

### Specification

Medium for the detection and enumeration of coliforms in milk and other dairy products, according to APHA and ICMSF, FIL-IDF and ISO Standards.

### Presentation

10 Prepared bottle  
Bottle 125 ml  
with:  $100 \pm 3$  ml

#### Packaging Details

1 box with 10 bottles 125 ml. Non injectable cap.  
metal cap.

#### Shelf Life

12 months

#### Storage

8-25°C

### Composition

Composition (g/l):

Yeast extract.....	3.000
Peptone.....	7.000
Bile salts No. 3.....	1.500
Lactose.....	10.00
Sodium chloride.....	5.000
Neutral red.....	0.030
Crystal violet.....	0.002
Agar.....	13.000

### Description /Technique

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The Violet Red Bile Agar corresponds to the classic formulation of standardized media for the screening of coliforms in milk and other dairy products. This medium has been adopted for the enumeration of coliforms as well as for differentiating between lactose-fermenting and non-lactose fermenting organisms, due to its contents of crystal violet and bile salts, whose inhibiting or selective properties have been widely confirmed.

#### Technique

To use, the contents of the bottle should be poured into plates. The melting of the culture medium should be carried out according to the manufacturer's instructions, either in a water bath (100°C) or microwave oven. Never apply direct heat to melt a medium. The melting temperatures and times depend on the shape of the container, the volume of medium and the heat source. Before melting any medium loosen the screwcap of the container to avoid breaking the container. The medium should be melted only once and used. Media with agar should not be melted repeatedly as their characteristics change with each remelting. Overheating should be avoided as much as prolonged heating, especially with regard to media with an acidic or alkaline pH.

Once melted pour the plates using aseptic techniques. To inoculate, follow standard laboratory methods or the applicable norms. Spiral plate method, streak plating, econometric methods, dilution banks, spread plating etc...

The recommended procedure is inoculation directly into Petri dishes, with the molten agar cooled to 45-47°C. Plates can be read after 24 hours of incubation at 30°C.

The size of the colonies ranges from 2 to 5 mm, depending on the amount per plate. If enterococci develop they will appear small in size and pink coloured. Lactose fermenting enterobacteria acquire a dark red colour with a clearing zone around them, while lactose non-fermenting ones form colourless colonies.

**Quality control****Physical/Chemical control**

Color : Violet-pink

pH: 7.4 ± 0.2 at 25°C

**Microbiological control**Melting - pour plates - inoculation Practical range 100±20 CFU; Min. 50 CFU (Productivity) / 10<sup>4</sup>-10<sup>6</sup> CFU (Selectivity)

Aerobiosis. Incubation at 30 ± 1 °C during 18-24 h

**Microorganism**

*Enterococcus faecalis* ATCC® 19433, WDCM 00009  
*Ps. aeruginosa* ATCC® 27853, WDCM 00025  
*Escherichia coli* ATCC® 8739, WDCM 00012  
*Escherichia coli* ATCC® 25922, WDCM 00013  
*Salmonella typhimurium* ATCC® 14028, WDCM 00031

**Growth**

Inhibited  
Good - Colourless colonies  
Good (≥50%)- Red purple colonies  
Good (≥50%)- Red purple colonies  
Good - Colourless colonies

**Sterility Control**

Incubation 48 hours at 30-35°C and 48 hours at 20-25°C: NO GROWTH

Check at 7 days after incubation in same conditions

**Bibliography**

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- MARSHALL, R.T. (1992) Standard Methods for the Examination of Dairy Products. 16th ed. APHA, Washington. DC.
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