Willis and Hobbs Medium Base

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

Summary
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. This makes detection and isolation of these organisms from food important. Willis and Hobbs Medium Base 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 under the specifications IS: 5887 (Part-IV) 1976.

Principle
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.

Formula*
Ingredients	**g/L**
Peptic Digest of Animal Tissue	10.0
Meat Extract	10.0
Sodium Chloride	5.0
Lactose	12.0
Neutral Red	0.032
Agar	10.0
Final pH (at 25°C)	7.0 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 23.51 g of the powder in 420 mL purified / distilled water.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
4. Cool to 50°C-55°C and aseptically add 15 mL Egg Yolk Emulsion (204050370100), 60 mL sterile skimmed milk* and rehydrated contents of one vial of Willis and Hobbs Supplement (204230080005).
5. Mix well and pour into sterile petridishes.

*10% solution is prepared from skim milk powder and sterilized separately by autoclaving at 121°C (15 psi) for 5 minutes as per validated cycle.

Quality Control

Dehydrated Appearance: Pale yellow to pink coloured, homogenous, free flowing powder.
Prepared Appearance: Basal medium: Red coloured, clear to slightly opalescent gel forms in petridishes.
After addition of sterile Egg Yolk Emulsion and sterile Skim milk solution, pinkish red coloured opaque gel forms in petridishes.
Cultural Response: Cultural characteristics observed when incubated anaerobically after an incubation at 35°C-37°C for 18-48 hours with added Egg Yolk Emulsion and sterile Skim milk solution and Willis and Hobb's supplement.

Organism (ATCC)	Growth	Lecithinase
Clostridium botulinum (25763)	Good	+
Clostridium perfringens (12919)	Good	+

Key:
+ = Positive reaction, opaque zone around the colony.

Interpretation of Results

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. Zones of clearing develop around proteolytic colonies.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

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.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
201230030500	Dehydrated Culture Media	500 g