

Violet Red Bile Glucose HiCynth™ Agar w/o lactose**MCD581****Intended Use:**

Recommended for detection and enumeration of *Enterobacteriaceae* in raw food samples.

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No.2*	7.000
HiCynth™ Peptone No.5*	3.000
Sodium chloride	5.000
Synthetic detergent	1.500
Dextrose (Glucose)	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

*Chemically defined peptones

Directions

Suspend 38.53 grams 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). Violet Red Bile Glucose HiCynth™ Agar w/o Lactose is a modification of Violet Red Bile Glucose Agar wherein animal or vegetable peptones are replaced with chemically defined peptone to avoid BSE/TSE/GMO risks associated with animal peptones. 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). Selectivity of VRBGA can be increased by incubation under anaerobic conditions and/ or at elevated temperature, i.e. equal to or above 42°C (5-7).

HiCynth™ Peptone No.2 and HiCynth™ Peptone No.5 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 (8).

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9-11). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (12).

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

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. Over incubation may result in reverting of reaction.
2. Further biochemical tests must be carried out for confirmation.
3. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.

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

Cultural characteristics was observed after an incubation at 35-37°C for 18-24 hours. Recovery rate is considered as 100% for bacteria growth on Tryptone Soya HiCynth™ Agar.

Organism	Inoculum (CFU)	Growth	Recovery	Colour of colony
<i>Escherichia coli</i> ATCC 25922 (00013*)	50 -100	good-luxuriant	≥50 %	pink-red
<i>Escherichia coli</i> ATCC 8739 (00012*)	50 -100	luxuriant	≥50 %	pink-red with bile precipitate
<i>Escherichia coli</i> NCTC 9002	50 -100	luxuriant	≥50 %	pink-red with bile precipitate
<i>Pseudomonas aeruginosa</i> ATCC 9027 (00026*)	50 -100	good-luxuriant	≥50 %	pink to red
<i>Salmonella</i> Enteritidis ATCC 13076 (00030*)	50 -100	good-luxuriant	≥50 %	light pink
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50 -100	good-luxuriant	≥50 %	pink-red
<i>Staphylococcus aureus</i> subsp.aureus ATCC 25923 (00034*)	≥10 ⁴	inhibited	0%	
<i>Staphylococcus aureus</i> subsp.aureus ATCC 6538 (00032*)	≥10 ⁴	inhibited	0%	

Key : (*) Corresponding WDCM numbers.

(#) - Formerly known as *Enterobacter aerogenes*

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (13,14).

Reference

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