

Laboratory Study guides DO NOT earn extra credit. You DO NOT need to turn them in. They ARE NOT mandatory.

They are, however, VERY useful...

Lab Topics for Review:

Intro& Safety

1. Why do we incubate some plates at room temperature and others at 37C? Why do you think we would use the refrigerator in micro lab?
2. What is “media” (in a micro lab context)? What forms can it take (e.g. what kinds of containers are used and what consistencies can media be)? What is the difference between general, enriched and differential media? What's a "fastidious" microorganism? What types of media have we used so far and which are general/enriched?
3. What information needs to be included when you label your microbiology experiment (like an agar plate)? WHERE does this information go?

At the end of lab, would you place each of the following?

- used petri plates that you're finished with
- used test tubes and their lids
- cultures
- your gloves (used that day)
- a disposable Pasteur pipet
- used slides

Aseptic Technique & Cultural Characteristics

Definitions:

aseptic

sterile

pure culture

mixed culture

contaminated culture

1. What does “aseptic” mean? Why do we use aseptic technique in the micro lab? Describe how you would perform an aseptic transfer of bacterial culture from one tube to another using aseptic techniques. Use as much detail as possible and then refer to your lab manual to check yourself.
2. What does “colony morphology” mean? Why do we care about the characteristics of a bacterial (or fungal) colony?

3. Is colony morphology always the same for a particular bacteria? If not, when is it different?
4. Be able to identify colony shape and size using the following terms: punctiform, small, medium, large, circular, irregular, filamentous.
5. Be able to use the following terms to describe colony margin: entire, undulate, lobate, wavy (pie crust).
6. Be able to use the following terms to describe colony pigment: color, water soluble, water insoluble (how do you tell the difference?).
7. Be able to use the following terms to describe colony texture: mucoid, moist, dry (or rough), smooth, concentric (swarming).
8. Explain the importance of using isolated colonies for experiments in the micro lab.
9. Explain the rationale for the 4-quadrant streak. Why do we do it? What is the point? Demonstrate the method on a sheet of paper.

Microscopy

1. What's the role of the condenser? The iris diaphragm? objective lenses? ocular lens? (also be able to identify them on the scope)
2. Which objective lens requires the use of immersion oil? Why? What does the oil do?
3. How can you estimate sizes of specimens under the microscope? (You should be able to estimate a specimen's size if the size of the field of view is provided and you know approximately how much of the field it spans)

Simple Staining

1. Why do we need to stain some microbes to observe them with a bright-field microscope? Which kinds do not need to be stained?
2. What are the characteristic bacterial cell morphologies? What is the difference between cell morphology and colony morphology?
3. What are the characteristic bacterial cell arrangements that we talked about in class? Why should you never base your identification of an unknown on the observed cell arrangement?
4. Describe the difference between a wet mount slide and a smear. Sometimes we use a broth (liquid) culture to make a smear, and sometimes we use a colony (from an agar slant or agar plate) to make a smear. Do we do anything differently to prepare a smear

from broth than we do to prepare a smear from a colony?

5. Why do we heat-fix the smear? WHEN do we heat fix?
6. What kind of information can you gather by doing a simple stain?
7. What's the difference between a basic dye and an acidic dye? What is the difference between the terms stain, dye and chromophore?
8. What's a negative stain? What type of dye/chromophore is found in a negative stain? Why is it necessary to use this technique to visualize capsules? Why don't we heat-fix the slide when doing a capsule stain?

Gram Stain & Endospore Stain

1. What's a differential stain? What information can you obtain from a Gram stain? What's the difference between a Gram positive cell and a Gram negative cell, in terms of their structure? You should know the steps in a Gram stain, and be able to explain the rationale for each step.
2. The last stain applied is safranin. Why do we use it? Why don't all cells end up looking red/pink at the end?
3. Why might old cells not give an accurate Gram stain result? Does it indicate that their cell wall structure has changed from Gram + to Gram -? What does it indicate about their cell walls?
4. What's an acid-fast stain? What type of bacteria are we looking for? What makes these bacteria "acid-fast"?
5. What is an endospore? How is it different from a vegetative cell? What sorts of bacteria make spores? Why do we care?
4. Please be familiar with the steps of the spore stain, and the rationale behind each step.