



Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin

M969

Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin is used to test toxicity type of *Clostridium botulinum* cultures.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	50.000
Peptic digest of animal tissue	5.000
Yeast extract	20.000
Dextrose	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 grams in 1000 ml 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.

Casein enzymic hydrolysate, peptic digest of animal tissue 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. Presumptive *C. botulinum* cultures are inoculated into Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin, for the non-proteolytic types and Cooked Meat 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.

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

M969: 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 below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

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.

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