بكتـريـا طبيـة 1

Bacterial Staining

Sawa University جامعـــــــة ســـــــاوة الأهليـة

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Second Stage المرحلـــــــــــــة / الثانيـــة

تدريسي المادة : دكتــور معيــن حســن

Practical الجانب العملي

محاضرة رقم 7

Lecture No. 7

Bacterial Staining

Purpose of staining

Q: Why do we stain microorganisms?

1. **To study their shape**
2. **To differentiate the bacterial species by using different stain.**
3. **To study internal components of the bacterial cell (Endospore, Capsule, flagella)**

Q/ What is the stain?

- **Stains or dyes are chemicals with two parts:**

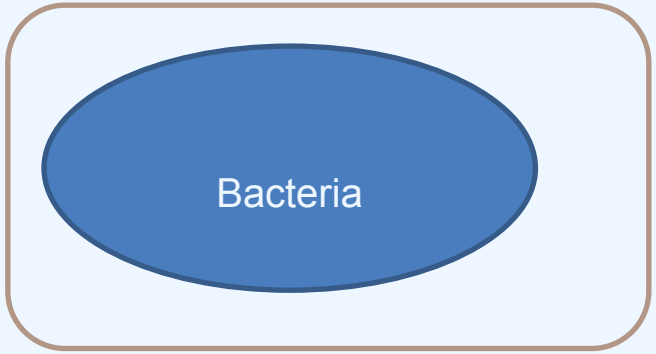
1-Chromophore: the part that has the color. Which may be charged either +ve charge or -ve charge.

2-Auxochrome: the part that helps in the coloring properties .

- **Types of stains**

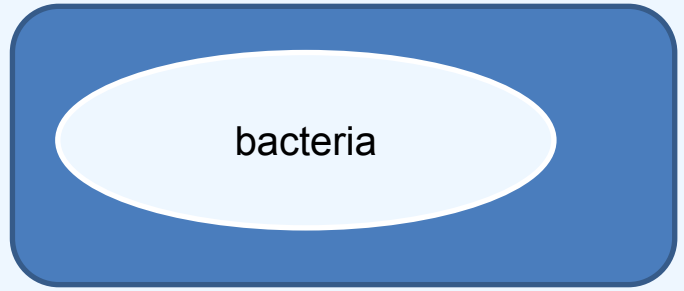
1- Basic stain: A positively charged (cationic dye):

- **Chromophore is a positive ion dye attracted by the bacteria so the cells of bacteria stained**
- **e.g. Crystal violet, methylene blue, safranine.**



2- Acidic stain: A negatively charged (anionic dye)

- **Chromophore is a negative ion dye rejected by the bacteria (bacteria are colorless) and background of slide is stained.(The glass of the slide will stain, but the bacterial cells will not)**
- **e.g. Eosin, India ink**



- **Bacteria are slightly negative (cell surface is –ve charge), so are attracted to positive chromophore of basic stain.**

Types of Staining techniques

1- **Simple staining:** Use of one stain. (e.g. Crystal violet, methylene blue).

- Use for visualization of morphological shape (Coccus, spiral, and bacillus) and arrangement (chains, clusters, pairs, and tetrads)

2- **Differential staining:** Use of two contrasting stains.

- Use for Separation into groups

  e.g. 1-Gram stain

  2-Acid-fast stain

3- **Special staining**

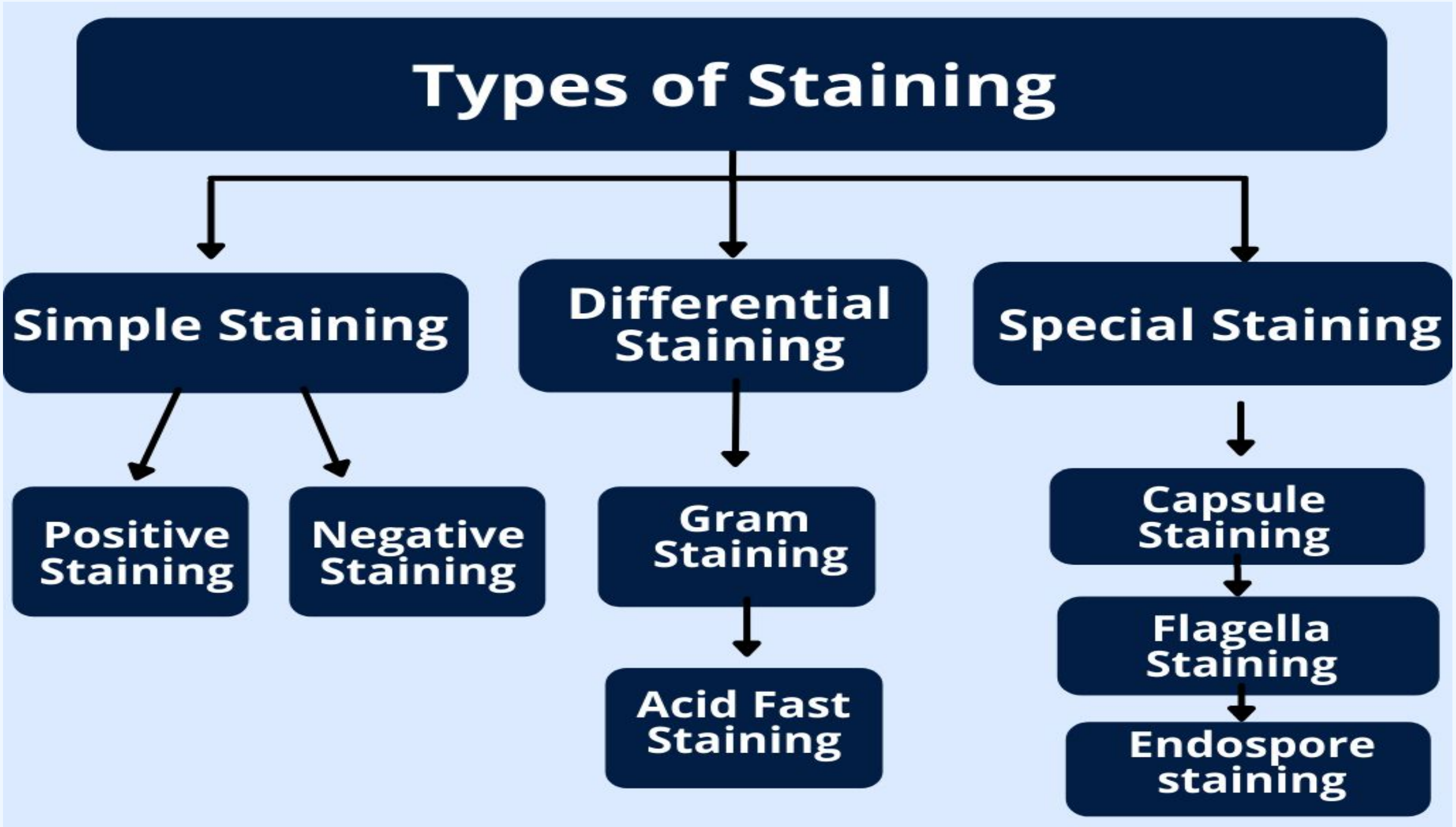
- Use for Visualization of structures

  e.g. 1- Flagella stain

  2- Capsule stain

  3-Spore stain

  4-Nuclear stain


Types of Staining
Simple Staining
Differential Staining
Special Staining
Positive Staining
Negative Staining
Gram Staining
Acid Fast Staining
Capsule Staining
Flagella Staining
Endospore staining

❖ Simple Staining:

- **It used to reveal the morphological features of most bacterial cell including size, shape and arrangement of bacterial cells.**

- **There are two groups of simple stain**

1-Basic or Alkaline stain: has positive charge, it is staining the acidic compounds (RNA,DNA) of the cell with a negative charge (Methylene blue , Crystal violet, safranin)

2-Acidic stain: has negative charge, it is staining the basic compounds (protein)of the cell with a positive charge(Eosin, India ink , acid fuchsin)

Steps of work

1- Smear preparation : It is spreading microbial cells on a clean glass slide and allowing it to air dry and then heat fix.

- **From broth: one loopful directly from broth on the slide**
- **From solid culture: put one drop of water in the center of the slide the take one touch from colony and mix well with the drop.**

2- Fixation

- **After air drying, the fixation will done by passing the slide rapidly across the flame three times the slide should be warm not hot , why? hot will change the shape of bacterial cell.**

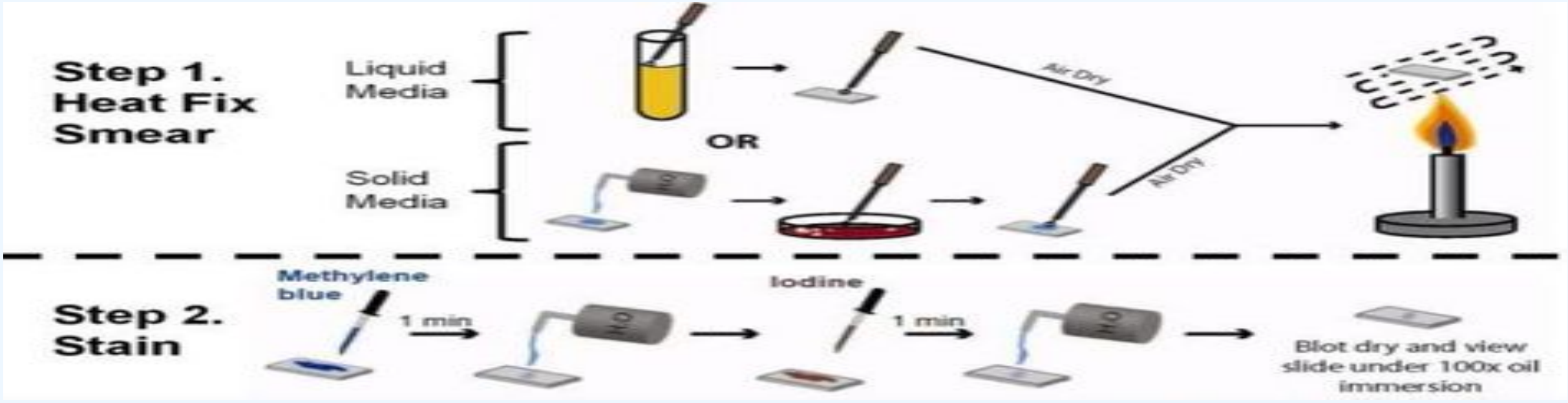
The fixation benefit in:

A. Kill the bacteria so the stain will penetrate without serious destruction of cell structure.

B. Fix the cell on the slide (by coagulation of their protein) So they are not removed during staining.

3- Staining with dye:

- **Put the slide on the staining rack and flood the smear with simple stain (Crystal violet or Safranin)for 1 min.**
- **Wash the slide with tap water gently and drain of excess water**
- **Air dry**
- **Examine with microscope under oil –immersion lens.**



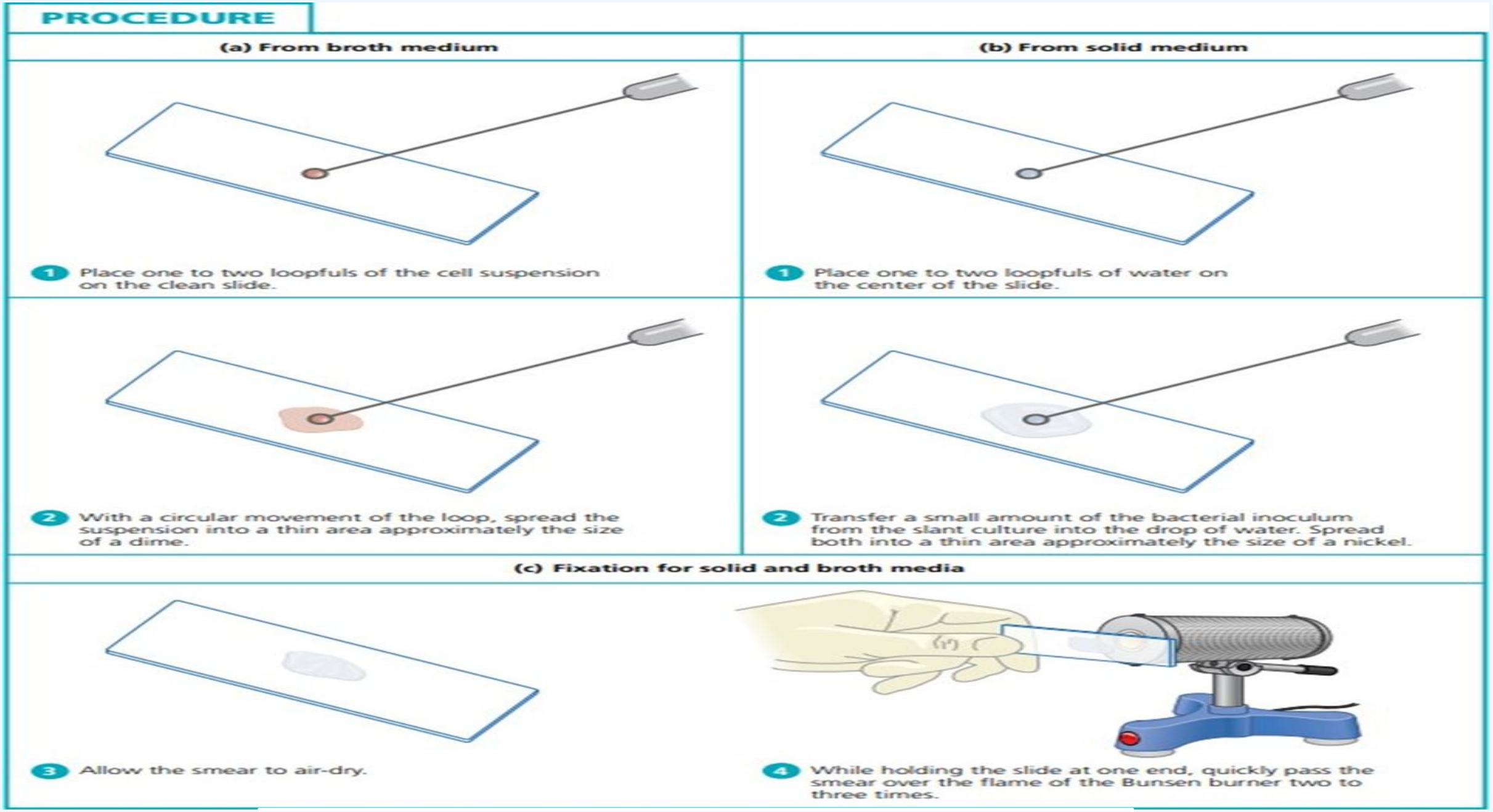


Figure: Procedure of smear preparation

Figure: Slide Smear.

QUIZ

Q1/ WHAT ARE PURPOSE OF STAINING.

Q2/ WHAT IS THE STAIN ?

Q3/ WHAT ARE THE TYPES OF STAINS?

Any questions?