Willis and Hobbs Medium Base

M1375

Willis and Hobbs Medium Base is used for isolation and identification of *Clostridium* from food.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Meat extract	10.000
Sodium chloride	5.000
Lactose	12.000
Neutral red	0.032
Agar	10.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.51 grams in 420 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 15 ml Egg Yolk Emulsion (FD045), 60 ml sterile skimmed milk* and rehydrated contents of one vial of Willis and Hobbs Supplement (FD156). Mix well and pour into sterile Petri plates.

* 10% solution is prepared from skim milk powder and sterilized separately by autoclaving at 15 lbs pressure (121°C) for 5 minutes.

Principle And Interpretation

Besides its ability to produce gastrointestinal tract-active toxins, *Clostridium* species possesses several other characteristics that significantly contribute to its ability to cause foodborne diseases. The heat resistance of its spores often allows *Clostridium* species to survive incomplete cooking of food, with the surviving bacteria then able to cause food poisoning (1). This makes detection and isolation of these organisms from food important. Willis and Hobbs Medium Base (2) formulated by Willis and Hobbs can be used for the identification of *Clostridium perfringens* on the basis of lecithinase reaction in egg yolk and lactose fermentation. This medium is prepared in accordance with Indian Standard (3) under the specifications IS: 5887 (Part-IV) 1976.

Peptic digest of animal tissue and meat extract in the medium provide nitrogenous source and other growth factors. Sodium chloride maintains the osmotic balance of the medium. Lactose is the energy and the carbon source. Species of *Clostridium* like *C.perfringens* and *C.botulinum* produce an opalescent zone around the colony in egg yolk containing media. The production of a precipitate in the medium and a layer having a “pearly” (iridescent) appearance adjacent to and covering the colonies (lipase activity) of the different types of *C.botulinum* on agar medium containing egg yolk has been used as an aid in the differentiation and isolation of this group of bacteria (2, 4). Zones of clearing develop around proteolytic colonies.

Quality Control

Appearance
Pale yellow to pink homogeneous free flowing powder

Gelling
Firm, comparable with 1.0% Agar gel.

Colour and Clarity of prepared medium
Basal medium : Red coloured clear to slightly opalescent gel. After addition of sterile Egg Yolk Emulsion and sterile Skim milk solution, pinkish red coloured opaque gel forms in Petri plates.

Reaction
Reaction of 4.7% w/v aqueous solution of base at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
M1375: Cultural characteristics observed when incubated anaerobically after an incubation at 35-37°C for 18-48 hours with added Egg Yolk Emulsion (FD045), sterile skimmed milk solution and Willis and Hobb's supplement (FD156).

Organism	Inoculum (CFU)	Growth	Recovery	Lecithinase
Cultural Response				
Clostridium botulinum ATCC 25763	50-100	luxuriant	>=50%	positive reaction, opaque zone around the colony
Clostridium perfringens ATCC 12919	50-100	luxuriant	>=50%	positive reaction, opaque zone around the colony

Storage and Shelf Life
Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry date on the label.

Reference
1.Doyle, Michael, Beuchat, Larry and Montville Thomas, Food Microbiology, Fundamentals and Frontiers, ASM Press, Washington D.C.
2.Willis A. T., Hobbs G., 1959, Journal of Pathology and Bacteriology, Vol. 77, 511-521.
3.Bureau of Indian Standards (BIS), 1976, IS: 5887 (Part IV).
4.McClung L. S. and Toabe R., 1947, J. Bacteriol., 53:139.

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