Rogosa SL HiCynth™ Agar

MCD130

Intended Use:

Recommended for selective cultivation of *Lactobacilli.*

Composition**

Ingredients	Gms / Litre
HiCynth™ Peptone No. 4*	10.000
HiCynth™ Peptone No. 5*	5.000
Dextrose (Glucose)	10.000
Arabinose	5.000
Saccharose (Sucrose)	5.000
Sodium acetate	15.000
Ammonium citrate	2.000
Potassium dihydrogen phosphate	6.000
Magnesium sulphate	0.570
Manganese sulphate	0.120
Ferrous sulphate	0.030
Polysorbate 80 (Tween 80)	1.000
Agar	15.000
Final pH (at 25°C)	5.4±0.2

**Formula adjusted, standardized to suit performance parameters

*Chemically defined peptones

Directions

Suspend 74.72 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Add 1.32 ml glacial acetic acid. Mix thoroughly, distribute into culture tubes or flasks. Heat to 90 - 100°C for 2-3 minutes. Cool to 45-50°C for direct inoculation. DO NOT AUTOCLAVE.

Principle And Interpretation

Rogosa SL HiCynth™ Agar also known as RMW Agar, is a modification of the media formulated by Rogosa, Mitchell and Wiseman (1,2). This media is used for isolation, enumeration and identification of Lactobacilli from foodstuffs and specimens (3,4). Accompanying bacterial flora is suppressed due to the low pH of the medium and also because of the high sodium acetate concentration. Rogosa SL HiCynth™ Agar is prepared by replacing animal and vegetable peptones with chemically defined peptones to avoid BSE/TSE risks associated with animal peptones.

HiCynth™ Peptone No. 4 and HiCynth™ Peptone No. 5 provide nitrogenous compounds, sulphur, trace elements and vitamin B complex, essential for growth of Lactobacilli. Dextrose, arabinose and saccharose are the fermentable carbohydrates. Polysorbate 80 is the source of fatty acids. Ammonium citrate and Sodium acetate inhibit moulds, Streptococci and many other organisms. Potassium dihydrogen phosphate provides buffering capability. Magnesium sulphate, manganese sulphate and ferrous sulphate are sources of inorganic ions. Low pH of the medium and addition of acetic acid makes the medium selective for Lactobacilli inhibiting other bacterial flora (3).

It is recommended that the plates should be incubated at 30°C for 5 days or at 37°C for 3 days in an atmosphere of 95% hydrogen and 5% carbon dioxide (5). If this is not possible, overlay the inoculated plates with a second layer of the agar before incubation. High acetate concentration and acidic pH suppress many strains of other lactic acid bacteria. All colonies should be checked by gram staining and by catalase test before further identification. The salt in the formulation makes the medium unsuitable for isolation of dairy Lactobacilli. e.g. *L. lactis, L. bulgaricus* and *L. helveticus* (2,3).

Type of specimen

Food samples

Specimen Collection and Handling:

For food samples follow appropriate techniques for handling specimens as per established guidelines (4).

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

Warning and Precautions

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

Limitations :

1. It is recommended that the plates should be incubated at 30°C for 5 days or at 37°C for 3 days in an atmosphere of 95% hydrogen and 5% carbon dioxide (7). If this is not possible, overlay the inoculated plates with a second layer of the agar before incubation.
2. High acetate concentration and acidic pH suppress many strains of other lactic acid bacteria.
3. All colonies should be checked by gram staining and by catalase test before further identification.

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

Cream to yellow homogeneous soft lumps which can be easily broken down to powder form.

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured opalescent gel forms in Petri plates

Reaction

Reaction of 7.5% w/v aqueous solution with 0.132% v/v acetic acid at 25°C. pH : 5.4±0.2

pH

5.20-5.60

Cultural Response

Cultural characteristics observed in presence of 5% Carbon dioxide (CO_2) and 95% H_2 after an incubation at 35-37° C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
Lactobacillus casei ATCC 9595	50-100	good - luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	good to luxuriant	>=50%
Lactobacillus leichmanni ATCC 4797	50-100	good to luxuriant	>=50%
Lactobacillus plantarum ATCC 8014	50-100	good-luxuriant	>=50%
Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)	>=10^4	inhibited	0%

Key : (*) Corresponding WDCM numbers.

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°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 (6,7).

Reference

1. Rogosa M., Mitchell J. A. and Wiseman R. F, 1951, J. Bacteriol., 62, 132-133.
2. Rogosa M., Mitchell J. A. and Wiseman R. F., 1951, J. Dental Res. 30:682.
3. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification- Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore. Md.
4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
5. Sharpe M. L. (Ed.), 1960, Lab-Practice, 9(4): 223.
6. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
7. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

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