

VRBG AGAR

ENUMERATION AND CONFIRMATION OF *ENTEROBACTERIACEAE*

1 INTENDED USE

Violet Red Bile Glucose Agar containing crystal violet and neutral red (VRBG Agar) was used by Mossel for the detection and enumeration of enterobacteria in dairy products, meat, prepared pork products and other food products. The media can also be used for the detection of Gram negative, bile resistant bacteria during the microbiological control of non-sterile products.

The typical composition corresponds to that defined in the standards NF EN ISO 21528-1 & 21528-2, NF V08-054 and in the European Pharmacopeia.

2 PRINCIPLES

The simultaneous presence of crystal violet and bile salts inhibit Gram-positive bacteria. The degradation of glucose to acid is shown by the red color of the pH indicator, neutral red.

3 TYPICAL COMPOSITION

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

For 1 liter of media:

- Enzymatic digest of animal tissues..... 7,0 g
- Yeast extract..... 3,0 g
- Glucose 10,0 g
- Bile salts 1,5 g
- Sodium chloride 5,0 g
- Neutral red..... 30,0 mg
- Cristal violet..... 2,0 mg
- Bacteriological agar 13,0 g

pH of the ready-to-use media at 25 °C : 7,4 ± 0,2.

4 PREPARATION

- Dissolve 39,5 g of dehydrated media (BK011) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Do not overheat; Do not autoclave.
- Cool the media and maintain it in a molten state at 44-47 °C.
- Use in the 4 hours following the preparation.

✓ **Reconstitution:**
39,5 g/L

✓ **Sterilization:**
Bring to a boil.

Use of ready-to-melt media:

- Heat the ready-to-melt media (BM075) for the minimum amount of time in order to achieve total liquefaction.
- Cool and maintain the media in a molten state at 44-47 °C.

Note

The media can be supplemented with MUG (4-methylumbelliferyl-β-D-glucuronide) in order to detect *Escherichia coli* (refer to the monograph on MUG 50 mg Supplement, BS024).

5 INSTRUCTIONS FOR USE

Enumeration of *Enterobacteriaceae* – Food Microbiology

(NF V08-054; NF EN ISO 21528-2):

- Transfer 1 mL of the suspension and its serial dilutions to empty, sterile Petri plates.
- Pour in about 15 mL of medium per plate.
- Homogenize by swirling and let solidify on a cold, flat surface.
- Overlay the solidified agar with about 5 mL of medium to form a second layer.
- Let solidify.
- Incubate at 37 ± 1 °C for 24 ± 2 hours.

✓ **Inoculation**
1 mL in a double layer

✓ **Incubation:**
24 ± 2 h at 37 °C

Note:

Alternatively, the temperature of 30 °C can be chosen when the enumeration of mesophilic *Enterobacteriaceae* takes place in the context of a sanitary control of a technological process.

Detection of *Enterobacteriaceae* (European Pharmacopeia, NF EN ISO 21528-1)

- Pour into sterile Petri plates and let solidify on a cold, flat surface.
- Dry the plates in an incubator, covers partially removed.
- Inoculate by streaking the enrichment broth obtained in the preceding steps.
- Incubate at 37°C for 24 ± 2 hours, as indicated in the standard NF EN ISO 21528-1 or for 18-24 hours at 30-35 °C, following the Pharmacopeia.

✓ **Inoculation :**
Surface streaking

✓ **Incubation :**
Acc. to the reference used

6 RESULTS

Enterobacteria form violet colonies whose diameter is equal to or greater than 0.5 mm, surrounded by a halo of precipitated bile salts.

7 QUALITY CONTROL

Dehydrated media: slightly pink powder, free-flowing and homogeneous.

Prepared media: red agar.

Typical culture response after 24 hours of incubation at 37°C, inoculation in depth (pour plates; NF EN ISO 11133) :

Microorganisms	Growth (Productivity Ratio : P_R)	Characteristics
<i>Salmonella</i> Typhimurium WDCM 00031	$P_R \geq 50$ %	Red-violet colonies, with a violet halo
<i>Salmonella</i> Enteritidis WDCM 00030	$P_R \geq 50$ %	Red-violet colonies, with a violet halo
<i>Escherichia coli</i> WDCM 00012	$P_R \geq 50$ %	Red-violet colonies, with a violet halo
<i>Enterococcus faecalis</i> WDCM 00087	Inhibited	-

Typical culture response after 18 hours of incubation at 30-35 °C, inoculum $\leq 10^2$ microorganisms:

Microorganisms	Growth (Productivity Ratio : P_R)	Characteristics
<i>Escherichia coli</i> WDCM 00012	$P_R \geq 50$ %	Red-violet colonies, with a violet halo
<i>Pseudomonas aeruginosa</i> WDCM 00026	Good	Orange to colorless colonies

8 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C.

Ready-to-melt media: 2-8 °C.

The expiration dates are indicated on the labels.

Prepared media in vials (*): Not recommended.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

9 PACKAGING

Dehydrated media:

500 g bottle..... BK011HA

5 kg drumBK011GC

Ready-to-melt media:

10 x 200 mL vials.....BM07508

10 BIBLIOGRAPHY

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Mossel, D.A.A., Wisser, M., and Cornelissen, A.M.R. 1963. The examination of foods for *Enterobacteriaceae* using a test of the type generally adopted for the detection of *Salmonellae*. J. Appl. Bact., 24: 444-452.

NF V08-054. Avril 2009. Microbiologie des aliments. Dénombrement des entérobactéries présumées par comptage des colonies obtenues à 30°C ou à 37°C.

NF EN ISO 21528-1. Juillet 2017. Microbiologie de la chaîne alimentaire. Méthodes horizontales pour la recherche et le dénombrement des *Enterobacteriaceae*. Partie 1 : Recherche des Enterobacteriaceae.

NF EN ISO 21528-2. Juillet 2017. Microbiologie de la chaîne alimentaire. Méthodes horizontales pour la recherche et le dénombrement des *Enterobacteriaceae*. Partie 2 : Méthode par comptage des colonies.

Pharmacopée Européenne.. Chapitre 2.6.13. Contrôle microbiologique des produits non stériles : Recherche de microorganismes spécifiés.

11 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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