

SELECTIVE AND DIFFERENTIAL MEDIA

Principle and Purpose

Microorganisms are generally studied after being grown on or in a microbiological medium. There is an expansive variety of useful media for this purpose including many types of general growth media that support a wide spectrum of organisms. Examples include defined media, such as glucose-salts, as well as rich, chemically complex media such as brain heart infusion agar and tryptic soy broth. However, these general growth media may permit the growth unwanted microorganisms from clinical or environmental samples that are not relevant to the purpose of the microbiological examination. To help resolve this issue, the components of different media are sometimes adjusted to enhance the growth of particular organisms or, alternatively, to retard the growth of unwanted microbes. In the clinical laboratory, this ability to preferentially grow one type of microbe over another is critical in determining the cause of an infection and perhaps the means by which to treat the disease. Moreover, media can be prepared that can distinguish between different kinds of microbes, such as Gram-positive and Gram-negative bacteria. Often, in a clinical setting, the most helpful type of medium is one that is both selective and differential.

Functionally, **selective media** permit certain types of organisms to grow while inhibiting the growth of other microbes. The selectivity can be accomplished by adding dyes, antibiotics, salts, particular chemicals or inhibitors which alter a microbe's metabolism. In the case of **differential media**, the addition of certain dyes or chemicals to the medium will cause microbes to produce characteristic changes or growth patterns that may be used for identification or differentiation.

Microbiologists employ a seemingly unending variety of selective and differential media, alone or in combination, to study specific microbes – especially those of economic or medical importance. In this exercise on four types of selective and/or differential media will be examined. Though this exercise is not a comprehensive study of selective and differential, the student will gain valuable exposure, as well as experience, in working with and understanding these special types of media.

Hektoen Enteric (HE) Agar

HE agar is a selective and differential medium used for the isolation and differentiation of Gram-negative enteric pathogens, i.e., disease-causing microbes typically found in the intestinal tract of humans and other animals. This medium was originally to help increase the recovery of *Salmonella* and *Shigella* species. HE agar is particularly useful in the isolation of *Shigella* species.

HE agar incorporates bile salts which allow for the selective nature of this medium by inhibiting Gram-positive bacteria, though these salts can also be toxic for certain Gram-negative strains,



Figure 1. Stool culture on Hektoen enteric agar: *Salmonella* (black colonies) mixed with normal fecal flora (orange-yellow colonies).

but not *Salmonella* and *Shigella* species. HE agar also contains salicin, sucrose, and lactose as fermentable carbohydrates. Bromothymol blue and acid fuchsin in HE agar serve as acid-base indicators, whereas the presence of ferric ammonium citrate and sodium thiosulfate enable the detection of H₂S, as noted by the formation of black-centered colonies.

After inoculation and incubation, colonies of *Salmonella* and *Shigella* species are green to bluish-green in color (Fig. 1). In addition, those *Salmonella* species that produce H₂S shall appear as blue-green colonies with black centers. Alternatively, non-*Salmonella* and non-*Shigella* species that ferment lactose, sucrose, or salicin shall give rise to yellow-orange to salmon colored colonies.

MacConkey Agar (MAC)

MAC a selective and differential medium for the isolation of Gram-negative bacilli, including coliforms and enteric pathogens. This medium was one of the earliest culture media for the cultivation and identification of enteric organisms and is also used in the isolation of pathogens from foods and coliforms in water samples. Like HE agar, MAC incorporates bile salts, in addition to sodium chloride, which inhibits the growth of most Gram-positive bacteria. Differentiation of enteric microorganisms is achieved by the fermentation of lactose in the presence of bile salts and a neutral red indicator. Lactose-fermenting microbes form pink colonies surrounded by a zone of precipitated bile salts (Fig. 2). The production of acid from lactose fermentation changes the neutral red pH indicator from colorless to red. The acid production is also responsible for the formation of bile salt precipitation. Non-lactose-fermenters (e.g., *Salmonella* and *Shigella* species) develop as transparent, colorless colonies with no surrounding bile precipitate.

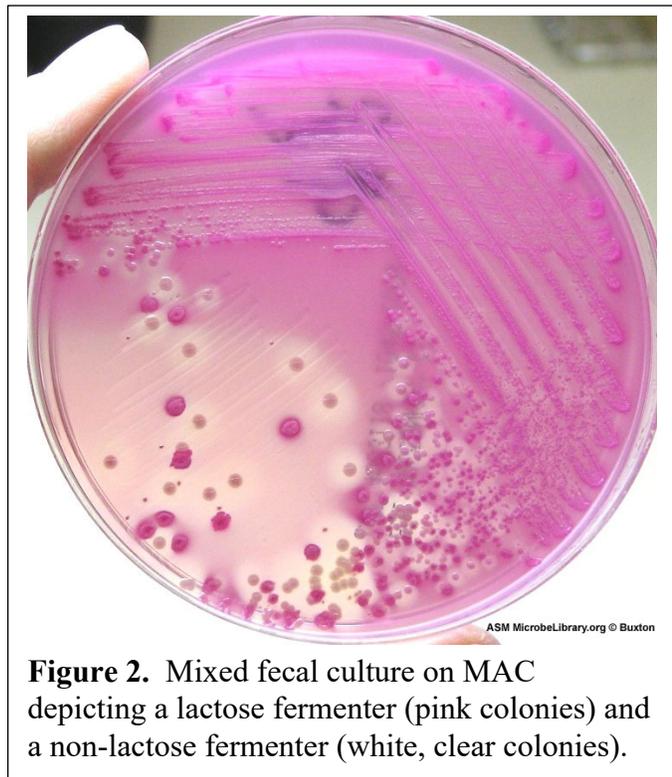


Figure 2. Mixed fecal culture on MAC depicting a lactose fermenter (pink colonies) and a non-lactose fermenter (white, clear colonies).

Eosin Methylene Blue (EMB) Agar

EMB agar was developed for the isolation of enteric bacilli. The incorporation of the dyes eosin and methylene blue inhibit the growth of Gram-positive bacteria in addition to serving as differential indicators for the fermentation of lactose. The production of acid from lactose fermentation results in an eosin-methylene blue dye complex being taken up by the microbial cell, thereby imparting a brown to blue-black colony appearance. EMB agar has often been used to detect fecal and non-fecal coliforms and is recommended for use in the microbiological examination of potable water, waste water, dairy products, and foods.

On EMB agar, isolated colonies of lactose-fermenting bacteria appear brown to blue-black in color. *Escherichia coli* grow as large, blue-black colonies that often possess a green metallic sheen (Fig. 3). However, not all *E. coli* strains produce a green metallic sheen. Hence, the green

metallic should not be used as the ultimate diagnostic for this microbe. Non-lactose-fermenting colonies, such as *Shigella* and *Salmonella* species, appear transparent and colorless, though some strains of these two genera fail to grow on EMB agar. Occasionally, some Gram-positive bacteria, such as *Enterococcus* and *Staphylococcus* will grow on this medium, but usually as pinpoint colonies. Non-pathogenic, non-lactose-fermenting organisms may grow on EMB agar as well.

Mannitol Salt Agar (MSA)

Koch used media containing 7.5% sodium chloride as a selective agent for the isolation of *Staphylococcus*. Eventually, this observation was combined with the fact that *Staphylococcus aureus* usually ferments mannitol. The result was the formulation of MSA, which is recommended for the enumeration of staphylococci in food and dairy products. In MSA, the sodium chloride concentration of 7.5% is nearly ten times that usually found in most microbiological media. This high salt concentration serves to inhibit most organisms except staphylococci and some halophilic marine microbes. Mannitol and phenol red in MSA help demonstrate the fermentation capabilities of the organism being examined. Mannitol fermenters, such as *Staphylococcus aureus*, appear as yellow colonies with yellow zones in the media. Non-mannitol fermenters, such as *Staphylococcus epidermidis*, shall form clear pink to red colonies with no yellow color change in the medium.

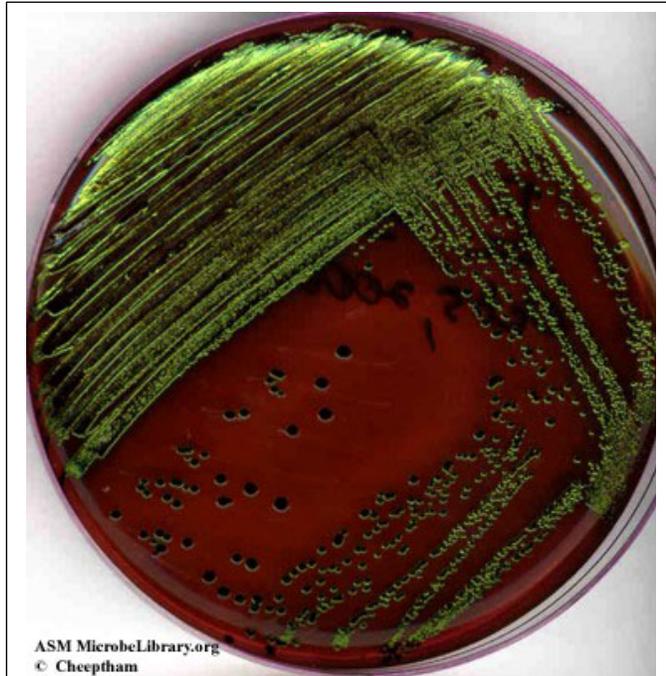


Figure 3. *Escherichia coli* growing on EMB.



Figure 4. MSA inoculated with *Staphylococcus aureus* (sector A), *Staphylococcus epidermidis* (sector B), and *Escherichia coli* (sector C).

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the underlying functional basis of the various media employed;
- Properly conduct testing with these media; and
- Accurately interpret the results generated after inoculating these media.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms – The following organisms should be provided as 24-48 hour TSB cultures:

- *Escherichia coli* (ATCC 25922) [abbreviated as *E. coli*]
- *Salmonella enterica* serovar Cholerasuis (ATCC 10708) [abbreviated as *S. cholerasuis*]
- *Shigella flexneri* (ATCC 12022) [abbreviated as *S. flexneri*]
- *Staphylococcus aureus* (ATCC 25923) [abbreviated as *S. aureus*]
- *Staphylococcus epidermidis* ATCC 12228) [abbreviated at *S. epidermidis*]
- *Streptococcus pyogenes* (ATCC 19615) [abbreviated at *S. pyogenes*]

Media

- Hektoen Enteric (HE) agar plates
- MacConkey (MAC) agar plates
- Eosin Methylene Blue (EMB) agar plates
- Mannitol Salt Agar (MSA) plates

Procedures

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

Microbial Growth on HE Agar Plates

- 1) Obtain two (2) HE agar plates. On the bottom of each plate, use a Sharpie marker to divide each plate into two halves.

Note: The bile salts in the HE agar may crystallize over time and appear as small spider-like entities within the medium. These will not affect the performance of the medium. No worries!!!

- 2) Within one half of one divided HE agar plate, label it as '*S. cholerasuis*'. Label the other half as '*S. aureus*'.
- 3) On the second of the two HE agar plates, within one half of the divided plate mark label it as '*E. coli*'. Label the other half as '*S. flexneri*'.
- 4) On the surface of each HE agar plate, use a sterile loop to streak a loopful of the appropriate bacterium in a single direction within the half that is marked for that particular species.
- 5) Incubate both plates inverted (i.e., agar side up) at 35-37°C for 18-24 hours.
- 6) Remove the plates from the incubator and examine each for growth to include colony morphology and color.

Record your observations on the report sheet attached to this exercise.

Microbial Growth on MAC Agar Plates

- 1) Obtain three (3) MAC agar plates.
- 2) On the bottom of one MAC agar plate, use a Sharpie marker to label it as '*E. coli*'.
- 3) On the bottom of a second MAC agar plate, use a Sharpie marker to label it as '*S. cholerasuis*'.



- 4) On the bottom of the third MAC agar plate, use a Sharpie marker to label it as '*S. aureus*'.
- 7) On the surface of each MAC agar plate, use a sterile loop perform a 3-phase streak of a loopful of the appropriate bacterium on the plate that is marked for that particular species.
- 8) Incubate all three plates inverted (i.e., agar side up) at 35-37°C for 18-24 hours.
- 9) Remove the plates from the incubator and examine each for growth to include colony morphology and color.

Record your observations on the report sheet attached to this exercise.

Microbial Growth on EMB Agar Plates

- 1) Obtain three (3) EMB agar plates.
- 2) On the bottom of one EMB agar plate, use a Sharpie marker to label it as '*E. coli*'.
- 3) On the bottom of a second EMB agar plate, use a Sharpie marker to label it as '*S. choleraesuis*'.
- 4) On the bottom of the third EMB agar plate, use a Sharpie marker to label it as '*S. aureus*'.
- 5) On the surface of each EMB agar plate, use a sterile loop perform a 3-phase streak of a loopful of the appropriate bacterium on the plate that is marked for that particular species.
- 6) Incubate all three plates inverted (i.e., agar side up) at 35-37°C for 18-24 hours.
- 7) Remove the plates from the incubator and examine each for growth to include colony morphology and color.

Record your observations on the report sheet attached to this exercise.

Microbial Growth on MSA Plates

- 1) Obtain two (2) MSA plates. On the bottom of each plate, use a Sharpie marker to divide each plate into two halves.
- 2) Within one half of one divided MSA plate, label it as '*S. aureus*'. Label the other half as '*E. coli*'.
- 3) On the second of the two MSA plates, within one half of the divided plate mark label it as '*S. epidermidis*'. Label the other half as '*S. pyogenes*'.
- 4) On the surface of each MSA plate, use a sterile loop to streak a loopful of the appropriate bacterium in a single direction within the half that is marked for that particular species.
- 5) Incubate both plates inverted (i.e., agar side up) at 35-37°C for 18-24 hours.
- 6) Remove the plates from the incubator and examine each for growth to include colony morphology and color.
- 7) Remove the plates from the incubator and examine each for growth to include colony morphology and color.

Record your observations on the report sheet attached to this exercise.

Student Name: _____

COMPLETE THE FOLLOWING TABLE BASED UPON YOUR OBSERVATIONS

Medium Tested: Hektoen Enteric Agar

Observations	Bacteria Tested			
	<i>Escherichia coli</i>	<i>Salmonella cholerasuis</i>	<i>Shigella flexneri</i>	<i>Staphylococcus aureus</i>
Growth (Yes or No)				
Color of Colonies				

Additional Notes:

Medium Tested: MacConkey Agar

Observations	Bacteria Tested		
	<i>Escherichia coli</i>	<i>Salmonella cholerasuis</i>	<i>Staphylococcus aureus</i>
Growth (Yes or No)			
Color of Colonies			

Additional Notes:

Medium Tested: Eosin Methylene Blue Agar

Observations	Bacteria Tested		
	<i>Escherichia coli</i>	<i>Salmonella cholerasuis</i>	<i>Staphylococcus aureus</i>
Growth (Yes or No)			
Color of Colonies			

Additional Notes:

Medium Tested: Mannitol Salt Agar

Observations	Bacteria Tested			
	<i>Escherichia coli</i>	<i>Streptococcus pyogenes</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
Growth (Yes or No)				
Color of Colonies				

Additional Notes: