C. botulinum Isolation Agar Base M911

C. botulinum Isolation Agar is recommended for selective isolation of *Clostridium botulinum* from food samples.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	40.000
Yeast extract	5.000
Dextrose	2.000
Disodium phosphate	5.000
Sodium chloride	2.000
Magnesium sulphate	0.010
Agar	20.000
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37 grams in 450 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50-55°C and aseptically add sterile 50 ml Egg Yolk Emulsion (FD045) and reconstituted contents of 1 vial of CBI Supplement (FD049). Mix well and pour into sterile Petri plates.

Principle And Interpretation

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 (1) for the selective isolation of C. botulinum from food samples.

The antibiotic supplement (FD049) 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 (2). 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. Casein enzymic hydrolysate 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.

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 (M149). 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 (M911)(1). Alternatively untreated enrichment cultures or stool can be streaked directly on C.botulinum Isolation Agar Base (1).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity 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

Reaction

Reaction of medium (7.4 gm in 90 ml distilled water) at 25°C. pH : 7.4±0.2

Cultural Response

M911: Cultural characteristics observed under anaerobic condition, with added Egg Yolk Emulsion(FD045) and CBI Supplement(FD049), after an incubation at 35-37°C for 48 hours.

Organism	**Inoculum (CFU)**	**Growth**	**Recovery**	**Lecithinase**		
Clostridium botulinum ATCC 25763	50-100	good-luxuriant	>=50%	positive reaction,opaque zone around the colony		

Reference

1. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of foods, 3rd Ed., APHA, Washington, D.C.
2. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Company, St. Louis.

Storage and Shelf Life

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.