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Readycult®
Coliforms 100

Contents: 20 tests (snap pack format)

One snap pack is sufficient for a 100 ml water sample.

EPA approved (40 CFR Part 141)

Application

Presence/Absence test for the simultaneous detection of total coliforms and E.coli, both of which have to be monitored under USEPA Total Coliform Rule (Sec. 141.21).

Principle

The high nutritional quality of the peptones and the incorporated phosphate buffer support rapid growth of coliforms. Laurylsulphate largely inhibits the accompanying flora, especially gram-positive organisms. The simultaneous detection of coliforms and E.coli is made possible by adding the chromogenic substrate X-GAL and the fluorogenic substrate MUG. A color change to blue-green in the broth indicates the presence of total coliforms. E.coli additionally creates a blue fluorescence under UV-light (365 nm).

Composition in g / snap pack

Tryptose 0.5; sodium chloride 0.5; sorbitol 0.1; tryptophan 0.1; di-potassium hydrogen phosphate 0.27; potassium dihydrogen phosphate 0.2; Laurylsulphate sodium salt 0.01; X-GAL 0.008; MUG 0.005; IPTG 0.01.

Procedure

Note: In case you cannot start testing the water sample right away. Test has to be done

- within 6 hours after receiving the water sample if stored at room temperature
- within 24 hours if stored in the refrigerator.

1. Take one snap pack, lightly tap to ensure the granules are at the bottom. Bend the upper portion of the snap pack until it breaks open.
   Do not touch the opening to avoid contamination!
2. Add the contents to a 100 ml water sample in a sterile, transparent **non-fluorescing** vessel with a minimum capacity of 120 ml.
3. Shake the caped vessel to dissolve granules completely.
4. Incubate the vessel at 35–37 °C up to 24 hours. If incubated at room temperature (+20–25 °C) the incubation time is prolonged up to 48 hours.

Interpretation of Results

No color change (the broth remains yellowish in color): absence of coliforms and E.coli.

Color change to blue-green

Total coliforms:
Any color change of the broth to blue-green, even if only in the upper section, confirms the presence of total coliforms (X-GAL reaction).

E.coli: Look for fluorescence in blue-green colored vessels using UV light (365 nm) in front of the vessel (towards the sample away from your eyes). A bluish fluorescence indicates presence of E.coli (MUG reaction).
Attention: Protect eyes from UV light!

Confirmation of E.coli (takes 30 seconds to do!)
There are other species of bacteria able to create a positive fluorescence with MUG. The simple indole reaction makes the difference!

To confirm E.coli in the vessel with positive fluorescence overlay the broth with KOVAC'S reagent.
A red ring confirms the presence of E.coli.

	Color change to blue-green	Fluorescence	**Confirmation:** Indole-Reaction
Total coliforms	+	–	–
E.coli	+	+	+
Negative	No color change		

Quality Control

Test strain	Growth	Color change	Fluorescence	Indole-Reaction
E.coli ATCC 11775	+	+	+	+
Citrobacter freundii ATCC 8090	+	+	–	–
Salmonella typhimurium ATCC 14028	+	–	–	–
Klebsiella pneumophila ATCC 31488	+	+	–	–
Pseudomonas aetuginosa ATCC 10145		–	–	

Disposal

Dispose in accordance with Good Laboratory Practices.

Storage

Store dry and tightly closed at +15 to +25 °C.
Protect from light.

Shelf life

See expiry date.

Additives

Merck Cat.No.	Product	Pack size
1.13203.0001	UV-Lamp	1 each
1.09293.0100	KOVAC'S Indole Reagent	100 ml
1.13311.0001	CULTURA Mini-Incubator 230 V	1 each
1.15533.0001	CULTURA Mini-Incubator 115 V	1 each

Approvals / References

USEPA: 40 CFR Part 141 (Sec.141.21) Federal Register/ Vol. 67, No. 209, Tuesday, October 29, 2002 / Rules and Regulations

Microbiology Manual for the Meat Industry, 3rd Edition April 2000, New Zealand, Chapter 11 Testing Potable Waters for Indicator Bacteria, A1.1 Chromogenic Substrate Methods for Total Collforms and E.coli

Ministry of Health, New Zealand, Drinking Water Standard for New Zealand 2000 (DWSNZ:2000), Enzyme Substrate Coliform Test

Ministry of Health, Colombia, Potable Water Quality Regulations, National Standard 475, 2001, Defined Substrate Technology for Total Coliforms and E.coli

Manafi, M.; Rosman, Evaluation of Readycult® Presence/Absence Test for the Detection of Total Coliforms and E.coli in Water. 98th American Society of Microbiology, Atlanta, 17–21th May 1998

Lee, J.V.; Lightfoot, N. F.; Tillett, H. E. An evaluation of presence/absence tests for coliforms and Escherichia coli. International Conference on Coliforms and E.coli: Problem or solution? 24–26th September 1995, University of Leeds, UK

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