

## User Quality Control

### Identity Specifications

#### Bacto™ Heart Infusion Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.5% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 2.5% Solution at 25°C:	pH 7.4 ± 0.2

### Cultural Response

#### Bacto™ Heart Infusion Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Escherichia coli</i>	25922	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Staphylococcus aureus</i>	25923	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Streptococcus pneumoniae</i>	6305	10 <sup>2</sup> -10 <sup>3</sup>	Good
<i>Streptococcus pyogenes</i>	19615	10 <sup>2</sup> -10 <sup>3</sup>	Good



## Principles of the Procedure

Infusion from beef heart and tryptose supply the nutritional requirements for growth of microorganisms in heart infusion media. Sodium chloride maintains the osmotic balance of the medium.

## Formula

#### Bacto™ Heart Infusion Broth

Approximate Formula* Per Liter	
Beef Heart, Infusion from 500 g	10.0 g
Tryptose	10.0 g
Sodium Chloride	5.0 g

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

## Directions for Preparation from Dehydrated Product

1. Dissolve 25 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

## Procedure

See appropriate references for specific procedures.

## Expected Results

Refer to appropriate references and procedures for results.

## References

1. Huntoon. 1918. *J. Infect. Dis.* 23:169.
2. Elliott, Kaysner, Jackson and Tamplin. 1995. *In* FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
3. Vanderzant and Splittstoesser (ed.). 1992. *Compendium of methods for the microbiological examination of foods*, 3rd ed. American Public Health Association, Washington, D.C.
4. Ruoff. 1995. *In* Murray, Baron Pfaller, Tenover and Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
5. Atlas. 1997. *Handbook of microbiological media*, 2nd ed. CRC Press, Inc., Boca Raton, Fla.

## Availability

#### Bacto™ Heart Infusion Broth

	BAM	COMP	EP	
Cat. No.	238400			Dehydrated – 500 g
	238100			Dehydrated – 2 kg
	292844			Dehydrated – 10 kg

# Hektoen Enteric Agar

## Intended Use

Hektoen Enteric (HE) Agar is a moderately 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.<sup>1-3</sup> 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

## User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

### 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
<i>Enterococcus faecalis</i>	29212	10 <sup>3</sup>	Marked to complete inhibition	–
<i>Escherichia coli</i>	25922	10 <sup>2</sup> -3 × 10 <sup>2</sup>	Partial inhibition	Salmon-orange, may have bile precipitate
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	10 <sup>2</sup> -3 × 10 <sup>2</sup>	Good	Greenish blue, w/black centers
<i>Shigella flexneri</i>	12022	10 <sup>2</sup> -3 × 10 <sup>2</sup>	Good	Greenish blue

### Identity Specifications

#### BBL™ Hektoen Enteric Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain some specks.
Solution:	7.5% solution, soluble in purified water upon boiling. Solution is dark, green trace blue to green to brown green, clear to slightly hazy.
Prepared Appearance:	Dark, green trace blue to green to brown green, clear to slightly hazy.
Reaction of 7.5% Solution at 25°C:	pH 7.6 ± 0.2

### Cultural Response

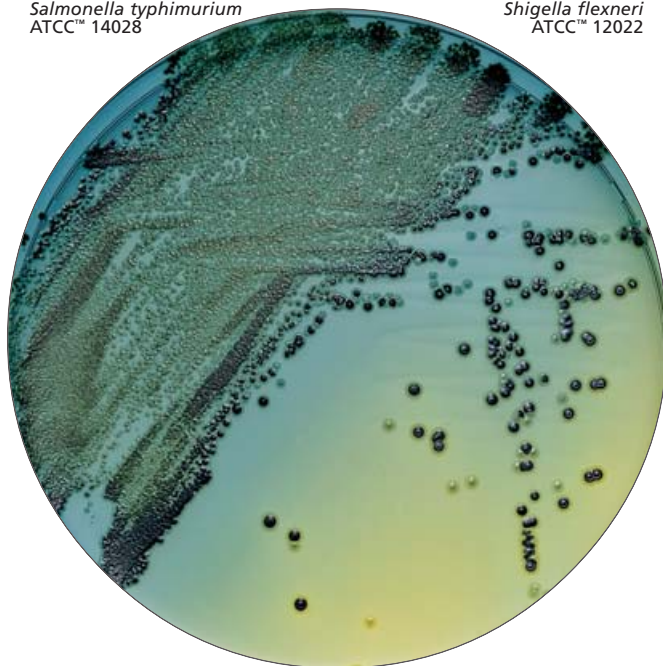
#### BBL™ Hektoen Enteric Agar

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

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Enterococcus faecalis</i>	29212	10 <sup>4</sup> -10 <sup>5</sup>	Partial to complete inhibition	Yellow
<i>Escherichia coli</i>	25922	10 <sup>4</sup> -10 <sup>5</sup>	Partial to complete inhibition	Yellow to salmon, may have bile precipitate
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	10 <sup>3</sup> -10 <sup>4</sup>	Good	Blue-green to blue w/black centers
<i>Shigella flexneri</i>	12022	10 <sup>3</sup> -10 <sup>4</sup>	Good	Green to blue-green

*Salmonella typhimurium*  
ATCC™ 14028

*Shigella flexneri*  
ATCC™ 12022



have been increased in order to offset the inhibitory effects of the bile salts.<sup>4</sup>

HE Agar is currently recommended as one of several plating media for the culture of *Enterobacteriaceae* from stool specimens.<sup>5</sup> 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*.<sup>6-9</sup>

## 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.

## Formulae

### 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

### BBL™ Hektoen Enteric Agar

Approximate Formula* Per Liter		
Peptic Digest of Animal Tissue .....	12.0	g
Yeast Extract .....	3.0	g
Bile Salts .....	9.0	g
Lactose .....	12.0	g
Sucrose .....	12.0	g
Salicin .....	2.0	g
Sodium Chloride .....	5.0	g
Sodium Thiosulfate .....	5.0	g
Ferric Ammonium Citrate .....	1.5	g
Agar .....	13.5	g
Bromthymol Blue .....	64.0	mg
Acid Fuchsin .....	0.1	g

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

## Directions for Preparation from Dehydrated Product

- Suspend the powder in 1 L of purified water:
  - Difco™ Hektoen Enteric Agar – 76 g;
  - BBL™ Hektoen Enteric Agar – 75 g.
 Mix thoroughly.
- Heat to boiling with frequent agitation to dissolve completely. Do not overheat. DO NOT AUTOCLAVE.
- Cool to 45-50°C and use immediately.
- 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

- King and Metzger. 1967. Abstr. M99, p. 77. Bacteriol. Proc. Am. Soc. Microbiol. 1967.
- King and Metzger. 1968. Appl. Microbiol. 16:577.
- King and Metzger. 1968. Appl. Microbiol. 16:579.
- MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
- Chapin and Murray. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
- Marshall (ed.). 1993. Standard Methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
- U.S. Food and Drug Administration. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, Md.
- Horwitz (ed.). 2000. Official methods of analysis of AOAC International, 17th ed., vol. I. AOAC International, Gaithersburg, Md.
- 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	BS10	CMPH	COMP	MCM7	SMD
Cat. No. 285340							
285310							
285320							

### BBL™ Hektoen Enteric Agar

	AOAC	BAM	BS10	CMPH	COMP	MCM7	SMD
Cat. No. 212211							
212253							
293327							

#### 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*
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### BBL™ Hektoen Enteric Agar//Salmonella Shigella Agar

Cat. No. 297426	Prepared I Plate™ Dishes – Pkg. of 20*
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### BBL™ Hektoen Enteric Agar//XLD Agar

Cat. No. 295646	Prepared I Plate™ Dishes – Pkg. of 20*
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\*Store at 2-8°C.