

## VIOLET RED BILE AGAR WITH LACTOSE (VRBL) ISO 4832

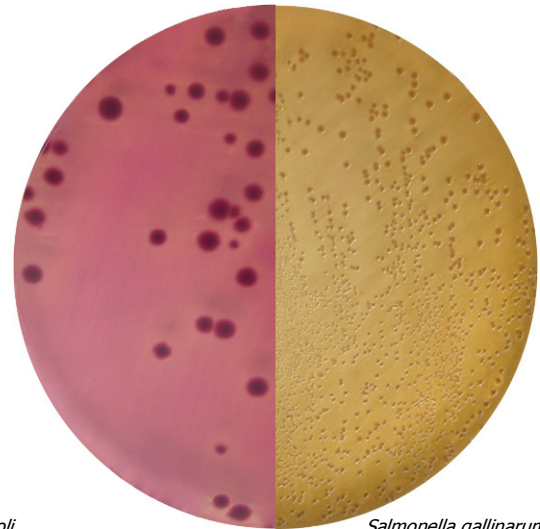
**CAT N°: 1093**

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

### FORMULA IN g/l

|                 |       |                      |       |
|-----------------|-------|----------------------|-------|
| Lactose         | 10.00 | Bile Salts           | 1.50  |
| Gelatin Peptone | 7.00  | Neutral Red          | 0.03  |
| Sodium Chloride | 5.00  | Crystal Violet       | 0.002 |
| Yeast Extract   | 3.00  | Bacteriological Agar | 15.00 |

**Final pH 7.4 ± 0.2 at 25°C**



*Escherichia coli*  
ATCC 25922

*Salmonella gallinarum*  
NCTC 9240

### 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 AUTOCLAVE. Cool to 45°C and use immediately. If desired, the can also be dispensed and sterilized in the autoclave at 118°C for 15 minutes, as Hartham demonstrated that media prepared only by boiling gave the same results as media autoclaved. The prepared medium should be stored at 8-15°C. The color of the medium is purple-red.

The dehydrated medium should be homogeneous, free-flowing and beige reddish in color. If there are any physical changes, discard the medium.

### 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 *Coli-aerogenes* 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 by placing 1 ml of the desired dilution in a sterile Petri dish, adding 15 ml of the medium, cooled to 45 – 50°C, and rotating gently before allowing to solidify. Once solidified, pour a second layer of the medium to a depth of 5 mm. Allow to solidify. Incubate at temperatures of 35 ± 2°C for 18 – 24 hours.

ISO 4832 recommends 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 h ± 2 h.

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

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 35 ± 2°C and observed after 18-24 hours\*.

| Microorganisms                           | Growth    | Colony Color    | Inoculum (cfu/ml)                | Recovery Rate(%) |
|--|-----------|-----------------|----------------------------------|------------------|
| <i>Escherichia coli</i> ATCC 25922       | Good      | Purple          | 10 <sup>3</sup> -10 <sup>5</sup> | ≥30              |
| <i>Enterobacter aerogenes</i> ATCC 13048 | Good      | Purple          | 10 <sup>3</sup> -10 <sup>5</sup> | ≥30              |
| <i>Salmonella gallinarum</i> NCTC 9240   | Good      | Colorless       | 10 <sup>3</sup> -10 <sup>5</sup> | ≥30              |
| <i>Staphylococcus aureus</i> ATCC 6538   | Inhibited |                 | >10 <sup>5</sup>                 | ≤0.01            |
| <i>Enterococcus faecalis</i> ATCC 29212  | Inhibited |                 |                                  |                  |
| <i>Pseudomonas aeruginosa</i> ATCC 27853 | Good      | Colorless-beige | 10 <sup>2</sup>                  | ≥80              |

\*According to ISO 4832. Incubation at 30 or 37°C and observed after 24 ± 2 h

## 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).

## STORAGE

Once opened keep powdered medium closed to avoid hydration.

