Calcium Carbonate Agar

M1900

Intended Use:

Recommended for the differentiation of microorganisms especially yeasts based on the production of acid from glucose.

Composition**

Ingredients	Gms / Litre
Calcium Carbonate (fine, granulated)	5.000
Dextrose (Glucose)	50.000
Yeast extract	5.000
Agar	15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 75 grams in 1000 ml purified / distilled water. Heat to boiling to digest the agar completely. DO NOT AUTOCLAVE. A residue of calcium may remain. Cool to 45-50°C. Mix well and pour into sterile Petri plates, by evenly distributing the residue.

Note: *Due to the presence of calcium carbonate, the prepared medium forms opalescent solution with a white precipitate.*

Principle And Interpretation

Yeasts and Moulds form a very large group of microorganisms,with most coming from the air, water or soil. Yeasts are unicellular, eukaryotic, budding cells that are generally round oval or elongate in shape (5). They multiply principally by the production of blastoconidia (buds) (1). Yeast colonies are moist and creamy or glabrous to membranous in texture and are considered opportunistic pathogens. Moulds are microscopic, plant-like organisms, composed of long filaments called hyphae. Calcium Carbonate Agar is differentiation agar recommended by Kurtzman and Fell (2) for the identification of yeasts. Yeast extract provide the nitrogen, vitamins and amino acids for growth. Glucose is the fermentable carbohydrate. Calcium carbonate serves as indicator as it makes the plate milky and turbid and in case of acid is produced the media clears up. The acid is produced due to characteristic fermentation of glucose, which along with calcium carbonate results in forming, calcium acetate, that gets soluble in water. Yeasts from the genus Dekkera (*Brettanomyces*) forms acetic acid and show a positive result. Sometimes the acid production is quite weak. Also some other yeasts like *Candida* species produce some citric acid and show a weak positive reaction.

Type of specimen

Soil sample, Water sample etc.

Specimen Collection and Handling

For soil samples, follow appropriate techniques for sample collection and processing as per guidelines (6).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1).
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. Some other yeasts like *Candida* species produce some citric acid and show a weak positive reaction.

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 free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

Cultural Response

Cultural characteristics observed after an incubation at 25-30°C for 24-72 hours with added Tetracycline at a final concentration of 10mcg/ml.

Organism	Inoculum (CFU)	Growth	Acid production	Recovery
Candida albicans ATCC 10231 (00054*)	50-100	good	Weakly positive	>=50%
Saccharomyces cerevisiae ATCC 9763 (00058*)	50-100	good	Negative	>=50%

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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.

2. C.P. Kurtzman, J.D. Fell (ed.), The yeast, a taxonomic study, 4th edition, Elsevier (1998)

3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.

4. 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.

5. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.) 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.

6. Subba Rao N. S., 1977, Soil Microorganisms and Plant Growth, Oxford and IBH Publishing Co., New Delhi.

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