Hektoen Enteric Agar

Intended Use
Hektoen Enteric Agar is a selective medium used for detection and isolation of pathogenic intestinal bacteria including *Salmonella* and *Shigella* species from clinical and non-clinical specimens.

Summary
King and Metzger developed H. E. Agar at the Hektoen institute in Chicago for selective isolation of *Shigella* and other pathogenic species from clinical specimens. They found in their comparative study that H. E. medium was more superior than SS agar for recovery of *Salmonella* and *Shigella* species.
The present formulation of H. E. agar has less amount of bile salt and deoxycholate is absent. High level of peptones and sugars reduce the inhibitory effect of bile salt and enable it to be moderately selective for *Salmonella* and *Shigella* species. H. E. agar is suitable for isolation of *Salmonella* and *Shigella* species from food, clinical, dairy and other specimens.

Principle
Peptone and Yeast extract serve as a source of nitrogen. Bile salts act as a selective agent by inhibiting Gram-positive and other than enteric organisms. Sodium chloride provides sodium ions for the membrane transport and maintains osmotic equilibrium of the medium. Salicin, Sucrose and Lactose provide differentiation of Gram-negative enteric pathogens. Bromothymol blue and Acid fuchsin are acid-base indicator. The additions of ferric ammonium citrate and sodium thiosulphate enable the detection of H_2S production. Agar is the solidifying agent.

Formula*

Ingredients	**g/L**
Proteose peptone	12.0
Yeast Extract	3.0
Lactose	12.0
Sucrose	12.0
Salicin	2.0
Bile Salts	9.0
Sodium Chloride	5.0
Sodium Thiosulphate	5.0
Ferric Ammonium Citrate	1.5
Bromothymol Blue	0.065
Acid Fuchsin	0.1
Agar	14.0
Final pH (at 25°C)	7.5 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Clinical samples – faeces
Food and Dairy samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 75.66 g of the powder in 1000 mL purified / distilled water and mix thoroughly.
2. Boil with frequent agitation to dissolve the powder completely.
3. AVOID OVERHEATING. DO NOT AUTOCLAVE.
4. Cool the medium to approximately 45°C-50°C, pour into sterile petridishes.

Quality Control

Dehydrated Appearance: Yellow coloured with tan cast, homogenous free flowing powder.
Prepared Appearance: Green coloured, clear to slightly opalescent gel forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation of 18-24 hours at 35°C-37°C.

Organism (ATCC)	**Growth**	**Colour of Colony**
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Greenish blue with black centre
Shigella flexneri serotype 2b (12022)	Good	Greenish blue
Escherichia coli (25922)	Partial Inhibition	Orange with bile precipitate
Enterococcus faecalis (29212)	Inhibited	-

Note: For Good growth - Growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.
For inhibition no growth of test microorganism should occur.

Interpretation of results

Typical colonial morphology on H E Agar

E. coli	Large, yellow to salmon color; some strains may be inhibited
Salmonella	Blue-green to blue; most strains with black center
Enterobacter/Klebsiella	Large, yellow to salmon color
Shigella	Green and moist, raised
Proteus	Variable, blue-green to blue or salmon, most strains with black center
Pseudomonas	Irregular, green to brown
Gram-positive bacteria	No growth to slight growth

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. Colonies of proteus may resemble *Salmonella* or *Shigella*.
2. It is preferable that biochemical and/or serological tests be performed on colonies from pure culture for complete identification

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. King, S., and W.I. Metzger. 1968. Appl. Microbiol.
2. Douglas S. R., 1922-23, Brit. J. Expt. Pathol., 3:263.
3. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201080020100	Dehydrated Culture Media	100 g
201080020500	Dehydrated Culture Media	500 g
201080022500	Dehydrated Culture Media	2.5 k