



## QUALITY CONTROL PROCEDURES

### I INTRODUCTION

Hektoen Enteric Agar is a moderately selective and differential medium for the isolation, cultivation and differentiation of gram-negative enteric microorganisms from both clinical and nonclinical specimens. It is of particular importance as a medium for the isolation of *Shigella* species.

### II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
  - a. Streak the plates for isolation. Use 18–24 h broth cultures of *Enterococcus faecalis* and *Escherichia coli* diluted to yield  $10^4$ – $10^5$  CFU/plate. For the remaining organisms, use 18–24 h broth cultures diluted to yield  $10^3$ – $10^4$  CFU/plate.
  - b. Incubate plates at  $35 \pm 2^\circ\text{C}$  in an aerobic atmosphere.
  - c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for growth, colony size, pigmentation and selectivity.
3. Expected Results

CLSI Organisms	ATCC™	Recovery	Colony Color
* <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Growth	Blue to green-blue with black centers
* <i>Shigella flexneri</i>	12022	Growth	Green to blue-green
* <i>Enterococcus faecalis</i>	29212	Inhibition (partial)	Yellow
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)	Yellow to salmon

\*Recommended organism strain for User Quality Control.

### III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of  $7.6 \pm 0.2$ .
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at  $35 \pm 2^\circ\text{C}$  for 72 h and examine for microbial contamination.

## PRODUCT INFORMATION

### IV INTENDED USE

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

### V 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. Those media which isolated a broader spectrum of enteric pathogens also 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 recovery 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 reduced. Additionally, the peptone concentrations 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. This is because of its moderate selectivity as well as for its differentiation property.<sup>5</sup>

### VI 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 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.

## VII REAGENTS

### Hektoen Enteric Agar

Approximate Formula\* Per Liter Purified Water

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
Bromthymol Blue .....	0.064	g
Acid Fuchsin .....	0.1	g
Agar.....	13.5	g

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

#### Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"<sup>6-9</sup> and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

## VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.<sup>5,10</sup> Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

## IX PROCEDURE

**Material Provided:** Hektoen Enteric Agar

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

**Test Procedure:** Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates, protected from light, at 35 ± 2°C in an aerobic atmosphere for 18–24 h.

**User Quality Control:** See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

## X 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.

Typical colonial morphology on Hektoen Enteric Agar is as follows:

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

## XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.<sup>5,10-14</sup>

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

## XII AVAILABILITY

Cat. No.	Description
221365	<b>BBL™</b> Hektoen Enteric Agar, Pkg. of 20 plates
221366	<b>BBL™</b> Hektoen Enteric Agar, Ctn. of 100 plates

## XIII REFERENCES

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