

PRODUCT CODE: 413746

Violet Red Bile Lactose Agar (VRBL) (ISO 4832) (Dehydrated Culture Media) for microbiology

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 45°C and use immediately. If desired, the medium can also be sterilized in the autoclave at 118°C for 15 minutes. 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 colour of the medium is purple-red. The dehydrated medium should be homogeneous, free-flowing and beige reddish in colour. 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 *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 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 ± 2h.

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

Composition

See in Data Sheet (TDS).

Microbiological Test

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of $35 \pm 2^\circ\text{C}$ and observed after 18-24 hours according to ISO 4832. Incubation at 30 or 37°C and observed after 24 ± 2 h.

Microorganism	Growth	Colony Colour	Inoculum (CFU/ml)	Recovery Rate (%)
<i>Escherichia coli</i> ATCC 25922	Good	Purple	$10^3 - 10^5$	≥ 30
<i>Enterobacter aerogenes</i> ATCC 13048	Good	Purple	$10^3 - 10^5$	≥ 30
<i>Salmonella gallinarum</i> NCTC 9240	Good	Colourless	$10^3 - 10^5$	≥ 30
<i>Staphylococcus aureus</i> ATCC 6538	Inhibited	-	$>10^5$	≤ 0.01
<i>Enterococcus faecalis</i> ATCC 29212	Inhibited	-	-	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	Good	Colourless-Beige	10^2	≥ 80

According to 11133 (24 h/30°C) Productivity, Selectivity and Specificity

Microorganism	Inoculum (CFU)	Productivity Quantitative	Selectivity Qualitative	Characteristic Colonies
<i>Escherichia coli</i> ATCC 25922	10^2	pr ≥ 0.5	-	-
<i>Salmonella</i> ssp	10^2	pr ≥ 0.8	-	-
<i>Enterococcus faecalis</i> ATCC 29212	$10^4/10^6$	-	Inhibited	-
<i>Pseudomonas aeruginosa</i> ATCC 27853	10^2	-	-	White-beige

Reference Media Productivity TSA

Storage

Once opened keep powdered medium closed to avoid hydration.

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