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 21 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 ± 3 hours in water destined for human consumption with respect to the reference method NF EN ISO 9308-1:2000. Please refer to the certificate available on the NF VALIDATION website for the validity end date of the method.

In case of NF Validation mark, refer to ISO 8199 : 2018 for media preparation and enumeration.

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 allows 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 ± 3 h of incubation at 36 ± 2 °C:

Microorganisms		Growth (Productivity Ratio P_R)	Colony aspect
Escherichia coli	WDCM 00179	$P_R \geq 50\%$	Blue violet
Escherichia coli	WDCM 00013	$P_R \geq 50\%$	Blue violet
Citrobacter freundii	WDCM 00006	$P_R \geq 50\%$	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 medium COMPASS® *cc* Agar in Petri plates (Ø 55 mm):
20 plates BM15308

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

Selective supplement for COMPASS® *cc* Agar (qsf 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 (indice 90-414): « Water quality - Detection and enumeration of Escherichia coli and coliform bacteria - Part 1: membrane filtration method ».

NF EN ISO 8199 : 2018. Water quality - General requirements and guidance for microbiological examinations by culture.

12 ADDITIONAL INFORMATION

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

Document code : COMPASS CC_v8(en).
Creation date : 03-2010
Updated : 06-2024
Origin of revision : Quality control correction

ANNEX 1: PHOTO SUPPORT

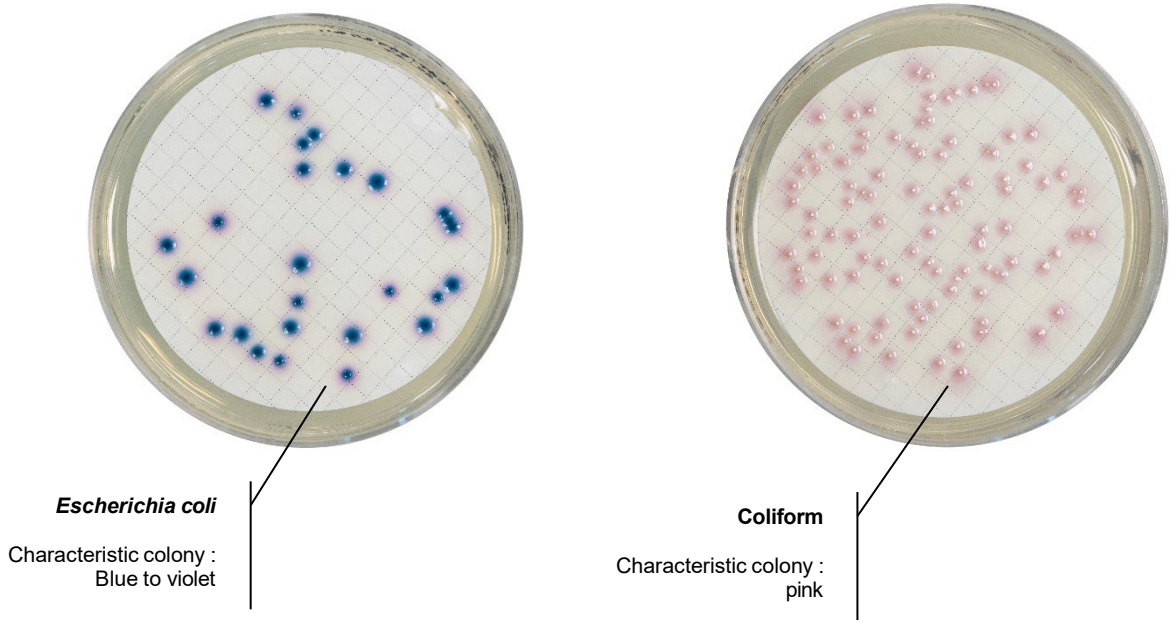
COMPASS® *cc* Agar

Validated method for the enumeration of *Escherichia coli* and coliforms in human drinking water in 24 ± 3 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:



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