## Also known as

VRB Agar; VRBA; VRBL

# Specification

Solid medium for the detection and enumeration of coliforms in milk and other dairy products, according to APHA and ICMSF, FIL-IDF and ISO standards.

# Formula \* in g/L

Yeast extract	3.000
Peptone	7.000
Bile salts No. 3	1.500
Lactose	
Sodium chloride	5.000
Neutral red	0.030
Crystal violet	0.002
Agar	

#### Final pH 7.4 ±0.2 at 25 °C

\* Adjusted and /or supplemented as required to meet performance criteria

#### Directions

Suspend 39,5 g in 1 L of distilled water. Heat and boil for 1 minute and pour into plates. The medium must to be used preferably on the same day of preparation. Do not autoclave.

#### Description

The Violet Red Bile Agar corresponds to the classic formulation of standardized media for the screening of coliforms in milk and other dairy products. This medium has been adopted for the enumeration of coliforms as well as for differentiating between lactose-fermenting and non-lactose fermenting organisms, due to its contents of crystal violet and bile salts, whose inhibiting or selective properties have been widely confirmed.

## Technique

The recommended procedure is inoculation directly into Petri dishes, with the molten agar cooled to 45-47 °C. Plates can be read after 24 hours of incubation à 37 °C or 30 °C.

The size of the colonies ranges from 2 to 5 mm, depending on the amount per plate. If enterococci develop they will appear small in size and pink coloured. Lactose fermenting enterobacteria acquire a dark red colour with a clearing zone around them, while lactose non-fermenting ones form colourless colonies.

## Quality control

# Incubation temperature: $30 \degree C \pm 1.0$ Incubation time: $24 \pm 2 h$

**Inoculum:** Practical range 100±20 CFU. min. 50 CFU (productivity)/ 10<sup>4</sup>-10<sup>6</sup> CFU (selectivity)/ ≥ 10<sup>3</sup> CFU (specificity), according to ISO 11133:2014/Amd 1:2018.

Microorganism	Growth	Remarks
Enterococcus faecalis ATCC <sup>®</sup> 29212	Inhibited	Selectivity
Pseudomonas aeruginosa ATCC <sup>®</sup> 27853	Poor to good (Specificity)	Colourless colonies w/o precipitate
Escherichia coli ATCC <sup>®</sup> 8739	Productivity > 0.50	Dark violet colonies w. precipitate zone
Escherichia coli ATCC <sup>®</sup> 25922	Productivity > 0.50	Dark violet colonies w. precipitate zone
Salmonella typhimurium ATCC <sup>®</sup> 14028	Good (Specificity)	Colourless colonies w/o precipitate

## References

- DOWNES, F.P. & K. ITO (2001). Compendium of Methods for the Microbiological Examination of Food. 4th ed. APHA, Washington. DC.
- FIL-IDF. (1998) Standard 73B. Enumeration of coliform bacteria. ICMSF (1978). Microorganisms in Food, University of Toronto Press.
- · ISO (1986) Standard 5541-1 Milk and Milk Products. enumeration of coliforms. Colony-count technique at 30°C.
- · ISO (2006) Standard 4832: 2006 (E) Microbiology of food and animal feeding stuffs Horizontal method for the enumeration of coliformes Colony-count technique.
- . ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- · MARSHALL, R.T. (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA, Washington. DC.
- · PASCUAL ANDERSON, Mª R. (1992) Microbiología Alimentaria. Díaz de Santos, S.A., Madrid.

## Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).