VIOLET RED BILE LACTOSE AGAR

Code: CM0107

A lactose-containing selective medium for the detection and enumeration of coliform organisms in water, food and dairy products.

Typical Formula*	**gm/litre**
Yeast extract	3.0
Peptone	7.0
Sodium chloride	5.0
Bile Salts No.3	1.5
Lactose	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.0
pH 7.4 ± 0.2 @ 25°C	

*** Adjusted as required to meet performance standards**

Directions
Suspend 38.5g in 1 litre of distilled water. Bring to the boil. Continue to boil for 2 minutes or for the minimum time necessary to dissolve completely and ensure that there are no remaining flecks of unmelted agar. No further sterilisation is necessary or desirable. Mix well before pouring.

Description
Violet Red Bile Lactose Agar is a selective medium for the detection and enumeration of coliform organisms. The medium has been used for the determination of the coli-aerogenes content of water, milk and other dairy products, dairy equipment, and food products etc [1,2].
Organisms which rapidly attack lactose produce purple-pink colonies surrounded by purple haloes. Non-lactose or late-lactose fermenters produce pale colonies with greenish zones. Other related Gram-negative bacteria may grow but can be suppressed by incubation at >42°C or by anaerobic incubation.

Druce *et al.*[3] found that Violet Red Bile Lactose Agar was as good an indicator of coli-aerogenes bacteria in milk as MacConkey Broth, and that the Oxoid medium was suitable for determining the coli-aerogenes content of milk.

Technique
Druce et al. recommended the following procedures:
For the routine determination of the coli-aerogenes content of raw milk, prepare pour-plates containing 1.0, 0.1 and 0.01ml of the sample in Violet Red Bile Lactose Agar, and incubate for 20-24 hours at 35°C.
For coli-aerogenes counts of pasteurised milk, employ 4 pour-plates of Violet Red Bile Lactose Agar.

Divide 10ml of the sample among three of the plates, and add 1ml of the sample to the remaining plate. Incubate for 20-24 hours at 30°C. Similarly the examination of rinses and swabs from dairy equipment and apparatus, should include the spreading of 10ml of solution on each of 3 plates and of 1ml on a single plate. Coliform organisms form purple-pink colonies which are 1 to 2mm in diameter, usually surrounded by a purple zone. Occasionally colonies may be considerably smaller (less than 0.5mm in diameter).
When preparing pour-plates the medium should be freshly made up, cooled to 47°C and used within 3 hours.
An overlay method is helpful to improve the specificity of the medium. In this case a thin layer of cooled molten medium is poured over the inoculated base layer and allowed to set before incubation. Incubation may be carried out at >42°C for 18 hours, 30°C for 24-48 hours or 4°C for 10 days, depending on the temperature characteristics of the organisms to be recovered. For *Escherichia coli* a temperature of 44 ± 1°C is specifically recommended [4].

Characteristic appearance of colonies
Round, purple-pink may be surrounded by purple haloes (lactose-positive organisms).
Pale, may have greenish haloes (lactose-negative organisms).
Confirmation of the identity of purple-pink colonies must be made by further tests.

Storage conditions and Shelf Life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared medium at 2-8°C and use as freshly as possible.

Appearance
Dehydrated medium: Straw-pink coloured, free-flowing powder
Prepared medium: Dark purple coloured gel

Quality control

Positive control:	**Expected results**
Escherichia coli ATCC® 25922 *	Good growth; purple-pink colonies with haloes
Negative controls:	
Staphylococcus aureus ATCC® 25923 *	No growth
Enterococcus faecalis ACTT®29212*	No growth

*** This organism is available as a Culti-Loop®**

Precautions
This medium is not completely specific for Enterobacteriaceae, other organisms e.g. *Aeromonas* and *Yersinia* species may give similar reactions.
The selectivity of the medium diminishes after 24 hours incubation and organisms previously suppressed may exhibit growth.

References

1. American Public Health Association (1978) *Standard Methods for the Examination of Dairy Products. 14th Edn. APHA Inc. Washington DC.*

2. American Public Health Association (1992) *Compendium of Methods for the Microbiological Examination of Foods 3rd Edition APHA Inc. Washington DC.*

3. Druce R. G., Bebbington N. N., Elson K., Harcombe J. M. and Thomas S. B. (1957) *J. Appl. Bact*. 20. 1-10.

4. Mossel D. A. A. and Vega C. L. (1973) *Hlth Lab. Sci*. 11. 303-307.

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