

## Laboratory 2- Smear Preparation, Simple Stain and Bacterial Morphology\*

\*Laboratory notes are to be used as a **study guide** only and *do not* represent the comprehensive information you will need to know for the exams.

### Aseptic technique:

When working with microbes, such as bacteria, it is important to practice *aseptic techniques*.

Aseptic – derived from *septikos* : to make putrid

*a* : prefix, without

*aseptic*: to not make putrid

*Aseptic techniques*: handle microbes so that unwanted organisms do not grow in the growth medium.

Microbes are all around us, it is important to keep the unwanted ones out. Aseptic technique is good practice when it is important to treat patients with a disease.

### Using an inoculating needle / inoculating loop:

1. Transfer the inoculum (cells) to the medium – inoculated. Use aseptic techniques. An inoculating loop or inoculating needle is used to transfer the bacteria. It is important to flame sterilize the inoculating loop and/or inoculating needle before and after use.

- flame the loop until **bright red - orange**, cool the loop, pick up the bacteria, inoculate the growth medium,

- then flame again. \* carefully follow this procedure\*

2. Once the inoculation is complete the culture will be incubated in an environment that will allow optimum growth.

- growth in a broth culture is detected by turbidity
- growth on a plate / slant is detected by colonies

### Smear Preparation:

Learn how to prepare a *smear* – spread organisms evenly across a glass microscope slide. Can use organisms from a liquid or solid medium. Use aseptic techniques.

Steps:

1. Obtain a clean glass slide.
2. Add a small drop of water on the slide.
3. Collect bacterial cells using aseptic techniques.
4. Mix bacterial cells with the drop of water on the slide, spread evenly.
5. Heat fix the cells to the slide by placing a clothes pin at the end of the slide and pass quickly through the flame 4 -5 times.
6. Add the appropriate dye to stain the cells.

### Staining:

A stain is a dye that has two functional chemical groups:

- i. chromophore, imparts color
- ii. auxochrome, assists in the solubility of the dye

The function of a stain is to impart color to cells to visually see them.

Types of stains:

- a. *simple stain*: help to visualize the whole cell, use one dye / stain.
- b. *differential stain*: based on a chemical and / or physical characteristic aspect of the cell, distinguish differences among cells.
- c. *special stain*: stain a unique physical characteristic of the cell

### Smear Preparation and simple staining:

- cells are transparent when viewed through the microscope after making a smear, therefore you must stain the cells to visualize them
  
- General stain procedure:
  1. *smear*: apply a thin layer of cells to the microscope slide
  2. *heat fix*: adhere the cells to the slide with heat
  3. *stain*: cover the slide with cell with a dye / stain
  
- *Simple stain*: will stain the entire cell one color, unable to differentiate among bacteria and specific structures, example, **methylene blue**. Simple staining can be useful to determine morphology and arrangement.

### Morphology and Arrangement:

1. Morphology: the shape of the cell, useful in classifying bacteria. The most common shapes of medically important bacteria:

- a. bacillus, bacilli / rod, examples, *Escherichia coli* and *Bacillus megaterium*.
- b. coccus, cocci / spherical, examples, *Staphylococcus aureus* and *Streptococcus pyogenes*.
- c. *spirillum*, *spirochete* / helical, twist, examples, *Treponema pallidum*.

2. Arrangement: how cells are grouped together. The most common grouping of medically important bacteria:

- a. diplo / pair, example, *Neisseria gonorrhoeae*.
- b. staph, staphy / "grape-like" clusters, example, *Staphylococcus aureus*.
- c. strep, strepto / chains, example, *Streptococcus pyogenes*

