



Leeming-Notman Agar (Modified)(Twin Pack)

M2189

Intended Use:

Recommended for primary isolation and cultivation of *Malassezia furfur* and other *Malassezia* species.

Composition**

Ingredients	g/ L
Part A	-
Peptone	10.000
Dextrose (Glucose)	10.000
Yeast extract	2.000
Bile Dessicated #	8.000
Agar	15.000
Part B	-
Glycerol	10.000ml
Olive oil	20.000ml
Glycerol monostearate	0.500
Tween 60	5.000ml
Final pH (at 25°C)	6.2±0.2

**Formula adjusted, standardized to suit performance parameters

- Equivalent to Ox bile Dessicated

Directions

Suspend 45.0 grams of Part A in 1000 ml purified/distilled water. Add 35.5ml of fluid Part B. Mix well and heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. (If desired, for more selectivity add 50mcg/ml of Chloramphenicol and 200mcg/ml of Cycloheximide). Mix well and pour into sterile Petri plates or dispense into tubes for slants.

Note: Warm the Part B to 60-70°C before adding to Part A solution

Principle And Interpretation

Malassezia is a lipophilic yeast commensal in areas rich in sebaceous glands of the human skin and other warm-blooded animals (1). LNA supported the highest growth of *Malassezia* species, due to its richness and nutrient balance because of ox bile, glycerol monostearate, glycerol and Tween 60 (2). It is also recommended by ATCC for culture revival of *Malassezia* species(3). *M. furfur* is a lipophilic yeast, therefore in vitro growth must be stimulated by natural oils or other fatty substances. With acidic pH, they are used for the isolation, cultivation and maintenance of yeast and moulds.

Yeast extract and peptone provides nitrogenous and carbonaceous compounds, long chain amino acids and other essential nutrients. Dextrose is the energy source. Bile dessicated inhibits accompanying microflora. Glycerol, olive oil, glycerol monostearate and Tween 60 serve as lipid source. Low pH favours fungal growth and inhibits contaminating bacteria from test samples (4,5).

Type of specimen

Clinical samples, pure isolates

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (6,7).

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

Limitations

1. Some pathogenic fungi may produce infective spores which are easily dispersed in air.
2. Further biochemical and serological tests must be performed 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 expiry period when stored at the recommended temperature.

Quality Control

Appearance

Part A : Light yellow to greenish yellow homogeneous free flowing powder

Part B: Colourless to pale yellow viscous, hazy solution

Gelling

Firm, comparable with 1.50% Agar gel.

Colour and Clarity of prepared medium

Brownish yellow coloured, opalescent gel with scum forms in Petri plates

Reaction

Reaction of 4.5% w/v aqueous solution of Part A and 3.55% v/v of Part B at 25°C. pH : 6.2±0.2

pH

6.00-6.40

Cultural Response

Cultural characteristics observed after an incubation at 30-35°C for 48-72 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Malassezia furfur</i> ATCC 14521	50-100	good-luxuriant	≥50%
<i>Candida albicans</i> ATCC 10231(00054*)	50-100	good-luxuriant	≥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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

Reference

1. Shah A, Koticha A, Ubale M, Wanjare S, Mehta P, Khopkar U. Identification and speciation of *Malassezia* in patients clinically suspected of having pityriasis versicolor. Indian J Dermatol 2013;58:239.
2. Medical Mycology, Volume 43, Issue 8, December 2005, Pages 699–704,
3. www.atcc.org. ATCC Medium: 2737 Leeming & Notman agar Modified MLNA media
4. Lars F. Westblade, Eileen M. Burd, Shawn R. Lockhart, Gary W. Procop, Larone's Medically Important Fungi: A Guide to Identification, 7th Edition.
5. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Tenover FC, Tenover FC (editors) 2003, Manual of clinical Microbiology, 8th ed., ASM, Washington, D.C.
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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