HEKTON 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 *Salmonella*. 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]

PRINCIPLE

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.

REAGENTS (FORMULA)

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
Bromthymol Blue	65.0	mg
Acid Fuchsin	0.1	g
Agar	14.0	g
Deionized Water	1000.0	ml

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.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	35 ± 2°C for 18-24 hours	Growth, Salmon-orange, may have bile precipitate

Shigella flexneri ATCC 12022	35 ± 2°C for 18-24 hours	Growth, Greenish blue

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