

TM 750 – L. D. AGAR

INTENDED USE

For identification and cultivation of fastidious anaerobic bacteria.

PRODUCT SUMMARY AND EXPLANATION

Organisms that grow in the absence of oxygen are termed as anaerobes. Depending upon their ability to tolerate oxygen, they are classified as either facultative or obligate anaerobes. The anaerobic gram-negative bacteria are part of the normal flora of the upper respiratory tract, mouth, intestinal tract and urinogenital tract of human and animals. The bile-resistant *Bacteroides fragilis* group is the most commonly recovered anaerobe in clinical specimens and is more resistant to antimicrobial agents than any other anaerobe. *Fusobacterium necrophorum* is a very virulent anaerobe that may cause severe infections, usually in children or young adults.

L. D. Medium or Lombard-Dowell Medium was developed by Dowell and Lombard for the cultivation and identification of fastidious anaerobic bacteria. L. D. Agar is used to evaluate the degree of growth of anaerobes and also to assess indole and catalase production by *Bacteroides* and *Fusobacterium* species.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	5.000
Yeast extract	5.000
Sodium chloride	2.500
Sodium sulphite	0.100
L-Cystine	0.400
L-Tryptophan	0.200
Vitamin K1	0.010
Hemin	0.010
Agar	20.000

PRINCIPLE

This medium is essentially a casein digest agar, enriched with hemin, vitamin K1, L-cystine and yeast extract. This medium contains various nutritious substances, which can promote the growth of fastidious anaerobic bacteria. Tryptone and yeast extract provide the necessary nitrogenous nutrients while hemin and vitamin K1 supply additional growth factors. L-cystine and L-tryptophan serve as the amino acid sources. Sodium sulphite is an antioxidant. Sodium chloride maintains osmotic balance of the medium. Catalase-positive reaction may not be evident uptill 30 seconds to 1 minute after application of 3% hydrogen peroxide.

INSTRUCTION FOR USE

- Dissolve 33.22 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 45-50°C.
- Mix well and pour into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS















Appearance of Powder : Cream to yellow homogeneous free flowing powder.

Appearance of prepared medium : Medium amber coloured clear to slightly opalescent gel forms in Petri plates.

pH (at 25°C) $: 7.4 \pm 0.2$

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganis m	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Indole reaction	Catalase	Incubation Temperature	Incubati on Period
Bacteroides fragilis	25285	50-100	Good- luxuriant	>=50%	Negative reaction	Positive reaction	35-37 °C	40 - 48 Hours
Bacteroides corrodens	23834	50-100	Fair-good	20-40%	Negative reaction	Negative reaction	35-37 °C	40 - 48 Hours
Fusobacteriu m necrophorum	25286	50-100	Good- luxuriant	>=50%	Positive reaction	Negative reaction	35-37 °C	40 - 48 Hours
Fusobacteriu m nucleatum	25586	50-100	Fair-good	20-40%	Positive reaction	Negative reaction	35-37 °C	40 - 48 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

- 1. Dowell V. and Lombard G., June 1977, U.S., DHEW, Center for Disease Control (CDC), Atlanta. Ga.
- 2. Finegold S. M., Baron E. J., Bailey and Scotts Diagnostic Microbiology, 8th Ed., 1990, The C.V. Mosby Company.
- 3. Koneman E., Allen S., Dowell V. and Sommers H., 1979, Colour Atlas and Textbook of Diagnostic Microbiology, J. B. Lippincott Co., Philadelphia.
- 4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
- 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.







































NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices. *For Lab Use Only

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