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Oxoid Dehydrated Culture Medium

Chromogenic Coliform Agar

REF **CM1205**

Intended Use
Thermo Scientific™ Oxoid™ Chromogenic Coliform Agar is recommended for the detection, enumeration and differentiation of coliforms and *E.coli* in water samples with low bacterial background flora.

Summary and Explanation
ISO 9308-1:2014[1] recommends Chromogenic Coliform Agar (CCA) for the enumeration and differentiation of *Escherchia coli* and coliforms. ISO 9308-1:2014 redefines the identification of coliform bacteria based upon the presence and activity of β-D-galactosidase. Further differentiation of *E.coli* is to be based on the presence and activity of β-D-glucuronidase activity.

Coliform bacteria produce pink to red colonies from the cleavage of the chromogen 6-Chloro-3 indoxyl-β-D-galactopyranoside by β-D-galactosidase. *E.coli* can be differentiated by the cleavage of the chromogen 5-Bromo-4-chloro-3-indoxyl-β-D-glucuronic acid by β-D-glucuronidase[2]. *E.coli* however, produces dark blue to violet colour colonies based upon the ability to cleave both 6-Chloro-3 indoxyl-β-D-galactopyranoside and 5-Bromo-4-chloro-3-indoxyl-β-D-glucuronic acid. Microorganisms unable to cleave either substrate produce colourless or naturally pigmented colonies.

Principle
Nutritional needs are met through the presence of enzymatic digest of casein peptone and yeast extract. Yeast extract supplies B vitamins that are essential for promoting growth. Agar is the solidifying agent.

Sodium chloride supplies electrolytes and maintains the osmotic equilibrium within the medium. Sodium dihydrogen phosphate and disodium hydrogen phosphate are buffering agents that create a stable pH. Sodium pyruvate promotes longevity of damaged cells. Tergitol® 15-S-7, a secondary alcohol ethyloxylate surfactant, reduces Gram positive bacterial growth.

6-Chloro-3 indoxyl-β-D-galactopyranoside and 5-Bromo-4-chloro-3-indoxyl-β-D-glucuronic are the two chromogens used as substrates to determine target colony identification based on colour produced. Isopropyl-β-D-thiogalactopyranoside (IPTG) induces the production of β-D-galactosidase.

Typical Formula*	grams per litre
Enzymatic digest of casein	1
Yeast extract	2
Sodium chloride	5
Sodium dihydrogen phosphate dehydrate $2H_2O$	2.2
Disodium hydrogen phosphate	2.7
Sodium pyruvate	1
Sorbitol	1
Tryptophan	1
Tergitol® 15-S-7	0.15
6-Chloro-3 indoxyl-β-D-galactopyranoside	0.2
5-Bromo-4-chloro-3-indoxyl-β-D-glucuronic acid	0.1
IPTG	0.1
Agar	13.55

*Formulation may be modified to meet performance criteria

Physical characteristics	
Colour	Straw
Colour on reconstitution	Straw
Moisture level	<7%
pH	6.8 ± 0.2 at 25 °C
Clarity	Clear
Gel strength	Firm, comparable to 13.55g/litre agar

Precautions
This product should only be used by trained individuals. This includes the safe disposal of used or unused reagents and as well as any other contaminated or potentially contaminate material. It is the responsibility of each laboratory to manage waste produced according to any federal, state and local applicable regulations.

Storage
Store dehydrated medium at 10-30°C and use before the expiry date on label.
Store prepared medium at 2-8°C and keep away from light.

Specimen Collection, Handling and Storage
Specimens should be collected and handled following the recommended guidelines.

Materials Required but Not Supplied
(1). Hotplates
(2). Sterile Petri dishes
(3). Incubator
(4). Water baths

Directions
Suspend 30g in 1 litre of distilled water. Bring to the boil to dissolve completely. Cool to 50°C. Mix well and pour into sterile Petri dishes.

Technique
Water Samples
Refer to the ISO standard[1] for the complete method.

1. Filter the appropriate amount of test water (for example 100 ml for drinking water).
2. Place the membrane filter onto the CCA plate ensuring that no air is trapped beneath the filter.
3. Invert the Petri dish and incubate at 36 °C ± 2 °C for 21 hours to 24 hours.
4. Examine the membrane and count all pink colonies i.e. those giving a positive β-D-galactosidase reaction as presumptive coliform bacteria (not *E. coli*). To confirm carryout an oxidase test.
5. Count all colonies producing a positive β-D-galactosidase and β-D-glucuronidase reaction (dark blue to violet colonies) as *E.coli*.

Quality control
Growth characteristics tested in accordance with ISO11133.
Positive control (21-24 hours incubation at 36 °C ± 2°C, using membrane filtration technique)

Escherichia coli	ATCC® 25922 WDCM 00013	0.5-2mm dark blue to violet colonies.
Escherichia coli	ATCC® 8739 WDCM 00012	0.5-2mm dark blue to violet colonies.
Enterobacter aerogenes	ATCC® 13048 WDCM 00175	0.5-2mm pink to red colonies.
Citrobacter freundii	ATCC® 43864 WDCM 00006	0.5-2mm pink to red colonies.

Negative control (21-24 hours incubation at 36°C ± 2°C, using diminishing sweep technique)

Pseudomonas aeruginosa	ATCC® 10145 WDCM 00024	0.5-2mm colourless/cream colonies.
Enterococcus faecalis	ATCC® 29212 WDCM 00087	No growth or pinpoint-2mm white colonies.
Enterococcus faecalis	ATCC® 19433 WDCM 00009	No growth or pinpoint-2mm white colonies.

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Note:
It is the responsibility of the user to perform Quality Control testing taking into account the intended use of the medium, and in accordance with any local applicable regulations (frequency, number of strains, incubation temperature etc.)

Performance
Performance was evaluated using the five bacterial strains; *Escherichia coli, Enterobacter aerogenes, Citrobacter freundii, Pseudomonas aeruginosa* and *Enterococcus faecalis.*

All organisms gave expected growth characteristics according to the current product specification.

Limitations
It should be noted that, as with all media, atypical organisms may give anomalous growth. A small number of atypical strains may give a weak growth or fail to grow, especially when low numbers and competitive flora are present in the sample. Membrane filters may affect the growth of bacteria; they should be suitable for the test and must not affect the recovery rate or colony colour[3].

Some strains of *E.coli* are β-D-glucuronidase negative[4] notably *Escherchia coli* O157 these will therefore appear as coliform bacteria (pink) on CCA. If the membrane is crowded it may be necessary to subculture presumptive coliform colonies to a non-selective medium to ensure that the oxidase test is carried out with pure colonies.

Packaging

Product code	Suffix	Weight (KG)
CM1205	B	0.5
	R	2.5
	T	5
	G	10
	K	25

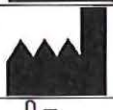
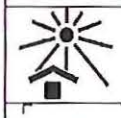
*Not all pack sizes will be available for every product.

Waste disposal
For waste disposal refer to the relevant Material Safety Data Sheet.

Bibliography

1. ISO International Standardisation Organisation. *Water quality – Enumeration of* Escherichia coli *and coliform bacteria. Part 1: Membrane filtration method for waters with low bacterial background flora.* ISO 9308-1:2014.
2. Hansen W. and Yourassowsky E. (1984) *J. Clin. Microbiol.* 20. 1177-1179.
3. ISO International Standardisation Organisation. ISO 7704 *Water Quality – Evaluation of membrane filters used for microbiological analyses.*
4. Ratnam S., March S.B., Almed R., Bezanson G.S. and Kasatiya S. (1988) *J. Clin. Microbiol.* 26. 2006-2012.

Symbol Legend

Symbol	Meaning
REF	Catalogue number
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
	Temperature limitation (storage temp.)
	Use by (expiration date)
LOT	Lot number
	Protect from light
	Consult instructions for use

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