MLAB 2434 Clinical Microbiology
Laboratory: Anaerobes
Skills= 12 points

Objective:

At the completion of the laboratory, the student will be able to:

1. Demonstrate aerotolerance and perform gram stains on selected bacteria.
2. Describe and compare the gram stain morphologies of *Clostridium perfringens, Bacteroides fragilis,* and *Bacillus sp.*
3. Presumptively identify these bacteria based on aerotolerance and gram stain results.
4. 5. Create and monitor special atmospheric conditions needed to support the growth of anaerobic bacteria.
6. Be able to classify bacteria as facultative, aerobic or anaerobic.

Materials:

4 Blood agar plates
2 Glass Microscope slides
Microscope
Immersion Oil
Inoculating loop
Gram stain materials
1 Anaerobic Biobag
Cultures of:
Clostridium perfringens
Bacteroides fragilis
Bacillus sp.

References:

1. Mahon and Manuselis, Textbook of Diagnostic Microbiology, Third Edition, Chapter 23
2. Kiser, K. M., Payne, W. C., & Taff, T. A. (2011). *Clinical Laboratory Microbiology: A Practical Approach*, Chapter 24, Upper Saddle River, NJ: Pearson Education, Inc.

Discussion:

Anaerobe morphology can present in a variety of shapes and sizes, both gram negative and gram-positive cocci and bacilli.

Because slow-growing anaerobes can take up to 48 hours to appear, many microbiology labs wait until 48 hours to examine the primary culture plates. Direct gram stains from clinical specimens can provide the physician with valuable information to begin treating patients with antibiotics long before culture results are obtained. In addition, the results of the gram stain can confirm specimen acceptability, guide media selection, and determine the probability of infection by observing indicators of inflammation.

One physical condition that is extremely important for microbial growth is the oxygen requirement. Some microorganisms cannot live in the absence of oxygen; we call these organisms **strict aerobes**. A strict aerobe must have oxygen present in order to live and produce. At the opposite extreme are those organisms that cannot live if oxygen is present; these are **strict** or **obligate anaerobes**. Oxygen is actually toxic to obligate anaerobes. Between the two extremes, we find the majority of organisms; these are **facultative anaerobes**. They grow either in the presence or absence of oxygen, but grow better if oxygen is available. Anaerobes exist as normal flora at many body sites (e.g., gastrointestinal tract, genital tract, mucous membranes of upper respiratory tract and skin).

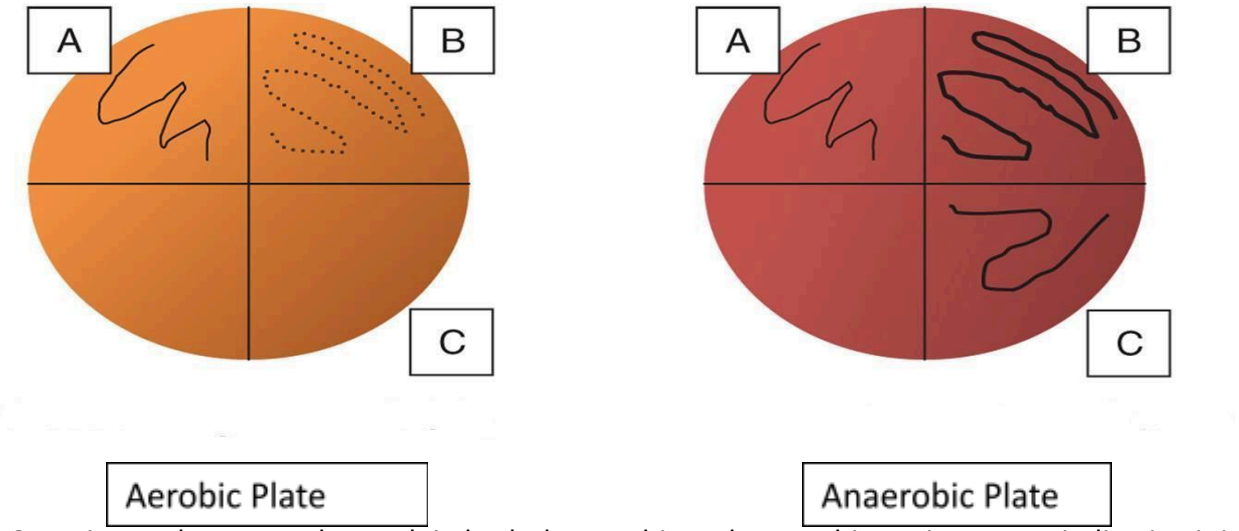
Identification of Anaerobes:

Determine the relationship of oxygen for various organisms. Prove that the organism on the anaerobic plate is an obligate anaerobe by incubating the organism aerobically and anaerobically. Aerotolerance tests are used to determine the oxygen requirements of the organism.

Aerotolerance Test
Procedure:

1. Working in pairs, take four (4) blood agar plates (BAP) from the supply bench.
2. On the first set of 2 BAPs, divide into two sections with a marking pen and label each section with the organism's name as follows:
   Clostridium perfringens
   Bacteroides fragilis
3. In each section respectively, streak with a loop the appropriate organisms on each plate. Touch one colony ONCE and use this to inoculate BOTH aerobic and anaerobic plates for that organism. The instructor will demonstrate the best technique for streaking.
4. Label one plate "AER" and incubate plate in ambient atmosphere in the 35°C, CO_2 incubator.
5. Label the other plate "ANA" and incubate in the anaerobic "Gas-Pak" jar or other anaerobic system at 35°C. Place in the non-CO_2 incubator.

6. On the second set of BAPs, label the plate with the organism's name as follows:
   Bacillus sp.
5.
6.
7. Label one plate "AER" and incubate plate in ambient atmosphere in the 35°C, CO_2 incubator.
8. Label the other plate "ANA" and incubate in the anaerobic "Gas-Pak" jar or other anaerobic system at 35°C. Place in the non-CO_2 incubator.
5. After 24 hours incubation, observe each plate for growth. If growth is observed, report as "G " (growth) and record results in the report form. If no growth is observed, record as "NG" (no growth).
6. Determine the relationship of the organism to oxygen and using the correct terminology (i.e., microaerophilic, facultative, obligate), record the aerotolerance results in the report form. Review the image below for interpretation of results.



Organism A shows equal growth in both the aerobic and anaerobic environment, indicating it is a facultative anaerobe. Organism B shows a growth pattern of either a microaerophilic organism or an aerotolerant anaerobe. Organism B is growing sparsely on the aerobic plate, but shows better growth in the enriched anaerobic environment. Organism C demonstrates the growth pattern of a true obligate anaerobe since it is not growing in the aerobic environment and is growing in the anaerobic environment.

Gram Stain Morphology
Procedure:

1. Prepare smears on all organisms, placing two organisms per slide.
2. Perform a gram stain on all isolates.
3. Determine the gram reaction and morphology.
4. Record results of the gram stain in the report form.

NAME _____ **DATE** _____

Report Form: Anaerobes
Points= 12

Organism	Presence/Absence of Growth on Aerobic Plate	Presence/Absence of Growth on Anaerobic Plate	Aerotolerance Results	Gram Reaction & Morphology
Clostridium perfringens				
Bacteroides fragilis				
Bacillus sp.				

Grading Rubric:
Each test result is worth = 1 point
Points will be deducted for incorrect notation of reactions

Name:_____
Date:_____

Lab: Anaerobes
Study Questions
Points= 11

Instructions:
Using the textbook, lecture notes, or lab complete this exercise.

1. Briefly explain the collection and transport of clinical specimens for anaerobic culture. (1 pt.)

2. List four (4) types of clinical specimens that are generally unacceptable for anaerobic culture. (2 pts.)

3. Identify three (3) methods of achieving anaerobic conditions for cultures in the laboratory. (3 pts.)

4. Which chemicals are used to make anaerobic conditions in the Gas Pak anaerobic jar system? (2 pts.)

5. **Thought question:** Why is it a good idea to do a gram stain on the clinical specimen for anaerobic culture even when a gram stain is not ordered? (1 pt.)

6. Describe the shape of the ends of *C. perfringens.* (1 pt.)

7. What is the purpose of incubating an aerobic sheep blood agar plate as wells as an anaerobic BAP when culturing anaerobes? (1 pt.)