Quick Guide

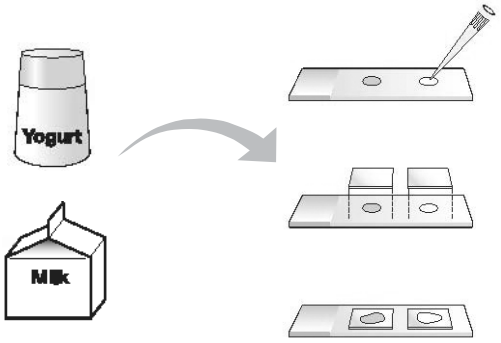
Lesson 1

Postulate 1: Identify possible pathogens

1. Compare yogurt and milk with respect to appearance, smell, and pH. Record observations.

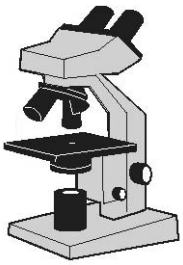
2. Label left hand edge of slide "yogurt" and right hand edge “milk”.

3. Dip toothpick in yogurt, mix with a drop of water on left hand side of slide, and cover with cover slip.



4. Add drop of milk to right hand side of slide and cover with cover slip.

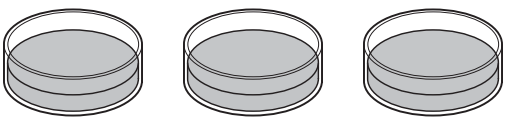
5. Observe yogurt and milk under the microscope. Describe and draw what you see.



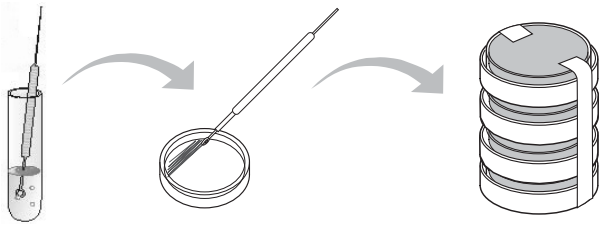
6. Repeat steps 1–5 with a different brand of yogurt.

Postulate 2: Isolate and culture suspected pathogens

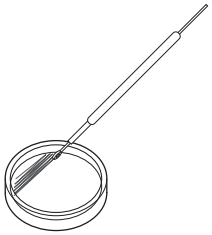
7. Label 3 LB sugar agar plates on the bottom (not the lid) with your initials and one as "milk", one as "yogurt", and the third as "*E. coli*".



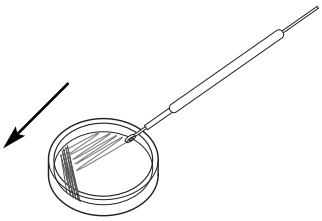
8. Streak milk onto milk plate for single colonies. Streak yogurt onto yogurt plate for single colonies as above. Streak *E. coli* onto *E. coli* plate for single colonies as above.



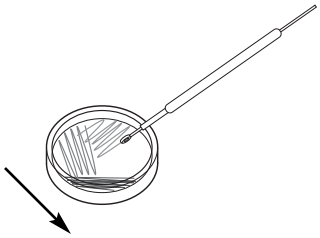
A) Streak for single colonies by gently rubbing the loop back and forth in the top left corner of the plate about 10 times. Stay in the top left quadrant of the plate and do not break the surface of the agar.



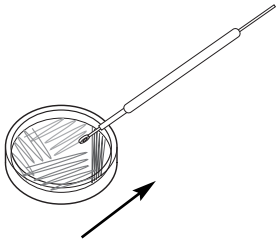
B) Rotate the plate 45° and using the same loop draw the loop through one end of the first streak. **Do not dip the loop back into the starting material**. Then rub the loop back and forth in the second quadrant about 10 times. Avoid passing the loop into the first streak.



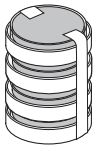
C) Rotate the plate 45° and using the same loop draw the loop through one end of the second streak and rub the loop back and forth in the third quadrant about 10 times. Avoid passing the loop into the first and second streaks.



D) Rotate the plate 45° and using the same loop draw the loop through one end of the third streak and rub the loop back and forth in the fourth quadrant about 10 times avoiding all previous streaks.



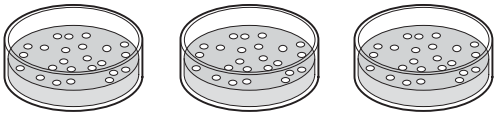
9. Invert the plates and place in incubator at 37°C for 24–48 h.



Lesson 2

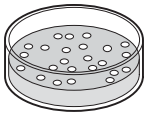
Postulate 2 continued: Isolate and culture suspected pathogens

1. Obtain plates from previous lesson. Count the individual colonies on each plate. Record results.



milk **yogurt** ***E. coli***

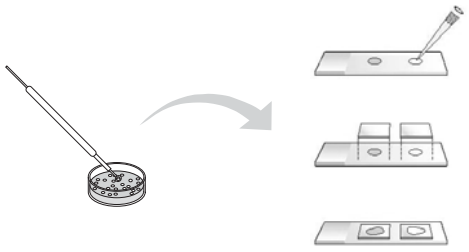
2. Observe colonies. Use a magnifying glass if available. Record how many different types of colonies you have on each plate. Use a marker to circle one of each type of colony and label with a number on the bottom of the plate.



3. Describe the appearance of each numbered colony.

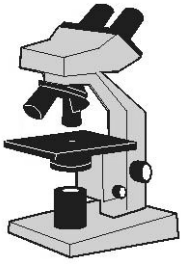
4. Label some slides according to your colony numbers. Use one slide for two samples as in the first lesson.

5. Pick a numbered colony from the yogurt plate, mix with a drop of water on right hand side of the appropriately numbered slide, and cover with a cover slip.



6. Repeat with the other numbered colonies from the yogurt, milk, and *E. coli* plates.

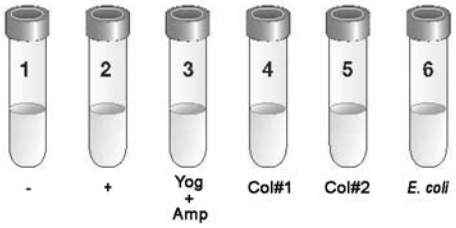
7. Observe colonies under the microscope. Describe and draw what you see.



8. Compare the bacteria with your descriptions of those observed in the yogurt in the first lesson.

Postulate 3: Inoculate healthy individual with pure culture of suspected pathogen

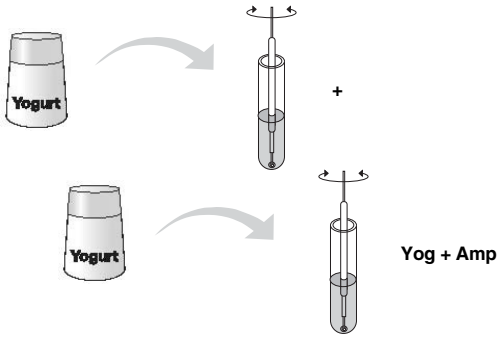
9. Label 6 tubes of milk as follows:
   Tube 1 Negative control
   Tube 2 Yogurt (positive control)
   Tube 3 Yogurt + amp
   Tube 4 Yogurt Colony #1
   Tube 5 Yogurt Colony #2
   Tube 6 *E. coli*



10. Add 10 µl or 1 drop of ampicillin to tube "Yogurt + amp".

11. Dip a fresh inoculation loop into the yogurt and swirl the loop into tube "positive control".

12. Use the same loop to dip into the yogurt again and swirl into the "Yogurt + amp" tube.

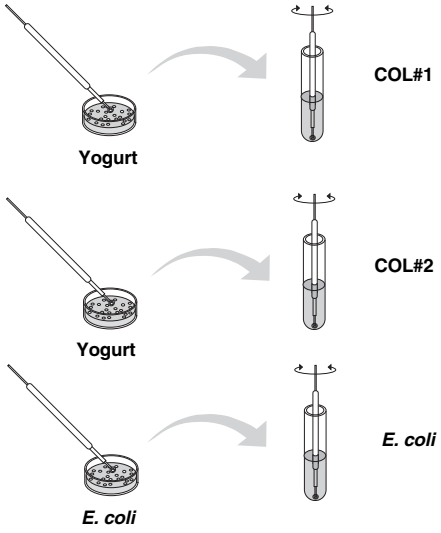


13. Identify two colonies on the yogurt agar plate that you investigated in the previous lesson of different types, if possible. Number the colonies 1 and 2 on the bottom of the plate and record which is which. If there is only one type of colony on your yogurt plate then number two similar colonies.

14. Using a fresh inoculation loop, pick colony #1 and transfer it to the tube "yogurt colony #1".

15. Using a fresh inoculation loop, pick colony #2 and transfer it to the tube "yogurt colony #2".

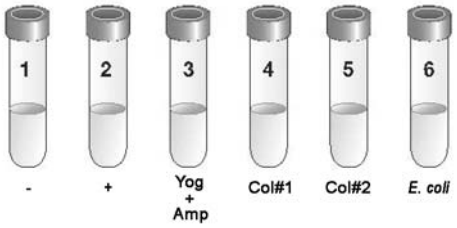
16. Using a fresh inoculation loop, pick an *E. coli* colony and transfer it to the tube "*E. coli*".



17. Place the tubes in an incubator or water bath at 37°C for 24–48 h.

Lesson 3

Postulate 4: Isolate and identify suspected pathogen from newly diseased individual

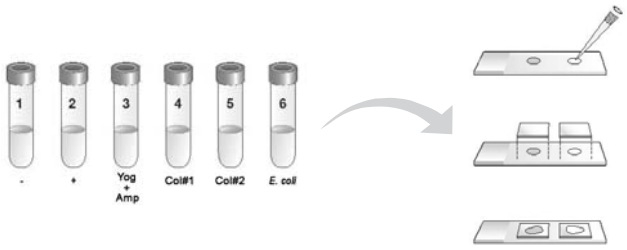


1. Obtain milk tubes and yogurt agar plate from previous lesson. Describe each milk culture with respect to appearance, smell, and pH.

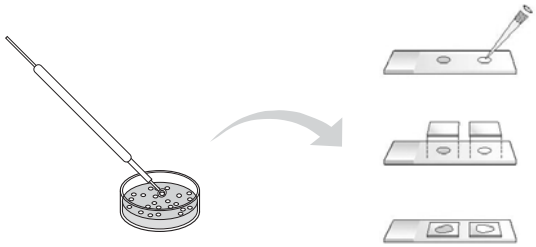
2. Label 3 slides according your milk tube labels. Use one slide for two samples on the right and the left as in the first lesson.

3. Label a fourth slide yogurt colony #1 on the right and yogurt colony #2 on the left.

4. Prepare slide samples of each milk culture for viewing under microscope as in previous lessons. For solid cultures, dip toothpick in culture and mix with a drop of water. For liquid cultures, add a drop to the slide. Cover with cover slip.

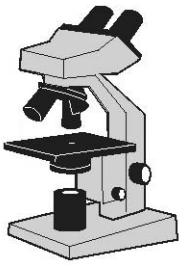


5. Pick a colony from the yogurt plate similar to that used to start the yogurt cultures in tube 4 (i.e. the same colony type as yogurt colony #1). Mix colony with a drop of water on right hand side of the appropriately numbered slide and cover with cover slip. Repeat with yogurt colony #2 on the left of the slide.



6. Observe slides under the microscope. Describe and draw what you see.

7. Using the microscope compare any bacteria in the newly infected cultures in milk tubes 4 and 5 with the pure bacteria used to inoculate these cultures. Are they the same?



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