 Hektoen Enteric Agar is a differential selective medium used for the isolation of *Shigella* and *Salmonella* species from enteric pathological specimens.

**Hektoen Enteric Agar**

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| REF: V.1/HE01.100.0100 100 gram REF: V.1/HE01.250.0250 250 gram |  REF: V.1/HE01.500.0500 500 gram  |

# CLINICAL SIGNIFICANCE

Hektoen Enteric (HE) Agar is a moderately selective differential medium used for the isolation and culture of gram-negative enteric microorganisms, particularly for the isolation of *Shigella* and *Salmonella* species from fecal (mixed flora) samples.

# METHOD PRINCIPLE

The increased concentration of carbohydrate and proteose peptone helps to reduce the inhibitory effect of bile salts and indicators and allows good growth of *Salmonella* and *Shigella* species while inhibiting the normal intestinal flora. The medium contains three carbohydrates i.e. lactose, sucrose and salicin for differentiation of enteric pathogens. The higher lactose concentration aids in the visualization of enteric pathogens and minimizes the problem of delayed lactose fermentation. Salicin is fermented by many coliforms including those that do not ferment lactose and sucrose. Combination of ferric ammonium citrate and sodium thiosulphate in the medium enables the detection of hydrogen sulfide production, thereby aiding in the differentiation process due to the formation of black centered colonies. The indicator system, consisting of acid fuchsin and bromothymol blue, has lower toxicity as compared to other enteric media, resulting in improved recovery of enteric pathogens. Hoben et al further enhanced the selectivity of the medium by addition of novobiocin at a concentration of 15 mg/litre, which inhibits *Citrobacter* and *Proteus* species. Taylor and Schelhaut found the medium valuable for differentiating pathogenic enteric organisms and for better growth of *Shigellae*.

# MEDIA COMPOSITION (4,5)

|  |  |
| --- | --- |
| **Item**  | **Formula in g/L**  |
| Protese peptoneYeast extractLactoseSucroseSalicinBile salts mixture Sodium chlorideSodium thiosulphateFerric ammonium citrateAcid fuchsinBromothymol blueAgar  | 123121229551.50.10.06515 |

## pH 7.5 ± 0.2 at 25°C

# PRECAUTIONS AND WARNINGS (2)

Media to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

* Wearing personnel protective equipment (overall, gloves, glasses,..).
* Do not pipette by mouth.
* In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries, seek medical advice immediately.
* Handle specimens and inoculated culture bottles as though capable of transmitting infectious agents. All inoculated culture bottles, specimen collection needles, and blood drawing devices should be decontaminated according to country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the Hektoen enteric Agar

material safety data sheet.

# MEDIA PREPARATION, STORAGE AND STABILITY

**Lab.Vie**. Hektoen enteric agar should be stored between 10-30°C in a firmly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

##  PROCEDURE

1. Suspend 50.0 grams in 1000 ml purified / distilled water.
2. Adjust pH to pH 7.1 ± 0.2 at 25°C.
3. Heat to boiling to dissolve the medium completely.
4. Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes.
5. Mix well and pour into sterile Petri plates.

## Deterioration

**Lab.Vie**. Hektoen Enteric Agar is cream to yellow with tancast homogeneous free flowing powder, dehydrated medium is green coloured to slightly opalescent gel. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

# SPECIMEN COLLECTION AND PRESERVATION

# For clinical samples follow appropriate techniques for handling specimens as per established guidelines (11,12). For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (9,10,13). After use, contaminated materials must be sterilized by autoclaving before discarding.

# TYPE OF SPECIMEN

# Clinical samples: Blood, urine, faeces;

# Foods, water samples

# EQUIPMENT REQUIRED NOT PROVIDED

# Sterile cups

# Sterile petri-dishes

# Incubator

# Autoclave

# QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact the technical support.

# PERFORMANCE CHARACTERISTICS

The following organisms are used by us as part of the quality assurance of the product. The total inoculum challenge for each test organism per bottle is 10 to 50 colony forming units (CFU’s).

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| --- | --- | --- | --- | --- |
| **Organism** | **Inoculum****(CFU)** | **Growth** | **Recovery** | **Colour of****colony** |
| Escherichiacoli ATCC 25922 | 50-100 | fair | 20-30% | orange (mayhave bile precipitate) |
| #Klebsiella aerogenesATCC 13048 | 50-100 | fair- good | 30-40% | salmon- orange |
| Enterococcusfaecalis ATCC 29212 | >=10³ | inhibited | 0% | None |
| Salmonella Enteritidis ATCC 13076 | 50-100 | luxuriant | >=50% | greenish blue may have black centres(H2Sproduction) |
| Salmonella Typhi ATCC 6539 | 50-100 | luxuriant | >=50% | greenish blue may have blackcentres(H2S production) |
| Salmonella Typhimurium ATCC 14028 | 50-100 | luxuriant | >=50% | greenish blue may have black centres(H2Sproduction) |
| Shigellaflexneri ATCC 12022 | 50-100 | luxuriant | >=50% | greenish blue |
| Escherichia coli ATCC8739 | 50-100 | Fair | 20-30% | orange (may have bileprecipitate) |

# REFERENCES

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| **SYMBOLS IN PRODUCT LABELLING**  |
|   IVD For in-vitro diagnostic use  |   Number of <n> test in the pack  |
|  LOT Batch Code/Lot number  | A black and white triangle with a exclamation mark  Description automatically generated Caution  |
|  REF Catalogue Number  | Do not use if package is damaged   |
|   Temperature Limitation   Expiration Date   Manufactured by  |  Consult Instruction for use      |

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