Violet Red Bile Glucose Agar without Lactose ISO

Intended Use
Violet Red Bile Glucose Agar without Lactose is used for the detection and enumeration of *Enterobacteriaceae* in food, animal feed and environmental samples in compliance with ISO 21528-1:2017 and ISO 21528-2:2017.

Summary
Violet Red Bile Glucose Agar without Lactose is a modification of Violet Red Bile Lactose Agar (VRBL) introduced by Mossel in 1978, was designed for the enumeration of *Enterobacteriaceae*. It is applicable to products intended for human consumption and the feeding of animals, and environmental samples in the area of primary production, food production and food handling, as described in ISO 21528-2:2017 and ISO 21528-1:2017.

Principle
The VRBGA formula modification replaces lactose with glucose to enable detection of non-lactose-fermenting *Enterobacteriaceae*. Glucose is fermented by all members of the *Enterobacteriaceae* thus gives a presumptive *Enterobacteriaceae* count. The rapid fermentation of glucose by *Enterobacteriaceae* results in acid production, which is indicated by the pH indicator neutral red. Peptone provides the essential vitamins, minerals, amino acids, nitrogen and carbon, while sodium chloride maintains the osmotic balance. Bile salts and crystal violet are used to inhibit Gram-positive and nonenteric organisms.
According to ISO 21528-1:2017 inoculation is performed after enrichment in Buffered Peptone Water (BPW) and according to ISO 21528-2:2017, inoculation is performed from a quantity of the initial suspension and decimal dilutions of the test sample. This medium conforms to the performance and formulation requirements of ISO 21528-1:2017 and ISO 21528-2:2017 standards. Further biochemical tests are necessary for positive identification.

Formula*

Ingredients	**g/L**
Peptone	7.0
Yeast extract	3.0
Sodium chloride	5.0
Bile salts mixture	1.5
Glucose (Dextrose)	10.0
Neutral red	0.03
Crystal violet	0.002
Agar	12.000
Final pH (at 25°C)	7.4 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Food and Dairy samples; Water samples.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 38.53 g of the powder in 1000 mL purified / distilled water.
2. Heat to boiling to dissolve the powder completely.
3. DO NOT AUTOCLAVE.
4. Cool to 45°C-50°C.
5. Mix well and pour into sterie petridishes.

Quality Control

Dehydrated Appearance: Light yellow to pinkish beige coloured, homogeneous, free flowing powder.
Prepared Appearance: Light pinkish purple coloured, clear to slightly opalescent gel forms in petridishes.
Growth Promotion Test: Growth promotion is carried out in accordance with the method of ISO and growth is observed after an incubation at 35-37°C for 18 to 24 hours.
Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism at 35-37°C for 18 hours.
Indicative Properties: The test results observed are within the specified temperature and time, inoculating ≤100 cfu of appropriate microorganism.
Inhibitory Properties: No growth of the test microorganism occurs for the specified temperature and not less than the longest period of the time specified, inoculating >100 cfu of the appropriate microorganism at 35-37°C for ≥ 24 hours.

Growth Promoting + Indicative

Organism (ATCC)	**Growth**	**Colour of Colony**	**Incubation Temperature**
Escherichia coli (8739)	Good	Pinkish red with bile precipitate	35-37°C
Escherichia coli (25922)	Good	Pinkish red with bile precipitate	35-37°C
Escherichia coli (NCTC 9002)	Good	Pink - red	35-37°C
Pseudomonas aeruginosa (9027)	Good	Pink - red	35-37°C
Salmonella Enteritidis (13076)	Good	Light Pink	35-37°C
Klebsiella aerogenes (13048)	Good	Pink - red	35-37°C
Staphylococcus aureus subsp. *aureus* (25923)	Inhibited	-	35-37°C
Staphylococcus aureus subsp. *aureus* (6538)	Inhibited	-	35-37°C

Note:

1. For good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.
2. For inhibition no growth of test microorganism should occur.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

1. Over incubation may result in reverting of reaction.
2. Further biochemical tests must be carried out for confirmation.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. ISO 21528-1:2017 Microbiology of the food chain -- Horizontal method for the detection and enumeration of *Enterobacteriaceae* -- Part 1: Detection of *Enterobacteriaceae*
2. ISO 21528-2:2017 Microbiology of the food chain -- Horizontal method for the detection and enumeration of *Enterobacteriaceae* -- Part 2: Colony-count technique
3. Mossel, D.A.A. Media for *Enterobacteriaceae* (1985) International Journal of Food Microbiology, 2 (1- 2), pp. 27-32.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
201220220500	Dehydrated Culture Media	500 g