

Violet Red Bile Glucose (VRBG) Agar (ISO) (NCM0041)

Intended Use

Violet Red Bile Glucose (VRBG) Agar (ISO) is a selective agar 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 21528-1:2017 and ISO 21528-2:2017. Violet Red Bile Glucose (VRBG) Agar (ISO) is not intended for use in the diagnosis of disease or other conditions in humans.

Description

A selective agar 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. Formulated according to ISO 21528-1:2017 and ISO 21528:2017 (all parts), VRBGA is a modification of Violet Red Bile Lactose Agar (NCM0089) introduced by Mossel in 1978. VRBL gives a 'coliform' count as it contains lactose which is fermented by members of the Coli-Aerogenes group. 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. Enzymatic digest of animal tissues provide 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 non-enteric organisms. The overlay procedure ensures anaerobic conditions and suppresses the growth of non-fermentative Gram-negative bacteria. 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.

Typical Formulation

Enzymatic digest of Animal Tissues	7.0 g/L
Yeast Extract	3.0 g/L
Bile Salts	1.5 g/L
Glucose	10.0 g/L
Sodium Chloride	5.0 g/L
Neutral Red	0.03 g/L
Crystal Violet	0.002 g/L
Agar	12.0 g/L

pH: 7.4 ± 0.2 at 25°C.

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 38.5 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.
4. Cool to 45-50°C.

Prepare the medium just before use. Use the molten medium within 4 hours of its preparation.

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Revision: 2

Technical Specification Sheet



Test Procedures

- For the detection of *Enterobacteriaceae* – Refer to ISO 21528-1:2017
- For the Colony-count technique – Refer to ISO 21528-2:2017

Quality Control Specifications

Dehydrated Appearance: Powder is homogenous, free flowing, and beige to pinkish to reddish-beige.

Prepared Appearance: Prepared medium is reddish purple, trace to slightly hazy.

Expected Cultural Response: Cultural response at 37 ± 1 °C for 24 ± 2 hours.

Microorganism	Approx. inoculum (CFU)	Expected Results	
		Recovery	Reaction
<i>Enterococcus faecalis</i> ATCC 29212	>10 ⁴	Complete Inhibition	N/A
<i>Enterococcus faecalis</i> ATCC 19433	>10 ⁴	Complete Inhibition	N/A
<i>Escherichia coli</i> ATCC 25922	50-200	Recovery ≥50%	Pink colonies w/ red precipitate
<i>Escherichia coli</i> ATCC 8739	50-200	Recovery ≥50%	Pink colonies w/ red precipitate
<i>Pseudomonas aeruginosa</i> ATCC 27853	4 Quad Streak	Growth	Colorless to grey
<i>Salmonella enteritidis</i> ATCC 13076	50-200	Recovery ≥50%	Pink colonies w/ red precipitate
<i>Salmonella typhimurium</i> ATCC 14028	50-200	Recovery ≥50%	Pink colonies w/ red precipitate
<i>Staphylococcus aureus</i> ATCC 25923	>10 ⁴	Complete Inhibition	N/A

The organisms listed are the minimum that should be used for quality control testing.

Results

Enterobacteriaceae ferment glucose and produce acid products. Acid production is indicated by the neutral red and crystal violet in the medium, resulting in pink to red or purple coloured colonies.

Enterobacteriaceae colonies are usually surrounded by a red/purple halo produced from a precipitate of bile salts.

Crystal violet and bile salts inhibit the growth of Gram-positive organisms.

Expiration

The dehydrated medium should be discarded if it is not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Certain *Enterobacteriaceae* may cause discoloration of their colonies or of the medium.

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Storage

Store dehydrated culture media at 2 – 30 °C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

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. ISO 11133:2014+A1:2018 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.

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620 Leshar Place • Lansing, MI 48912 • 800-234-5333 (USA/Canada) • 517-372-9200
1 Quest Park • Heywood, BL9 7JJ, UK • +44 (0)161 820 3833
foodsafety@neogen.com • foodsafety.neogen.com