# Pre-Lab Questions

1. Biochemical and differential tests can be incorporated into growth media or performed on isolated colonies after they have been cultured. Give three examples of a differential test that is incorporated into media and three examples that are applied after the colonies have been cultured.
2. Using a textbook or a reputable internet source, find two Gram-positive pathogenic bacteria and two Gram-negative pathogenic bacteria that may be classified as being facultative anaerobes. What tissues or organs do these bacteria target in the human patient? Why is it to their advantage to be able to use either aerobic respiration or anaerobic respiration/fermentation?

**Experiment 1: Oxidase Assay For Respiration**

## Data Tables

Table 1: Experiment 1 Assay Results

| Colony Sample | Color Change? (Yes or No) | Oxidase Positive or Negative? |
| --- | --- | --- |
| Sink |  |  |
| Shoe |  |  |
| Phone |  |  |

## Post-Lab Questions

1. Which bacterial samples have cytochrome c oxidase as the terminal enzyme of their ETC? Which samples do not have cytochrome c oxidase as the terminal enzyme of their ETC?
2. What are the possible types of respiration that oxidase-positive samples might be capable of? Oxidase-negative?
3. Research and discuss a scenario in a lab or clinical setting in which you would need to perform an oxidase test.
4. Research and discuss how mitochondria in eukaryotic cells are similar to bacteria that use aerobic respiration.
5. What reaction would you expect when performing a positive control in the oxidase assay? What would it mean if a known oxidase-positive bacterium did not cause the expected reaction?

**Experiment 2: Catalase Assay**

## Data Tables

Table 2: Experiment 2 Assay Results

| Petri Dish Section | Colony | Bubbles? (Yes or No) | Catalase Positive or Negative? |
| --- | --- | --- | --- |
| Sink  | 1 |  |  |
| Sink | 2 |  |  |
| Shoe | 1 |  |  |
| Shoe | 2 |  |  |
| Phone | 1 |  |  |
| Phone | 2 |  |  |

## Post-Lab Questions

1. What are the possible types of respiration that catalase-positive samples might be capable of? Catalase-negative?
2. Did the colonies from each plate show the same reaction? Why or why not? How can you interpret this?
3. Research and discuss a scenario in a lab or clinical setting in which you would need to perform a catalase test.
4. What reaction would you expect when performing a positive control in the catalase assay? What would it mean if a known catalase-positive bacteria did not produce the expected reaction?

**Experiment 3: Antibiotic Sensitivity**

## Data Tables

Table 3: Zone of Inhibition for Skin Sample

| Antibiotic Disk | Diameter of Zone (mm) |
| --- | --- |
| AM10 |  |
| K30 |  |
| Te30 |  |

Table 4: Zone of Inhibition for Local Sample

| Antibiotic Disk | Diameter of Zone (mm) |
| --- | --- |
| AM10 |  |
| K30 |  |
| Te30 |  |

Table 5: Zone of Inhibition for Yeast Sample

| Antibiotic Disk | Diameter of Zone (mm) |
| --- | --- |
| AM10 |  |
| K30 |  |
| Te30 |  |

## Post-Lab Questions

1. Table 6 outlines interpretive standards of antibiotic resistance based on the size of inhibition zones. For example, many bacterial species that are resistant to Kanamycin have an inhibition zone of 13 mm or smaller. Of course, inhibition zones may also be affected by the antibiotic concentration present within the disk. Use Table 6 to determine whether the microbial samples you tested are sensitive (S), moderately resistant (MR), or resistant (R) to each of the antibiotic disks. Remember, you will not know the exact species of bacteria on your “Skin” and “Local” plates. The table is a guide to compare your results to a standard.
2. Is the bacterial lawn sampled from your skin more resistant to one antibiotic than the others? What about the local sample? The yeast sample? Use your results from Question 1 to support your answer.
3. What is the significance of the size of the zone of inhibition with respect to the microbe samples?
4. Research and describe the relationship between the minimum inhibitory concentration (MIC) and the zone of inhibition. What can you determine about the MIC and the effectiveness of the antibiotics used in this experiment? Note, disk potencies are listed per antibiotic in the Materials section before the experimental procedure.
5. Use a reputable internet source to determine and describe which cellular component in the bacterial cell is targeted by each of the antibiotics used in this experiment.