

## OF Basal Medium (Hugh And Leifson)

Cat. 1500

For the identification of non-fermenting bacilli of medical and sanitary importance

### Practical information

| Applications    | Categories                              |
|-----------------|---|
| Differentiation | Non fermentative gram negative bacteria |

Industry: Clinical / Antimicrobial susceptibility testing



### Principles and uses

OF Basal Medium (Hugh And Leifson) is a semisolid medium, prepared according to Hugh and Leifson's formula, and is used to determine the metabolism (oxidation, fermentation) of Gram-negative bacteria. It is useful for *Pseudomonas*, *Salmonella*, *Shigella* and *Alcaligenes*.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Bromothymol blue is the pH indicator. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Dipotassium phosphate acts as a buffer system. Bacteriological agar is the solidifying agent.

### Formula in g/L

|                 |      |                       |     |
|-----------------|------|-----------------------|-----|
| Bromthymol blue | 0,03 | Bacteriological agar  | 2,5 |
| Casein peptone  | 2    | Dipotassium phosphate | 0,3 |
| Sodium chloride | 5    |                       |     |

### Preparation

Suspend 9,8 grams of the medium in one litre of distilled water. Heat with frequent agitation until dissolved. Sterilize in autoclave at 121 °C for 15 minutes. Add 10 ml of 10% glucose (or any suitable sugar) solution, sterilized by filtration, to 100 ml of liquid medium. Mix and aseptically dispense 5 ml per tube. If preferred, add 1,0 grams of carbohydrate directly to 100 ml. of medium and sterilize in the autoclave at 118 °C for 10 minutes to avoid the degradation of the sugar.

### Instructions for use

- Inoculate 2 fresh tubes by stabbing with a fresh culture of the organism in study. If the medium has been prepared and stored, remelt in a water bath to expel the dissolved gases.
- After inoculation, add a layer of 4 to 5 mm of paraffin oil to one of the tubes. It is not recommended to use mineral oil.
- Incubate both tubes at 35°C for 48 hours or more, up to 7 days with the caps loose. -To facilitate the identification of Gram-negative non-fermenting bacilli, also use Indole Nitrate Medium (Cat. 1504).

Results:

- 1) Fermentation: Yellow color in both tubes with or without gas.
- 2) Oxidation: Yellow color only in tube without oil.
- 3) No oxidation/fermentation: No change in the color of the tubes. The carbohydrates have not been fermented or oxidized. Inert microorganisms, e.g. *Alcaligenes faecalis*.

### Quality control

| Solubility | Appearance  | Color of the dehydrated medium | Color of the prepared medium | Final pH (25°C) |
|------------|-------------|--------------------------------|------------------------------|-----------------|
| w/o rests  | Fine powder | Beige with a greenish tint     | Green-bluish                 | 7,1 ± 0,2       |

## Microbiological test

Incubation conditions: (35±2 °C / 48-72 h)

K = Alkaline, green (without change)

A = Acid, yellow

G = Gas, sometimes perceptible

| Microorganisms                           | Specification | Characteristic reaction with sacrose | Characteristic reaction w/o sugar | Characteristic reaction with glucose | Characteristic reaction with lactose |
|--|---------------|--------------------------------------|-----------------------------------|--------------------------------------|--------------------------------------|
| <i>Shigella flexneri</i> ATCC 12022      | Good growth   | Opened: K Closed: K                  | Abierto: K Cerrado: K             | Opened: A Closed: A                  | Opened: K Closed: K                  |
| <i>Salmonella enteritidis</i> ATCC 13076 | Good growth   | Opened: K Closed: K                  | Opened: K Closed: K               | Opened: AG Closed: AG                | Opened: K Closed: K                  |
| <i>Escherichia coli</i> ATCC 25922       | Good growth   | Opened: K Closed: K                  | Opened: K Closed: K               | Opened: AG Closed: AG                | Opened: AG Closed: AG                |
| <i>Pseudomonas aeruginosa</i> ATCC 27853 | Good growth   | Opened: K Closed: K                  | Opened: K Closed: K               | Opened: K Closed: K                  | Opened: K Closed: K                  |
| <i>Alcaligenes faecalis</i> ATCC 8750    | Good growth   | Opened: K Closed: K                  | Opened: K Closed: K               | Opened: K Closed: K                  | Opened: K Closed: K                  |

## Storage

Temp. Min.: 2 °C

Temp. Max.: 25 °C

## Bibliography

Hugh, R. and Leifson, E.J. Bact. 66:24-26. 1953. Lisenko J. Gen. Microbiol., 35:379, 1961. Edwards y Ewing Identification of Enterobacteriaceae. Burgess Publ. Co. Minneapolis, Minn., 1972.