

VRBL/Agar (Violet - Red - Bile - Lactose)

355-4799
356-4594

DEFINITION

Selective medium used for the enumeration of coliforms and thermotolerant coliforms in food products.

STANDARDS

FOOD MICROBIOLOGY

- **NF ISO 4832 (July 2006):** Food Microbiology - Horizontal method for the enumeration of coliforms - Colony count technique.
- **FIL 73A (1985):** Milk and dairy products - Enumeration of coliforms - Colony-count technique and Most-Probable-Number technique at 30°C.

PRINCIPLE

The principle of the medium relies on the ability of coliforms to ferment lactose.

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 355-4799**
- **Dehydrated**
500 g **code 356-4594**

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
Lactose	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
- Vortex type shaker
- 25 ml Pyrex bottles
- Sterile Petri dishes (ø = 90 mm)
- Sterile pipettes (0.1 ml, 1 ml, etc)
- Sterile Pasteur pipettes (**code 355-0751**) or inoculating loop
- Sterile spreaders
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM

Always shake before use.

Dissolve 38 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. If necessary, adjust the pH to 7.4 ± 0.2.

Dispense. **DO NOT AUTOCLAVE.**

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

Reconstitution ratio: 38 g/l.

500g of powder makes 13.1 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 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 on a cool, completely horizontal surface.

Pour a second layer (approximately 4 ml) of this medium, maintained at 44°C-47°C, and leave to dry again.

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When completely solidified, turn the dishes over and incubate in this position at:

- 30°C, 35°C or 37°C ± 1°C for 24 h ± 2 hours for the detection and enumeration of coliforms.

and/or

- 44°C ± 1°C for 24 h ± 2 hours for the detection and enumeration of thermotolerant coliforms.

READING AND INTERPRETATION

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

Coliforms form pink-red colonies (Lactose positive) with a diameter of 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.

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.

PERFORMANCES / QUALITY CONTROL OF THE TEST

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

STRAINS	Results after 24 h culture at 30°C	
Productivity test		
<i>Escherichia coli</i> ATCC 25922	Lactose fermentation	Positive. Pink to red colonies with or without precipitation halo.
	Diameter	≥ 0.5 mm
	PR*	≥ 0.5
Specificity		
<i>Pseudomonas aeruginosa</i> ATCC 27853	Lactose fermentation	Negative. Colorless to beige colonies
	Growth	Good
Selectivity		
<i>Enterococcus faecalis</i> ATCC 19433	Growth	No growth

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

KEY WORDS

VRBL / Coliforms / Food products / Enumeration / Crystal Violet / Bile salts / Lactose / Fermentation / MPN / Medium.

BIBLIOGRAPHY

American Public Health Association (1978): Standard methods for the examination of dairy products. 14th Edn. APHA Inc. Washington DC.