

VRBG/Agar (Violet - Red - Bile - Glucose)

355-4239 / 356-4584

DEFINITION

Medium used for the detection and enumeration of enterobacteria in food products.

STANDARDS

FOOD MICROBIOLOGY

- **NF ISO 21528-1 (December 2004):** Food Microbiology Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 1: Detection and enumeration by MPN technique with pre-enrichment.
- **NF ISO 21528-2 (December 2004):** Food Microbiology Horizontal methods for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony count method.

PRINCIPLE

The principle of the medium relies on the ability of *Enterobacteriaceae* to ferment glucose. Due to the simultaneous presence of crystal violet and bile salts, the medium inhibits Gram-positive bacteria and some Gram-negative bacteria.

Neutral red is an indicator of the pH.

PRESENTATION

- **Ready-to-use**
100 ml x 6 bottles **code 355-4239**
- **Dehydrated**
500 g **code 356-4584**

STORAGE

- Ready-to-use: + 2°C to 8°C.
- Dehydrated: +15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Peptone	7 g
Yeast extract	3 g
Sodium chloride	5 g
Bile salts	1.5 g
Glucose	10 g
Neutral red	30 mg
Crystal violet	2 mg
Agar	12 g
Distilled water	1,000 ml

Final pH (25°C) = 7.4 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- 125 ml Pyrex bottles
- Sterile Petri dishes (Ø = 90 mm)
- Sterile pipettes (1 ml, etc)
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator
- All usual laboratory equipment

PREPARATION OF DEHYDRATED MEDIUM

Always shake before use

Dissolve 38,5 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix thoroughly until a homogenous suspension is obtained.

Heat gently swirling frequently, then bring to boiling point until completely dissolved. Dispense. **DO NOT AUTOCLAVE.**

This medium can be used up to 3 hours after preparation.

**Reconstitution ratio: 38,5 g/l.
500 g of powder makes 13 liters of medium.**

PROTOCOL

• Preparation of samples

According to the standards or recommendations applicable to the product concerned.

• Inoculation and incubation

Inoculate 1 ml of the product to be analyzed, or its decimal dilutions, into sterile Petri dishes.

Pour about 15 ml of medium, melted and cooled to 44°C - 47°C, homogenize and leave to solidify.

Pour a second layer (approximately 2 cm thick) of this medium, maintained at 44°C - 47°C, and leave to dry again.

Incubate at 30°C, 35°C or 37°C (± 1°C) for 24 hours (± 2 h).

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READING AND INTERPRETATION

After 24 hours incubation, enumerate typical *Enterobacteriaceae* colonies on dishes containing between 15 and 150 colonies.

Enterobacteriaceae form pink-red colonies (Glucose-positive) with a diameter equal to or exceeding 0.5 mm or more, with or without a zone of bile precipitation.

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10⁻¹ dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- **DO NOT AUTOCLAVE.**
- Comply with Good Laboratory Practice.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Result after 24H at 37°C		
	Glucose fermentation	Diameter	PR*
Productivity test			
<i>Escherichia coli</i> ATCC 25922	Positive Pink to red colonies with or without precipitation halo	≥ 0.5 mm	≥ 0.5
<i>Salmonella Typhimurium</i> ATCC 14028	Black colonies	≥ 0.5 mm	≥ 0.5
Selectivity			
<i>Enterococcus faecalis</i> ATCC 19433	No growth		

* PR = Total colony count obtained on 2 plates of VRBG/total colony count on 2 plates of TCS agar.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

KEY WORDS

VRBG / *Enterobacteriaceae* / Food products / Detection / Enumeration / Crystal Violet / Bile salts / Glucose / Fermentation / MPN / Medium.

BIBLIOGRAPHY

MOSSEL D.A.A., MENGERINK W.H.J. and SCHOLTS H.H. (1962): Use of a modified MacConkey agar medium for the selective growth and enumeration of all *Enterobacteriaceae*. Journal of Bacteriology 84: 381.