



CCA Agar Chromogenic ISO 9308-1:2014

Application

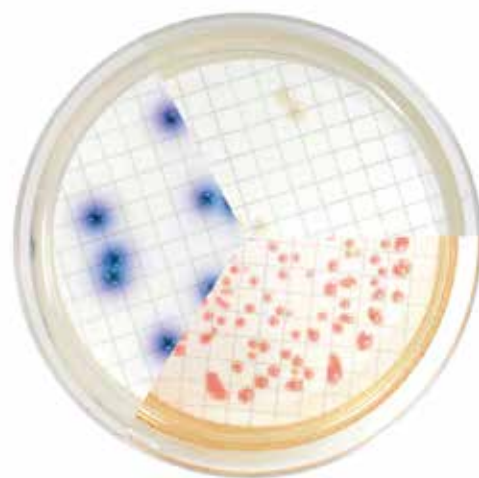
A major revision of the ISO 9308-1 standard has come into force in late 2014. This states that the TTC Agar (Chapman or Tergitol-7 Agar) is replaced by the CCA Chromogenic Agar as a culture medium for the enumeration of *Escherichia coli* and **coliform** bacteria after membrane filtration stage.

The CCA is based on **enzymatic reactions** that give **colour to the colonies** of target organisms for simultaneous detection of coliforms and *E. coli*.

This medium is suitable for **samples with low microbial load** as drinking water, swimming pools, disinfected waters and water treatment plants at the end of the treatment.

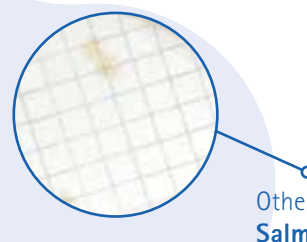
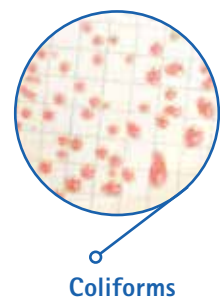
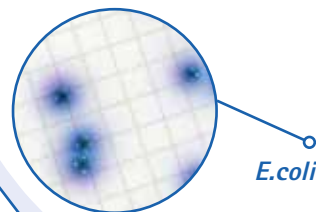
Interpretation

E. coli → Colonies from blue to violet
Coliforms → Pink salmon to red
Other bacteria (primarily Gram-negative) → Colourless (except those with a weak glucuronidase activity but not galactosidase, producing light blue or turquoise colonies).



Main advantages

- Very good recovery.
- Ideal for detection and collection of *E. coli* and coliforms in water with low contamination.
- Good colour contrast that facilitates the interpretation.
- Available in dehydrated media and 55 mm plates.



Technique

1. The water sample is filtered through a membrane filter of 0.45 µm pore diameter, validated according to the ISO Standard 7704:1985 (*).
2. The membrane is then placed on the surface of the CCA medium avoiding entrapment of air bubbles between the membrane and agar surface.
3. The petri dish with the membrane is incubated for 18–24 hours at 36 ± 2°C. If in 18 h there is growth of red or colourless colonies, extend the incubation until 24 h to include late reactions of β-galactosidase or β-glucuronidase.

(*) When the Chromogenic Agar for Coliform is used with the membrane filter method, the colour and growth of the colonies can be modified by the characteristics of the membrane filter. It is advisable to perform a validation of the membrane filter type used.

Composition (g/l)

Enzymatic digest of casein	1.00
Yeast extract	2.00
Sodium chloride	5.00
Monosodium phosphate	2.20
Disodium phosphate	2.70
Tryptophan	1.00
Sodium pyruvate	1.00
Tergitol®7	0.15
Sorbitol	1.00
6-Chloro-3-indoxyl-β-D-galactopyranoside	0.20
5-Bromo-4-chloro-3-indoxyl-β-D-glucuronide	0.10
IPTG	0.10
Agar	13.00
pH: 6.8 ±0.2	

Results

Count β-galactosidase positive colonies and β-glucuronidase negative colonies (all colonies coloured from salmon-rose to red) as Coliform bacteria different from *E. coli*.

Count β-galactosidase positive colonies and β-glucuronidase positive colonies (all colonies coloured from deep blue to violet) as *E. coli*.

Total Coliform count is obtained by the addition of the salmon-rose to red colonies plus the deep blue to violet colonies.

Calculate the concentration of Coliform bacteria and *E. coli* in 100 mL from the initial volume of water filtered and the number of characteristic colonies counted on the membrane. The results are expressed as Colony Forming Units per 100 mL (CFU/100 mL).

Confirmation

To confirm the *E. coli* colonies in this medium a small amount of tryptophan is included verifying indole production:

Coat the blue-violet colonies with a drop of Kovacs' Reagent. If the reagent turns a cherry-red colour in a few seconds this confirms the production of indole and hence the presence of *E. coli*.

Code	Description	Package
417153.1210	CCA Coliforms Chromogenic Agar (ISO 9308-1) (Dehydrated Culture Media) for microbiology	500 g
447153.0922	CCA Coliforms Chromogenic Agar (ISO 9308-1) (Prepared Plate (Ø 55 mm)) for microbiology	30 plates
Also available CCA Agar according to Spanish regulation SCO/778/2009, of 17 March (not ISO) (Ideal for analysis of waste water and very contaminated samples)		
446910.0922	CCA Coliforms, Chromogenic Agar (Prepared Plate (Ø 55 mm)) for microbiology	20 plates
Auxiliary Reagent		
252908.1608	Kovacs' Reagent	100 ml

IP-012

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