

Biochemicals and Gram-Positive Organisms Identification Course Facilitator Guide

Introduction

The Basic Microbiology Curriculum: Biochemicals and Gram Positive Organisms ID Course is a blended learning activity that includes both eLearning and hands-on laboratory exercises. Both components of the course are equally important in providing knowledge and actual laboratory experience to the participant. This facilitator guide is meant to serve as a manual for the supervisor/mentor that will be overseeing the completion of the laboratory exercises after the eLearning activity has been completed. The manual contains instructions for the overall laboratory exercise components, objectives, laboratory setup, a supply list, laboratory exercises, instructions and answer key as well as job aids.

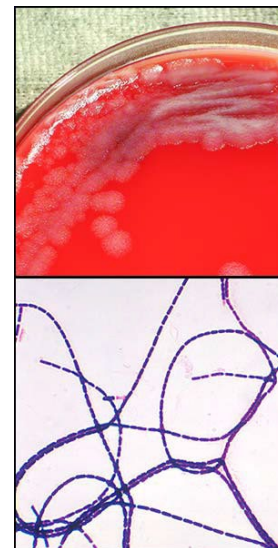
The goal of these exercises is to allow the participant to use the information and procedures learned during the eLearning portion of the course and apply them using hands-on laboratory exercises.

Please note: These laboratory exercises may be edited according to your laboratory's standard operating procedures or guidelines, if necessary.

The laboratory exercises were created with the forethought that laboratory procedures may vary from laboratory to laboratory and therefore, may need to be edited according to the procedures or protocols followed within that laboratory.

The participant of the course is strongly recommended to complete the laboratory exercises to transfer the didactic content of the course to experiential knowledge gained through hands-on laboratory exercises with the equipment from their laboratory. The supervisor/mentor should work with the participant to develop these laboratory skills as well as confirm that these exercises have been completed. The number and types of exercises completed will be at the discretion of the supervisor/mentor based on procedures followed within their laboratory.

After the laboratory exercises are completed and discussed with the supervisor/mentor, the supervisor/mentor should then follow-up the exercises with instruction related to your laboratory's specific procedures or guidelines.



Biochemicals and Gram Positive Organisms Identification Course Facilitator Guide, page 2

Laboratory Exercise Objectives

After completing the laboratory exercises, the participant will be able to:

- Identify different types of bacterial morphology seen on a Gram stain.
- Explain commonly encountered problems with performing a Gram stain.
- Identify the different types of colonial characteristics.
- Interpret results of a tube motility test.
- Correlate results with commonly encountered problems.

Initial Planning for the Laboratory Exercises

1. Communicate with the participant and schedule days/times to complete the laboratory exercises.
2. Collect the supplies and culture media necessary to complete the exercises (see supply and culture media list).
3. Prepare ahead subcultures of microorganisms to be used for the Gram stains, morphology demonstration and tube motility test.
4. Label all slides, plates and tubes with the number corresponding to the numbers in each exercise.

Day(s) of Scheduled Laboratory Exercises

1. Set up supplies and equipment for the exercises.
2. Remind the participant about the use of proper PPE and laboratory equipment according to your laboratory's procedures and safety manual.
3. Have Gram stains ready to be read.
4. Pull culture plates and tubes out of the incubator.
5. Participants should have a copy of the laboratory exercises and job aids as a printout from the eLearning course.
6. Have participants complete each exercise with your approval. Please feel free to instruct participant as they work or after the exercise is completed. Exercises may be completed all at once or as time permits.
7. Relay to the participant any information that is needed to comply with your laboratory's standard operating procedures (SOPs) or safety procedures.

Supply List for Preparing the Exercises

1. Personal protective equipment (PPE) and laboratory equipment
2. Loops (sterile plastic or metal)
3. Inoculating needles (sterile plastic or metal)
4. Incinerator or Bunsen burner (if using metal loops and for heat fixing the slides)
5. Microscope slides

Biochemicals and Gram Positive Organisms Identification Course Facilitator Guide, page 3

Supply List for Preparing the Exercises Cont'd

6. Labelling pen
7. Biohazard waste container
8. Sharps container
9. Incubator
10. Gram stain kit
11. Microscope and immersion oil

Culture Media and Microorganism List for Preparing the Exercises

1. 18 hour subcultures of the microorganisms to be Gram stained and inoculated onto plates and into tubes.
2. Suggested Microorganisms:
 - Group A streptococci
 - Alpha-hemolytic streptococci (Example: *Streptococcus pneumoniae* ATCC 49619)
 - Gamma-hemolytic streptococci (Example: *Enterococcus faecalis* ATCC 29212)
 - *Staphylococcus aureus*
 - *S. epidermidis* (or other non-hemolytic Staphylococci)
 - Diphtheroids
 - *E. coli* ATCC 25922 for Gram stain and motility test (motile microorganism)
 - *K. pneumoniae* ATCC 13883 or 27736 - non-motile microorganism for motility test
3. Culture media: BAP and tube motility with or without TTC.

Supply List for the Exercises

1. Personal protective equipment (PPE) and laboratory equipment
2. Biohazard waste container
3. Microscope and immersion oil

Culture Media and Microorganism List for the Exercises

1. Previously prepared Gram stains for observation.
2. Previously inoculated media for observation.
3. Previously inoculated tube motility media that had problems (to be used for demonstration and problem solving).

Biochemicals and Gram Positive Organisms Identification Course Facilitator Guide, page 4

Laboratory Exercise I

After completing this laboratory exercise, the participant will be able to:

- Identify different types of bacterial morphology seen on a Gram stain
- Explain commonly encountered problems with performing a Gram stain.

Exercise

- Observe Gram stains 1-6 and identify the appropriate morphology.
- Observe Gram stains 7-10 and identify what is wrong with the Gram stain and explain how the problem may have occurred.
- Using the microorganisms listed in the supplies list prepare the following Gram stains:
 1. QC slide
 2. Gram positive cocci in pairs
 3. Gram positive cocci in chains
 4. Gram positive cocci in clusters
 5. Gram positive rods showing palisades and some pleomorphic rods
 6. Gram negative rods
 7. Gram stain that is too thick to determine the morphology
 8. Gram stain that is too thin to find the microorganism
 9. Gram stain of any of the Gram positive microorganisms that is over decolorized
 10. Gram stain of any of the Gram negative microorganisms that is under decolorized
- Ask the participant to observe Gram stains 1-6 and identify the morphology on the Gram stains correctly.
- Ask the participant to observe Gram stains 7-10 and identify what is wrong and what could have caused the problem.

Notes:

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____
9. _____
10. _____

Mentor/Supervisor Date

Biochemicals and Gram Positive Organisms Identification Course Facilitator Guide, page 5

Laboratory Exercise II

After completing this laboratory exercise, the participant will be able to:

- Identify the different types of colonial characteristics

Exercise

Obtain prepared plates from your supervisor/mentor.

For this exercise, you will observe the plates and identify the appropriate colonial characteristics. Use the Colonial Characteristics Job Aid.

- Using the microorganisms listed in the supplies prepare the following plates and incubate them for 18-24 hours:
 1. BAP demonstrating alpha, beta and gamma hemolysis (be sure to get isolated colonies).
 2. BAP demonstrating small colonies (recommend using diphtheroids).
 3. BAP demonstrating mucoid colonies.
 4. BAP demonstrating large grey/white colonies.
 5. BAP demonstrating a mixed culture using *S. aureus* and *S. epidermidis*.
- Ask the participant to observe the plates and identify the appropriate colonial characteristics.

Notes:

1. _____
1. _____
1. _____
2. _____
3. _____
4. _____
5. _____

Mentor/Supervisor Date

Biochemicals and Gram Positive Organisms Identification Course Facilitator Guide, page 6

Laboratory Exercise III

After completing this laboratory exercise, the participant will be able to:

- Interpret results of a tube motility test.
- Correlate results with commonly encountered problems.

Exercise

Observe motility tubes for commonly encountered problems that may arise.

- Using the *E. coli* and *K. pneumoniae* isolates prepare the following motility tubes and incubate them for 18-24 hours. Include an uninoculated tube as a comparison:
 1. Tube demonstrating positive motility test (*E. coli*).
 2. Tube demonstrating negative motility test (*K. pneumoniae*).
 3. Tube demonstrating false positive motility test by inoculating it down the side and hitting the bottom (*K. pneumoniae*).
 4. Tube demonstrating false positive motility test by over inoculating it very heavily and not making a simple straight line down (*K. pneumoniae*).
 5. Tube demonstrating false negative motility test by inoculating it with a sterile needle and not having any growth.
 6. Tube demonstrating false positive motility test by inoculating the tube at an angle so it appears to be positive (*K. pneumoniae*).
- Ask the participant to observe each tube for possible problems.
- Ask the participant to report the problem to you and discuss with him/her what you think may have caused the problem and what should be done to fix the problem.

Notes:

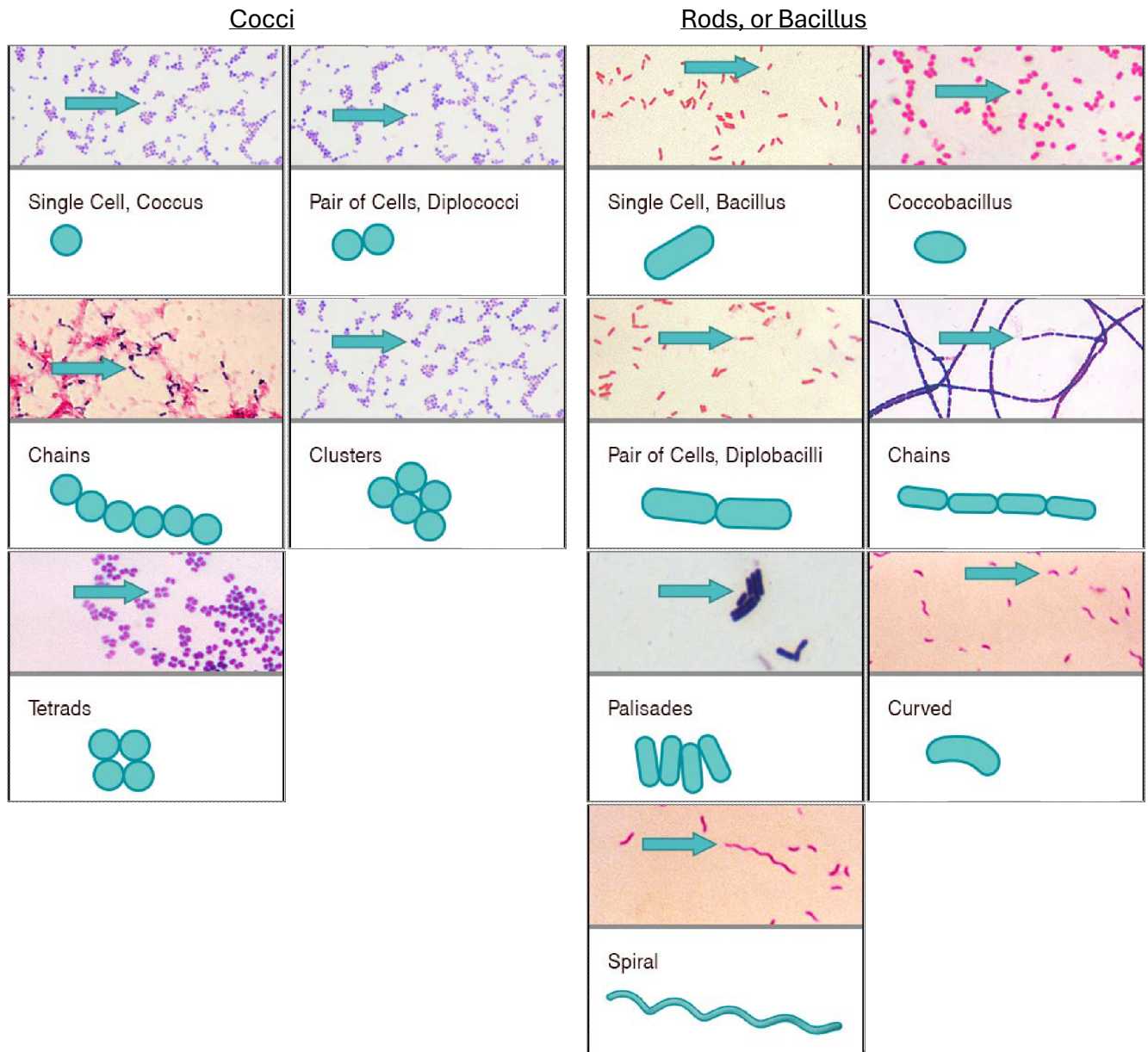
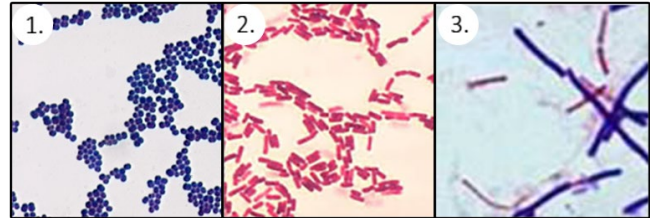
1. _____
2. _____
3. _____
4. _____
5. _____
6. _____

Mentor/Supervisor Date

Gram Stain Cell Morphology

Gram stain results:

1. Gram Positive Cocci
2. Gram Negative Rods
3. Gram Variable Rods



Colonial Characteristics

Introduction

Colony morphology is the visual culture characteristics of a bacterial colony on an agar plate. Observing colony morphology is an important skill used in the microbiology laboratory to identify microorganisms. Colonies need to be well isolated from other colonies to observe the characteristic shape, size, color, surface appearance, and texture. Another important characteristic of a bacterial colony is hemolysis. Hemolysis is the result of the lysis of the blood cells that are a component of Blood Agar.

Colony Morphology Characteristics:

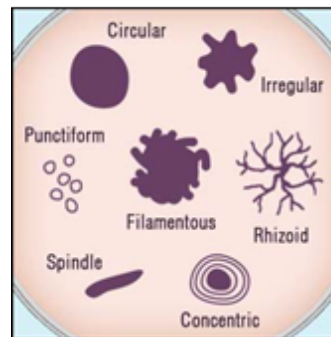
Size:

General Guidelines:

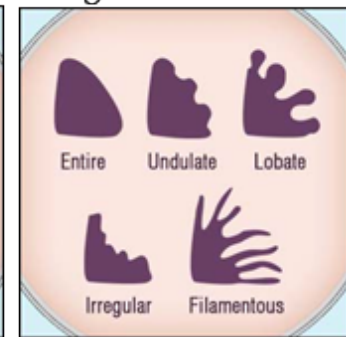
- large (>1mm)
- medium (approximately=1mm)
- small (<1mm)
- pinpoint (<0.5mm)

Shape:

Form



Margin



Surface Appearance:

- smooth
- rough
- glistening
- dull
- wrinkled
- spreading
- mucoid

Color:

- pigmentation (white, pink, purple, etc.)
- opaque
- transparent

Hemolysis:

- Alpha (α)
- Beta (β)
- Gamma (γ)

