**Guided Work 02**

**I- Revue questions**

1. What are the different techniques used to observe bacterial cells?

2. What is the difference between direct examination in the fresh state and observation after staining?

3. Why is the fresh state particularly used to observe the shape and motility of bacteria?

4. Explain the importance of the methylene blue staining technique. What type of bacteria are stained with this technique?

5. What staining method is used to identify pathogenic bacteria?

6. What are the essential steps in the process of fixation and staining of bacterial smears?

7. What staining technique is used to identify bacterial spores, and why is this treatment necessary?

8. What are the particularities of observing fungi in the fresh state?

**II- Exercices:**

Bacteria, being unicellular organisms of very small size, require specific observation techniques to be seen under a microscope. Depending on their size, shape, and cell composition, different staining and observation methods are used to study them.

1- Define the following terms: Gram-positive bacteria, Gram-negative bacteria, Bacilli, Cocci, Spirilla, Acid-fast

2- Describe the steps of Gram staining. Explain what happens at each step of the process and how this method helps differentiate bacterial types.

3- Why is fixation a crucial step before any staining? Explain the objectives of fixation, What consequences could result from a lack of fixation of a smear?

4- Comparison between Gram staining and Ziehl-Nielsen staining: How do these two staining methods differ? Which bacteria are targeted by each method? Provide examples of bacteria that require each of these stains.

5- Interpret the following results from a microscope observation after staining: a) Bacteria appear purple and in clusters. b) Bacteria appear pink and in chains. c) Bacteria are only visible in small spiral colonies. For each case, indicate:

* + The type of staining performed.
  + The shape and arrangement of the bacteria.
  + The likely classification of the bacteria.

1. Case Study: You observe two different samples under the microscope after Gram staining:
   * Sample A: The bacteria are purple and form irregular clusters.
   * Sample B: The bacteria are pink and form regular chains.

Based on your microbiological knowledge, identify the type of each bacteria (Gram-positive or Gram-negative) and their arrangement. Justify your answers.

1. Explain the color difference observed in Gram staining between Gram-positive and Gram-negative bacteria.
   * Why do Gram-positive bacteria remain purple after decolorization, while Gram-negative bacteria turn pink?
2. What is an acid-fast cell?
   * Give examples of acid-fast bacteria.
   * Explain why certain bacteria need Ziehl-Nielsen staining to be identified.