TM 1154 – C. BOTULINUM ISOLATION AGAR BASE

INTENDED USE

For isolation of *Clostridium botulinum* from food and clinical samples.

PRODUCT SUMMARY AND EXPLANATION

Clostridium botulinum is an anaerobic, spore forming bacteria that produces a neurotoxin protein botulin. Severe food poisoning results from the consumption of this protein (toxin), which may be produced in foods contaminated with *Clostridium botulinum*. C. botulinum Isolation Agar Base is formulated as per the recommendation of APHA for the selective isolation of *C. botulinum* from food samples.

The antibiotic supplement containing the broad spectrum antibiotics namely cycloserine, sulphamethoxazole and trimethoprim makes the medium very selective. Egg yolk emulsion helps in detecting lecithinase, lipase and proteolytic activity. Lecithinase degrades lecithin present in the egg yolk producing an insoluble, opaque precipitate in the medium surrounding the growth. Lipase break down free fats present in the egg yolk causing an iridescent (oil on water) sheen to form on the surface of the colonies.

Botulinal toxin is heat-labile. Therefore, the test samples and cultures should be maintained at refrigeration temperatures. The pH of the toxic material should also be maintained at a slightly acidic pH since botulinal toxin is less stable at alkaline pH. Inoculate 1-2 grams of solid or 1-2 ml of liquid food per 15 ml of enrichment broth. The enrichment broth employed is Cooked Meat Medium. After an incubation at 35°C for 7 days, observe for turbidity, gas production and meat digestion. Carry out gram staining and spore staining. To isolate *C.botulinum* mix enrichment broth with equal amount of sterile ethanol (alcohol treatment). The alcohol treated culture is further streaked on *C.botulinum* Isolation Agar Base. Alternatively untreated enrichment cultures or stool can be streaked directly on *C.botulinum* Isolation Agar Base.

COMPOSITION

Ingredients	Gms / Ltr
Tryptone	40.000
Yeast extract	5.000
Dextrose (Glucose)	2.000
Disodium hydrogen phosphate	5.000
Sodium chloride	2.000
Magnesium sulphate	0.010
Agar	20.000

PRINCIPLE

Tryptone and yeast extract supply amino acids and other nitrogenous substances and vitamin B complex. Dextrose is the fermentable carbohydrate. Disodium phosphate helps in buffering the medium while magnesium sulphate helps for the sporulation of the organisms. Sodium chloride maintains the osmotic equilibrium of the medium.

INSTRUCTION FOR USE

- Dissolve 37.0 grams in 450 ml purified / 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 sterile 50 ml Egg Yolk Emulsion and reconstituted contents of 1 vial of CBI Supplement.

- 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 : Basal medium: Yellow coloured clear to slightly opalescent gel. After addition of egg yolk emlusion : Light yellow coloured, opaque 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	Lecithinase	Incubation Temperature	Incubation Period
Clostridium botulinum	25763	50-100	Good-luxuriant	>=50%	Positive reaction, opaque zone around the colony	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 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.

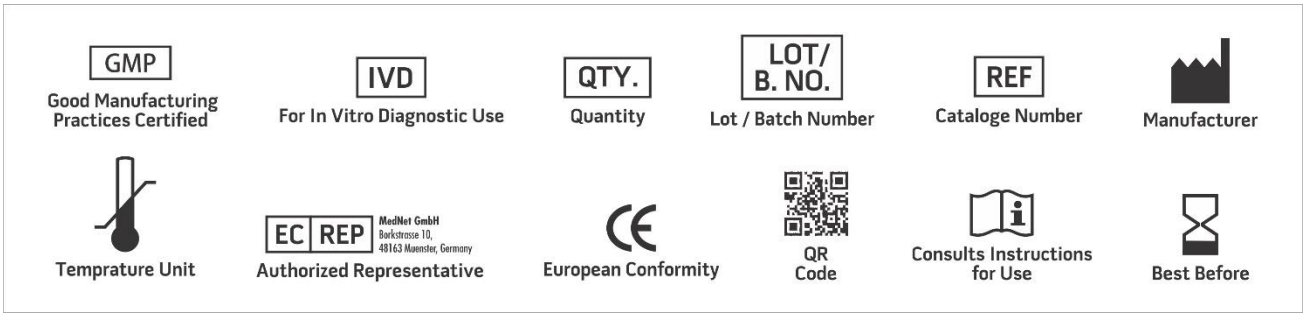
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

1. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Company, St. Louis.
2. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, American Public Health Association, Washington, D.C.



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

***For Lab Use Only**

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