

Hektoen Enteric (HE) Agar (NCM0006)

Intended Use

Hektoen Enteric Agar is used for the isolation and differentiation of enteric pathogens. Hektoen Enteric Agar is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Hektoen Enteric Agar was developed in 1967 by King and Metzger. Compared to other enteric differentiating media commonly used, Hektoen Enteric Agar increased the isolation rate of *Salmonella* spp. and *Shigella* spp. This was accomplished by increasing the carbohydrate and peptone content of the medium in order to counteract the inhibitory effects of bile salts and indicators. King and Metzger formulated a medium that slightly inhibited growth of *Salmonella* and *Shigella*, while inhibiting Gram-positive microorganisms.

Principles of the Procedure

Enzymatic Digest of Animal Tissue provides nitrogen, carbon, and amino acids required for organism growth. Yeast Extract is a vitamin source. Bile Salts Mixture and Acid Fuchsin inhibit Gram-positive organisms. Lactose, Sucrose, and Salicin are fermentable carbohydrates. Sodium Chloride maintains the osmotic balance of the medium. Ferric Ammonium Citrate, a source of iron, allows the detection of hydrogen sulfide (H₂S) produced from Sodium Thiosulfate. H₂S-positive colonies have black centers. Bromothymol Blue is added as the pH indicator. Agar is the solidifying agent.

Typical Formulation

Enzymatic Digest of Animal Tissue	12.0 g/L
Yeast Extract	3.0 g/L
Bile Salts Mixture	9.0 g/L
Lactose	12.0 g/L
Sucrose	12.0 g/L
Salicin	2.0 g/L
Sodium Chloride	5.0 g/L
Sodium Thiosulfate	5.0 g/L
Ferric Ammonium Citrate	1.5 g/L
Bromothymol Blue	0.065 g/L
Acid Fuchsin	0.1 g/L
Agar	13.5 g/L

pH: 7.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Preparation

1. Suspend 75 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. DO NOT AUTOCLAVE.

Test Procedure

For isolation and identification of pathogenic *Enterobacteriaceae* refer to appropriate references.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light greenish beige.



Technical Specification Sheet



Prepared Appearance: Prepared medium is trace to slightly hazy and light to dark green.

Expected Cultural Response: Cultural response on Hektoen Enteric Agar at 35 ± 2°C after 18 - 24 hours incubation.

Microorganism	APPROX. INOCULUM (CFU)	Growth	Reaction
<i>Escherichia coli</i> ATCC® 25922	>10 ⁵	Suppressed growth to complete inhibition	yellow to salmon-orange colonies
<i>Enterococcus faecalis</i> ATCC® 29212	>10 ⁵	Complete inhibition	---
<i>Salmonella enteritidis</i> ATCC® 13076	50-200	≥70%	Green w/ black center
<i>Salmonella typhimurium</i> ATCC® 14028	50-200	≥70%	Green w/ black center
<i>Shigella flexneri</i> ATCC® 12022	50-200	≥50%	Green colonies
<i>Shigella sonnei</i> NCTC 8574	50-200	≥50%	Green colonies

The organisms listed are the minimum that should be used for quality control testing.

Results

Refer to appropriate references and procedures for results.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Do not autoclave medium because excessive heat may alter ingredients.
2. *Proteus* spp. may resemble salmonellae or shigellae. Further testing should be conducted to confirm the presumptive identification or organisms isolated on this medium.
3. Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. King, S., and W. I. Metzger. 1968. A new plating medium for the isolation of enteric pathogens. *Appl. Microbiol.* 16:577-578.
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Technical Specification Sheet



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