

## Violet Red Bile Agar with Lactose (VRBL) ISO

Cat. 1093

Selective medium for the detection and enumeration of coliforms in dairy products, water and food.

### Practical information

Applications	Categories
Selective enumeration	Coliforms

Industry: Water / Food / Dairy products

Regulations: ISO 11133 / ISO 4832

### Principles and uses

Violet Red Bile Agar with Lactose (VRBL), containing Bile and Violet Red dye, is based on MacConkey Agar (Cat. 1052) for the detection and enumeration of lactose-fermenting bacteria and the differentiation of coliforms or Coliaerogenes group from non-lactose fermenting organisms in dairy products, water and foods.

Peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is a source of vitamins, particularly of the B-group. Lactose is the fermentable carbohydrate providing carbon and energy. Bile salts and crystal violet inhibit Gram-positive bacteria. Neutral red is a pH indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Bacteriological agar is the solidifying agent.

It is convenient to use the pour plate method.

Lactose fermenters form red colonies with red-purple halos. Occasionally the cocci of the intestinal tract can develop as small, punctiform red colonies.

The purplish red colonies with a diameter of at least (sometimes surrounded by a reddish zone of precipitated bile) are considered as typical colonies of coliforms and do not require further confirmation.

Atypical colonies (e.g. of smaller size), and all colonies derived from milk products that contain sugars other than lactose, may result in colonies with an appearance that looks similar to the typical coliforms. These colonies should be confirmed in tubes of Brilliant Green Bile Broth (Cat 1228).

### Formula in g/L

Bacteriological agar	15	Bile salts	1,5
Crystal violet	0,002	Neutral red	0,03
Sodium chloride	5	Yeast extract	3
Enzymatic digest of animal tissues	7	Lactose monohydrate	10

### Preparation

Suspend 41,5 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. DO NOT OVERHEAT. Cool to 44-47 °C and use immediately.

### Instructions for use

For the enumeration of coliforms according to ISO 4832:

- It is recommended the preparation of two dishes for the liquid product and/or from each dilution chosen.
- With a sterile pipette transfer 1 ml of liquid product or the appropriate dilutions to the center of each dish. Use another sterile pipette to inoculate each dilution into the dishes.
- Pour about 15 ml of VRBL medium, at 44 °C to 47 °C, into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10-1 dilution if the product is liquid) and the moment when the medium is poured into the dishes should not exceed 15 min.
- Carefully mix the inoculum with the medium and allow the mixture to solidify with the Petri dishes standing on a cool horizontal surface.
- Also prepare a control plate with of the medium for checking its sterility.
- After complete solidification, pour about 4 ml of VRBL medium, at 44 °C to 47 °C, onto the surface of the inoculated medium. Allow to solidify as described above.
- Invert the prepared dishes and incubate them in the incubator set at 30 °C or 37 °C (as agreed) for 24 ± 2 h.

- In order to confirm the presumptive coliform colonies, inoculate five colonies of each atypical type, if available, into tubes of Brilliant Green Lactose Bile Broth (Cat. 1228).
- Incubate the tubes in the incubator set at 30 °C or 37 °C (as agreed) for 24±2 hours.
- Consider as coliforms colonies that show gas formation in the Durham tube.

## Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige reddish	Purple-red	7,4 ± 0,2

## Microbiological test

According to ISO 11133:

Incubation conditions: Productivity, Specificity, Selectivity (24±2 h/ 30±1 °C).

Condiciones de inoculación: Productivity quantitative (100±20. Min.50 CFU) / Selectivity (10<sup>4</sup>-10<sup>6</sup> CFU) / Specificity (10<sup>3</sup>-10<sup>4</sup> CFU).

Reference media: TSA

Microrganisms	Specification	Characteristic reaction
Escherichia coli ATCC 25922	Good growth >50%	Purplish-red colonies with or without precipitation halo
Pseudomonas aeruginosa ATCC 27853		Colourless to beige colonies
Enterococcus faecalis ATCC 29212	Total inhibition (0)	

## Storage

Temp. Min.:2 °C  
Temp. Max.:25 °C

## Bibliography

ISO 4832. Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of coliforms - Colony-count technique.

Collins, J. Milk and Food Tech 18:169, 1955.

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Speck, M.L. (ed) 1976. Compendium of Methods for the Microbiological Examination of Foods (APHA).