TECHNICAL DATA SHEET

COMPASS® *CC* AGAR

ENUMERATION OF *ESCHERICHIA COLI* AND COLIFORMS

1 INTENDED USE

COMPASS® *cc* Agar allows the direct enumeration of *Escherichia coli* and coliforms in water by membrane filtration in 24 hours, without the typical confirmation tests as oxidase detection or indole production from tryptophan.

BKR 23/08-06/12
ANALYSIS METHOD FOR WATER

http://nf-validation.afnor.org/

COMPASS® *cc* Agar is adaptable to either water control of treated water and other potable water sources which contain only a small number of bacteria or to much more highly contaminated water sources containing high concentration of interfering bacteria.

COMPASS® *cc* Agar is certified NF VALIDATION for the enumeration of *E. coli* and coliforms in 24 hours in water destined for human consumption.

2 HISTORY

The classification of coliforms is traditionally founded on their capacity to ferment lactose with a corresponding production of acid. Slow lactose or lactose negative strains are known to exist within the coliform genera & species. Traditional media ignore these β-galactosidase-positive but permease-negative biotypes. In 1989, Leclerc & Mossel proposed that the presence of β-galactosidase with coliforms be used as the main criteria for classification. The use of a synthetic chromogenic substrate, insensitive to variations in the permeability of lactose, allows the use of this enzyme by a colorimetric reaction.

Buehler *et al.,* in 1949, was the first to identify the presence of a β-D-glucuronidase with *Escherichia coli*. Since then, numerous studies have demonstrated that 94 to 97% of *Escherichia coli* possess a β-D-glucuronidase activity and that the same activity is only rarely encountered with other species.

3 PRINCIPLES

The simultaneous presence of two chromogenic substrates allow the detection of two types of specific enzymatic activity : β-galactosidase and β-glucuronidase.

Bacteria belonging to the group coliforms are distinguished by their production of a β-galactosidase (β-gal). This enzyme reacts with the chromogenic substrate mixture to form a pink precipitate.

All the strains of E. coli possess the β-galactosidase activity and 94 to 97% of them equally possess a β-glucuronidase (GUD) activity. The presence of this second enzyme is revealed by the visualization of a blue color. The simultaneous action of the two enzymes give rise to purple colonies with *Escherichia coli*.

In light of the principles used, the method reveals bacteria that are lactose negative but β-galactosidase positive.

Microorganisms	Typical phenotype	Colony color
Escherichia coli	GUD $^{+}$ / β-gal $^{+}$	Blue-violet
Coliforms non *Escherichia coli*	GUD $^{-}$ / β-gal $^{+}$	pink
Other Gram negative bacteria	GUD $^{-}$ / β-gal $^{-}$	white

The special mixture of peptones favors the excellent growth of coliforms and the selective system inhibits potentially interfering microflora.

A buffering system allows the enzymatic reactions to take place in the most optimal conditions.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of complete media :

- Special peptone mixture ..... 18,40 g
- Growth activators ..... 3,55 g
- Buffering system ..... 5,80 g
- Chromogenic mixture ..... 0,44 g
- Inhibiting agents ..... 1,61 g
- Bacteriological agar ..... 11,0 g

pH of the ready-to-use media at 25°C : 6,8 ± 0,2.

5 PREPARATION

- Dissolve 40,8 g of dehydrated media (BK210) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Distribute into vials at 100 mL per vial.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- After cooling the medium to 44-47°C, aseptically add 1 mL of selective supplement reconstituted with 5 mL of sterile water (BS084).
- Pour into Petri plates (Ø 55 mm).

NOTE : It is imperative to shield the plates from light.

✓ **Reconstitution :**
40,8 g/L

✓ **Sterilization :**
15 min at 121°C

✓ **Add reconstituted selective supplement**

6 INSTRUCTIONS FOR USE

- Aseptically filter through a membrane a determined volume of sample to be tested.
- Deposit the membrane on the surface of the plates, filtered side up and taking care to keep the membrane and the agar in close contact.
- Incubate at 36 ± 2 °C for 24 ± 3 hours.

✓ **Inoculation :**
Membrane filtration

✓ **Incubation :**
24 h at 36°C

7 RESULTS

Enumerate characteristic colonies.
Coliforms other than *Escherichia coli* present pink colonies.
Colonies of *E. coli* are blue to violet and may sometimes present a pink diffuse halo around the colonies.

NOTE : Reading can be performed through the back of the plates.

(See Annex 1 : PHOTO SUPPORT)

8 QUALITY CONTROL

Prepared media : amber agar.
Typical culture response after 24 h of incubation at 36 °C :

Microorganisms		Growth (Productivity Ratio R_2)	Colony aspect
Escherichia coli	WDCM 00179	$66\% \leq R_2 \leq 150\%$	Blue violet
Escherichia coli	WDCM 00013	$66\% \leq R_2 \leq 150\%$	Blue violet
Citrobacter freundii	WDCM 00006	$66\% \leq R_2 \leq 150\%$	Pink
Staphylococcus aureus	WDCM 00035	inhibited	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
Complete, pre-poured media in Petri plates : 2-8 °C, **shielded from light.**
Selective supplement : 2-8 °C.
The expiration dates are indicated on the labels.

Base media in vials (*) : 180 days at 2-8 °C, **shielded from light.**
Rehydrated supplement (*) : 30 days at 2-8 °C.
Complete, prepared media in plates (*) : 30 days at 2-8 °C, **shielded from light**.
(*)Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Complete, pre-poured media in Petri plates (Ø 55 mm) :
20 plates..... BM15308

Dehydrated base media COMPASS® *cc* Agar
500 g bottle BK210HA

Selective supplement for COMPASS® *cc* Agar (qsp 500 mL) :
10 vials BS08408

11 BIBLIOGRAPHY

MANAFI, M., KNEIFEL, W., and BASCOMB, S.. 1991. Fluorogenic and chromogenic substrates used in bacterial diagnostics. Microbiological Reviews, **55** : 335-348.

NF EN ISO 9308-1 : 2000 « Qualité de l'eau : Recherche et dénombrement des *Escherichia coli* et des bactéries coliformes – Partie 1 : Méthode par filtration sur membrane ».

12 ADDITIONAL INFORMATION

COMPASS® is a trademark of SOLABIA S.A.S.

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : COMPASS CC_EN_V5.
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Origin of revision : General update

ANNEX 1 : PHOTO SUPPORT

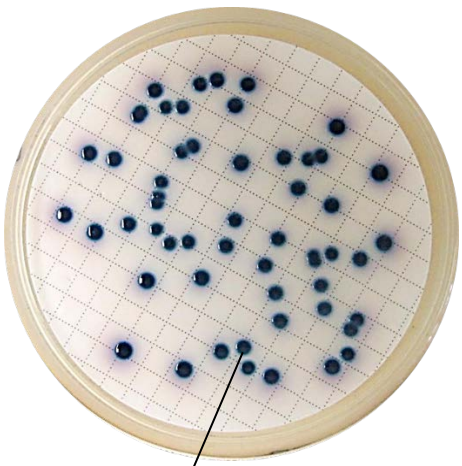
COMPASS® *cc* Agar

Validated method for the enumeration of *Escherichia coli* and coliforms in human drinking water in 24 hours.

Methodology :

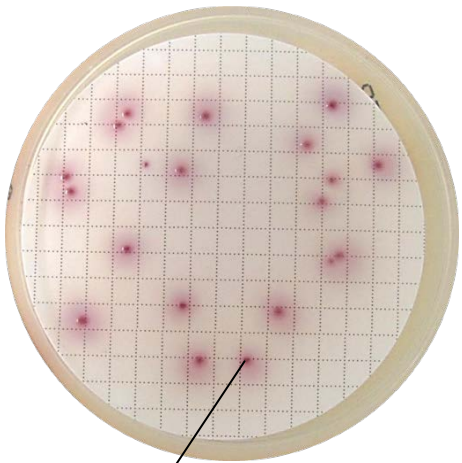
- Aseptically filter through a membrane a pre-determined volume of the sample to test.
- On the surface of plates, deposit the membrane, filtered side up, insuring a close contact with the agar surface.
- Incubate at (36 ± 2)°C for (24 ± 3) hours.

Results and enumeration :



Escherichia coli

Characteristic colony :
Blue to violet



Coliform

Characteristic colony :
pink

Microorganisms	Typical phenotype	Colony colors
Escherichia coli	GUD $^{+}$ / β-gal $^{+}$	Blue - violet
Coliforms non *Escherichia coli*	GUD $^{-}$ / β-gal $^{+}$	Pink
Other Gram negative bacteria	GUD $^{-}$ / β-gal $^{-}$	White

The enumeration of *E. coli* is the result of the sum of the **blue to violet colonies**.
The enumeration of total coliforms is the result of the **pink colonies** and the **blue to violet colonies**.

Product code :

BM15308 : Pre-poured Petri plates (Ø 55 mm) - 20 plates