Technical Information

Lactose Lecithin Agar

Product Code: DM 2047

Application: - Lactose Lecithin Agar is recommended for isolation and differentiation of histotoxic clostridia from clinical specimens.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	12.650
Peptone	5.500
Meat hydrolysate #	3.300
Yeast extract	3.850
Corn starch	1.100
Sodium chloride	5.500
Lactose	10.000
Sodium azide	0.200
Neomycin sulphate	0.150
L-Cysteine hydrochloride	0.500
Calcium chloride	0.050
Egg lecithin	0.660
Bromocresol purple	0.025
Agar	15.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance
Equivalent to Pancreatic digest of heart muscles

Principle & Interpretation

Clostridium species are widely distributed in nature and are also associated with humans, either as non-pathogens at a variety of anatomic locations or at infected sites. Diseases caused by members of the genus *Clostridium* generally fall into one of the three categories:

a. non-invasive disease in which toxin(s) is responsible for all the symptoms

b. invasive (histotoxic) disease in which a progressive infections process and tissue destruction occur and

c. purulent disease in which a closed-space mixed infection involving multiple organisms is present (1).

Histotoxic clostridia can be isolated on egg yolk containing medium, as demonstrated by McClung and Toabe (2). This medium was further supplemented with additional milk and lactose to differentiate clostridia on the basis of lecithinase production, casein hydrolysis and lactose fermentation (3). Selectivity was obtained by the incorporation of neomycin sulphate. Subsequently, eggs were replaced by purified lecithin, to obtain an egg-free medium (4). This egg-free medium was further modified with reduced concentration of neomycin and additional sodium azide, which enhanced the selective properties of the medium (5). This refined medium was designated as Lactose Lecithin Agar, which is used for isolation and differentiation of histotoxic clostridia from clinical specimens.

Casein enzymic hydrolysate, pepone and meat hydrolysate provide carbonaceous and nitrogenous compounds essential for the growth of bacteria. Lactose is the fermentable carbohydrate with bromocresol purple being the pH indicator. L-cysteine helps to create anaerobic conditions. Yeast extracts supplies vitamin B-complex nutrients. Corn starch neutralizes toxic fatty acids if any, present in the medium. Neomycin and sodium azide inhibit accompanying gram-negative and gram-positive organisms.

Before inoculation, prepared media plates should be pre-reduced by placing under anaerobic conditions for 18-24 hours. Specimens should be inoculated on these pre-reduced plates. A non-selective media should be inoculated simultaneously (1, 6). An opalescent zone surrounding the colonies indicates lecithinase production. Yellow colour around colonies indicates lactose fermentation.

Methodology

Suspend 58.48 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat to boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Shake well before pour into sterile Petri plates.

Warning: *Sodium azide has a tendency to form explosive metal azides with plumbing materials. It is advisable to use enough water to flush off the disposables.*

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity
Light purple coloured slightly opalescent gel forms in Petri plates

Reaction
Reaction of 5.85% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH Range
6.60-7.00

Cultural Response
DM2047: Cultural characteristics observed under anaerobic condition, after an incubation at 35-37°C for 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery	Lactose Fermentation	Lecithinase production	Lipase activity
Clostridium difficile ATCC 17857	50-100	luxuriant	>=50%	negative reaction	negative	negative
Clostridium histolyticum ATCC 19401	50-100	luxuriant	>=50%	negative reaction	Negative	Negative , no irridescent sheen on the colony surface and medium
Clostridium perfringens ATCC 12924	50-100	luxuriant	>=50%	Positive reaction,yellow coloured zones surrounding colonies due to acid production	positive reaction, opaque zone around the colony	negative
Clostridium sordellii ATCC 9714	50-100	luxuriant	>=50%	negative reaction	positive reaction, opaque zone around the colony	negative
Clostridium sporogenes ATCC 11437	50-100	luxuriant	>=50%	negative reaction	negative	positive, irridescent sheen on the colony surface and medium

Clostridium tetani ATCC 10709	50-100	luxuriant	>=50%	negative reaction	negative	variable, usually negative

Storage and Shelf Life

Dried Media: Store dehydrated powder and prepared medium at 2-8°C in tightly closed container. Use before expiry date on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. McClung L. S. and Toabe R., 1947, J. Bacteriol., 53:139.
3. Willis A. T. and Hobbs G., 1959, J. Pathol. Bacteriol., 77:511.
4. Willis A. T., 1960, J. Pathol. Bacteriol., 80:379.
5. Ellner P. D. and O. Donnell D., 1971, Am. J. Clin. Pathol., 56:197.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria., Vol. 1, Williams and Wilkins, Baltimore.

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
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