Hektoen Enteric Agar

Intended Use

Hektoen Enteric (HE) Agar is a modrately selective medium used in qualitative procedures for the isolation and cultivation of gram-negative enteric microorganisms, especially *Shigella*, from a variety of clinical and nonclinical specimens.

Summary and Explanation

Through the years many media have been devised for the isolation of enteric pathogens. These various formulations have differed in their degree of selectivity for the pathogenic species. Some were designed to isolate and differentiate *Shigella* species whereas others were formulated for the selective isolation of the salmonellae. Media that isolated a broader spectrum of enteric pathogens were less inhibitory to members of the nonpathogenic intestinal flora.

Hektoen Enteric Agar was developed in 1967 by King and Metzger of the Hektoen Institute in order to increase the frequencies of isolation of *Shigella* and *Salmonella* organisms when compared with their recovery on other media frequently utilized in clinical laboratories at that time.[1-3] This medium is considered to be moderately selective, and is particularly useful in the isolation of *Shigella* species. The present formulation differs from that of the original in that sodium desoxycholate has been eliminated and the concentration of bile salts is reduced. Additionally, the peptone concentrations have been increased in order to offset the inhibitory effects of the bile salts.[4]

HE Agar is currently recommended as one of several plating media for the culture of *Enterobacteriaceae* from stool specimens.[5] Foods containing poultry, eggs or dairy products are the most frequent vehicles for foodborne salmonellosis, and a variety of procedures have been developed using Hektoen Enteric Agar as part of the multi-step procedure to isolate *Salmonella*.[6-9]

Principles of the Procedure

The selective nature of Hektoen Enteric Agar is due to the incorporation of bile salts in the formulation. These substances inhibit gram-positive organisms but also can be toxic for some gram-negative strains.

This medium contains three carbohydrates, lactose, sucrose (saccharose) and salicin, for optimal differentiation of enteric pathogens by the color of the colonies and of the medium adjacent to the colonies. The lactose concentration is higher than in many other media used for enterics in order to aid in the visualization of enteric pathogens and minimize the problem of delayed lactose fermentation. Ferric ammonium citrate and sodium thiosulfate in the medium enable the detection of hydrogen sulfide production, thereby aiding in the differentiation process due to the production of black-centered colonies. The indicator system, consisting of acid fuchsin and bromthymol blue, has a lower toxicity than that of many other enteric media, resulting in improved recovery of enteric pathogens.

User Quality Control

Identity Specifications

Difco™ Hektoen Enteric Agar

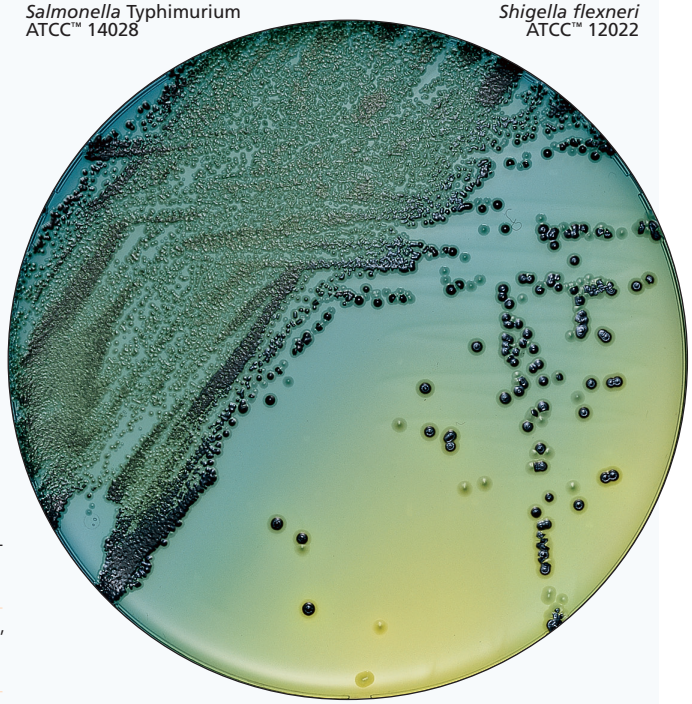
Dehydrated Appearance:	Light beige, may have a slight green cast, free-flowing, homogeneous.
Solution:	7.6% solution, soluble in purified water upon boiling. Solution is brown with greenish cast, slightly opalescent.
Prepared Appearance:	Green with yellowish cast, slightly opalescent.
Reaction of 7.6% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Difco™ Hektoen Enteric Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–
Escherichia coli	25922	10^2-3×10^2	Partial inhibition	Salmon-orange, may have bile precipitate
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-3×10^2	Good	Greenish blue, w/black centers
Shigella flexneri	12022	10^2-3×10^2	Good	Greenish blue



Formula

Difco™ Hektoen Enteric Agar

Approximate Formula* Per Liter		
Proteose Peptone	12.0	g
Yeast Extract	3.0	g
Bile Salts No. 3	9.0	g
Lactose	12.0	g
Saccharose	12.0	g
Salicin	2.0	g
Sodium Chloride	5.0	g
Sodium Thiosulfate	5.0	g
Ferric Ammonium Citrate	1.5	g
Agar	14.0	g
Bromthymol Blue	65.0	mg
Acid Fuchsin	0.1	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 76 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat to boiling with frequent agitation to dissolve completely. Do not overheat. DO NOT AUTOCLAVE.
3. Cool to 45-50°C and use immediately.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours.

Expected Results

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Limitation of the Procedure

Proteus species may resemble salmonellae or shigellae. Further testing should be conducted to confirm the presumptive identification of organisms isolated on this medium.

References

1. King and Metzger. 1967. Abstr. M99, p. 77. Bacteriol. Proc. Am. Soc. Microbiol. 1967.
2. King and Metzger. 1968. Appl. Microbiol. *16*:577.
3. King and Metzger. 1968. Appl. Microbiol. *16*:579.
4. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. l. Williams & Wilkins, Baltimore, Md.
5. Murray, Baron, Jorgensen, Landry and Pfaller, (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. Wehr and Frank. (ed.). 2004. Standard Methods for the examination of dairy products, 17th ed. American Public Health Association, Washington, D.C.
7. U.S. Food and Drug Administration. 2001. Bacteriological analytical manual, online. AOAC International, Gaithersburg, Md.
8. Horwitz (ed.). 2007. Official methods of analysis of AOAC International, 18th ed., online. AOAC International, Gaithersburg, Md.
9. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Hektoen Enteric Agar

AOAC BAM BS12 CCAM CMPH2 COMPF MCM9 SMD

Cat. No.	285340	Dehydrated – 500 g
	285310	Dehydrated – 2 kg
	285320	Dehydrated – 10 kg

BBL™ Hektoen Enteric Agar

AOAC BAM BS12 CCAM CMPH2 COMPF MCM9 SMD

United States and Canada

Cat. No.	221365	Prepared Plates – Pkg. of 20*
	221366	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254009	Prepared Plates – Pkg. of 20*
	254075	Prepared Plates – Ctn. of 120*

Mexico

Cat. No.	224450	Prepared Plates – Pkg. of 10*

BBL™ Hektoen Enteric Agar//Salmonella Shigella Agar

Cat. No. 297426 Prepared **I Plate**™ Dishes – Pkg. of 20*

BBL™ Hektoen Enteric Agar//XLD Agar

Cat. No. 295646 Prepared **I Plate**™ Dishes – Pkg. of 20*

**Store at 2-8°C.*