

# TECHNICAL DATA SHEET

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

#### 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.
- Use in the 4 hours following the preparation.

#### Note

The media can be supplemented with MUG (4-methylumbelliferyl-β-D-glucuronide) in order to detect *Escherichia coli* (refer to the technical data sheet of 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  $37 \pm 1$  °C for  $24 \pm 2$  hours.

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

✓ **Incubation:**  
 $24 \pm 2$  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* (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  $24 \pm 2$  hours.

✓ **Inoculation:**  
Surface streaking

✓ **Incubation:**  
 $24 \pm 2$  h at 37 °C

## 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	-

## 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

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### Dehydrated media:

500 g bottle.....	BK011HA
5 kg drum .....	BK011GC

### Ready-to-melt media:

10 x 200 mL vials .....	BM07508
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## 10 BIBLIOGRAPHY

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## 11 ADDITIONAL INFORMATION

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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 : VRBG-AGAR\_BK011\_BM075\_V16(en)  
Creation date : 11-2000  
Updated : 04-2024  
Origin of revision : Review of bibliography