

TM 1962 – ANAEROBIC EGG AGAR BASE

INTENDED USE

Anaerobic Egg Agar Base supplemented with egg yolk emulsion is recommended for detection of *Clostridium perfringens* in foods.

PRODUCT SUMMARY AND EXPLANATION

Clostridium species ranked behind *Salmonella* and *Staphylococcus aureus*, has been the third most common etiological agent of food-borne diseases. *Clostridium botulinum* is an anaerobic, rod-shaped spore forming bacterium that produces a botulinum toxin with characteristic neurotoxicity. *Clostridium* food poisoning results from consumption of contaminated food. Anaerobic Egg Agar Base supplemented with egg yolk emulsion is recommended for detection of *Clostridium botulinum* in foods and also to detect the lipase activity of *Yersinia* sp. in accordance with FDA BAM, 1998. The major virulence factor of *C. botulinum* is botulinum toxin, which is secreted upon invasion of the host gut, which contributes to food poisoning, gastrointestinal illnesses and even death.

Yersinia sp. degrades lecithin of egg yolk, forming an insoluble opaque precipitate. Lipase breaks down free fats present in the egg yolk causing iridescent sheen to form on the colony surface. Proteolysis is indicated by clear zones in the medium surrounding the growth.

Representative samples of food (1-2 g solid or 1-2ml liquid) are enriched in Cooked Meat Media up to 5days at 35°C under anaerobic conditions. Vegetative cells are destroyed either by alcohol treatment or by heat treatment wherein an aliquot of 1-2ml is heated at 80°C for 10-15min. These treated cultures are streaked to either Liver-Veal-Agar Base, Modified or Anaerobic Egg Agar Base. Typical *C.botulinum* colonies may be raised or has smooth or rough surfaces. They show spreading and have an irregular edge. On egg yolk medium, *C.botulinum* colonies usually exhibit surface iridescence when examined by oblique light. This luster zone often referred to as a pearl layer, usually extends beyond and follows the irregular contour of the colony. Colonies of *C. botulinum* types C, D, and E are ordinarily surrounded by a wide zone (2-4 mm) of yellow precipitate. Colonies of types A and B generally show a smaller zone of precipitation. Further biochemical tests need to be performed to differentiate it from other species of *Clostridium*.

COMPOSITION

Ingredients	Gms / Ltr
Yeast Extract	5.000
Tryptone	5.000
Proteose peptone	20.000
Sodium chloride	5.000
Agar	20.000

PRINCIPLE

Casein enzymic hydrolysate and proteose peptone supply amino acids and other complex nitrogenous nutrients. Yeast extract provides essential B-complex vitamins. Egg yolk emulsion is added to the medium by which the lipase and lecithinase activity can be observed. Agar acts as the solidification agent. Sodium chloride maintains the osmotic equilibrium of the cells.

INSTRUCTION FOR USE

- Dissolve 55 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 and aseptically add 80 ml sterile Egg Yolk Emulsion.



- Mix thoroughly before pouring into sterile Petri plates.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium	: Basal medium -Light yellow coloured, clear to very slightly opalescent gel. After addition of Egg Yolk Emulsion -Light yellow coloured, opaque gel forms in Petri plates.
pH (at 25°C)	: 7.0±0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Lecithinase	Lipase	Incubation Temperature	Incubation Period
<i>Clostridium perfringens</i>	12924	50-100	Good-luxuriant	>=50%	Positive reaction, opaque zone around the colony	Negative reaction	35-37°C	18-24 Hours
<i>Clostridium sporogenes</i>	11437	50-100	Good-luxuriant	>=50%	Negative reaction	Positive reaction, iridescent sheen on the colony	35-37°C	18-24 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 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

- Centre for Disease Control, 1982, CDC Surveillance Summaries, 35:7SS-16SS, 1986.
- Czczulin, J. R., Hanna, P. C. and McClane, B. 1993. Infect. Immun., 61: 3429-3439.
- FDA, U.S. 1998. Bacteriological Analytical Manual. 8 ed. Gaithersburg, MD: AOAC International.
- Finegold. and Baron. 1986. Bailey and Scott's Diagnostic Microbiology. 7 ed. St. Louis: The C.V. Mosby Company.
- Murray, P. R., Baron, E. J., Jorgensen, J. H., Tenover, M. C. and Tenover, R. H. 2003. Manual of Clinical Microbiology. 8 ed. Washington, D.C: ASM.



 GMP Good Manufacturing Practices Certified	 IVD For In Vitro Diagnostic Use	 QTY. Quantity	 LOT/ B. NO. Lot / Batch Number	 REF Catalogue Number	 Manufacturer
 Temperature Unit	 EC REP Authorized Representative <small>MedNet GmbH Buckstrasse 10 48163 Muenster, Germany</small>	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

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

***For Lab Use Only**
Revision: 08 Nov., 2019