C. botulinum Isolation HiVeg™ Agar Base

MV911

Intended use

Recommended for selective isolation of *Clostridium botulinum* from food samples.

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	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
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 37.0 grams in 450 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 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. C. botulinum Isolation HiVeg™ Agar Base is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. HiVeg™ 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).

Type of specimen

Food samples.

Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,3,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to nutritional variations, some strains may show poor growth.

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 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

pH

7.20-7.60

Cultural Response

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

Storage and Shelf Life

Store between 10-30°C in a tightly closed container 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. 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.

2. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Company, St. Louis.

3. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.

4. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

5. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
6. 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.

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