

**Also known as**

HE Agar; HEA

**Specification**

Solid, selective and differential culture medium for isolation of pathogenic enterobacteria from contaminated samples according to ISO 21567 standard.

**Formula \* in g/L**

|                         |       |                                   |
|-------------------------|-------|-----------------------------------|
| Meat peptone.....       | 12.00 |                                   |
| Yeast extract.....      | 3.00  | Ammonium ferric citrate..... 1.50 |
| Bile salts.....         | 9.00  | Acid fuchsin..... 0.10            |
| Lactose.....            | 12.00 | Bromothymol blue..... 0.06        |
| Sucrose.....            | 12.00 | Agar..... 15.00                   |
| Salicin.....            | 2.00  |                                   |
| Sodium chloride.....    | 5.00  | Final pH 7,5 ±0,2 at 25 °C        |
| Sodium thiosulfate..... | 5.00  |                                   |

\* Adjusted and /or supplemented as required to meet performance criteria

**Directions**

Suspend 77 g of powder in 1 L of distilled water and let it soak. Heat stirring constantly, until boiling. Cool to 55-60°C and pour into sterile plates. Do not autoclave. This medium is very thermolabile and overheating should be avoided.

**Description**

This culture medium, originally developed by King and Metzger, has a high nutrient content, peptones, fermentable sugars and combination of indicators. All these characteristics and the bile salts make it a very selective and effective medium.

**Technique**

In order to avoid the spreading of *Proteus*, it is necessary that the agar surface be perfectly dry at the moment of inoculation. Inoculation must be carried out by surface streaking, directly from rectal swabs or faeces. If colonies are well separated after 18 hours of incubation, the first characteristic appearances or colony morphology may be observed:

- *Shigella* spp., *Proteus* *inconstans*: Raised colonies, green colour.
- *Salmonella* spp.: Green-blue colonies, with or without black centre.
- *Pseudomonas* spp.: Irregular colonies, plain, green or brown.
- Companion and non pathogenic bacteria: Salmon coloured colonies.

**Quality control**
**Incubation temperature:** 37°C ±1,0

**Incubation time:** 24 ± 3 h

**Inoculum:** Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) / 10<sup>4</sup>-10<sup>6</sup> CFU (Selectivity) according to ISO 11133:2014/Amd 1:2018 .

| Microorganism                             | Growth              | Remarks                              |
|---|---------------------|--------------------------------------|
| <i>Enterococcus faecalis</i> ATCC® 29212  | Inhibited           | Light Pink small colonies            |
| <i>Escherichia coli</i> ATCC® 25922       | Inhibited           | -                                    |
| <i>Proteus mirabilis</i> ATCC® 43071      | Productivity > 0.50 | Black colonies, Greenish-Blue medium |
| <i>Salmonella enteritidis</i> ATCC® 13076 | Productivity > 0.50 | Black colonies, Greenish-Blue medium |
| <i>Salmonella typhimurium</i> ATCC® 14028 | Productivity > 0.50 | Black colonies, Greenish-Blue medium |
| <i>Shigella sonnei</i> ATCC® 25931        | Productivity > 0.50 | Green to blue colonies               |
| <i>Shigella flexneri</i> ATCC® 12022      | Productivity > 0.50 | Green to blue colonies               |

**References**

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- HORWITZ, W. (2000). Official Methods of Analysis of the AOAC International 17th ed. Gaithersburg Md. USA.
- ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
- ISO 21567 Standard (2004) Microbiology of food and animal feeding stuffs.- Horizontal method for the detection of *Shigella* spp.
- KING S. and METZGER W. Y. (1968). A new plating method for the isolation of the enteric pathogens. Appl. Microbiol. 16:577.
- MURRAY, P.R., E.J. BARON, J.H. JORGENSEN, M.A. PFALLER & R.H. YOLKEN (Eds) (2003) Manual of Clinical Microbiology 8th ed. ASM Press. Washington DC, USA.
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**Storage**

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).