

Tryptone Peptone Glucose Yeast Extract HiVeg™ Broth Base w/o MV969 Trypsin

Intended Use:

Recommended to test toxicity type of *Clostridium botulinum* cultures from various samples.

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	50.000
HiVeg™ peptone	5.000
Yeast extract	20.000
Dextrose (Glucose)	4.000
Sodium thioglycollate	1.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.0 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Refrigerate the sterile medium until use. Before inoculation add 1.5% filter sterilized trypsin solution to a final concentration of 0.1% if desired.

Principle And Interpretation

Clostridium botulinum is a species of anaerobic, spore-forming, rod-shaped bacteria that produces a protein with a characteristic neurotoxicity. *C. botulinum* cultures fall into three distinct groups by properties other than the toxin type they produce, with each group composed only of strains having similar cultural and physiological characteristics. Proteolysis i.e. ability to digest coagulated egg white or meat, is one of the differentiating characteristic.

Tryptone Peptone Glucose Yeast Extract (TPGY) Broth is formulated as per recommendation of APHA (1), for the determination of toxicity of *Clostridium botulinum* cultures in food. Tryptone Peptone Glucose Yeast Extract HiVeg™ Broth Base w/o Trypsin is prepared by completely replacing animal based peptone with vegetable peptones to avoid BSE/TSE risks associate with animal peptones. HiVeg™ hydrolysate, HiVeg™ peptone and yeast extract provide nitrogenous, carbonaceous substances, vitamin B complex and other essential growth nutrients. Dextrose serves as fermentable carbohydrate and sodium thioglycollate serves as a reducing agent. Trypsin activates toxins of the non-proteolytic types.

Type of specimen

Isolated microorganism from food samples

Specimen Collection and Handling:

Presumptive *C. botulinum* cultures are inoculated into Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin, for the non-proteolytic types and Cooked M Medium (M149) for the proteolytic types. Incubate inoculated tubes for 7 days and then test for toxin (1) If there is no growth after 7 days of incubation, incubate for an additional 10 days to permit possible delayed germination of spores of *C. botulinum* before discarding. Toxins of non-proteolytic types do not manifest maximum potential toxicity until they are activated. Therefore food supernatant, liquid food, TPGY Broth or cooked meat cultures are treated with trypsin for activation. Toxins of proteolytic types do not need such activation. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Well isolated colonies must be used.

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

Colour and Clarity of prepared medium

Yellow coloured clear solution without significant precipitate.

Reaction

Reaction of 8.0% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed under anaerobic condition, after an incubation at 26-28°C for upto 7 days.

Organism	Inoculum (CFU)	Growth
<i>Clostridium botulinum</i> ATCC 25763	50-100	luxuriant

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 15-25°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 (2,3).

Reference

1. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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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