Evaluation of Oxoid Selective E. coli/Coliform Chromogenic Medium Using Pure Cultures

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INTRODUCTION

The recovery and enumeration of *Escherichia coli* and other coliforms is an important indicator of environmental and food hygiene.[1]

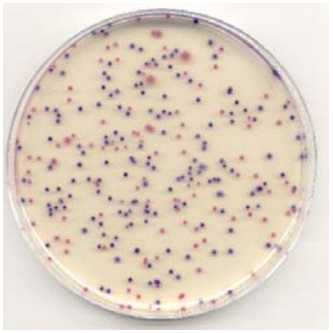
In recent years, specific and rapid identification of these indicator organisms has been greatly improved by incorporating of specific substrates into culture media that visualise enzyme mediated chromogenic reactions.

Oxoid Selective E. coli/Coliform Chromogenic Medium is a medium incorporating chromogens that can be used for the isolation and presumptive identification of *E. coli* and total coliforms in environmental and food samples (Figure 1).

The four chromogenic media used in this evaluation each contained two chromogenic substrates, one for glucuronidase activity (*E. coli*) and the other for galactosidase activity (coliforms). The respective chromogenic reactions allow the user to differentiate between *E. coli* and other coliforms and therefore enumerate both simultaneously.

In this study pure cultures were used to evaluate the performance of Oxoid chromogenic medium in comparison to three competitors' media. Oxoid Plate Count Agar (PCA) was used to validate the counts obtained from these media.

Figure 1: Typical colours of organisms in a pour plate of Oxoid Selective E. coli/ Coliform Medium (CM1046)



***E. coli* – purple**
Coliforms - pink

METHODS

Media tested and preparation

- Selective E. coli/Coliform Chromogenic Medium (Oxoid)
- Chromocult Coliform Agar (Merck)
- Harlequin E. coli/Coliform Medium (LabM)
- E. coli/Coliform CHROMagar ECC (CHROMagar)
- PCA (Oxoid)

All media were prepared according to the manufacturer's instructions.

Preparation and testing of pure inoculum

25 *E. coli* cultures, 14 other coliform cultures and 11 other organisms; which included; *Escherichia* spp., *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Hafnia alvei*, *Shigella* spp., *Proteus* spp., *Salmonella* spp., *Aeromonas* spp., *Pseudomonas aeruginosa* and *Lactobaccillus* spp.; were grown overnight in Nutrient Broth (NB) at 37ºC. Each broth was serially diluted to 10^{-6} using Maximum Recovery Diluent (MRD). Miles and Misra[2] counts were performed in duplicate on these dilutions using all 5 media. The expected colony colours for each chromogenic medium are shown in Table 1.

Table 1: Pure culture colony colour specification, as stated in the product literature

MEDIUM	Colony Colour		
	E. coli	coliforms	Others
PCA	White	White	White
Selective E. coli/Coliform Chromogenic Medium (Oxoid)	Purple	Pink	Colourless or no growth
Chromocult Coliform Agar (Merck)	Purple	Pink	No information given
Harlequin E. coli/Coliform Medium (LabM)	Blue/Purple	Rose/Pink	Usually no growth
E. coli/Coliform CHROMagar® ECC (CHROMagar)	Blue	Red	No information given

RESULTS

Table 2: Pure culture colony colours on chromogenic media

ORGANISM	No. tested	Colony colour on selective media				
		PCA	Oxoid	Merck	LabM	CHROMagar
E. coli	25	White	Purple (20) Green/purple (2) Purple/pink (2) NG (1)	Purple (22) Green/purple (2) NG (1)	Blue (12) Pale Blue (2) Blue/ Purple (2) Green/Blue (2) Purple (5) Pale Purple (1) NG (1)	Pale Blue (1) Very Pale Blue (1) Green (22) NG (1)
Escherichia spp.	3	White	Pink	Pink	Purple (2) Blue/Purple (1)	Pink
Citrobacter spp.	3	White	Pink	Pink	Purple (2) Purple/Pink (1)	Pink (2) Pink/Purple (1)
Klebsiella spp.	3	White	Pink	Pink	Purple	Pink
Enterobacter spp.	5	White	Pink	Pink	Purple (4) Green/Blue (1)	Pink (4) Pink/Purple (1)
Serratia spp.	3	White (2) NG (1)	Pink (2) NG (1)	Pink (2) NG (1)	Pink (1) Pink/Purple (1) NG (1)	Pink (2) NG (1)
Hafnia alvei	1	White	White	White	White	White
Salmonella spp	2	White	White	White	White	White
Shigella boydii	1	White	Green	Green	Green	Green
Proteus vulgaris	1	White	Cream/White	Orange	Cream	Pink
Aeromonas hydrophila	1	White	Pink	NG	White	NG
Pseudomonas aeruginosa	1	Green	White	White	Green	Green
Lactobacillus plantarum	1	White	NG	NG	NG	NG

NG = no growth

Table 3: Total count productivity ratios (PR) of the chromogenic media compared to PCA

	PCA	Oxoid Selective *E. coli*/ Coliform Chromogenic Medium	Merck Chromocult Coliform Agar	LabM Harlequin E. coli/Coliform Medium	E. coli/Coliform CHROMagar ECC
E. coli PR (n=25)	1.0	0.95 – 1.08*	0.95 – 1.06*	0.94 – 1.09*	0.96 – ≥1.17*
Coliform PR (n=14)	1.0	0.95 – 1.05	0.96 – 1.05	0.94 - ≥1.17	0.96 – 1.08
Other organisms PR (n=11)	1.0	0.96 – 1.12**	0.98 – ≥1.06**[a]	≤0.85 – 1.02**	0.94 – 1.01**[a]

Table 3 cont.: * 1 *E. coli* strain was not recovered by all four chromogenic media tested yielding a PR of ≤0.71 at the lowest dilution plated.
** 1 *Lactobacillus plantarum* was not recovered by all four chromogenic media at the lowest dilution plated yielding a PR of ≤0.30.
[a] 2 chromogenic media did not recover *Aeromonas hydrophila* at the lowest dilution plated yielding a PR of ≤0.33.
NG = no growth

DISCUSSION AND CONCLUSIONS

The productivity ratios (ratio of total cfu count of the test medium against total cfu count of the reference medium) showed good agreement for pure culture counts obtained from all chromogenic media by comparison to PCA.[3] However, one *E. coli* was not recovered at the lowest dilution plated by all four chromogenic media (Table 3). *Lactobacillus plantarum* was not recovered by all four chromogenic media (Table 2). *L. plantarum* frequently causes problems because it competes with the growth of target organisms on isolation plates. However, the inclusion of lauryl sulphate in the Oxoid medium effectively eliminates the growth of *Lactobacillus* spp. and other non-target organisms. Recovery on PCA confirmed that it was a viable culture.

The colours of pure cultures showed some variations within species (Table 2). This was particularly apparent on all chromogenic media inoculated with *E.coli*. Colour variations with other species were more apparent with LabM and CHROMagar media. Enzyme levels do vary between species, with factors such as inducer levels, types of permease and other cell wall transport systems present in different species playing an important role in enzyme activity.[1]

Serratia spp. yielded pink, false positive (FP) coliform reactions on all chromogenic media tested. *Proteus vulgaris* yielded a FP coliform reaction on CHROMagar and only *Aeromonas hydrophila* yielded FP coliforms on Oxoid medium (Table 2). *Aeromonas* spp. are known to give FP reactions on chromogenic media and can be distinguished from the Enterobacteriaceae by the oxidase test, i.e. they are oxidase positive.[1]

Overall, the results show that the performance of Oxoid E. coli/coliform Chromogenic Medium was equivalent to that of other chromogenic media.

ACKNOWLEDGMENTS

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REFERENCES

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