

## HEKTOEN ENTERIC (HE) AGAR

**PRODUCT:**

**Plate Media:**<sup>a</sup>

Hektoen Enteric Agar, item no. P1700

<sup>a</sup>see catalog for ordering options

**PURPOSE:**

Hektoen Enteric (HE) Agar is a selective and differential medium for the isolation of enteric pathogens from fecal material and food products.

**PRINCIPLE:**

Hektoen Enteric Agar was developed by King and Metzger<sup>1</sup> in an effort to increase the recovery of *Shigella* species over the previously formulated Salmonella-Shigella (SS) Agar. By incorporating larger amounts of peptone to offset the inhibitory effects of bile salts, additional carbohydrates for better differentiation of enteric pathogens, and a less toxic system, the inhibitory effect was reduced, allowing for good recovery of *Shigella*. Three fermentable carbohydrates are present: salicin, sucrose, and lactose. An increased concentration of lactose aids in the recognition of slow lactose fermenters. Coliforms capable of overcoming the moderately inhibitory qualities of the medium will develop into orange or salmon-pink colonies in the presence of the bromthymol blue indicator. *Shigella* species develop into green-colored colonies with darker blue-green centers. *Salmonella* species appear as blue-green colonies with or without black centers. Producers of H<sub>2</sub>S will form black-centered colonies in the presence of the ferric ammonium citrate indicator.

**FORMULAS:**

Approximate, per liter of deionized filtered water.

Peptic Digest of Animal Tissue .....	12.0 g
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
Agar .....	14.0
Acid Fuchsin .....	0.1
Bromthymol Blue .....	65.0 mg
Final pH 7.6 ± 0.2 at 25°C	

**PRECAUTIONS:\***

For in vitro diagnostic use. Observe approved biohazard precautions.

**Storage:** Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

**Limitations:** *Proteus* may or may not be inhibited and colonies may resemble *Salmonella* or *Shigella*. Additional biochemical testing must be done to confirm pathogen identification.

Processing delays in excess of 2-3 hours (see "Specimen Collection") of an unpreserved stool specimen greatly jeopardizes the recovery of most shigellas and many salmonellas. These organisms are very susceptible to the acidic change that occurs with a drop in temperature of the feces.

**PROCEDURE:**

**Specimen Collection:** Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and should be delivered to the laboratory within 2-3 hours. Stool specimens require special attention. Specimens should be collected early in the course of the disease, and stool specimens need to be cultured within two hours after collection. *Shigella* species are delicate and are best recovered by inoculating the media directly at the bedside. If there is delay, suitable transport media such as Cary-Blair Transport Medium or Enteric Pathogen Transport must be used to maintain the viability of the organisms.

**Method of Use:** Prior to inoculation, the medium should be brought to room temperature. Directly inoculate the specimen or sample from transport media onto the agar using standard microbiological procedures. Streak the inoculum so as to obtain isolated colonies. Incubate aerobically for 18-24 hours at 35°C. Further incubation up to 48 hours will improve differentiation between *Salmonella* and *Shigella*. Select nonfermenting colonies with or without H<sub>2</sub>S production for additional biochemical and serological testing.

**Interpretation:**

<i>Salmonella</i> species	Blue to blue-green colonies, with or without black centers
<i>Shigella</i> species	Green with blue-green centers
<i>Escherichia coli</i> (coliforms)	Inhibited - if growth, yellow colonies surrounded by orange or salmon-pink zones.
<i>Citrobacter</i> species	Inhibited - if growth, small blue-green colonies
<i>Proteus</i> species	If growth - small glistening blue-green colonies with or without black centers.
<i>Pseudomonas aeruginosa</i>	If growth - small flat, green-brown colonies

**Materials Required but Not Provided:** Standard microbiological supplies and equipment such as loops, needles, incubators, and incinerators are not included.

**QUALITY CONTROL:\***

**Microorganisms Used (ATCC#):**

*Salmonella choleraesuis* ssp. *choleraesuis* (14028)  
*Shigella flexneri* (12022)  
*Escherichia coli* (25922)  
*Enterococcus faecalis* (29212)

**Expected Results:**

Growth  
Growth  
Inhibition, partial to complete  
Inhibition  
Key: See "Interpretation"

**User Quality Control:** Check for signs of contamination and deterioration.

**BIBLIOGRAPHY:**

- King, S., and W. I. Metzger, *Proc. Am. Soc., Microbiol.*, 1967, p. 77.
- King, S., and W. I. Metzger, *Appl. Microbiol.*, 16:579-581, 1968.
- Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 3rd ed., J. B. Lippincott, Philadelphia, 1988.
- Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1988.
- MacFaddin, J. F., *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*, vol. 1, Williams and Wilkins, Baltimore, 1985.

\*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

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## SALMONELLA-SHIGELLA (SS) AGAR

**PRODUCT:**

**Plate and Tube Media:<sup>a</sup>**

Salmonella-Shigella Agar, item no. P2350 (plate), T7480 (tube)

<sup>a</sup>see catalog for ordering options

**PURPOSE:**

Salmonella-Shigella (SS) agar is a highly selective and differential medium primarily used for the isolation of *Salmonella* species from stool or food samples.

**PRINCIPLE:**

Original investigators of SS Agar modified Deoxycholate Citrate Agar, described by Leifson<sup>3</sup>, by adding mixtures of bile salts to inhibit gram-positive and coliform bacteria. Almost all *Salmonella* species grow well in the presence of brilliant green and bile salts; however, many strains of *Shigella* grow poorly or not at all. *Salmonella* and other lactose nonfermenters appear as clear, translucent colonies, some with black centers indicating H<sub>2</sub>S production.

In addition to its use as a selective and differential medium for enteric pathogens growth on SS Agar is also used as a method of differentiating between various gram-negative nonfermenting organisms, including *Pseudomonas* species.

**FORMULA:**

Approximate, per liter of deionized filtered water.

Beef Extract .....	5.00 g
Peptic Digest of Animal Tissue .....	2.50
Pancreatic Digest of Casein .....	2.50
Lactose .....	10.00
Bile Salts Mixture .....	8.50
Sodium Citrate .....	8.50
Sodium Thiosulfate .....	8.50
Ferric Citrate .....	1.00
Agar .....	13.50
Neutral Red .....	25.00 mg
Brilliant Green .....	0.33
Final pH 7.0 ± 0.2 at 25°C	

**PRECAUTIONS:\***

For in vitro diagnostic use. Observe approved biohazard precautions.

**Storage:** Upon receipt store, at 2-8°C in the dark. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

**Limitations:** Studies by Taylor and Harris<sup>7</sup> found SS Agar supported the growth of only 25% of *Shigella* species, with the commonly isolated enteric pathogens *S. sonnei* and *S. dysenteriae* being completely inhibited. For this reason, the use of SS Agar as a single selective medium for enteric pathogen isolation is not recommended.

Occasional coliforms and other lactose fermenters may grow and develop into pink or red colonies. Some *Proteus* may form black-centered colonies.

Processing delays in excess of 2-3 hours (see "Specimen Collection") of an unpreserved stool specimen greatly jeopardizes the recovery of most shigellas and many salmonellas. These organisms are very susceptible to the acidic change that occurs with a drop in temperature of the feces.<sup>8</sup>

SS Agar is very light-sensitive. Protect from all sources of light.

Salmonella-Shigella Agar in a tube is used for identification purposes only; direct inoculation of specimens may lead to erroneous results.

**PROCEDURE:\***

**Specimen Collection:** Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and delivered to the laboratory within 2-3 hours. If there is delay, suitable transport media such as Cary-Blair or Enteric Pathogen Transport must be used to maintain the viability of the organisms.

**Method of Use, Plate:** Prior to inoculation, the medium should be brought to room temperature. Directly inoculate stool or well-mixed specimen from the transport media onto the agar using standard microbiological procedures. Streak the inoculum so as to obtain isolated colonies. Incubate aerobically at 35°C for 18-24 hours.

Select lactose nonfermenting colonies with or without H<sub>2</sub>S production for additional biochemical and serological testing.

**Method of Use, Tube:** Prior to inoculation, the medium should be brought to room temperature. Inoculate from a pure 18- to 24-hour culture onto the slant using a fishtail motion. Incubate aerobically at 35°C and examine after 24-48 hours for growth.

**Interpretation:**

*Salmonella* species

**Colonial Morphology:**

Colorless colonies with or without black centers

*Shigella flexneri*

Colorless colonies

*Shigella sonnei*

No growth

*Shigella dysenteriae*

No growth

*Escherichia coli*

Inhibited; if growth, salmon-pink colonies

*Pseudomonas* species

Inhibited; if growth, small transparent colony

*Proteus* species

Inhibited; if growth, colorless colonies with or without gray-black centers

*Citrobacter* species

**Materials Required but Not Provided:** Standard microbiological supplies such as incubators, loops, incinerators, and transport medium are not provided.

**QUALITY CONTROL:\***

**Microorganisms Used (ATCC#):**

*Salmonella choleraesuis* ssp. *choleraesuis* (14028)

*Shigella flexneri* (12022)

*Escherichia coli* (25922)

*Enterococcus faecalis* (29212)

**Expected Results:**

Growth

Growth

Inhibition, partial (colonies salmon pink) to complete

Inhibition

**User Quality Control:** Check for signs of contamination and deterioration.

**BIBLIOGRAPHY:**

1. Finegold, S. M., and E. J. Baron, *Bailey and Scott's Diagnostic Microbiology*, 7th ed., C. V. Mosby, St. Louis, 1986.
2. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 3rd ed., J. B. Lippincott, Philadelphia, 1988.
3. Leifson, E. J., *J. Pathol. Bacteriol.*, 40:581, 1935.
4. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.
5. MacFaddin, J. F., *Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria*, vol. 1, Williams and Wilkins, Baltimore, 1985.
6. Sack, R. B., et al., *Cumitech 12*, American Society for Microbiology, Washington, D. C., 1980.
7. Taylor, W. I., et al., *Am. J. Clin. Pathol.*, 35:476, 1965.

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