



## A Performance Comparison of RAPID'*E.coli* 2 and Chromocult Coliform Agars for the Enumeration of Total Coliform Bacteria and *Escherichia coli* in Drinking Water

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### Abstract

RAPID'*E.coli* 2 Agar allows for the direct and simultaneous enumeration of total coliform bacteria and *Escherichia coli* in water by membrane filtration. This report documents results obtained from a side-by-side comparison of RAPID'*E.coli* 2 Agar and Chromocult Coliform Agar for enumeration of coliforms and *E. coli* in dechlorinated municipal drinking water as described by the International Organization for Standardization (ISO) 9308-1:2014 standard. We found that RAPID'*E.coli* 2 Agar demonstrated high selectivity against interfering hydric flora and that color contrast eliminated the need for further confirmation tests.

### Introduction

In 2010, the United Nations General Assembly (UNGA) explicitly recognized the human right to water and sanitation: everyone has the right to sufficient, continuous, safe, acceptable, physically accessible, and affordable water for personal and domestic use (UNGA 2010). One of the most essential steps in ensuring this right is to protect public health by regularly testing water for indicators of fecal pollution. Coliform bacteria are a commonly used bacterial indicator of the sanitary quality of water.

Coliform bacteria are an exclusively gram-negative, non-spore forming, facultative anaerobic, and rod-shaped nontaxonomic group of bacteria that ferment lactose to produce acid and gas. The group is composed of enteric bacteria such as *Escherichia*, *Citrobacter*, *Klebsiella*, and *Enterobacter*. Coliform bacteria are abundant in the feces of warm-blooded animals and can also be found in soil, aquatic environments, and vegetation. Unlike other coliform bacteria, *Escherichia coli* is almost exclusively of fecal origin and can be detected in elevated densities in human and animal feces, along with sewage and water subjected to recent fecal pollution. Therefore, *E. coli* is considered the best indicator of fecal pollution and the possible presence of pathogens. While these bacteria are generally nonpathogenic, they can cause infection in young, old, and immunocompromised hosts. Most probable number methods for enumerating coliform bacteria can be laborious and costly. However, the use of chromogenic substrates in media has led to faster and easier methods for detecting, differentiating, and enumerating target bacteria.

The principle of the complete supplemented RAPID'*E.coli* 2 Agar Plates for Water Testing (REC2; Bio-Rad Laboratories, Inc., catalog #3563982) relies on simultaneously detecting activities of two enzymes,  $\beta$ -D-glucuronidase (GLUC) and  $\beta$ -D-galactosidase (GAL), using two chromogenic substrates. Cleavage of the GAL-specific substrate forms a precipitate that gives a green color to the colonies positive for  $\beta$ -D-galactosidase (coliforms). Cleavage of the GLUC-specific substrate forms a precipitate that gives a pink color to the colonies positive for  $\beta$ -D-glucuronidase (*E. coli*). Therefore, coliforms (GAL+/GLUC-) produce green colonies, whereas *E. coli* (GAL+/GLUC+) produces blue to violet colonies from the superposition of both colors. The selective mixture in the supplement inhibits the main interfering flora in water. The media is certified by NF Validation and approved by the United States Environmental Protection Agency (U.S. EPA).

### Methods

A total of 200 water samples were examined. Dechlorinated municipal drinking water served as the test matrix. Test samples were prepared by inoculating the drinking water with secondary sewage effluents obtained from ten wastewater treatment facilities geographically dispersed across the United States. This inoculation method was chosen to simulate natural contamination and achieve approximately 1–150 CFU of total coliform bacteria or *E. coli* per 100 ml of test sample.

After filtration of 100 ml portions of each inoculated drinking water sample, filters were deposited onto the surfaces of the chromogenic REC2 and Chromocult Coliform Agars (Millipore Sigma, catalog #110426). The plates were inverted and incubated for 22–24 hr at 36 ± 1°C. After incubation, the plates were examined for the presence of both total coliform bacteria and *E. coli*.

On Chromocult Agar, all dark blue to violet colonies were counted as *E. coli*, all pink to red colonies as presumptive total coliform bacteria (other than *E. coli*), and colorless to tan colonies as nontarget interfering flora (Figure 1). Presumptive total coliform bacteria were confirmed by performing an oxidase test on ten representative colonies (or all presumptive colonies if fewer than ten were observed) before calculating the final confirmed total coliform bacteria count. When conducting the oxidase tests to confirm total coliform bacteria,

subcultures of presumptive colonies to nonselective agar media were required to ensure pure growth when plates contained high levels of interfering nontarget flora. The total coliform bacteria count was then determined by adding the dark blue to violet colonies (*E. coli*) and the oxidase-negative pink to red colonies (non-*E. coli* total coliform bacteria) together.

On REC2 Agar, blue to violet colonies (with or without a violet halo) were counted as *E. coli*, green colonies as total coliform bacteria (other than *E. coli*), and colorless to tan colonies as nontarget interfering flora (Figure 2). No further confirmation steps were conducted to confirm total coliform bacteria colonies (per the NF Validation certification). The total coliform bacteria count was determined by adding dark blue to violet and green colonies together.

Fig. 1. Interpretation of Chromocult Agar Plates.

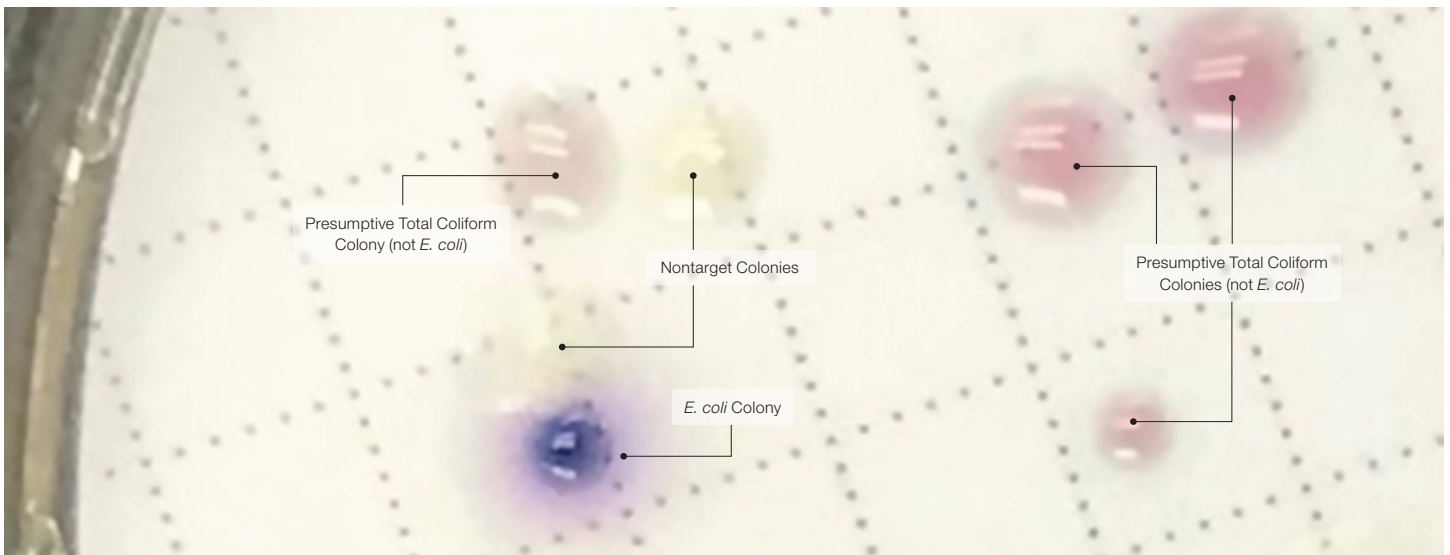
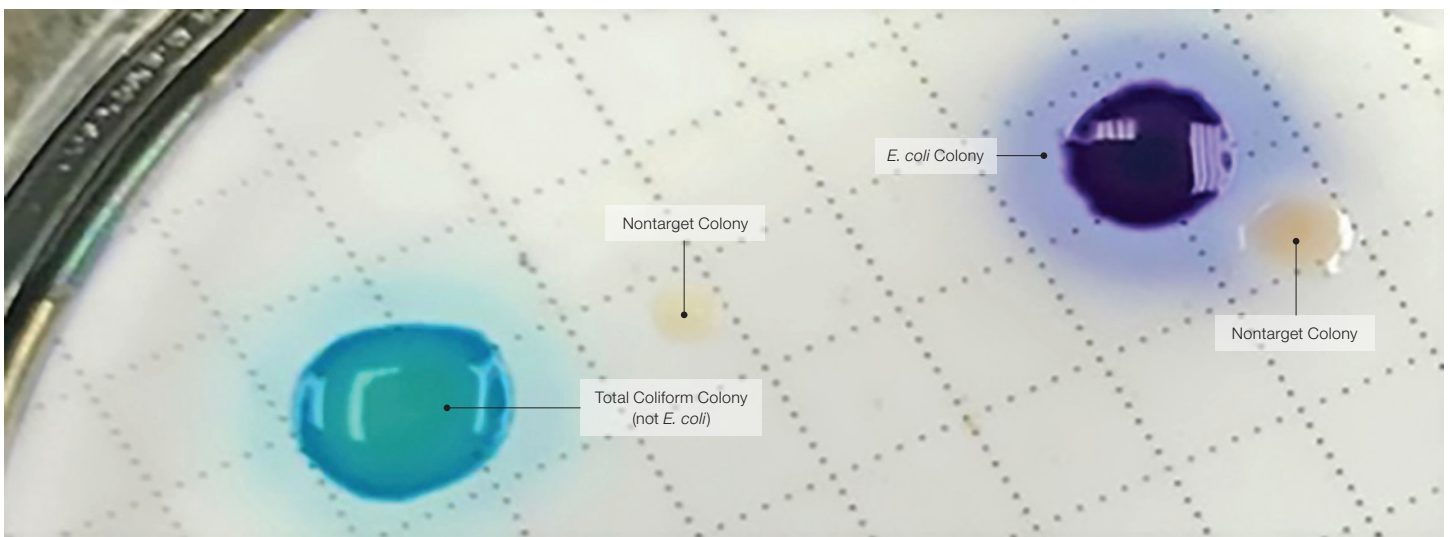


Fig. 2. Interpretation of REC2 Agar Plates.



**Table 1. Total coliform bacteria and *Escherichia coli* results comparison.\***

Target	Samples, n	REC2 Mean Count, CFU/100 ml	Chromocult Confirmed Mean Count, CFU/100 ml	Mean Relative Difference, x	Standard Deviation, S <sub>x</sub>	Half-Width Confidence Interval, W	Lower Confidence Limit, x <sub>L</sub>	Upper Confidence Limit, x <sub>U</sub>
Total coliform bacteria	236	16.7	17.5	-2.31%	57.44%	7.46%	-9.78%	5.15%
<i>Escherichia coli</i>	146	16.0	10.9	2.99%	77.15%	12.73%	-9.74%	15.72%

\* Statistical analyses did not include data points in which either or both methods yielded “zero” or “too numerous to count” (TNTC) results. Predetermined stipulated limit (2L) = 10%.

**Results and Discussion**

REC2 Agar performed comparably to Chromocult Agar for the enumeration of both total coliform bacteria and *E. coli*. It was generally noted that REC2 Agar Plates allowed for a more straightforward interpretation than Chromocult Agar. REC2 Agar yielded larger and more distinctly colored total coliform bacteria and *E. coli* colonies and generally exhibited a higher degree of selectivity against interfering flora.

A one-sided statistical evaluation was performed as described in ISO 17994, demonstrating that REC2 Agar yielded quantitative results that were either not statistically different or lower than those obtained with Chromocult Agar (Table 1). These analyses revealed that both the total coliform bacteria and *E. coli* counts obtained from REC2 Agar were not statistically different from those from Chromocult Agar. In both cases, the lower confidence limits were not less than -10%, and the upper confidence limits were greater than 0%.

**Conclusions**

According to the criteria defined in ISO 17994, our results demonstrated that REC2 Agar provides statistically equivalent results to Chromocult Coliform Agar while allowing for clearer interpretation of results and eliminating the need to conduct further

time-consuming isolation or confirmation steps. The streamlined REC2 workflow enables testing laboratories to operate with greater efficiency and higher productivity. Specifically, rapid and efficient detection of coliform bacteria can allow for prompt decisions and necessary corrective actions. Detection of coliform bacteria or *E. coli* in water indicates recent fecal contamination, which may pose an immediate health risk to anyone consuming the water. RAPID'E.coli 2 Agar for Water Testing is an innovative and easy-to-use media that allows simultaneous detection and enumeration of *E. coli* and total coliforms in 18 hr — without the need for further confirmation for all drinking water samples.

**References**

ISO 17994 (2014). Water quality — Requirements for the comparison of the relative recovery of microorganisms by two quantitative methods. [iso.org/standard/56617.html](https://www.iso.org/standard/56617.html), accessed July 30, 2024.

ISO 9308 (2014). Water quality — Enumeration of *Escherichia coli* and coliform bacteria. Part 1: Membrane filtration method for waters with low bacterial background flora. [iso.org/standard/55832.html](https://www.iso.org/standard/55832.html), accessed July 30, 2024.

UNGA (2010). The human right to water and sanitation: resolution. [refworld.org/legal/resolution/unga/2010/en/76535](https://www.refworld.org/legal/resolution/unga/2010/en/76535), accessed July 30, 2024.

**Additional Resource**

Bio-Rad Laboratories, Inc. (2023). RAPID'E.coli 2 Agar (for Water Testing) User Guide. Document 10000134990.

Visit [bio-rad.com/RAPIDecoli](https://www.bio-rad.com/RAPIDecoli) for more information.

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