

TECHNICAL DATA SHEET

VRBL AGAR

ENUMERATION OF TOTAL COLIFORMS

1 INTENDED USE

Violet Red Bile Agar (VRBL) is a selective medium used for the detection and enumeration of coliform and thermotolerant coliforms food products.

The typical composition corresponds to that defined in the standards NF V08-050, NF V08-060 and NF ISO 4832.

2 HISTORY

A large number of researchers have studied this medium. MacCraday in 1932 for the Committee of Standard Methods of Milk Analysis of the American Public Health Association, Bartram and Black for the isolation of coliform bacteria in raw and pasteurized milk, and also Miller and Prickett in a note concerning the recontamination of milk. All these authors found the medium satisfactory since complete results were obtained within 24 hours of incubation.

3 PRINCIPLES

Lactose fermentation results in acidification of the medium, shown by the red color of the pH indicator (neutral red) and by the precipitation of bile acids around the colonies.

The simultaneous presence of crystal violet and bile salts inhibit Gram-positive bacteria.

4 TYPICAL COMPOSITION

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

For 1 liter of media :

- Peptic digest of meat.....	7,0 g
- Yeast extract	3,0 g
- Lactose.....	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.....	12,0 g

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

5 PREPARATION

Preparation from dehydrated media :

- Dissolve 38,5 g of dehydrated media (BK152) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring until complete dissolution. Continue to boil for 2 minutes.
- Do not autoclave.
- Cool and maintain the media in a molten state at 44-47 °C.
- Use in the 4 hours following the preparation.

✓ **Reconstitution :**
38,5 g/L

✓ **Sterilization :**
Bring to boiling 2 minutes.

Use of ready-to-melt media :

- If the media was prepared in advance, or when using the ready-to-melt references (BM034 or BM035), heat the media with the minimum amount of time necessary in order to achieve total liquefaction.
- Cool and maintain the media in a molten state at 44-47 °C.

6 INSTRUCTIONS FOR USE

- Transfer 1 mL of the product to analyze and its serial dilutions to empty, sterile Petri plates.
- Pour in roughly 15 mL of medium per plate.
- Homogenize by swirling and Let solidify on a cold surface.
- Overlay the solidified agar with 5 mL of medium.
- Let solidify.
- Incubate for 24 ± 2 hours at 30 ± 1°C or at 44 ± 1°C according to the protocol or standard being followed.

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

✓ **Incubation :**
24 ± 2 h at 30 or 44 °C

NOTE : In the framework of the NF ISO 4832 standard, overlay the solidified agar with 4 mL of medium and incubate at 30 or 37 °C as agreed.

7 RESULTS

Coliform bacteria form violet colonies whose diameter is equal to or greater than 0.5 mm and often surrounded by a red zone due to the precipitation of bile.
Lactose-negative enterobacteria are colorless.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

Dehydrated media : beige to pinkish-beige powder, free-flowing and homogeneous.

Prepared media : red agar.

Typical culture response after 24 hours of incubation at 30 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
<i>Escherichia coli</i>	WDCM 00012	$P_R \geq 50 \%$	Violet-red colonies
<i>Escherichia coli</i>	WDCM 00013	$P_R \geq 50 \%$	Violet-red colonies
<i>Pseudomonas aeruginosa</i>	WDCM 00025	Good, score 2	Beige-pink colonies
<i>Enterococcus faecalis</i>	WDCM 00087	Inhibited	-

Typical culture response after 24 hours of incubation at 44 °C (NF V 08-060) :

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
<i>Escherichia coli</i>	WDCM 00012	$P_R \geq 50 \%$	Violet-red colonies
<i>Pseudomonas aeruginosa</i>	WDCM 00025	Good, score 2	Beige-pink colonies
<i>Enterococcus faecalis</i>	WDCM 00087	Inhibited	-

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

Ready-to-melt media in vials : 2-8 °C.

The expiration dates are indicated on the labels.

Prepared media from dehydrated powder (*) : Not recommended

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

10 PACKAGING

Dehydrated media :

500 g bottle	BK152HA
5 kg drum	BK152GC

Ready-to-melt media :

10 x 100 mL vials	BM03408
10 x 200 mL vials	BM03508

11 BIBLIOGRAPHY

NF ISO 4832. Juillet 2006. Microbiologie des aliments. Méthode horizontale pour le dénombrement des coliformes. Méthode par comptage des colonies.

NF V08-050. Avril 2009. Microbiologie des aliments. Dénombrement des coliformes présumés par comptage des colonies obtenues à 30°C.

NF V08-060. Avril 2009. Microbiologie des aliments. Dénombrement des coliformes thermotolérants par comptage des colonies obtenues à 44°C.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau - Préparation, production, stockage et essais de performance des milieux de culture (Tirage 2 (2016-01-01)).

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.

Document code : VRBL_ENv8
Creation date : 01-2004
Updated : 10-2018
Origin of revision : Correction of instructions for use, quality control.

ANNEX 1 : PHOTO SUPPORT

VRBL Agar

Detection and enumeration of coliforms.

Results :

Growth obtained after 24 hours of incubation at 30 °C

