

HEKTOEN ENTERIC AGAR (7138)

Intended Use

Hektoen Enteric Agar is used for the isolation and differentiation of enteric pathogens.

Product Summary and Explanation

Hektoen Enteric Agar was developed in 1967 by King and Metzger.^{1,2} Compared to other enteric differentiating media commonly used in clinical laboratories, 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.^{1,2}

Hektoen Enteric Agar is used to isolate and differentiate *Salmonella* spp. and *Shigella* spp., both of which cause a variety of serious human gastrointestinal illnesses.³ Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and or neurological damage.³ This infection can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella* spp. U.S. federal guidelines require various poultry products to be routinely monitored before distribution for human consumption. A variety of procedures have been developed using Hektoen Enteric Agar as part of the multi-step procedure to isolate *Salmonella* spp. from food samples.⁴⁻⁷

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 production of hydrogen sulfide (H₂S) present from Sodium Thiosulfate. H₂S-positive colonies have black centers. Bromothymol Blue is added as the pH indicator. Agar is the solidifying agent.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	16.5 g
Yeast Extract.....	3 g
Bile Salts Mixture	4.5 g
Lactose	12 g
Sucrose.....	12 g
Salicin	2 g
Sodium Chloride	5 g
Sodium Thiosulfate	5 g
Ferric Ammonium Citrate.....	1.5 g
Bromthymol Blue.....	0.065 g
Acid Fuchsin	0.1 g
Agar	13.5 g

Final pH: 7.6 ± 0.2 at 25°C

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

Precautions

1. For Laboratory Use.
2. **HARMFUL.** May be harmful if swallowed or inhaled. Irritating to eyes, respiratory system, and skin.

Directions

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.

Quality Control Specifications

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

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°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
<i>Escherichia coli</i> ATCC® 25922	partial to complete inhibition	yellow to salmon colonies
<i>Enterococcus faecalis</i> ATCC® 29212	inhibited	---
<i>Shigella flexneri</i> ATCC® 12022	growth	green colonies
<i>Salmonella typhimurium</i> ATCC® 14028	growth	green to green-blue colonies with black centers

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

Test Procedure

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

Results

Refer to appropriate references and procedures for results.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

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.

Packaging

Hektoen Enteric Agar	Code No.	7138A	500 g
		7138B	2 kg
		7138C	10 kg

References

1. **King, S., and W. I. Metzger.** 1968. A new plating medium for the isolation of enteric pathogens. *Appl. Microbiol.* **16**:577-578.
2. **King, S., and W. I. Metzger.** 1968. A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with S and EMB Agar. *Appl. Microbiol.* **16**:579-581.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
4. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytic Chemists.** 1996. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.
6. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** FDA Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Flowers, R. S., W. H. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products. *In* Marshall, R. T. (ed.). Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.