



# Technical Data

## Violet Red Bile Glucose Agar w/o Lactose

M581

### Intended Use:

Recommended for enumeration of *Enterobacteriaceae* in raw food and clinical samples. The composition and performance criteria are in accordance with ISO 21528-1&2:2017 and ISO 11133:2014 /Amd.2 : 2020 (E).

### Composition\*\*

#### ISO Specifications-Violet Red Bile Glucose

##### Agar w/o Lactose

Ingredients	g / L
Enzymatic digest of animal tissues	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts No.3	1.500
Glucose	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	9.000-18.000
Final pH ( at 25°C)	7.4±0.2

#### Violet Red Bile Glucose Agar w/o Lactose

M581

Ingredients	g / L
Peptone \$	7.000
Yeast extract	3.000
Sodium chloride	5.000
Bile salts mixture	1.500
Glucose (Dextrose)	10.000
Neutral red	0.030
Crystal violet	0.002
Agar	12.000
Final pH ( at 25°C)	7.4±0.2

\*\*Formula adjusted, standardized to suit performance parameters

\$ -Equivalent to Enzymatic digest of animal tissues

### Directions

Suspend 38.53 gram in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. **DO NOT AUTOCLAVE**. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Violet Red Bile Agar, a modification of MacConkey original formulation (1) is used for the enumeration of coli-aerogenes bacterial group. Violet Red Bile Glucose Agar w/o Lactose, a modification of VRBA (M049), was designed for the enumeration of *Enterobacteriaceae* (2). It employs the selective inhibitory components crystals violet and bile salts and the indicator system glucose and neutral red. Sought bacteria will dissimilate glucose and produce purple zones around the colonies (3). ISO committee has also recommended this medium (4,5). Selectivity of VRBGA can be increased by incubation under anaerobic conditions and/or at elevated temperature, i.e. equal to or above 42°C (6-8).

Peptone and yeast extract serve as sources of carbon, nitrogen, vitamins and other essential growth nutrients. Glucose is the fermentable carbohydrate, utilization of which leads to the production of acids. Neutral red indicator detects the acidity so formed. Crystal violet and bile salts mixture help to inhibit the accompanying gram-positive and unrelated flora. Sodium chloride maintains the osmotic equilibrium. Further biochemical tests are necessary for positive identification (9).

### Type of specimen

Clinical samples - faeces ; Food and dairy samples; Water samples

### Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (10,11).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (4,5,12-14).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines & local standards (15).

After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions

In Vitro diagnostic use. For professional use only. Read the label before opening the container. Wear protective gloves/ protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

Please refer disclaimer Overleaf.

2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Over incubation may result in reverting of reaction.
4. Further biochemical tests must be carried out on colonies of pure culture for confirmation.

## Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

## Quality Control

### Appearance

Light yellow to pinkish beige homogeneous free flowing powder

### Gelling

Firm, comparable with 1.2% Agar gel.

### Colour and Clarity of prepared medium

Reddish purple coloured clear to slightly opalescent gel forms in Petri plates.

### Reaction

Reaction of 3.85% w/v of aqueous solution at 25°C. pH : 7.4±0.2

### pH

7.20-7.60

### Cultural Response

**Productivity :** Cultural characteristics was observed after an incubation at 35±1°C for 24±2 hours. Recovery rate is considered as 100% for bacteria growth on Soyabean Casein Digest Agar.

**Selectivity:** Cultural characteristics was observed after an incubation at 35±1°C for 24±2 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<b>Productivity</b>				
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	luxuriant	≥50 %	pink to red colonies with or without precipitation zone
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant	≥50 %	pink to red colonies with or without precipitation zone
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	luxuriant	≥50 %	pink to red colonies with or without precipitation zone
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50 -100	luxuriant	≥50 %	pink to red colonies with or without precipitation zone
<b>Selectivity</b>				
<i>Enterococcus faecalis</i> ATCC 29212 (00087*)	≥10 <sup>4</sup>	inhibited		
<i>Enterococcus faecalis</i> ATCC 19433 (00009*)	≥10 <sup>4</sup>	inhibited		

Key : (\*) Corresponding WDCM numbers.

## Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

## Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (10,11).

## Reference

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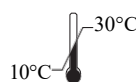
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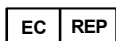
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