HealthLink
800-638-2625

Product No. 1062
Revision No. 01

PRODUCT INFORMATION AND QUALITY CONTROL SHEET

HEKTOEN ENTERIC AGAR

I. INTENDED USE

Hektoen Enteric Agar is a moderately selective and differential medium for the isolation and differentiation of gram negative enteric bacilli, especially *Shigella* and *Salmonella* species.

II. SUMMARY AND EXPLANATION

Hektoen Enteric Agar was originally developed by King and Metzger in 1967. Through increased carbohydrate and peptone concentration in the media, the inhibitory effects of the bile salts and indicators were reduced thereby minimally affecting the growth of *Shigella* and *Salmonella* species while still inhibiting other microbial flora. This media is recommended as one of several for the culture and evaluation of stool specimens for enteric pathogens.

III. PRINCIPLES OF THE PROCEDURE

The selective property of this medium is due to the presence of bile salts which inhibit the growth of gram positive organisms but are also toxic to some gram negative organisms. Lactose fermenting versus non lactose fermenting colonies are differentiated by the combination of two indicators; brom thymol blue and acid fuchsin. Two additional carbohydrates, sucrose and salicin, which are fermented more easily than lactose, are present for further organism differentiation. Thiosulfate in combination with ferric ammonium citrate detects those organisms which produce hydrogen sulfide by producing black centered colonies.

IV. TYPICAL FORMULA AND APPEARANCE

Appearance =green with yellowish cast, slightly opalescent
(Approximate formula* per liter of processed water)

Peptic Digest of Animal Tissue		12.0g
Yeast Extract		3.0
Bile Salts	9.0	
Lactose		12.0
Sucrose		12.0
Salicin		2.0
Sodium Chloride		5.0
Sodium Thiosulfate	5.0	
Ferric Ammonium Citrate		1.5
Brom Thymol Blue		0.064
Acid Fuchsin		0.1
Agar		13.5

*adjusted and/or supplemented to meet performance criteria.

V. PRECAUTIONS

This product is for IN VITRO diagnostic use only. Culture specimens may contain microorganisms which can be potentially infectious to the user. Strict adherence to aseptic techniques and established precautions against microbiological hazards should be followed throughout the procedure. Carefully dispose of all items which contact patient specimens or isolated bacteria. (See Material Safety Data Sheet for further information.)

VI. STORAGE/SHELF LIFE

Plated media should be stored at 2-8°C (36-46°F), media side up, in the unopened or resealed package protected from light. DO NOT FREEZE OR EXPOSE TO HIGH TEMPERATURES. Allow unopened plates to warm to room temperature prior to inoculation. Prior to and during inoculation procedures, plates should be handled in a manner that minimizes product exposure to the environment. Product which has exceeded the assigned expiration date noted on the label should not be used.

Do not use plates that exhibit evidence of drying, cracking, discoloration, microbial contamination or any other signs of deterioration. The presence of excessive condensate may indicate plates which have been damaged by exposure to temperature extremes.

VII. SPECIMEN COLLECTION

The quality of culture results depends primarily on the adequacy and condition of the specimen submitted for examination. Proper specimen collection techniques must be followed to ensure the most accurate culture results. Sterile swabs and collection containers should be used. Plates should be inoculated promptly after specimen collection. If a delay in inoculation is unavoidable, transport medium should be employed. Specimens should be collected prior to the initiation of antimicrobial therapy.

Detailed information on proper specimen collection may be obtained from microbiology reference materials.

VIII. MATERIALS PROVIDED

Hektoen Enteric Agar Plates and lot specific Quality Control Certificate.

IX. MATERIALS REQUIRED BUT NOT PROVIDED

Incubator maintaining 33-37°C.
Ancillary culture media, reagents and laboratory equipment as required.

X. PROCEDURE

Inoculate the specimen as soon as possible after it is received in the laboratory. The streak plate method is used primarily to isolate pure cultures from specimens containing mixed flora. If material is being cultured directly from a swab, roll the swab over a small area of the plate surface at the edge (approximately 1/4 to 1/3 of the plate); then streak in a zig-zag fashion with a sterile loop from this inoculated area in two sections to cover the entire agar surface; flaming or flipping loop between sections. Avoid applying excessive pressure to the agar surface during inoculation to prevent gouging and splitting of the agar media. Incubate plates media side up at 33-37°C for 18-24 hours. (Note: Agar surfaces should be smooth and moist but free of excessive moisture which could cause confluent growth patterns.)

A non-selective medium, such as Tryptic Soy Agar with 5% sheep blood, should also be considered for inoculation to increase the chance of recovering gram negative bacteria present in low numbers and to reflect other bacterial flora present in the specimen.

XI. EXPECTED RESULTS

NCCLS CONTROL ORGANISMS (ATCC STRAINS)

Salmonella typhimurium (ATCC 14028)	Growth, blue to green-blue colonies with black centers
Shigella flexneri (ATCC 12022)	Growth, green to blue-green colonies

(continued)

NCCLS CONTROL ORGANISMS (ATCC STRAINS)

Enterococcus faecalis (ATCC 29212)	Inhibition (partial; yellow colonies)
Escherichia coli (ATCC 25922)	Inhibition (partial to complete; yellow to salmon colonies)

XII. LABORATORY RESULTS

This medium is intended to be used as a primary isolation medium. Colonies exhibiting morphologies suspicious of gram negative enteric pathogens require further testing for definitive identification.

Typical colonial morphology on Hektoen Enteric Agar:

E. coli	Yellow to salmon (some strains may be inhibited)
Enterobacter/ Klebsiella sp.	Yellow to salmon
Proteus sp.	Variable, blue-green to blue or salmon, most strains with black centers
Salmonella sp.	Blue-green to blue, most strains with black centers
Shigella sp.	Green to blue-green
Pseudomonas sp.	Irregular, green to brown

Additional biochemical information may be obtained from reference microbiology texts.[1,2,3]

XIII. LIMITATIONS

The ability to detect microorganisms by culture techniques can be affected by the following factors: improper specimen collection, storage and inoculation , initiation of antiinfective therapy prior to specimen collection, improper culture incubation temperatures and atmospheres, improper length of culture incubation, and improper storage and handling of culture media.

XIV. REFERENCES

1. Finegold, S.M. and W.S. Martin. 1982. Bailey and Scott's Diagnostic Microbiology, 6th ed. C.V. Mosby Company, St, Louis.

2. Koneman, E.S., S.D.Allen, V.R. Dowell, Jr. and H. M. Sommers. 1983. Color Atlas and Textbook of Microbiology, 2nd ed. J.B. Lippincot Company, Philadelphia.

3. Lennette, E.H., ed. 1985. Manual of Clinical Microbiology, 4th ed. American Society for Microbiology, Washington, D.C.

USER QUALITY ASSURANCE/ QUALITY CONTROL PROCEDURES AND INFORMATION

HealthLink recommends that the following quality assurance and quality control procedures be performed on each batch of product.

I. QUALITY ASSURANCE

The following quality assurance procedures must be performed to assure the product will perform according to its intended use within the assigned expiry date:

1. Daily, document that product storage refrigerator maintains temperature within the recommended range: 2-8°C.
2. Daily, document that laboratory incubator maintains temperature within the recommended range: 22-35°C.

II. QUALITY CONTROL

The following incoming inspection procedures must be performed for each batch (batch = same lot, same shipment) of culture media received in the laboratory :

1. Inspect plates according to instructions contained on the "Quality Control Log Sheet." (See also Section VI "STORAGE/SHELF LIFE")
2. Peel off the lower portion of a product bag label (Quality Control Certificate) for the lot being accepted into the laboratory and affix it to the Quality Control Log Sheet.
3. Initial and date the Quality Control Log Sheet.

Note: Notify Technical Service immediately if media does not meet the inspection criteria.

TECHNICAL SERVICE

HealthLink provides a toll free technical service line (1-800-638-2625) to assist with product usage. To receive additional product information, procedural instructions, QA/QC log sheets, Material Safety Data Sheets, or to have technical questions answered; please call between the hours of 9:00 am to 5:00 pm EST.

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1-800-638-2625

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