

# HEKTOEN ENTERIC (HE) AGAR

## INTENDED USE

Remel Hektoen Enteric (HE) Agar is a solid medium recommended for use in qualitative procedures for the isolation and differentiation of gram-negative enteric pathogens.

## SUMMARY AND EXPLANATION

Hektoen Enteric (HE) Agar was developed by King and Metzger to produce a medium simultaneously conducive to growth of *Salmonella* and *Shigella*, and inhibitory to normal intestinal flora.<sup>1</sup> This was accomplished by increasing the amount of carbohydrate and peptone in the medium to counteract the inhibitory effects of bile salts and indicators. The HE Agar in use today represents a slight modification of the original formulation in that the bile salts concentration has been lowered and sodium desoxycholate has been eliminated.

## PRINCIPLE

Meat peptone supplies essential nutrients to support bacterial growth. Sucrose, lactose, and salicin are added to help differentiate pathogens by the color of the colonies and the medium surrounding them. The lactose concentration is higher than in many media to minimize delayed lactose fermentation. If hydrogen sulfide is produced from sodium thiosulfate, it reacts with ferric ammonium citrate in the medium to form a precipitate in the center of the colony. The indicator system consists of acid fuchsin and bromthymol blue which are low in toxicity. The bile salts in HE Agar inhibit the growth of gram-positive organisms.

## REAGENTS (CLASSICAL FORMULA) \*

Meat Peptone .....	12.0 g	Yeast Extract.....	3.0 g
Lactose .....	12.0 g	Salicin .....	2.0 g
Sucrose .....	12.0 g	Ferric Ammonium Citrate .....	1.5 g
Bile Salts No. 3.....	9.0 g	Acid Fuchsin .....	0.1 g
Sodium Chloride.....	5.0 g	Bromthymol Blue.....	0.064 g
Sodium Thiosulfate.....	5.0 g	Agar .....	14.0 g
		Demineralized Water.....	1000.0 ml

pH 7.6 ± 0.2 @ 25°C

\*Adjusted as required to meet performance standards.

## PROCEDURE

1. An enrichment broth, such as GN Broth (REF R060992), may be used in conjunction with HE Agar and a nonselective medium to increase the potential for recovery of enteric pathogens.
2. Inoculate and streak the specimen as soon as possible after it is received in the laboratory.
3. Incubate plate aerobically at 33-37°C for 18-24 hours.
4. Examine plate for typical colony morphology. *Salmonella* produces blue green to green colonies with or without black centers; colonies of *Shigella* are greener than those of *Salmonella* with the color fading toward the periphery of the colonies.<sup>3</sup>

## QUALITY CONTROL

All lot numbers of Hektoen Enteric (HE) Agar have been tested using the following quality control organisms and have been found to be acceptable. This quality control testing conforms with or exceeds CLSI standards.<sup>2</sup> Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

## CONTROL

\**Salmonella enterica* serovar Typhimurium ATCC® 14028

\**Shigella flexneri* ATCC® 12022

\**Enterococcus faecalis* ATCC® 29212

\**Escherichia coli* ATCC® 25922

\*CLSI recommended organism

## INCUBATION

Aerobic, 18-24 h @ 33-37°C

Aerobic, 18-24 h @ 33-37°C

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Aerobic, 18-24 h @ 33-37°C

## RESULTS

Growth, green colonies w/ black centers

Growth, green colonies

Inhibition (partial to complete), yellow colonies

Inhibition (partial to complete), colonies yellow to salmon

## LIMITATIONS

1. Growth of colonies that resemble *Salmonella* or *Shigella* (e.g., *Proteus* spp.) may occur; further biochemical testing is required to confirm the identification of isolates as enteric pathogens.<sup>3</sup>
2. Over time and during the shelf-life, bile salts in HE Agar may crystallize and form a precipitate in the medium. This does not affect the performance of the medium.<sup>3</sup>
3. Selective and nonselective media should be inoculated to increase the chance of recovering enteric pathogens when the population is low and to provide isolation of other organisms present in the specimen.
4. The color of HE Agar may shift from green to brownish during shipment. This is normal and should not affect the performance of the medium. Placing plates in refrigerated conditions (2-8°C) upon receipt overnight will result in the medium returning to a normal green appearance.

## BIBLIOGRAPHY

1. King, S. and W.I. Metzger. 1968. Appl. Microbiol. 16:577-581.
2. Clinical and Laboratory Standards Institute (CLSI). 2004. Quality Control for Commercially Prepared Microbiological Culture Media; Approved Standard, 3<sup>rd</sup> ed. M22-A3. CLSI, Wayne, PA.
3. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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