TM 1215 – LACTOSE LECITHIN AGAR

INTENDED USE

For isolation and differentiation of histotoxic *Clostridia* from clinical specimens.

PRODUCT SUMMARY AND EXPLANATION

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

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

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

COMPOSITION

Ingredients	Gms / Ltr
Casein enzymic hydrolysate	12.650
Peptone	5.500
Pancreatic digest of heart muscles	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

PRINCIPLE

This medium contains Casein enzymic hydrolysate, peptone and Pancreatic digest of heart muscles which 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.

INSTRUCTION FOR USE

- Dissolve 58.48 grams in 1000 ml 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.
  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 SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Light purple coloured slightly opalescent gel forms in Petri plates.
pH (at 25°C) : 6.8 ± 0.2

INTERPRETATION

Cultural characteristics observed under anaerobic condition after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Lactose Fermentation	Lecithinase production	Lipase activity	Incubation Temperature	Incubation Period
Clostridium difficile	17857	>=10^4	Luxuriant	>=70%	Negative reaction	Negative	Negative	35-37°C	48 Hours
Clostridium histolyticum	19401	50-100	Luxuriant	>=70%	Negative reaction	Negative	Negative , no irridescent sheen on the colony surface and medium	35-37°C	48 Hours
Clostridium perfringens	12924	50-100	Luxuriant	>=70%	Positive reaction,yellow coloured zones surrounding colonies due to acid production	Positive reaction, opaque zone around the colony	Negative	35-37°C	48 Hours
Clostridium sordellii	9714	50-100	Luxuriant	>=70%	Negative reaction	Positive reaction, opaque zone around the colony	Negative	35-37°C	48 Hours
Clostridium sporogenes	11437	50-100	Luxuriant	>=70%	Negative reaction	Negative	Positive, irridescent sheen on the colony	35-37°C	48 Hours

							surface and medium		
Clostridium tetani	10709	50-100	Luxuriant	>=70%	Negative reaction	Negative	variable, usually negative	35-37°C	48 Hours

PACKAGING:
In pack size of 500 gm bottles.

STORAGE
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 2-8°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. 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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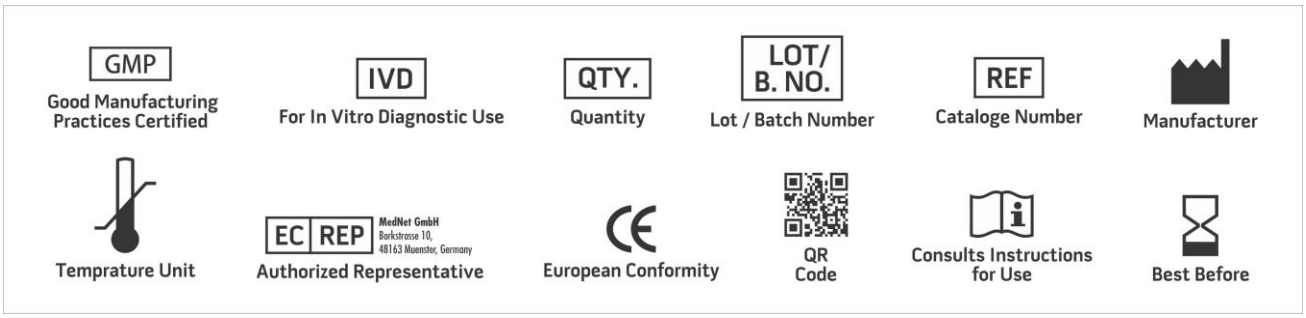
5. Ellner P. D. and O. Donnell D., 1971, Am. J. Clin. Pathol., 56:197.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria., Vol. 1, Williams and Wilkins, Baltimore.



NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.
***For Lab Use Only**
Revision: 08 Nov., 2019