



# Thermo Scientific Chromogenic Coliform Agar (ISO) Demonstrates Superior Detection of Coliforms From Waters With Low Bacterial Numbers

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## Key Words

Thermo Scientific Chromogenic Coliform Agar (ISO), Coliform, water testing

## Goal

To compare performance of Thermo Scientific™ Chromogenic Coliform Agar (ISO) (Thermo Fisher Scientific) and Chromocult™ Coliform Agar (Merck); two culture media formulated to **ISO 9308-1:2014** - Enumeration of *Escherichia coli* and coliform bacteria, Part 1: Membrane filtration method for waters with low bacterial background<sup>1</sup>.

## Abstract

Thermo Scientific Chromogenic Coliform Agar (ISO) (see figure 1) was assessed alongside Chromocult Coliform Agar to compare the performance of the two media for detection of coliforms. Coliform organisms were spiked into water samples which were then vacuum filtered onto a membrane, placed onto each media and incubated as per ISO 9308-1:2014. Non-coliform organisms were streaked directly onto the two media. Thermo Scientific Chromogenic Coliform Agar (ISO) proved to effectively isolate coliforms from water samples.

## Introduction

Chromogenic coliform agar (ISO) is a medium for the detection and enumeration of coliforms in food and water samples; the formulation is described in ISO 9308-1:2014. Publication of ISO 9308-1:2014 replaced ISO 9308-1:2000, which reduced the time to result and included the use of a chromogenic medium. The method is based on membrane



**Figure 1. Thermo Scientific Chromogenic Coliform Agar (ISO) growing *E. coli* (pink to red) and coliform (dark blue/violet) colonies on membrane filter.**

filtration, subsequent culture on chromogenic coliform agar medium, and calculation of the number of target organisms in the sample. The method is suitable for waters with low bacterial numbers that will cause less than 100 total colonies on chromogenic coliform agar medium. These may be drinking (potable) water, disinfected pool water, or finished drinking water from water treatment plants.

## The Study

### Organism preparation

Thirty seven coliform organisms (including 5 *Citrobacter freundii*, 1 *Enterobacter aerogenes*, 2 *Enterobacter cloacae*, 21 *Escherichia coli*, 4 *Klebsiella aerogenes* and 4 *Klebsiella pneumoniae*) and 17 non-coliform organisms

(including 2 *Aeromonas hydrophilia*, 2 *Bacillus* spp., 1 *Candida albicans*, 3 *Enterococcus* spp., 1 *Pseudomonas aeruginosa*, 2 *Salmonella* spp., 4 *Staphylococcus* spp. and 2 *Streptococcus* spp.) were included in the study.

All organisms were cultured from -80 °C freezer storage onto Tryptone Soya Agar; cultures were incubated at 36±2 °C for 18-24 hours. Three to five colonies were selected from each culture plate and inoculated into Nutrient Broth No. 2; the broth was then incubated at 36±2 °C for 18-24 hours. Following incubation, inoculated nutrient broths were diluted in sterile saline to produce a 0.5 McFarland standard suspension.

### Coliform testing

One hundred ml volumes of saline were inoculated with each coliform organism to achieve an inoculum level of 50-150 cfu in 10 ml as per the following method:

- a) Transfer 150 µl of each 0.5 McFarland suspension into the first 100 ml saline bottle (bottle A). Invert bottle A three times.
- b) Transfer 1 ml from bottle A into a second 100 ml saline (bottle B). Invert bottle B three times.
- c) Transfer 1 ml from bottle B into a third 100 ml saline (bottle C). Invert bottle C three times.

Ten ml samples of each organism from bottle C were vacuum filtered using 47 mm diameter 0.45 µm cellulose nitrate membrane filters as per ISO 9308-1:2014. Membrane filters were placed centrally onto the surface of Thermo Scientific Chromogenic Coliform Agar (ISO), Chromocult Coliform Agar plus Tryptone Soya Agar plates in duplicate. The vacuum filtration equipment was cleaned after each 10 ml sample filtration by vacuum filtering 25 ml sterile saline between samples.

Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar plates were incubated at

36±2 °C for 20-24 hours. Following incubation, colony counts, colony size and colony colour were recorded.

### Non-coliform testing

A 10 µl loop of each 0.5 McFarland standard suspension was inoculated directly onto Thermo Scientific Chromogenic Coliform Agar (ISO), Chromocult Coliform Agar plus Tryptone Soya Agar plates. All media were incubated at 36±2 °C for 20-24 hours. Following incubation, amount of growth, colony size and colony colour were recorded.

### Results

Percentage recovery of all coliform isolates was calculated using the Tryptone Soya Agar as the control plate; colony counts from Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar were compared to colony counts from the Tryptone Soya Agar. Percentage recovery of all coliforms plus percentage recovery of *E. coli* only was calculated for both chromogenic media; see table 1.

Coliform colony size on Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar was observed during the study. The average colony size (in mm) was calculated for all coliforms and for *E. coli* only; see table 2.

Coliform colony colour on Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar was also observed. *E. coli* are expected to grow as dark blue/violet colonies on both Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar. All other coliforms are expected to grow as pink to red colonies on Thermo Scientific Chromogenic Coliform Agar (ISO) and as salmon red on Chromocult Coliform Agar.

Of the 21 *E. coli* tested, 19 isolates were correctly described as growing as blue or purple colonies on both Thermo Scientific Chromogenic Coliform Agar (ISO) and

**Table 1.** Percentage recovery of coliform isolates on Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar.

Organism Group	Thermo Scientific Chromogenic Coliform Agar (ISO) total average percentage recovery (%)	Chromocult Coliform Agar total average percentage recovery (%)
All coliforms (n=37)	83.5 (95% CI* = 77.2 - 89.8)	70.4 (95% CI = 62.7 - 78.1)
<i>E. coli</i> (n=21)	83.7 (95% CI = 77.5 - 89.9)	65.2 (95% CI = 57.2 - 73.3)

\*95% Confidence Interval

Chromocult Coliform Agar. The two remaining *E. coli* isolates were found to be  $\beta$ -D-glucuronidase negative and therefore, (as advised in ISO 9308-1:2014) will appear as coliform bacteria on all chromogenic coliform agar. This observation was confirmed during this study: of the two  $\beta$ -D-glucuronidase *E. coli*, one was described as growing as pink-purple and the other as pink colonies on Thermo Scientific Chromogenic Coliform Agar (ISO). The same two  $\beta$ -D-glucuronidase *E. coli* were described as growing as pink-purple and pink-red colonies respectively on Chromocult Coliform Agar.

The other 16 coliform isolates tested were correctly described as growing as pink colonies on both Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar with the exception of one *Enterobacter cloacae* which was described as growing as purple on Thermo Scientific Chromogenic Coliform Agar (ISO) and dark blue on Chromocult Coliform Agar.

Of the 17 non-coliform isolates tested, 2 *A. hydrophilia* isolates grew as peach-coloured colonies on both Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar. An additional *Enterococcus faecium* isolate also grew as pink colonies on Chromocult Coliform Agar. Thus, exclusivity of Thermo Scientific Chromogenic Coliform Agar (ISO) was 88.2% and 82.4% for Chromocult Coliform Agar.

### Discussion

Thermo Scientific Chromogenic Coliform Agar (ISO) showed statistically significantly greater percentage recovery of all coliform isolates ( $p = 0.03$ ) and *E. coli* isolates ( $p = 0.01$ ) from the membrane filtration method than Chromocult Coliform Agar. Colony colour on Thermo Scientific Chromogenic Coliform Agar (ISO) more accurately met the expected colony colour as per ISO 9308-1:2014 than Chromocult Coliform Agar.

Colony size of all coliforms and *E. coli* isolates was statistically significantly larger (both  $p = <0.01$ ) on Thermo Scientific Chromogenic Coliform Agar (ISO) than Chromocult Coliform Agar.

### Conclusion

Thermo Scientific Chromogenic Coliform Agar (ISO) proved to give superior recovery of coliform isolates while giving a more reliable colony colour compared to Chromocult Coliform Agar.

### References

1. ISO 9308-1:2014 Water quality — Enumeration of *Escherichia coli* and coliform bacteria Part 1: Membrane filtration method for waters with low bacterial background flora

**Table 2.** Average coliform colony size on Thermo Scientific Chromogenic Coliform Agar (ISO) and Chromocult Coliform Agar.

Colony size	Thermo Scientific Chromogenic Coliform Agar (ISO) total average colony size (mm)	Chromocult Coliform Agar total average average colony size (mm)
All coliforms (n=37)	1.06	0.68
<i>E. coli</i> (n=21)	1.00	0.59

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LT2327A  
 993-218  
 February 2017

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