TECHNICAL DATA SHEET

COMPASS® *ENTEROCOCCUS* AGAR

ENUMERATION OF ENTEROCOCCI

1 INTENDED USE

COMPASS® *Enterococcus* Agar is a selective media used for the enumeration of enterococci in food and water.

2 HISTORY

Enterococci are used as indicator organisms for fecal contamination and it has been proven that the majority of these bacteria produce a β-D glucosidase. The majority of media frequently used until today have contained esculin (6-7 dihydroxycoumarine-6-glucoside) which is hydrolyzed by the β-D glucosidase into two compounds : esculetin and glucose. Esculetin produces a brown component in presence of ferric ions present in the media, leading to a positive reaction with enterococci, but also with other unrelated secondary microorganisms. Indeed, a certain number of contaminating microorganisms can lead to a relatively high level of false positives, requiring a number of confirmation tests for all the colonies presenting a characteristic aspect.

The use of chromogenic and fluorgenic substrates for the detection of glucosidic activity, to specifically dectect enterococci, has been studied by numerous authors as Dufour in 1980, Littel & Hartman in 1983, Hernandez *et al*. in 1993. The association between X-β-glucoside and the inhibiting nature of the formulation of COMPASS® *Enterococcus* Agar allows the direct enumeration by colony count of characteristic colonies, after only 24 hours of incubation and without confirmation.

3 PRINCIPLES

Peptones, yeast extract and Tween 80 stimulate the growth of enterococci.

Yeast extract is a source of complex vitamin B.

Sodium chloride maintains the osmotic pressure.

The choice of an incubation temperature at 44 °C, associated with an judicious mixture of inhibitors, allows the high selectivity of the media and the inhibition of contaminating microflora.

The X-β-glucoside insures the chromogenic revelation of the β-glucosidase activity of the enterococci. These colonies present a blue coloration after hydrolysis of the substrate (5-bromo-4-chloro-3-indolyl-β-D-glucoside).

4 TYPICAL COMPOSITION

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

For 1 liter of media :

- Peptones ..... 27,5 g
- Yeast extract ..... 5,0 g
- Sodium chloride ..... 5,0 g
- Tween 80..... 1,0 g
- Inhibiting mixture ..... 0,3 g
- X-β-glucoside ..... 0,1 g
- Bacteriological agar..... 14,0 g

pH of the ready-to-use media at 25 °C : 7,5 ± 0,2.

5 PREPARATION

- Dissolve 52,9 g of dehydrated media (BK183) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Divide into tubes or vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47 °C.
- For use in surface inoculation or membrane filtration, pour plates and let solidify on a cool, flat surface.

✓ **Reconstitution :**
52,9 g/L

✓ **Sterilization :**
15 min at 121 °C

Use of the ready-to-melt media :

- Melt the media (if it is prepared in advance) or the ready-to-melt media (BM116) for the minimum amount for time required to achieve total liquefaction.
- Cool and maintain at 44-47 °C.

6 INSTRUCTIONS FOR USE

Food microbiology

- Transfer 1 mL of the sample suspension and its serial dilutions to empty, sterile Petri plates.
- Pour roughly 15 mL of molten media.
- Mix well by swirling and let solidify on a flat, cold surface.
- Incubate at (44 ± 1) °C for (24 ± 2) hours.

✓ **Inoculation :**
1 mL in pour plates

✓ **Incubation :**
24 ± 2 h at 44 ± 1 °C

Water analysis

- Aseptically filter through a membrane a specific volume of sample water to test.
- To the surface of plates prepared as above, or to pre-poured media (BM157) brought to room temperature, place the membrane filtered side up and insure that the membrane and agar surface are in close contact.
- Incubate at (44 ± 1) °C for (24 ± 2) hours.

7 RESULTS

The enterococci are characterized by blue to blue-green colonies on the agar.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : whitish powder, free-flowing and homogeneous.
Prepared media : amber agar.

Typical culture response after 24 hours of incubation at 44 °C :

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
Enterococcus faecalis	WDCM 00087	$P_R \geq 50$ %	Blue colonies
Enterococcus faecium	WDCM 00010	$P_R \geq 50$ %	Blue colonies
Escherichia coli	WDCM 00013	Inhibited	-
Staphylococcus aureus	WDCM 00034	Inhibited	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.
Ready-to-melt media in vials : 2-8 °C.
Pre-poured media in plates : 2-8 °C, shielded from light.
The expiration dates are indicated on the labels.

Prepared media in tubes or vials (*) : 180 days at 2-8 °C, shielded from light.
Prepared media in 55 mm Petri plates (*) : 30 days at 2-8 °C, shielded from light.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :
500 g bottle BK183HA

Ready-to-melt media :
10 x 100 mL BM11608

Pre-poured media in Petri plates (Ø 55 mm) :
20 plates BM15708

11 BIBLIOGRAPHY

Microbiolgy, Washington, D.C., USA. Abstr. Q-69 : 205.

Littel, K.J., and Hartmann P.A.. 1983. Fluorogenic selective and differential medium for isolation of fecal streptococci. Applied and Environmental Microbiology, **45(2)** : 622-627.

Hernandez, J.F., Pourcher, A.M., Delattre, J.M., Oger, C., and Loeuillard J.L.. 1993. MPN Miniaturized procedure for the enumeration of faecal enterococci in fresh and marine waters, The must procedure. Water Research, **27** : 597-606.

12 ADDITIONAL INFORMATION

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.

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ANNEX 1 : PHOTO SUPPORT

COMPASS® *Enterococcus* Agar

Enumeration of *Enterococcus* spp.

Results :

Growth obtained after 24 hours of incubation at 44 °C (pour plate inoculation).

Enterococcus faecalis

Colony characteristic :
blue color
(hydrolysis of X-β-D-glucoside)