Selective and Differential Media Testing
(SM-0202-00-01)

Exploration Overview

Estimated Completion Times

- **Exploration** 30 min
- **Experimentation** 1-2 day incubation + 2 hrs
- **Evaluation**

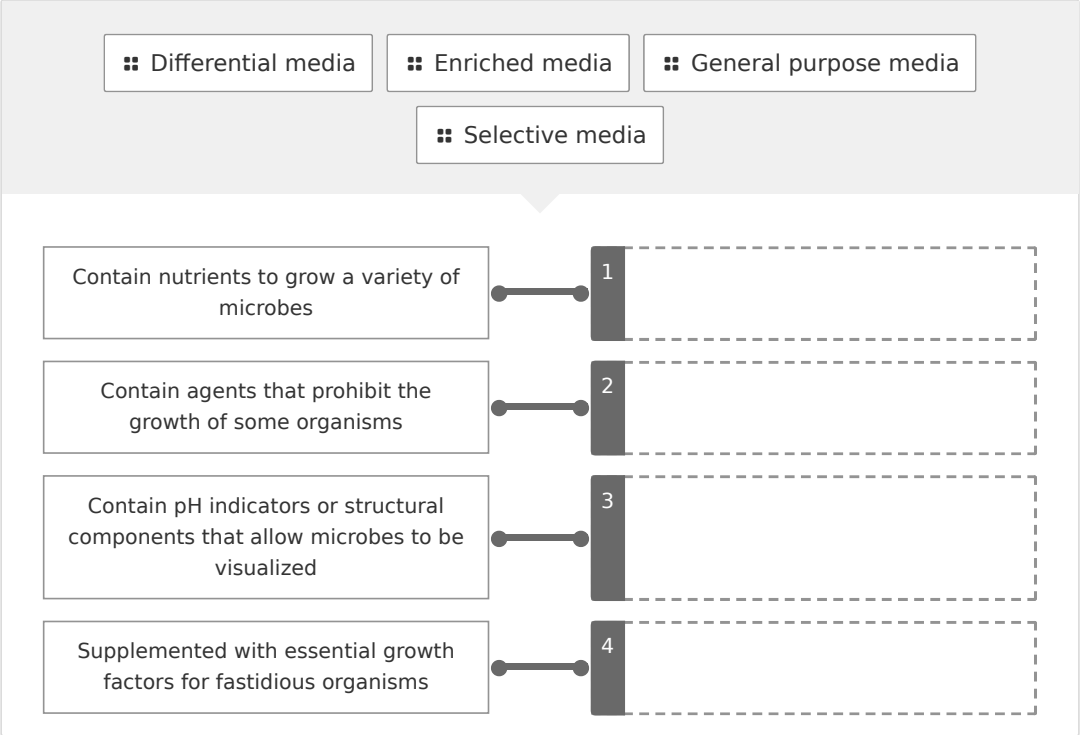
Exploration Summary

This Exploration contains targeted background content to prepare you for performing the exercises in this lesson.

Learning Objectives

- Describe the purpose of general purpose, functional, selective, and differential media.
- Discuss the functional properties of MacConkey and Levine eosin-methylene blue agars.

Match each term to the best description.



Correct answers:

1 General purpose media

2 Selective media

3 Differential media

4 Enriched media

Classify each statement as true or false.

MacConkey (MAC) and Levine eosin-methylene blue (EMB) agars are examples of functional media with both selective and differential properties.

MacConkey agar inhibits the growth of most Gram-negative bacteria.

Both MAC and EMB agars are used in laboratories to differentiate enteric species living in the guts of humans and other animals.

Levine EMB agar turns lactose fermenting bacteria pink.

True	False
1	2

Correct answers:

1

MacConkey (MAC) and Levine eosin-methylene blue (EMB) agars are examples of functional media with both selective and differential properties.

Both MAC and EMB agars are used in laboratories to differentiate enteric species living in the guts of humans and other animals.

2

MacConkey agar inhibits the growth of most Gram-negative bacteria.

Levine EMB agar turns lactose fermenting bacteria pink.

Explore Media Types

Microbiology media used to grow microorganisms in the laboratory may be classified as general purpose or functional. **General purpose media** contain nutrients that grow a variety of microbes and include the nutrient broth and tryptic soy agar (TSA) supplied in Science Interactive kits. **Functional media** contain compounds that facilitate the identification of physical and metabolic characteristics of microbes. Functional media may be divided into three categories: selective media, differential media, and enriched media. Select each type of functional media in the interactive below to explore its properties and uses.

Tryptic soy agar (TSA) is an example of a differential media.

- ○ True
- ○ False ✔

Explore MacConkey and Levine Eosin-Methylene Blue Agars

MacConkey and Levine eosin-methylene blue agars are examples of functional media with both selective and differential properties. Both agars contain dyes that select against many Gram-positive bacteria and pH indicators that differentiate between lactose fermenting Gram-negative bacteria. (A review of the Gram stain theory is provided **here**.) **MacConkey (MAC) agar** is formulated with lactose, bile salts, crystal violet, neutral red, and general purpose nutrients. The bile salts and crystal violet dye of MAC agar inhibit the growth of many Gram-positive bacteria, and the lactose and neutral red indicator stain Gram-negative bacteria that vigorously ferment lactose a pink color. See Figure 1A. **Levine eosin-methylene blue (EMB) agar** is formulated with lactose, sucrose, methylene blue, eosin Y, and general purpose nutrients. The methylene blue dye of EMB agar inhibits the growth of most Gram-positive bacteria, and the lactose and eosin Y indicator produce a green sheen on Gram-negative bacteria that vigorously ferment lactose. See Figure 1B. Bacteria that do not ferment lactose may grow on both MAC and EMB agar, but these microbes will remain translucent because they are not stained by the pH indicators in each formulation. In general, both MAC and EMB agars are used in laboratories to differentiate enteric species living in the guts of humans and other animals.

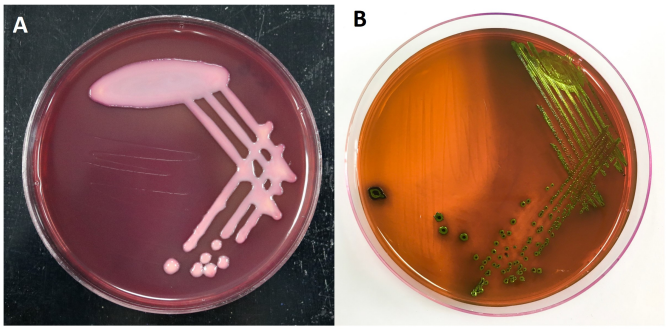


Figure 1. MacConkey (MAC) and Levine eosin-methylene blue (EMB) agar plates. **A.** MAC with pink-stained lactose fermenting bacteria. **B.** EMB agar with lactose fermenting bacteria displaying a green sheen.

Did you know?

Methylene blue is an organic chloride salt with numerous applications. Aside from an antimicrobial in functional media, methylene blue is used as an antidepressant, a cardioprotective agent, a histological dye, and a neuroprotective agent. Historically, methylene blue was widely used in Africa to treat malaria before chloroquine and other drugs were developed for this specific purpose.

_____ agar is formulated with lactose, bile salts, crystal violet, neutral red, and general purpose nutrients.

- Levine eosin-methylene blue (EMB)
- MacConkey (MAC) ✔
- Tryptic soy (TSA)
- Motility

The _____ dye of EMB agar inhibits the growth of most Gram-positive bacteria.

- lactose
- eosin Y
- natural red
- methylene blue ✔

Experimentation Overview

- **Exploration** 30 min

Estimated Completion Times

- **Experimentation** 1-2 day incubation + 2 hrs
- **Evaluation**

Experimentation Summary

This section will guide you through competency-building exercises. You will be challenged with applying the knowledge you gained in the Exploration to complete the activities successfully.

Learning Objectives

- Culture *S. epidermis* and *E. coli* on TSA, MAC, and EMB agar plates.
- Relate experimental results to the cell wall structure and metabolic properties of microbes.

Materials

Read through the procedures listed in the exercises on the next pages before beginning. Then, gather all of the materials listed below and begin Exercise 1.

Note: The packaging and/or materials in your kit may differ slightly in appearance from images in the experimental procedures.

Student Supplied

1	Active culture broth - S*. epidermidis*
1	Active culture broth - *E. coli*
1	Bottle of bleach
1	Bottle of hand soap
1	Bottle of isopropyl alcohol
1	Digital camera or smartphone
1	Large cooking pot (at least 8" deep)
1	Pen or pencil
1	Roll of paper towels
1	Sheet of paper
1	Source of tap water
1	Stove or hotplate

Science Interactive Supplied

1	Apron
1	Face mask with ear loops
2	Inoculating loop
1	Levine (EMB) agar, 18 mL tube
1	MacConkey agar (MAC), 18 mL tube
1	Matches
1	Pair of gloves

1	Pair of safety goggles
1	Permanent marker
3	Petri dish, 90 mm
1	Plastic cup, 9 oz
1	Resealable plastic bag
1	Ruler
1	Tea candle
1	Test Tube rack, 6 x 21 mm
1	Test tube clamp
1	Thermometer
1	Tryptic soy agar (TSA), 18 mL tube

Exercise 1 - Comparison of Media Types

In this exercise, you will inoculate TSA, MAC, and EMB agar plates with pure cultures of *S. epidermis* and *E. coli*. You will then examine and characterize developed cultures.

Note: View the following video for a demonstration of how to prepare agar plates before you continue the procedures.

Procedure

1. Wash and thoroughly dry your hands.
2. Put on your safety goggles, gloves, face mask, and apron.
3. Prepare one 90 mm tryptic soy agar (TSA) plate, one 90 mm MacConkey (MAC) agar plate, and one 90 mm Eosin Methylene Blue Levine (EMB) agar plate.

Note: One TSA pour tube should be used to fill each of the 90 mm Petri dishes. Download the Pouring Agar Plates supplemental document for detailed instructions.

4. Allow the agar plates to cool for 30 minutes or until the appearance of the agar changes from translucent to opaque.
5. Select and clear a non-porous work surface.
6. Disinfect the work surface by wiping with isopropyl alcohol and a paper towel or by using a disinfecting wipe.
7. Fill a sealable plastic bag 1/2 full of undiluted bleach and seal the bag.
8. Fill a clean plastic cup 1/4 full of 70% isopropyl alcohol.
9. Invert the plates so that the agar side (bottom) is facing upwards.
10. Use the permanent marker and ruler to divide the bottom of the agar plates into halves.
11. Label one half of each plate "E. coli" and the other half of each plate "S. epi." See Figure 2.

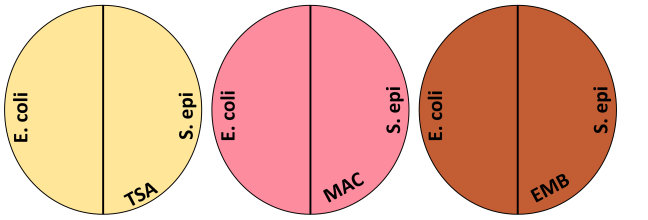


Figure 2. Labeled TSA, MAC, and EMB agar plates.

12 Place a tea candle on the work surface and light the candle.

13 Place the TSA agar plate on the work surface near the candle.

14 Place the *E. coli* culture broth tube on the work surface near the candle.

15 Invert the broth tube two times to disperse the microbes within the broth.

16 Submerge the end of an inoculating loop into the isopropyl alcohol for 30 seconds.

17 Remove the inoculating loop and position the end approximately 15 cm above the candle flame so that the residual alcohol evaporates from the surface of the loop.

Note: Ensure that the loop does not come closer than 10 cm to the flame, or it could melt.

18 Unscrew the lid from the tube and secure in your hand.

Note: Do not place the lid of the broth tube on the work surface. Continue holding the lid until instructed to replace it on the tube.

19 Lower the opening of the broth tube to within 3 cm of the candle flame. See Figure 3.

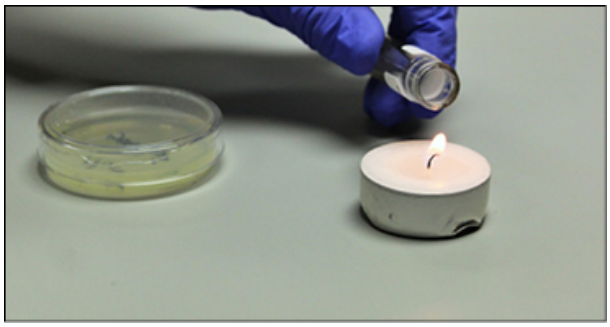


Figure 3. Sterilizing tip of broth tube. Note proximity of agar plate.

20 Heat the opening for 3 seconds.

21 Raise the opened tube to 15 cm above the flame and insert the sterile inoculating loop into the broth, being careful not to touch the rim with the loop or your gloves.

22 Submerge the end of the inoculating loop into the broth and carefully remove the loop so that the broth adheres to the surfaces of the loop.

23 Lower the opening of the broth vial to within 3 cm of the candle flame to heat the opening, while holding the inoculating loop in your other hand.

Note: Do not place the loop on the work surface.

24 Secure the lid on the broth tube and place the sealed tube on the work surface, while continuing to hold the inoculating loop.

25 Partially lift the lid of the TSA agar plate with your free hand and immediately rub the loop across the agar surface of the E. coli section in a zig zag motion until it is completely streaked, ensuring that the loop does not enter the S. epi section. See Figure 4.

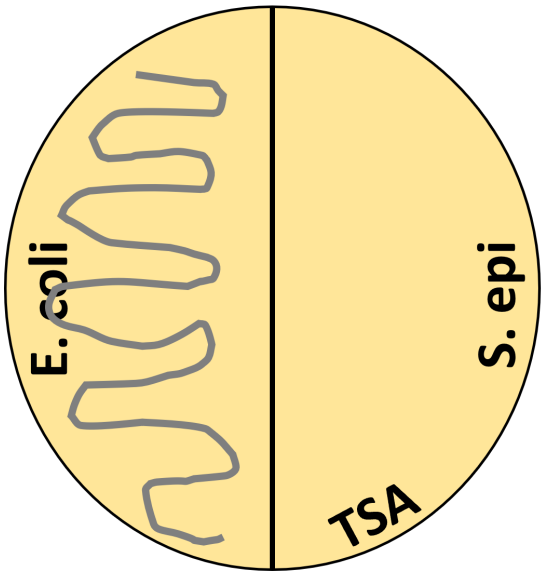


Figure 4. Streaking E. coli section of TSA plate.

Note: Apply only light pressure to the inoculating loop to avoid gouging the surface of the agar.

26 Remove the loop and close the lid of the agar plate.

27 Repeat steps 16-26 two additional times using the MAC and EMB agar plates.

28 Place the loop in the bag of bleach and seal the bag.

29 Repeat steps 13-28 using the *S. epidermidis* culture broth tube and the S. epi labeled section of each agar plate.

30 Extinguish the candle.

31 Return the *E. coli* and *S. epidermidis* culture broths to the refrigerator for use in future exercises.

32 Use the thermometer to locate a warm, dark, protected area that is at least 21^{o}C to incubate the inoculated plates.

Note: The incubation location should be protected from children, pets, direct light, and drafts. Cabinets located in warm locations inside your home or office are ideal for culturing microbes. The location must be at least 21^{o}C for cultures to properly develop.

33 Incubate the plated cultures agar side up for 24 hours.

34 Observe the agar surface of each plate, without removing the lid, for colony growth.

Note: If TSA plate shows no signs of growth after 24 hours, incubate for an additional 24 hours. One or more sections of the MAC and EMB plates may lack growth due to the selective properties of the agar.

35 Record the presence of growth and appearance of the colonies for each plate in **Data Table 1**.

36 Write your name and today's date on a sheet of paper.

37 Place the TSA plate lid side up on the sheet of paper with your name and date.

38 Take a photo of the plate focusing on the microbe colonies on the agar surface.

Note: For this exercise only, it may be necessary to remove the lid to properly capture the color/sheen of the colonies in the image. Wear you face mask if openning the lid and immediately replace the lid after taking the photo.

39 Upload the image into **Photo 1**.

40 Repeat steps 37-39 two additional times using the MAC and EMB agar plates and uploading the images in **Photos 2-3**.

41 Place the agar plates in the bag of bleach and seal the bag.

Clean up:

- Place the bag containing the bleached inoculating loops and agar plates in the trash.
- Wipe down the work surface with isopropyl alcohol and a paper towel or use a commercial disinfecting wipe.
- Remove your goggles, face mask, and apron and return them to the lab kit for future use.
- Remove your gloves and dispose of them in the trash.
- Wash and thoroughly dry your hands.

Exercise 1 - Questions

Question 0

How did the agars used in this exercise perform as either general purpose or functional media? Reference your results recorded in Data Table 1 and Photo 1 in your explanation. (SAMPLE ANSWER BELOW)

The TSA agar performed as a general purpose media as both *E. coli* and *S. epidermidis* grew equally and had similar appearances as recorded in Data Table 1 and Photo 1. Both the MAC and EMB agars performed as functional media as *E. coli* and *S. epidermidis* grew and appeared differently on these agars as recorded in Data Table 1 and Photo 1.

Question 1

Data Table 1: Colony Presence and Appearance

(SAMPLE ANSWER BELOW)

Plate	*E. coli* growth and appearance	*S. epidermidis* growth and appearance
TSA	Yes. Medium size, opaque, circular colonies	Yes. Small, white, circular colonies
MAC	Yes. Medium size, pink, circular colonies	No growth
EMB	Yes. Medium size, green sheen, circular colonies	Yes, Small, white, circular colonies

Question 2

Were the bile salts and methylene blue dyes present in the MAC and EMB agars, respectively, effective at selecting for either *E. coli* or *S. epidermidis*? Reference your results recorded in Data Table 1 and Photo 1 in your explanation.

(SAMPLE ANSWER BELOW)

Note to instructors: different bacterial strains and EMB/MAC agar formulations may impact students results. Student answers should match results recorded in Data Table 1 and Photo 1. From internal testing, the bile salts in MAC agar were successful in selecting for *E. coli* whereas the methylene blue in EMB agar permitted the growth of both *E. coli* and *S. epidermidis*.

Question 3

Photo 1: TSA Results

(SAMPLE ANSWER BELOW)

Question 4

What can you conclude about the cell wall structure and metabolic pathways of *E. coli* based on the results of this exercise? (SAMPLE ANSWER BELOW)

E. coli can be concluded to have a Gram-negative cell wall structure because the microbe grew well on both EMB and MAC plates which select for Gram-negative bacteria. *E. coli* can also be concluded to vigorously ferment lactose because the microbe was stained by the pH indicators on both MAC and EMB agars.

Question 5

Photo 2: MAC Results

(SAMPLE ANSWER BELOW)

Question 6

Photo 3: EMB Results

(SAMPLE ANSWER BELOW)

Evaluation Overview

Estimated Completion Times

- **Exploration** 30 min
- **Experimentation** 1-2 day incubation + 2 hrs
- **Evaluation**

Before You Proceed

- Did you complete all of the required exercises and assessments in this lesson? If not, please return to the previous section to finalize your work.
- Are you confident that you've achieved the learning objectives listed below? If not, please review the background content and your responses to the assessments and activities.

Learning Objectives

- Describe the purpose of general purpose, functional, selective, and differential media.
- Discuss the functional properties of MacConkey and Levine eosin-methylene blue agars.
- Culture *S. epidermis* and *E. coli* on TSA, MAC, and EMB agar plates.
- Relate experimental results to the cell wall structure and metabolic properties of microbes.

Competency Review

_____ is a category of functional media.

- Selective media
- Differential media
- Enriched media
- All of the above ✔

Examples of _____ media include salt agars and penicillin fortified agar.

- general purpose
- differential
- enriched
- selective ✔

_____ agar contains the pH indicator neutral red, which causes Gram-negative bacteria that vigorously ferment lactose to turn pink.

- Levine eosin-methylene blue (EMB)
- MacConkey (MAC) ✔
- Tryptic soy (TSA)
- Motility

Bacteria that do not ferment lactose may grow on both MAC and EMB agar, but these microbes will remain translucent because they are not stained by the pH indicators in each formulation.

- True ✔
- False

Inoculated EMB and MAC plates should be incubated until all microbes show signs of growth.

- True
- False ✔

***E. coli* produces colonies with a _____ sheen on EMB agar.**

- [x] green
- [ ] yellow
- [ ] blue
- [ ] red

***E. coli* grows on _____ agar.**

- [ ] EMB
- [ ] MacConkey
- [ ] Tryptic Soy
- [x] All of the above

Functional media testing results for *E. coli* using MAC and EMB agar suggest the microbe is a Gram-negative lactose fermenter.

- [x] True
- [ ] False

Extension Questions

Mannitol salt agar (MSA) is a selective and differential agar that prohibits the growth of most Gram-negative bacteria while selecting for Gram-positive, mannitol fermenting pathogens which appear as yellow stained colonies. Apply your knowledge of selective and differential media testing of *E. coli* to predict how the microbe would grow on an MSA plate. (SAMPLE ANSWER BELOW)

E. coli is a Gram-negative microbe that is selected for on MAC and EMB plates. Therefore, *E. coli* would be predicted to be inhibited on a MSA plate and show no signs of growth.