TRYPTONE PEPTONE GLUCOSE YEAST EXTRACT BROTH BASE W/O TRYPSIN
TM 1467

For used to test toxicity type of *Clostridium botulinum* cultures

Composition

Ingredients	Gms/Ltr.
Casein enzymatic hydrolysate	50.00
Yeast extract	20.00
Peptic digest of animal tissue	5.00
Dextrose	4.00
Sodium thioglycollate	1.00

* Dehydrated powder, hygroscopic in nature, store in a cool - dry place in tightly- sealed containers below 25°C and protect from direct sunlight.

Instructions for use

Dissolve 80.00gms in 1000ml distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15 psi (at 121°C) for 15 minutes. Cool to 45-50ºC and before inoculation add 1.5% filter sterilized trypsin solution (3231) to a final concentration of 0.1% as desired.

Appearance: Yellow colour, clear solution
pH (at 25°C): 7.0 ± 0.2

Principle

TRYPTONE PEPTONE GLUCOSE YEAST EXTRACT BROTH BASE W/O TRYPSIN is used for used to test toxicity type of *Clostridium botulinum* cultures. *Clostridium botulinum* is a Gram-positive, rod-shaped bacterium that produces several toxins. The best known are its neurotoxins, subdivided in types A-G, that cause the flaccid muscular paralysis seen in botulism. Botulism poisoning can occur due to improperly preserved or home-canned, low-acid food that was not processed using correct preservation times and/or pressure. *Clostridium botulinum* is a soil bacterium. The spores can survive in most environments and are very hard to kill. They can survive the temperature of boiling water at sea level, thus many foods are canned with a pressurized boil that achieves an even higher temperature, sufficient to kill the spores. The medium contains Casein enzymatic hydrolysate and Peptic digest of animal tissue provides the nitrogen and carbon source to the medium. Growth of the bacterium can be prevented by high acidity, high ratio of dissolved sugar, high levels of oxygen, and very low levels of moisture or storage at temperatures below 3°C (38°F) for type. Dextrose serves as fermentable carbohydrate. 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 (TM 702) for the proteolytic types. Incubate inoculated tubes for 7 days and then test for toxin. 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. Optimum temperature for growth and toxin production of proteolytic strains is close to 35°C; for nonproteolytic strains it is 26-28°C. Nonproteolytic types B, E, and F can produce toxin at refrigeration temperatures (3-

4°C). Toxins of the nonproteolytics do not manifest maximum potential toxicity until they are activated with trypsin; toxins of the proteolytics generally occur in fully (or close to fully) activated form. These and other differences can be important in epidemiological and laboratory considerations of botulism outbreaks.

Interpretation

Cultural characteristics observed after inoculating (10^3 CFU / ml), on incubation at 35°C for proteolytic strains for nonproteolytic strains it is 26-28°C.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth
Clostridium botulinum	25763	10^3	Good
Escherichia coli	25922	10^3	Inhibited

References

1. Vanderzant C. and Splittstoesser D. F., (Eds.), Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C. (1992).
2. Binz, T., H. Kuranzono, M. Wille, J. Frevert, K. Wernars, and H. Niemann. *J. Biol. Chem.* **265,** 9153-9158. (1990).
3. Craven, K. E., J.L. Ferreira, M.A. Harrison, and P. Edmonds. *JOAC* **85** (5):1025-1028. (2002).
4. East, A.K., P.T. Richardson, D. Allaway, M. D. Collins, T. A. Roberts, and D.E. Thompson. *FEMS Microbiol. Lett.* **75**:225-230. (1992).
5. Ferreira, J.L., M.K. Hamdy, S.G. McCay, and B.R. Baumstark. *J. Rapid Methods and Automation in Microbio.* **1**:29-39. (1992).