TM 960 – L.D. ESCULIN AGAR

INTENDED USE
For identification of anaerobic bacteria like *Bacteroides* species on the basis of esculin hydrolysis.

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. Esculin Agar is used to determine esculin hydrolysis, hydrogen sulfide and catalase production of *Bacteroides* and *Fusobacterium* species.

COMPOSITION

Ingredients	Gms / Ltr
Casein enzymic hydrolysate	5.000
Yeast extract	5.000
Sodium chloride	2.500
L-Tryptophan	0.200
Vitamin K1	0.010
L-Cystine	0.400
Hemin	0.010
Esculin	1.000
Ferric citrate	0.500
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. Casein enzymic hydrolysate 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. Esculin is hydrolyzed by the organisms to form esculetin and dextrose. The esculetin reacts with the iron salt of ferric citrate to produce a dark brown to black complex. Also L-cystine is a sulphur-containing amino acid and hence H_2S production in combination with ferric citrate gives black colouration to the colonies. Vitamin K1 and hemin are the additional growth factors. Black colour of H_2S positive colonies is rapidly lost after exposure to air; hence plates should be observed in anaerobic glove box or immediately upon exposure to air. Catalase positive reaction may not be evident up till 30 seconds to 1 minute after application of 3% hydrogen peroxide.

INSTRUCTION FOR USE
- Dissolve 34.62 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.
- 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 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	H_2S Production	Catalase	Esculin hydrolysis	Incubation Temperature	Incubation Period
Bacteroides saccharolyticus	25260	50-100	Luxuriant	>=70%	Negative reaction	Negative reaction	Negative reaction	35-37 °C	24 - 48 Hours
Bacteroides fragilis	25285	50-100	Luxuriant	>=70%	Negative reaction	Positive reaction, effervescence seen on addition of 3% hydrogen peroxide	Positive reaction, brown black precipitate around the colonies	35-37 °C	24 - 48 Hours
Fusobacterium mortiferum	9817	50-100	Luxuriant	>=70%	Positive reaction, blackening of colonies	Negative reaction	Positive reaction, brown black precipitate around the colonies	35-37 °C	24 - 48 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

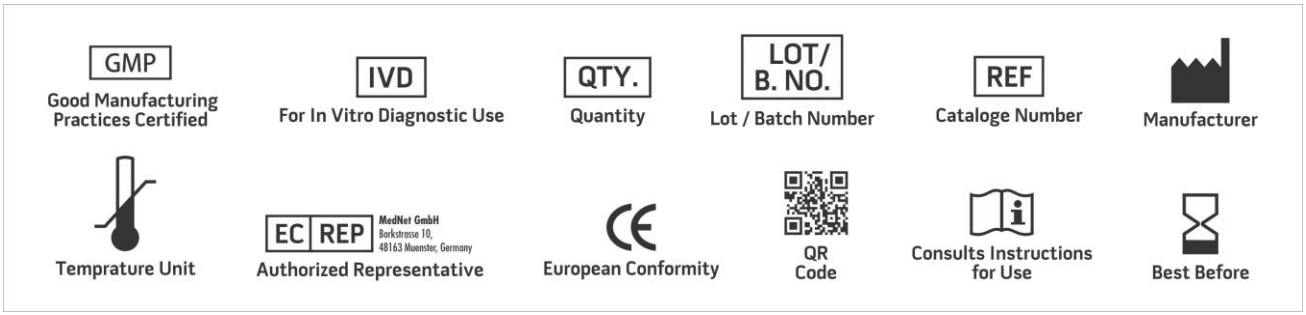
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided 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. 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.
4. Koneman E., Allen S., Dowell V. and Sommers H., 1979, Colour Atlas and Textbook of Diagnostic Microbiology, J. B. Lippincott Co., Philadelphia.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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