64.0% of the samples positive for *E.coli*. PCR assay had confirmed the presence of *E.coli* in those *E.coli* mMPN positive wells.

E.coli ("difficult" strain)







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