

## **TECHNICAL DATA SHEET**

### **Hugh Leifson Glucose Medium**

#### **Principle**

Hugh Leifson Medium was formulated by Hugh and Leifson (1953), media is taxonomically significant for fermentative and oxidative metabolism of carbohydrates in gram-negative bacteria. The media is composed of peptone, yeast extract, sodium chloride, glucose, bromocresol purple and agar. Peptone and yeast extract provides nitrogen, carbon, and amino acids required for organism growth. Glucose, serves as carbon source. Sodium chloride is used in high concentration which makes media very selective. The bromocresol purple is pH indicators. Less concentration of agar enables the determination of motility and aids in distribution of acid throughout the tube produced at the surface of medium. The high concentration of carbohydrate and low concentration of peptone avoid the possibility of an aerobic organism utilizing peptone and producing an alkaline condition help to neutralize slight acidity produced by an oxidative organism. Oxidative organisms produce acid in aerobic condition with little or no growth and no acid formation. Whereas, fermentative organisms produce acid in both aerobic and anaerobic conditions. The aerobic and anaerobic conditions are produced by sealing and unsealing the tubes. The bacterium capable of fermenting glucose produces yellow- or orange coloration. While the non-fermenters will produce blue-green color.

**Use:** For differentiation of Staphylococci from Micrococci by anaerobic fermentation of glucose.

#### **Contents\***

| <b>Ingredients</b>        | <b>Gram/Litre</b> |
|---------------------------|-------------------|
| <b>Peptone</b>            | <b>2.000</b>      |
| <b>Yeast extract</b>      | <b>0.500</b>      |
| <b>Sodium Chloride</b>    | <b>30.000</b>     |
| <b>Glucose</b>            | <b>10.000</b>     |
| <b>Bromocresol purple</b> | <b>0.015</b>      |
| <b>Agar pH</b>            | <b>3.000</b>      |
| <b>at 25°C</b>            | <b>7.4±0.2</b>    |

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

**Directions:** Dissolve 45.50 grams in 1000 ml distilled water. Boil to dissolve the medium completely and distribute in test tubes. Sterilize by autoclaving at 15 lbs pressure (121 °C) for 20 min, cool it to 42-45 °C and place it in upright position. Ensure solidification and inoculate test sample aseptically.

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## Specimens' types analyzed

Pharmaceutical samples, clinical and non-clinical samples etc.

## 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                        | Light yellow to bluish green colored free flowing, homogeneous powder |
| Reaction of 4.55 % solution       | 7.4 $\pm$ 0.2 at 25 °C                                                |
| pH                                | 7.20- 7.60                                                            |
| Gelling                           | Firm comparable with 0.3 % agar gel                                   |
| Color and clarity of ready medium | Purple colored clear to slightly opalescent gel                       |
| Growth Promotion properties       | Best at $\leq$ 100 CFU at 32-37 °C for 18-72 h                        |
| Indicative properties             | Optimum at $\leq$ 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 and incubate at 35±2°C for 1824 hours. Inoculum 50-100 CFU.**

| Organism                     | ATCC  | Aerobic fermentation | Anaerobic fermentation |
|------------------------------|-------|----------------------|------------------------|
| <i>Staphylococcus aureus</i> | 25923 | Yellow color         | Yellow color           |
| <i>Micrococcus luteus</i>    | 10204 | Yellow color         | Pink purple            |

**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. Difco Manual (1998). 11<sup>th</sup> Edition. Difco Laboratories., Division of Becton Dickinson and Company, Sparks, Maryland, USA.
3. Hugh R. and Leifson E. (1953). The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. *Journal of bacteriology*, 66(1), 24– 26.

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