Lactose Lecithin Agar

M1047

Intended Use:

Recommended for isolation and differentiation of histotoxic *Clostridia* from clinical specimens.

Composition**

Ingredients	g / L
Tryptone	12.650
Peptone	5.500
HM 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 anhydrous	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 parameters
Equivalent to Pancreatic digest of heart muscles

Directions

Suspend 58.48 grams in 1000 ml purified/distilled water. 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 . Mix well and pour into sterile Petri plates.

Principle And 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.

Tryptone, Peptone and HM 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.

Type of specimen

Clinical- stool, abscess

Specimen Collection and Handling

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. After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. 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. Ensure that the clinical samples are properly transported under anaerobic conditions.
2. Proper anaerobic conditions must be maintained for optimal recovery of organisms

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 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

6.60-7.00

Cultural Response

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 ac id production	positive reaction, opaque zone around the colony	negative
#*Paeniclostridium 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

Key : (#)- Formerly known as *Clostridium sordellii*

Storage and Shelf Life

Store dehydrated and the prepared medium at 2-8°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 (7,8).

Reference

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.
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3. Willis A. T. and Hobbs G., 1959, J. Pathol. Bacteriol., 77:511.
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8. 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.

Revision : 05/2024

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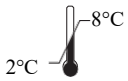
IVD *In vitro* diagnostic medical device



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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www.cepartner4u.eu

CE CE Marking

Do not use if package is damaged

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