HEKTOEN MEDIUM

Principle and Purpose

Species of the Gram-negative bacteria *Salmonella* and *Shigella* cause disease in both humans and other animals. Most often they are associated with contaminated food or water resulting in local, and sometimes regional, epidemics. It is critical to quickly identify these disease agents to begin proper treatment as well as for preventative and epidemiological purposes.

A selective and differential medium mainly used to isolate *Salmonella* and *Shigella* species from clinical or environmental samples is Hektoen enteric agar (HEA; Fig. 1). This medium, which distinguishes *Salmonella* and *Shigella* from other members of the *Enterobacteriaceae*, was developed in 1968 by Sylvia King and William I. Metzger at the Hektoen Institute in Chicago (hence, the name Hektoen enteric agar to honor their place of employment). Prior to the development of HEA, *Salmonella-Shigella* (SS) agar was often used for this purpose, but this medium is not ideal for the isolation of *Shigella* species. Therefore, King and Metzger sought to improve the isolation *Shigella* from mixed cultures and clinical specimens. Their version of HEA possessed extra amounts of carbohydrates and peptones as well as the dyes bromothymol blue and acid fuchsin in place if brilliant green and neutral red. The additional carbohydrates and peptones helped to offset the inhibitory effects of the bile salts used in the medium. The new dyes were less toxic than others, thus improving the recovery of pathogens.

The present composition of HEA incorporates the fermentable carbohydrates salicin, sucrose, and lactose. However, *Salmonella* and *Shigella* cannot use these carbohydrates, instead relying on peptone as a carbon source. The metabolism of peptone by *Shigella* and *Salmonella* causes HEA to become alkaline, thereby turning the medium blue in color due to by the pH indicator, bromothymol blue. In addition, the medium contains sodium thiosulfate and ferric ammonium citrate. The former provides a source of sulfur for sulfur-reducing bacteria. The latter provides a source of iron that forms a black precipitate upon the production of hydrogen sulfide. Hence, *Salmonella* species, which does produce hydrogen sulfide, appear as black colonies on HEA. In contrast, *Shigella* species, which do not produce hydrogen sulfide, appear as green colonies.

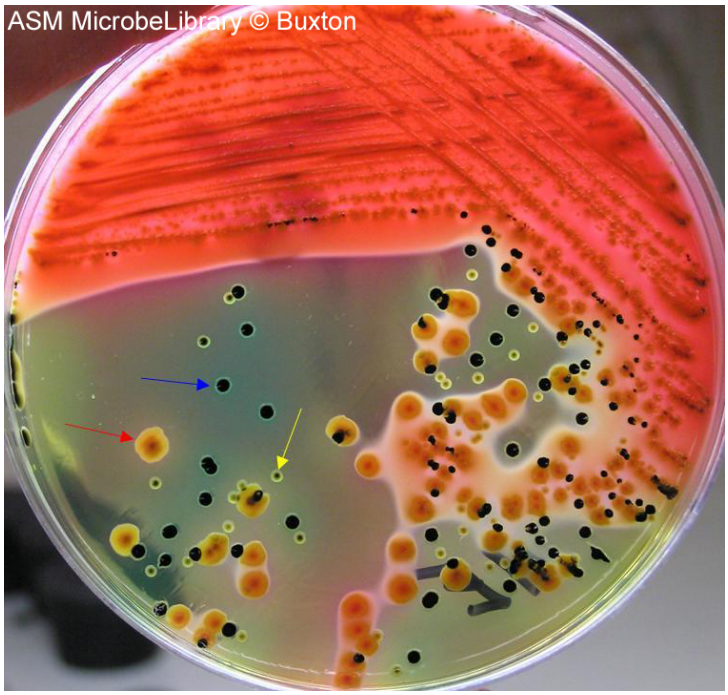

Figure 1. HEA streak plate of mixed flora. Isolated colonies include *Escherichia coli* (red arrow), *Salmonella* (blue arrow), and *Proteus vulgaris* (yellow arrow).

The selective nature of HEA is due to the presence of bile salts. The bile salts inhibit growth of Gram-positive bacteria and are less toxic to Gram-negative bacteria. The higher concentration of lactose and the addition of sucrose to HEA, compared to SS agar, enables the differentiation of slow lactose

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fermenters. Since most bacteria prefer to use sugars over peptone, the medium becomes acidic, causing the pH indicator, acid fuchsin, to turn the medium yellow or red.

Few sulfur-reducing bacteria, other than *Salmonella*, can be isolated from the intestines. Most of these are inhibited by bile salts. Rarely is a black colony that is not *Salmonella* isolated on HEA. However, in those unusual circumstances, such colonies appear red or yellow colonies with a black center. The red or yellow color indicates sugar fermentation which is most atypical of *Salmonella*.

The following exercise provides a background experience for students in the use and understanding the purpose of differential and selective media.

Learning Objectives

Upon completion of the following exercises, a student will be able to demonstrate the ability to:

- Understand the underlying chemical/physiological mechanisms that make Hektoen medium both a selective and differential medium;
- Properly use Hektoen medium to distinguish *Salmonella* and *Shigella* from other types of bacteria; and
- Accurately interpret the results of these tests.

Materials Required

Note of Precaution: Some of the bacteria used in the following exercises are potential pathogens. Use appropriate handling procedures with these cultures.

The following materials are necessary to successfully conduct this exercise:

Organisms: – these cultures should be 24-48 hours old and prepared as TSA slants

- *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
- *Staphylococcus epidermidis* (ATCC 12228) [abbreviated as *S. epidermidis*]
- *Shigella flexneri* (ATCC 12022) [abbreviated as *S. flexneri*]
- *Salmonella enterica* serovar Choleraesuis (ATCC 10708) [abbreviated as *S. enterica*]

Medium:

- Hektoen Enteric Agar (HEA) plates

Procedures

Students shall review and use the BIOL 3702L Standard Practices regarding the labeling, incubation, and disposal of materials.

1) Obtain two (2) Hekteon Enteric Agar (HEA) plates. On the bottom (agar side), label them accordingly.
2) In addition, on the bottom of each plate, divide it in half. On one plate, label one side as *E. coli* and the other as *S. flexneri*. On the second plate, label one side as *S. epidermidis* and the other as *S. enterica*
3) Inoculate the media as indicated below:

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a) Using a microbiological loop, aseptically transfer a minute amount of cells from a TSA slant culture of *E. coli* to the appropriately labeled portion of the HEA plate. Streak the section in an attempt to generate isolated colonies.

b) Using a microbiological loop, aseptically transfer a minute amount of cells from a TSA slant culture of *S. flexneri*to the appropriately labeled portion of the HEA plate. Streak the section in an attempt to generate isolated colonies.

c) Using a microbiological loop, aseptically transfer a minute amount of cells from a TSA slant culture of *S. epidermidis* to the appropriately labeled portion of the HEA plate. Streak the section in an attempt to generate isolated colonies.

d) Using a microbiological loop, aseptically transfer a minute amount of cells from a TSA slant culture of *S. enterica* to the appropriately labeled portion of the HEA plate. Streak the section in an attempt to generate isolated colonies.

4) Incubate all the plates at 37°C for 18-24 hours.

Note: Be sure to make your observations within 24 hours. Observations beyond this time frame may not be valid.

5) Remove both plates from the incubator.

Record any observations on the data report sheet attached to this document.

Interpretation of Results: The following table provides a guide to the interpretation of the results using HEA plates.

Observation	Interpretation
Growth on the HE agar plate	The organism is not inhibited by bile salts
Yellow or orange precipitate around the colonies	Bile salts have been precipitated by the organism. Typical of some nonpathogens
Yellow	Fermentation of lactose, sucrose, or salicin; not likely to be an enteric pathogen
Salmon to orange	Fermentation of salicin, not likely to be an enteric pathogen
Yellow, salmon to orange with black centers	Fermentation of one of the carbohydrates plus the production of H_2S; not likely to be an enteric pathogen (other than the rare lactose-fermenting *Salmonella*)
Greenish-blue, light green, or transparent	No fermentation present, suspect *Shigella;* confirm with additional tests
Greenish-blue, light green, or transparent with black centers	No fermentation present, H_2S production present, suspect *Salmonella,* confirm with additional tests

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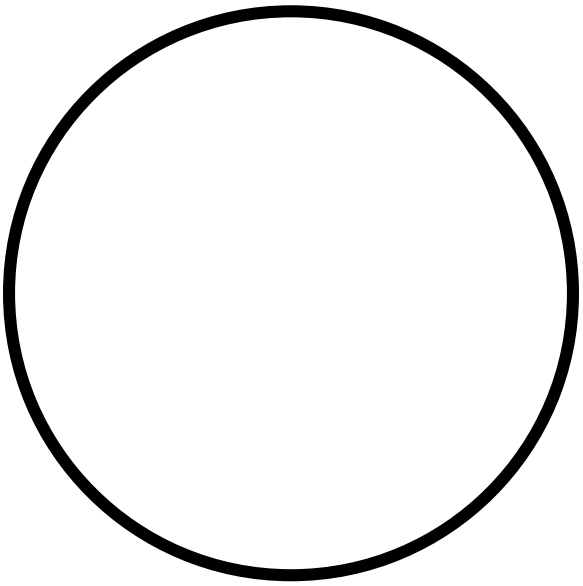
Student Name: _____

Colony Appearance of Selected Bacteria on Hektoen Enteric Agar

Organism	Colony Appearance and Interpretation
Salmonella enterica	
Staphylococcus epidermidis	
Shigella flexneri	
Escherichia coli	

GROWTH RESPONSE

Draw and label the observed growth responses of the selected bacteria on HEA.



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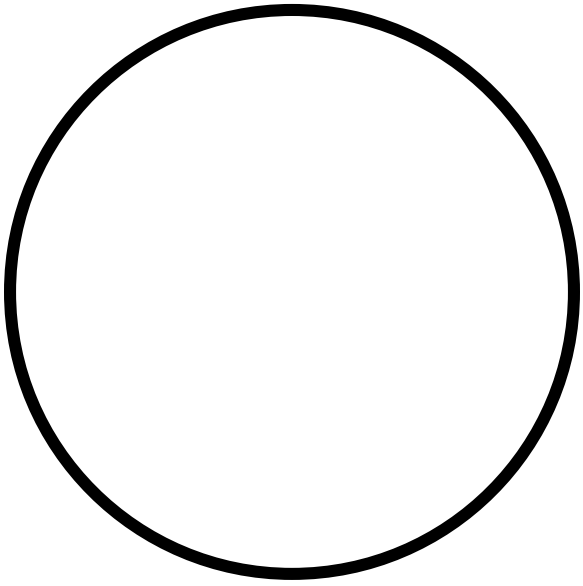

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