

MICROBIOLOGY LABORATORY - REVIEW QUESTIONS

1. Aside from the three morphological forms (cocci, bacilli & spirilli), are there other special morphological forms?

Aside from cocci, bacilli, and spirilli, the other special morphological forms are mainly actinomycetes, mycoplasma, spirochetes, and vibrios which all have varying characteristics. Actinomycetes refer to thin rod-like structure with long branching filaments owing to the presence of a rigid cell wall. Meanwhile, mycoplasma is oval/round in shape interlacing with filaments and is known as a bacteria that does not have a stable morphology due to its cell wall deficient nature. Furthermore, spirochetes are morphologically spiral ranging from loose coils to a rigid corkscrew shape while vibrios is known for its comma shaped curved rods.

2. Enumerate and give the functions of the reagents used in Gram's and Acid Fast Stain

1. **Crystal violet (primary stain)** - It refers to purple stain which is commonly used to differentiate between Gram-Positive and Gram-Negative bacteria. The crystal violet molecules are positively charged and bind to negatively charged bacterial cell wall structures, staining the cells in purple.
2. **Gram's Iodine (mordant)** - It acts as a dye-fixator (mordant) that fixes the purple stain in both Gram-positive and Gram-negative bacteria. As it is insoluble in water, it functions to ensure that the crystal violet dye remains trapped in the bacterial cell wall.
3. **95% Alcohol (decolorizer)** - It functions as a dehydration agent in the peptidoglycan layer by shrinking and tightening it. The large crystal violet-iodine complex is unable to penetrate this tightened peptidoglycan layer, and remains trapped in the cell in Gram-positive bacteria.
4. **Safranin (counter stain)** - It refers to a red dye that stains bacteria that has been decolorized. The Gram-positive bacteria turns purple while the Gram-negative bacteria turns red. Through this reagent, gram-negative bacteria can be easily distinguished from gram-positive bacteria.

3. What is the purpose of steaming the bacterial smear before staining?

Steaming the bacterial smear before staining is important as it soaks up the waxy layer and helps with the entry of the primary stain inside the cell. Moreover, steaming before staining also kills the bacteria in the smear which enables better adherence of the bacterial smear to the slide. All these help in long-term preservation of the smear as well as ensures that the cells do not slip during the staining procedure.

4. What is the factor behind an organism being gram (+) or gram (-)?

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