

TECHNICAL DATA SHEET

Willis And Hobb's Medium Base

Principle

Willis and Hobb's Medium Base is recommended for the isolation and identification of *Clostridium* from foods. *Clostridium* is a Gram-positive, obligate anaerobe capable of producing endospores. It has the ability to produce gastrointestinal tract-active toxins that are capable of causing foodborne diseases.

The media is composed of peptic digests of animal tissue and meat extract, which provide nitrogen and carbon sources along with other growth factors. Sodium chloride helps to maintain the osmotic balance of the medium. Lactose serves as an energy and carbon source. Neutral red is an indicator dye. Agar is used as a solidifying agent. With the help of sterile egg yolk emulsion and skim milk, the *Clostridium* species produces an opaque zone around the colony that has positive lecithinase activity. This medium is prepared in accordance with Indian Standard under the specifications IS: 5887 (Part-IV) 1976.

Use: Isolation & identification of *Clostridium* from foods.

Contents*

Ingredients	Gram/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
pH at 25°C	7.0±0.2

*** Formula adjusted for optimum performance and parameters**

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Directions: Dissolve 47.03 grams in 840 ml distilled water. Boil to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121 °C) for 15 min, cool it to 42-45 °C and aseptically add 30 ml egg yolk emulsion, 120 ml sterile skim milked* and 2 vial of Willis and Hobbs supplement (Neomycin sulphate 33 mg). Distribute aseptically in petri plates. Ensure the complete solidification and inoculate test sample aseptically.

* 10% skim milked solution sterilize by autoclaving at 15 lbs pressure (121 °C) for 5 min.

Specimens types analyzed Food samples.

Precautions to be taken

These microbial media are intended for the in-vitro use only. All the handling, experiments, storage, and discarding should be performed with the help of skilled and knowledgeable technicians and as per the established guidelines. The material should be disposed only after proper sterilization by autoclaving. Please go through the MSDS of the media to avoid any accidents or in emergency.

Performance and Evaluation

The expected performance of the medium is liable to use as per the direction on the label when stored at optimum conditions and within expiry date.

Quality Control

Appearance	Beige to pinkish colored, free flowing, homogeneous powder
Reaction of 4.7 % solution	7.00 ±0.2 at 25 °C
pH	6.80-7.20
Gelling	Firm comparable with 1.0% agar gel
Color and clarity of ready medium	Basal medium: Red colored, clear to slightly opalescent gel. After addition of sterile Egg Yolk Emulsion and sterile Skim milk solution: Pinkish red colored, opaque gel
Growth Promotion properties	Best at ≤ 100 CFU at 32-37 °C for 18-72 h
Indicative properties	Optimum at ≤ 100 CFU at 32-37 °C for 18-48 h
Negative control	Performed using sterile distilled water

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Different Microbial Response: Prepare media as per the label directions. Inoculate (Inoculum 50-100 CFU) and incubate at $35 \pm 2^{\circ}\text{C}$ for 18-48 hours.

Organism	ATCC	Growth	Recovery	Lecithinase activity
<i>Clostridium perfringens</i>	3624	Luxuriant	$\geq 60\%$	Positive, opaque zone around the colony

Storage and Shelf Life: The product is highly hygroscopic; keep the container tightly closed at all times and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Note: Sterilize media immediately after reconstitution.

Disposal: To avoid the contamination or propagation of any hazardous microbes the used, unusable or modified preparation of this product must be disposed after autoclaving after completion of task.

Reference

1. Atlas, R. M. (2005). Handbook of media for environmental microbiology. CRC press.
2. Willis A. T., Hobbs G., 1959, Journal of Pathology and Bacteriology, Vol. 77, 511-521.
3. Difco Manual (1998). 11th Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
4. Bureau of Indian Standards (BIS), 1976, IS: 5887 (Part IV).

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