

Violet red bile glucose agar (ISO)**Code 84603.0500****Also known as**

VRBD acc. to Mossel, VRBG.

Intended useFor the detection and enumeration of *Enterobacteriaceae* (ISO 21528)**Formula * - Composition in g/L**

Enzymatic digest of animal tissues.....	7.000
Yeast extract.....	3.000
Bile salts No. 3	1.500
Glucose.....	10.000
Sodium chloride.....	5.000
Neutral red.....	0.030
Crystal violet	0.002
Agar.....	15.000

* Adjusted and/or supplemented as required to meet performance criteria

Final pH 7.4 ± 0.2 at 25 °C.

Instructions for preparation

Dissolve 41.5 g in 1 litre of purified water by bringing to the boil with frequent shaking. Do not sterilise the medium in the autoclave.

Principle of the method and general information

Violet red bile glucose agar (VRBG) was designed by Mossel et al. for the enumeration of *Enterobacteriaceae*. It contains enzymatic digest of animal tissues and yeast extract to supply carbon, nitrogen, essential minerals, and vitamins to stimulate the growth of bacteria. Glucose supplies energy for growth and metabolism. Bile salts and crystal violet inhibit the growth of Gram-positive bacteria. Neutral red is added as a pH indicator. Microorganisms that dissimilate glucose will produce red to purple colonies with red-purple halos, demonstrating bile precipitation in the presence of neutral red.

Violet red bile glucose agar, prepared according to the formulation proposed by ISO 21528, can be used for:

- Enumeration of *Enterobacteriaceae* in foodstuffs with pre-enrichment in non-selective medium, enrichment in selective liquid medium and isolation and selection for confirmation. ISO 21528-1 describes two procedures recommended when the number of *Enterobacteriaceae* is expected to be in the range 1 to 100/ml or g: detection method and MPN method.
- Enumeration of *Enterobacteriaceae* in foodstuffs with plate count technique without pre-enrichment. This procedure, described by ISO 21528-2 is recommended when the number of *Enterobacteriaceae* is expected to be greater than 100/ml or g.

Instructions for use

For laboratory use only.

Enumeration of *Enterobacteriaceae* in foodstuffs, with plate count technique without pre-enrichment.

- Take two sterile Petri dishes and transfer by means of a sterile pipette to each dish 1 ml of the test sample, if the product is liquid, or 1 ml of the initial suspension in the case of other products.
- Take two other sterile Petri dishes and transfer by means of a other sterile pipette to each dish 1 ml of the first decimal dilution (10^{-1}) of the test sample, if the product is liquid, or 1 ml of the first decimal dilution (10^{-2}) of the initial suspension in the case of other products.
- If necessary repeat the procedure with the further dilutions using a fresh sterile pipette for each decimal dilution.
- Pour about 15 ml of VRBG cooled to 45°C into each Petri dish.

- Carefully mix the inoculum with the medium by rotating the plates and allow to solidify on a cool horizontal surface. Pour about 10 to 15 ml of VRBG cooled to approximately 45°C on the surface of inoculated medium to prevent spreading growth and to obtain semi-anaerobic conditions. Allow to solidify.
- Invert the prepared Petri dishes and place them in the incubator at 37 °C for 24 hours.
- Do not stack the dishes more than six high. Stacks of the dishes should be separated from one each other and from the walls and the top of the incubator.
- Count the colonies on the plates containing less than 150 typical pink to violet-red colonies (with or without precipitation halo) of diameter 0.5 mm or more.
- Select five typical colonies for biochemical confirmation (oxidase test, glucose fermentation).

Enumeration of *Enterobacteriaceae* in foodstuffs with pre-enrichment

- Prepare the test sample using the pre-enrichment Buffered peptone water (ART. N° 84600.0500): 1 g of test sample +10 ml of liquid medium
- Incubate the initial suspension at 37°C for not less than 16 hours and not more than 20 hrs.
- Transfer 1 ml of the culture to 10 ml of EE Broth Mossel (Art. N° 84689.0500) and incubate at 37°C for 18-24 hrs.
- Streak the incubated EE Broth Mossel onto the surface of VRBG and incubate the plate for 24 hours at 37°C.
- From each of the incubated plates on which typical pink or red-violet colonies with or without halo, choose five colonies for biochemical confirmation (oxidase test, glucose fermentation).

Consult the quoted ISO Standards for the details of the procedure for enumeration, results interpretation and confirmation of *Enterobacteriaceae*.

Quality Control

Physical characteristics:

Appearance of powder	Green-violet, fine, homogeneous, hygroscopic powder
Appearance of prepared medium	Violet, limpid
pH (25°C)	7.4 ± 0.2

Microbiological characteristics:

Test Strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity ratio
Growth promoting test according to ISO/TS 11133-2				
<i>E. coli</i> ATCC 25922	37°C / 24 h / AE	QT / 80-120 CFU	Good growth with typical pink-red colonies with red halo	PR ≥ 0.5
<i>S. Typhimurium</i> ATCC 14028	37°C / 24 h / AE	QT / 80-120 CFU	Good growth with colourless colonies	PR ≥ 0.5
Test for inhibitory properties, according to ISO/TS 11133-2				
<i>E. faecalis</i> ATCC 19433	37 °C / 24 h / AE	MM / ≥ 10 ⁴ CFU	Growth inhibited	
Other quality control tests				
<i>Y. enterocolitica</i> ATCC 23715	37 °C / 24 h / AE	EC	Good growth red-violet colonies	
<i>S. aureus</i> ATCC 25923	37 °C / 24 h / AE	MM	Growth inhibited	

Notes

PR (Productivity Ratio): CFU obtained on culture medium under test / CFU obtained on Tryptic Soy Agar

Incubation atmosphere AE: aerobic incubation

Inoculation method QT : quantitative surface plating method; EC: semi-quantitative, ecometric technique; MM: modified Miles-Misra surface drop method

Microbiological characteristics tested in accordance to ISO/TS 11133-2 and Ph. Eur.

ATCC is a registered trade mark of American Type Culture Collection

References

- ISO 21528-1:2004 Microbiology of food and animal feeding stuffs -- Horizontal methods for the detection and enumeration of *Enterobacteriaceae* -- Part 1: Detection and enumeration by MPN technique with pre-enrichment.
- ISO 21528-2:2004 Microbiology of food and animal feeding stuffs -- Horizontal methods for the detection and enumeration of *Enterobacteriaceae* -- Part 2: Colony-count method.
- Mossel, D.A.A., Eelderink, M. Koopmans and F. van Rossem. 1978. Lab. Pract. 27, 1049-1050

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+10°C to 30°C and <60% RH).

Ordering information

84603.0500

Violet red bile glucose agar (ISO)

Bottle of 500 g