

THE UBIQUITY OF BACTERIA

Bacteria far outnumber all other life forms on the planet. In fact, in your large intestine alone you harbor more bacterial cells than the total number of human cells in your body. It is estimated that only 3% of bacteria are pathogenic for man and animals. Bacteria are found in a wide variety of environments---in or on animals and plants, in water, in soil, in air, or on rock. Generally, they are contributors to the environment, decaying nutrients and recycling the minerals (for use by plants and other organisms). Bacteria are both metabolically diverse as well as structurally diverse.

Your body is actually its own ecosystem, containing vast numbers of bacterial species. Most of them are part of a commensalistic symbiotic relationship (they benefit, you are neither harmed nor do you benefit) or a mutualistic (both partners gain) symbiotic relationship. These indigenous bacteria, living in and on your body, are part of your normal flora.

Two forms of bacteriological media will be used to culture the microorganisms---agar medium and broth medium. The difference is the presence or absence of the complex polysaccharide called agar agar, a solidifying agent purified from red algae. Commonly, it is added as a 1.5-2% agar for the solidified media, giving a solid surface for the bacteria to grow on. Agar agar is not a nutrient: it cannot be utilized by the organisms. In a broth culture, the presence of bacteria will produce a turbidity (sterile broth medium will be clear). On an agar plate clusters of daughter bacterial cells, piled on top of each other in a discrete area of the plate, will produce colonies on the agar surface. Each colony started with one mother cell deposited on the agar surface, then dividing continuously. You will undoubtedly see mixed cultures from most of your specimens---different colony sizes, shapes, colors, etc.

OBJECTIVES:

Compare the growth of bacteria from different environments.
Identify the ways that bacteria grow on and in bacteriological media.
Become familiar with the use of various forms of media.

MATERIALS NEEDED:

2 TSA (trypticase soy agar) agar plates per table (1 for environment, 1 for body)
1 TSB (trypticase soy broth) per table (table doing a sneeze plate do not use TSB)

THE PROCEDURES:

Each table will sample 2 habitats---the body and the environment.

ISOLATION OF BACTERIA FROM THE ENVIRONMENT

1. Label the **bottom** of your agar plates with your name, lab section, date, and location of specimen.
2. Pick an environmental specimen to sample (see table below)
3. For an **OBJECT SURFACE** (toilet, doorknob, cellphone, etc.):

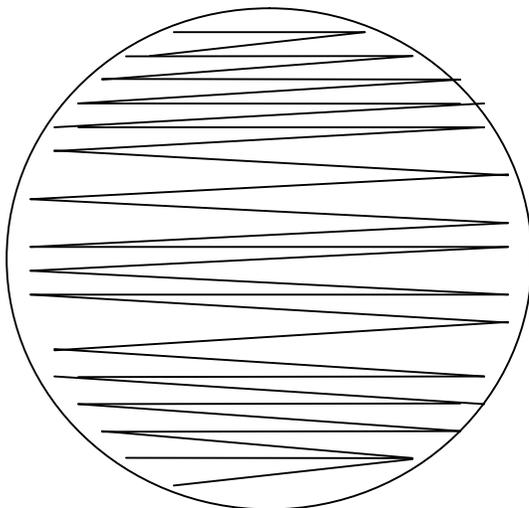
- a. Take a sterile cotton swab from the package, dip it into a tube of sterile saline (0.9% NaCl) and squeeze the swab against the glass wall of the tube to reduce excess fluid.
- b. Roll the swab around on the assigned environmental area.
- c. Rub the swab on an agar plate, using the **zig-zag procedure** shown below.
4. For **AIR SPECIMENS**, expose your TSA plate to your assigned area by uncovering the agar cover.
5. Incubate the agar plate at room temperature, 25 degrees Celsius.

ENVIRONMENTAL SPECIMENS	BODY SPECIMENS
Air in lab for 30 minutes	Palm of hand swabbed
Air in toilet stall (at base of toilet) for 30 minutes	Sole of foot swabbed
Air outside of building for 30 minutes	Sneeze onto plate
Toilet bowl inside or outside swabbed	Cheek swabbed
Cell phone speaker or earpiece swabbed	Between fingers swabbed
Various coins placed on medium	Armpit swabbed
Paper currency pressed onto agar medium	Inside ear swabbed
Hair shaken over plate	Inside nose swabbed
Doorknob swabbed	Inside mouth, back teeth swabbed

ISOLATION OF BACTERIA FROM THE HUMAN BODY (see table above)

You will culture from the same body part, left and right sides, onto an agar plate medium and a broth medium.

1. Label the **bottom** of your agar plate with your name, lab section, date, and location of specimen. Do the same with a tube of TSB, being sure to write on masking tape to label the tube.
2. Taking a sterile cotton swab from the package, dip it into a tube of sterile saline (0.9% NaCl) and squeeze the swab against the glass wall of the tube to reduce excess fluid.
3. Roll the swab around on the assigned area of your body.
4. Rub the swab on an agar plate, **using the zig-zag procedure** shown below.
5. Repeat steps 2 and 3, using a new swab and the same area of the body on the opposite side.
6. Place the swab into a tube of sterile TSB and cap the tube.
7. Incubate the agar plate and broth tube at room temperature, 25 degrees Celsius.



sneeze done on agar plates only, no TSB.

DATA COLLECTION:**BACTERIAL COUNTS FOR AGAR MEDIA**

0 = no growth

+1 growth = 10 bacterial colonies or less

+2 growth = 10-100 colonies

+3 growth = >100 colonies

1. Check for the number of bacterial colonies.
2. Try to differentiate different species of bacteria by colony shapes, size, and pigment.

GROWTH IN BROTH MEDIA

0 = clear

+1 = light turbidity

+2 = medium turbidity

+3 = very turbid, cannot see through broth

1. Record the amounts of growth in broth cultures and agar plate cultures.
2. Check for the number of bacterial colonies.
3. Try to differentiate different species of bacteria by colony shapes, size, and pigment.
4. The entire class's data will be listed on the board, along with the source of the specimen.

ENVIRONMENTAL SPECIMENS	Amount of growth	Different species #
Air in lab for 30 minutes		
Air in toilet stall (at base of toilet) for 30 minutes		
Air outside of building for 30 minutes		
Toilet bowl inside swabbed		
Toilet bowl outside swabbed		
Various coins placed on medium		
Paper currency pressed onto agar medium		
Hair shaken over plate		
Doorknob		
Cell phone speaker/earpiece swabbed		
BODY SPECIMENS		
Palm of hand		
Sole of foot		
Inside elbow		
Cheek		
Between fingers		
Armpit		
Inside ear		
Inside nose		
Inside mouth, back teeth		
Sneeze onto plate		

LABORATORY REPORT SHEET

QUESTIONS:

1. Can you determine the number of different bacterial species in a broth culture? Explain.
2. Which of the environmental habitats had the highest counts?
3. Which of the body areas had the highest counts?
4. Did any habitats result in no growth?
5. Of the specimens taken from the body, which one gave the great variation in bacterial species?