VIOLET RED BILE AGAR WITH LACTOSE (VRBL)

ABVL-00I-500

- **Principle**

Violet Red Bile Agar with Lactose (VRBL), containing Bile and Violet Red dye, is based on MacConkey Agar (AGMC-IEP-500) 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.

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 like the typical coliforms. These colonies should be confirmed in tubes of Brilliant Green Bile Broth (BRGR-00I-500).

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 4832 as well as the BAM reference methodology.

- **Composition**

Ingredients	**g/L**
Bacteriological agar	15.00
Crystal violet	0.002
Neutral red	0.03
Yeast extract	3.00
Bile salts	1.50
Lactose monohydrate	10.00
Sodium chloride	5.00
Enzymatic digest of animal tissues	7.00

- **Preparation**

Suspend 41.5 grams of the medium in one litre 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.

- **Applications and 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 centre 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.
- To confirm the presumptive coliform colonies, inoculate five colonies of each atypical type, if available, into tubes of Brilliant Green Lactose Bile Broth (BRGR-00I-500).
- 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	w/o rests
Appearance	Fine powder
Colour of the dehydrated medium	Beige reddish
Colour of the prepared medium	Purple-red
Final pH (25°C)	7.4±0.2

- **Microbiological test**

According to ISO 11133:

- Incubation conditions: Productivity, Specificity, Selectivity (24±2 h/ 30±1 °C).
- Inoculation conditions: Productivity quantitative (100±20. Min.50 CFU) / Selectivity (10^4-10^6 CFU) / Specificity (10^3-10^4 CFU).

Reference media: TSA

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

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **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.

Hartman, J. Milk and Food Tech 23:43. 1960.

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

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.