

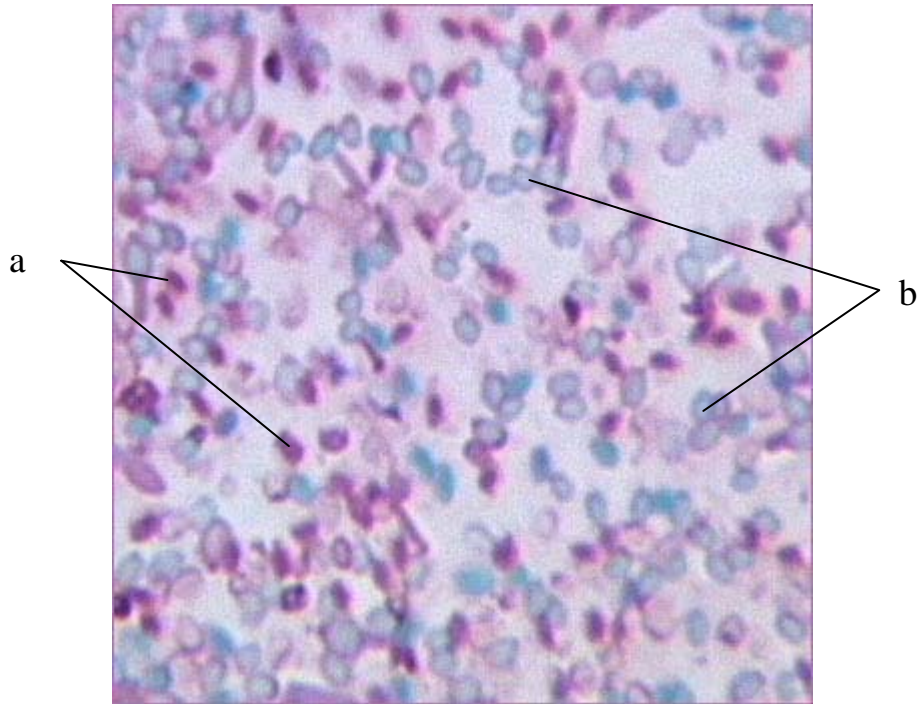
Exercises 7/8/9/10/17

Endospore Stain: Exercise 7

Name two medically important genera of bacteria that produce endospores.

Bacillus- which is aerobic

Clostridium- which is anaerobic



What will appear red and rod shaped when using Schaffer-Fulton Stain (see photo)?

a. Vegetative cells

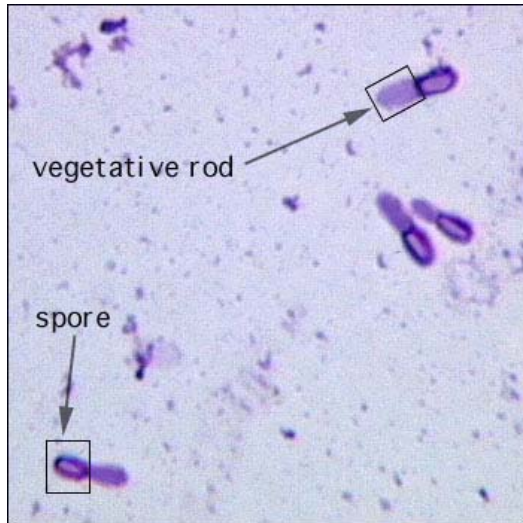
What will appear in green in a Schaffer-Fulton spore stain (see photo)?

b. Spores

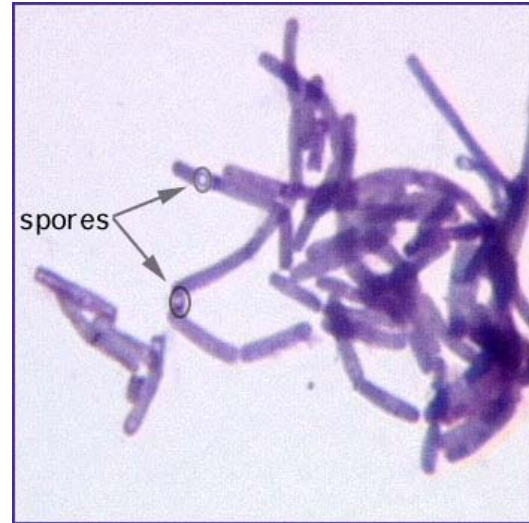
What is Schaffer-Fulton staining referred to due to its colors?

Christmas Tree Stain

Which is Bacillus and which is Clostridium?



a.



b.

- a. Clostridium – Tennis racket shaped
- b. Bacillus

Describe the process used to stain the relatively impermeable endospores.

- 1) Clean slide and draw a circle and label "BS" to indicate Bacillus subtilis on it
- 2) Put drop of water on slide, sterilize loop and put Bacillus subtilis taken from slant on slide.
- 3) Air dry
- 4) Heat fix slide, use Bacticinerator for 20 seconds, if using heat plate 1 minute 30 seconds.
- 5) Flood slide with 7.5% malachite green. Allow to sit on slide for 10 min.
- 6) Wash with distilled water
- 7) Flood with Gram's safranin. Allow to sit on slide for 2 min.
- 8) Wash with distilled water
- 9) Blot with bibulous paper or paper towel

Gram Stain: Exercise 8

Gram stain procedure separates almost all bacteria into what two large groups?

Gram-positive and Gram negative

What type of Gram stain is blue?

Gram positive

What type of Gram stain is pink?

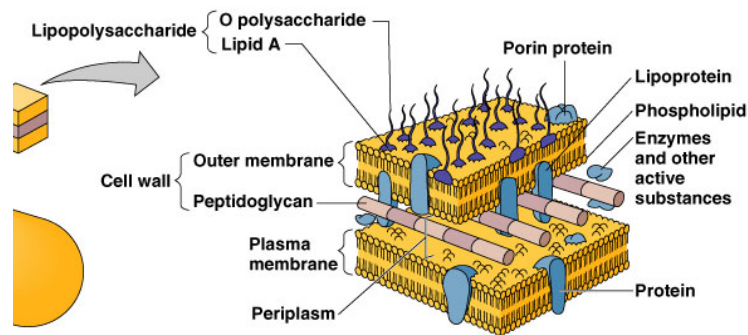
Gram negative

Why are Gram stains different?

Because their cell wall compositions are different

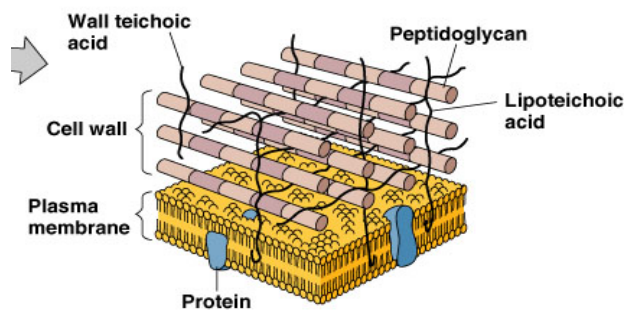
What Gram stain has a high lipid content with its thin peptidoglycan cell walls, resulting in violet dye to leak out of the cell walls?

Gram negative



What Gram stain has a thick peptidoglycan layer and contains teichoic acids, resulting in the cell walls retaining violet dye?

Gram positive



What are the three stains used in the Gram stain procedure?

Crystal violet, Gram's iodine, and safranin (*know how to spell "saf-ran-in")

What is used to decolorize the cells in the Gram stain procedure?

Acetone-Alcohol

How many dyes are used in a simple stain?

One (methylene blue, basic fuchsin, or crystal violet)

Simple stains allow one to distinguish the _____ of the bacteria.

Shape

Differential stains are more complex than simple ones and use one/more than one stain to differentiate cellular components.

More than one

What are two types of differential stains?

- Gram stains
- Acid-Fast stains

They are used to examine _____ of bacterial groups.

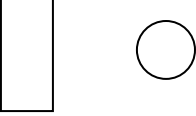
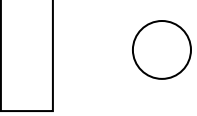
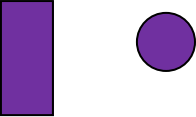
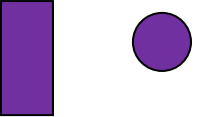
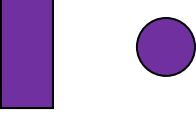
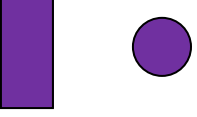
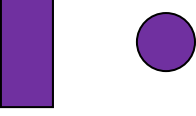
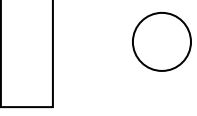
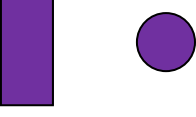
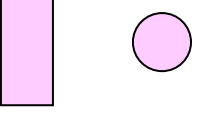
Structure

What is the purpose of heat-fixing a bacterial smear?

- To prevent the microorganism from being washed off from the slide.
- Kills organisms

List, in order, the reagents used in the traditional gram stain procedure.

Crystal Violet, Iodine, Acetone-alcohol, and Safranin.

Gram Positive	Type of Reagent	Gram Negative
 Colorless	Bacteria on Slide	 Colorless
 Stain purple	Primary stain / Buffer Crystal Violet/Biocarbonate	 Stain purple
 Remain purple	Mordant Iodine	 Remain purple
 Remain purple	Decolorizer Acetone/ethanol	 Lose purple dye
 Remain purple	Counterstain safranin	 Take up pink dye

State the function of each reagent in chart below.

Reagent	Function
Crystal violet	
Iodine	
Acetone-Alcohol	
Safranin	

Answers

Reagent	Function
Crystal violet	Primary Stain
Iodine	Mordant (binds to Gram + = purple color)
Acetone-Alcohol	Decolorizer
Safrinin	Counter-Stain (Pink color)

Cultures more than ____ hours old may lose their ability to _____ the crystal violet-iodine complex.

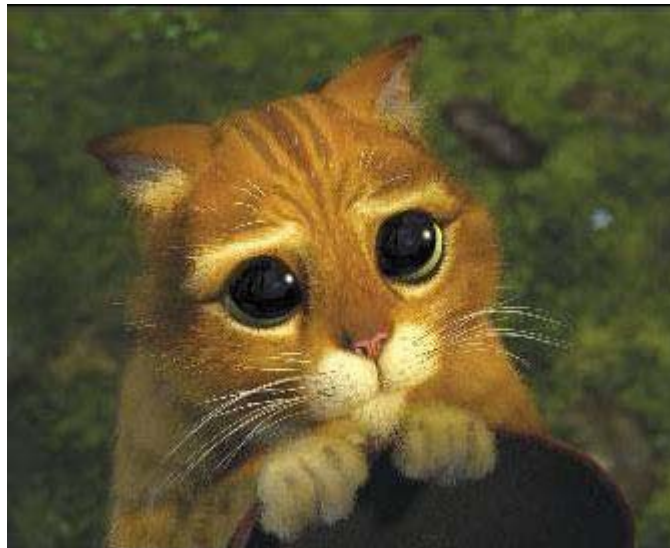
24 hours/retain

If the decolorizer is left on too long, what will happen?

A Gram positive will appear as a Gram negative (pink)

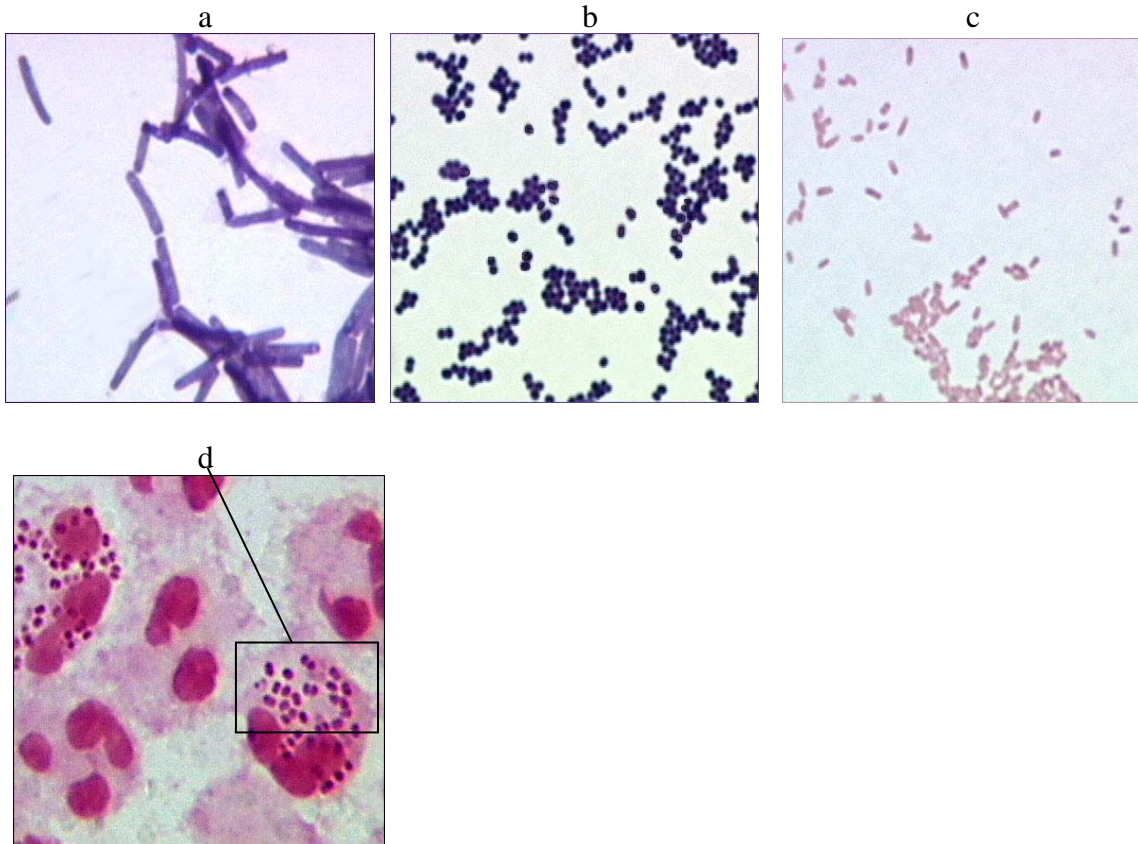
If the decolorizer is not left on long enough, what will happen?

A Gram negative will appear as a Gram positive (purple)



“Can you help me study for the test?”

State if Gram positive / Gram Negative and if bacilli / cocci in chart below.



	Gram positive / negative	Bacilli / Cocci
a		
b		
c		
d		

Answers

	Gram positive / negative	Bacilli / Cocci
a	Positive (Purple)	Bacilli (Rod shaped)
b	Positive (Purple)	Cocci (Sphere shaped)
c	Negative (Pink)	Bacilli (Rod shaped)
d	Negative (Pink)	Cocci (Sphere shaped)

Negative Stain: Exercise 9

31. Differentiate between a negative stain and a positive stain.

(**Different from Gram negative and Gram positive. Don't confuse Gram staining with Negative or Positive staining)

What type of staining procedure stains the object or structure you wish to see?

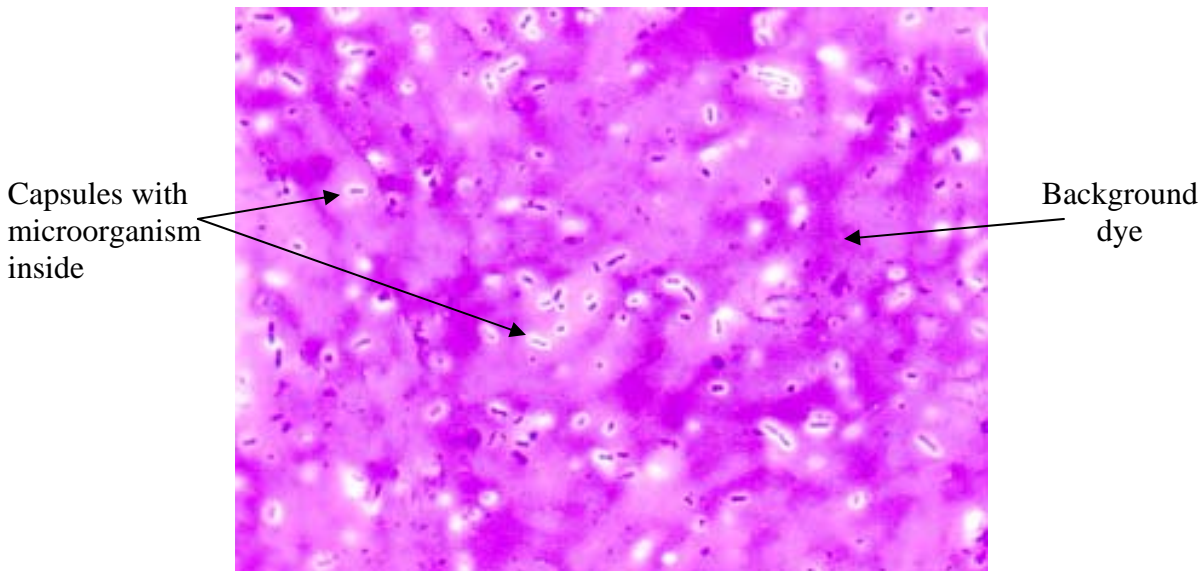
Positive staining

What type of staining procedure stains everything except what you want to see?

Negative staining

32. Identify a bacterial capsule in a negative stain.

To see capsules, the negative stain produces a dark blue background with light blue-violet or whitish capsules and a dark blue microorganism inside the capsule.



33. Describe the appearance of the bacteria and background of a negative-stained bacterial smear.

Everything but what we want to see will be stained. The negative stain will stain everything except what you want to see. The organism will stand out as transparent or retractile structure against a contrasting dark background.

34. State two advantages of using a negative stain versus a positive stain.

- In negative staining, the organism is not heat fixed, and will not be severely distorted or damaged. In positive staining heat fixing will damage the organism.
- When an organism won't stain using positive staining, the organism will stand out against contrasting dark background when using negative staining.

Acid Fast Stain: Exercise 10

23-State the diagnostic value of the acid –fast stains.

What is the diagnostic value of acid fast stain?

- Pathogens retain color even in presence of acid
- Distinguishes *Mycobacterium* (cause of tuberculosis and leprosy) with rod shapes and *Nocardia* (cause of a pulmonary disease called nocardiosis) with branching, filamentous shapes.

Acid fast microbes are pathogenic for humans, therefore observing them is critical. The diagnostic value of acid-fast stain is that pathogens retain color even in presence of acid. For example tuberculosis would retain the red dye carbolfuchsin even after it was washed with a powerful solvent made of a mixture of acid and alcohol.

24-Distinguish acid-fast organisms from non-acid fast organisms under the microscope?

What color is acid-fast microorganism?

Red color

What color is non-acid fast microorganism?

Blue color

Non acid fast microorganisms are decolorized by acid-alcohol mixture used in the acid-fast stain but they retain the blue dye.

Acid fast microorganisms retain their red dye color.

25-Explain why certain organisms cell structure are “acid –fast”?

What type of lipid is found in large amounts in *Mycobacterium* and many species of *Nocardia* (both acid-fast bacteria), making it difficult for the bacteria to absorb dye into the cell?

Mycolic acid

Certain organisms cell structure are acid fast because they contain large amounts of a type of lipid called mycolic acid. These lipids make it difficult for the bacteria to absorb dye into the cell.

26-Differentiate between the decolorizer used in the acid –fast stain and the gram stain?

What is the decolorizer in acid fast stain?

Acid/Alcohol









What is the decolorizer in gram stain?

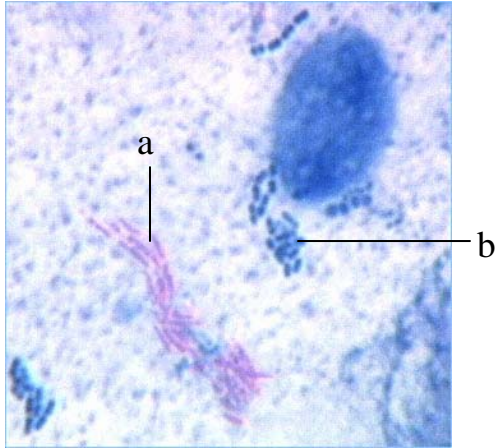
Acetone/Ethanol

The decolorizer in acid fast stain is acid/alcohol whereas the decolorizer in gram stain is acetone/ethanol.

27-State the three reagents used in the acid-fast stain?

- 1) Carbofuchsin
- 2) Acid-alcohol
- 3) Methylene blue

Acid-Fast	Type of Reagent	Non Acid-Fast
 Colorless	Bacteria on Slide	 Colorless
 Stain red	Primary stain Carbofuchsin	 Stain red
 Remain red	Decolorizer Acid-alcohol	 Lose red dye
 Remain red	Counterstain Methylene blue	 Take up blue dye



Name the type of stain for (a).

Acid fast

Name the type of stain for (b).

Non acid fast

Effect of Oxygen on Bacterial Growth: Exercise 17

Remember:

- Aerobes need oxygen!
- Anaerobes do not like oxygen!

What type of bacteria absolutely requires oxygen in order to grow?

Obligate aerobes

What type of bacteria can grow with or without oxygen?

Facultative anaerobes

What type of bacteria requires only a small amount of oxygen and its growth is enhanced by carbon dioxide?

Microaerophilic organisms

What type of bacteria cannot grow in the presence of oxygen (Oxygen is a toxic poison to it)?

Obligate anaerobes

* * *

What type of incubating device has very little oxygen and increased carbon dioxide presence?

Candle jar

What type of incubating device has absolutely no presence of oxygen in it?

Gas pak jar

What substance is chemically combined with free oxygen, reduces the concentration of oxygen in media and is useful for culturing anaerobes?

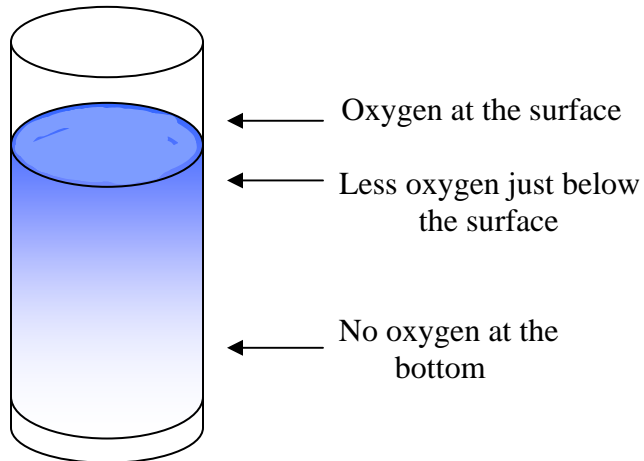
Reducing agents

What type of reducing agent is commonly used with resazurin dye, and eliminates oxygen at the bottom of the test tube but not at the surface (causing varied concentrations of oxygen in the tube)?

Thioglycollate broth

What color shows the presence of oxygen when using Resazurin dye?

Pink (colorless when reduced oxygen)



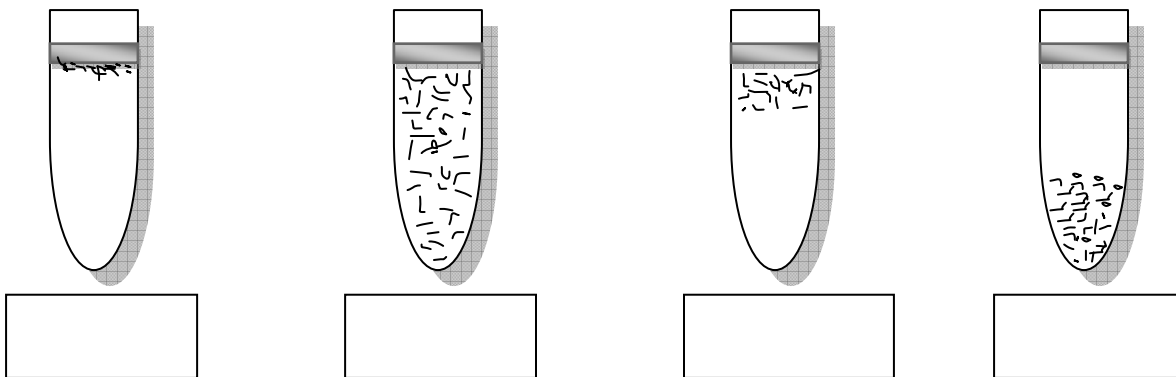
Test your knowledge! Complete the chart.

Under each bacteria type, indicate Good growth or little/no growth:

	Obligate aerobes	Facultative anaerobes	Microaerophilic organisms	Obligate anaerobes
Candle jar				
Gas pak jar				

Under each bacteria type, indicate where growth in tube:

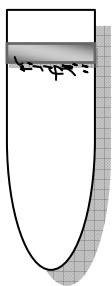
	Obligate aerobes	Facultative anaerobes	Microaerophilic organisms	Obligate anaerobes
Thioglycollate broth				



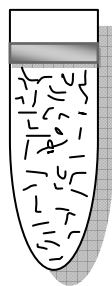
How did you do? Compare your answers.

	Obligate aerobes	Facultative anaerobes	Microaerophilic organisms	Obligate anaerobes
Candle jar (very little oxygen)	Very little growth	Good growth	Good growth	No growth
Gas pak jar (no oxygen)	No growth	Good growth	No growth	Good growth

	Obligate aerobes	Facultative anaerobes	Microaerophilic organisms	Obligate anaerobes
Thioglycollate broth	Only found on the surface of broth	Found all throughout broth	Found just below the surface of the broth	Only found on the bottom of the jar



Obligate aerobes



Facultative anaerobes



Microaerophilics



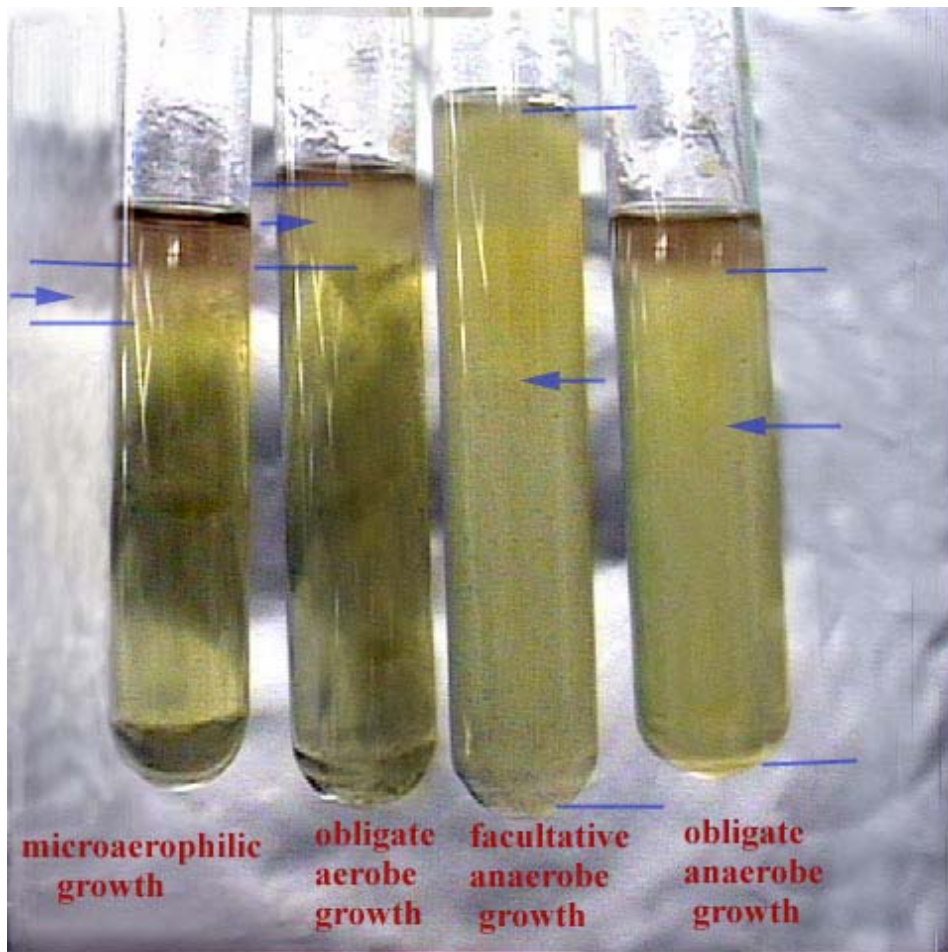
Obligate anaerobes



Candle Jar



Gas Pak Jar



**microaerophilic
growth**

**obligate
aerobe
growth**

**facultative
anaerobe
growth**

**obligate
anaerobe
growth**