Sawa University

DIAGNOSTIC MICROBIOLOGY

Sawa University

College of health and medical techniques

Department of Medical Laboratories

4 Stage

جامعـــــــة ســـــــــاوة الاهلية

كليـــــــة التقنيـــات الصحيـة والطبيـة

قسم تقنيات المختبرات الطبية

المرحلـــــــــــــــة/الرابعة

محاضرة رقم:8

Lecture No 8:

تدريسي المادة : م.م عذراء هادي

Fixation

Fixation is the process by which the internal and external structures of cells are preserved and fixed in position. It also inactivates the enzymes that might disrupt cell morphology. It toughens (hardens) cell structure so that they do not change during staining. It kills and fixes the cells on to the .slide

Aseptic Smear Preparation in Laboratories

1. Sterilization of tools

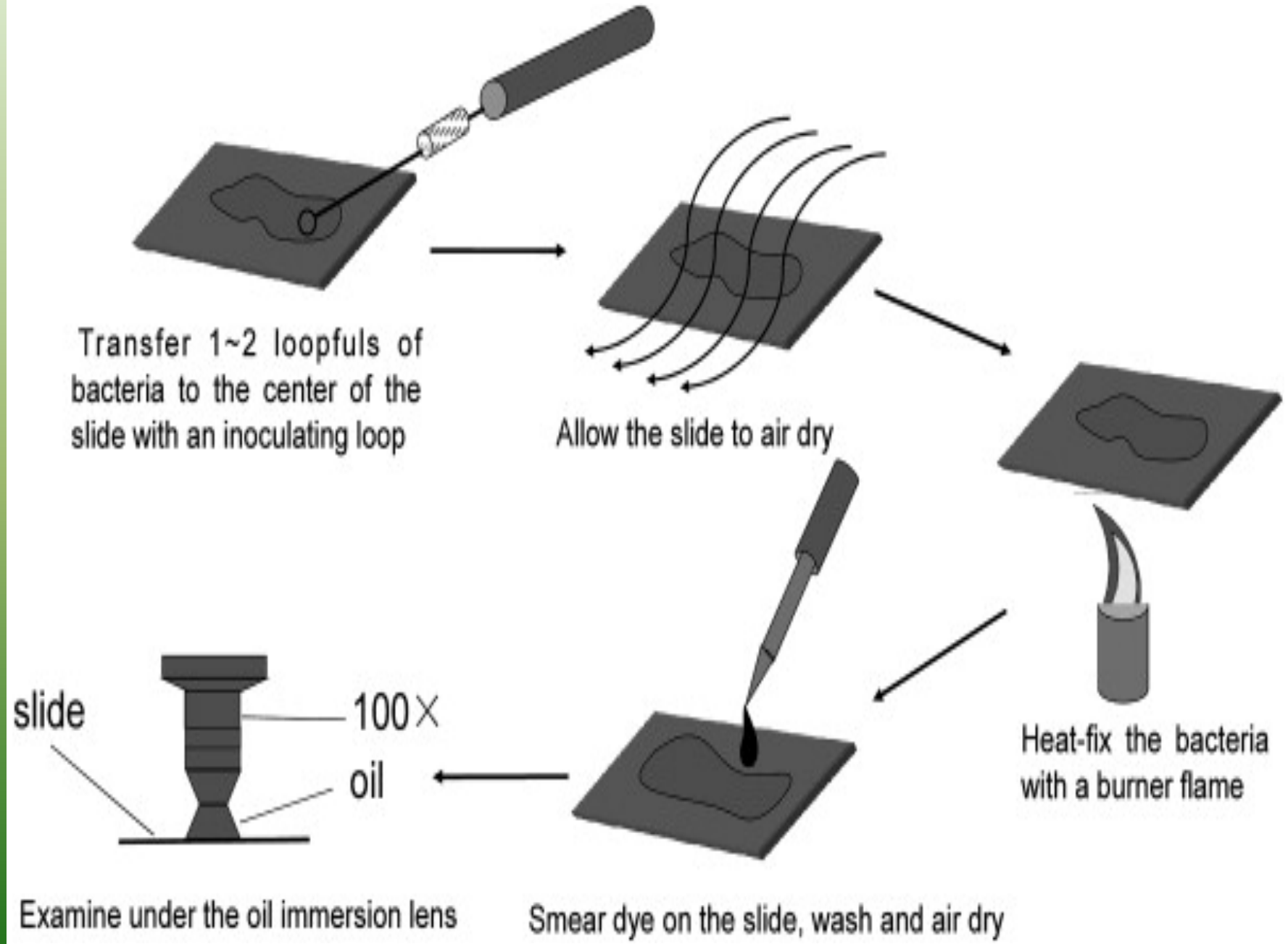
2. Sample collection

3. Smear preparation

4. Air-drying

5. Heat fixation

Aways work near a flame or in a laminar airtow cabinet.


Transfer 1~2 loopfuls of bacteria to the center of the slide with an inoculating loop
Allow the slide to air dry
Heat-fix the bacteria with a burner flame
Smear dye on the slide, wash and air dry
Examine under the oil immersion lens
slide
100×
oil

:There are two types of fixation as follows

Heat fixation in microbiology is a crucial step, usually involving passing a dried bacterial-1 smear on a slide through a Bunsen burner flame to **kill the microbes**, **adhere them firmly to the slide**, and **alter them to better accept stains**, preventing them from washing off during staining and microscopy, though it

.preserves overall morphology but not internal structures like capsules

Purpose of Heat Fixation

.**Adhesion:** Attaches the cells to the slide so they don't wash away-

Kills Microorganisms: Inactivates pathogens and enzymes, preventing autolysis-

.(self-digestion)

Enhances Staining: Makes cells more permeable to dyes-

.


heat fixing bacteria
flame
incinerator

Chemical fixation in microbiology is the process of using chemical agents to rapidly kill-**2** microorganisms and preserve their cellular components and morphology in a "lifelike" state for microscopic examination. This process is crucial for preventing the natural degradation of the .specimen through autolysis (self-digestion by enzymes) and putrefaction (bacterial decomposition)

Mechanism and Purpose

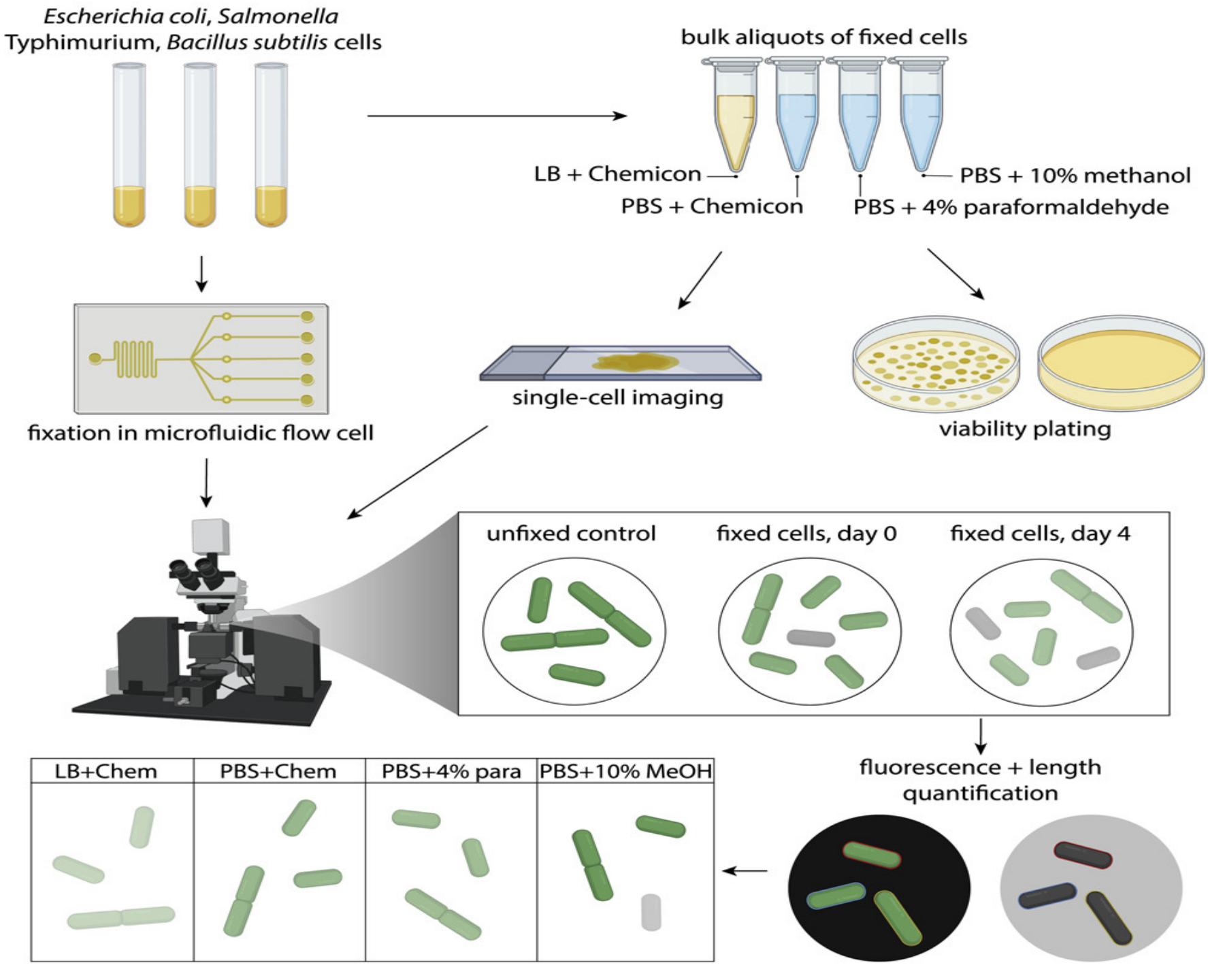
The primary goal is to stabilize proteins, lipids, and nucleic acids to maintain the structural integrity .of the cell during subsequent processing steps such as staining, dehydration, and microscopy

:The main mechanisms of chemical fixation are

Cross-linking: Certain chemicals form covalent bonds between proteins and other-1 .macromolecules, creating a stable, insoluble gel network that anchors cellular components in place

Denaturation/Coagulation: Organic solvents remove water and disrupt hydrophobic and hydrogen-2 .bonds, causing proteins to precipitate and lose their tertiary structure

Fixation also enhances the specimen's affinity for stains, improves optical differentiation, and inactivates pathogens, making the sample safer to handle


Escherichia coli, *Salmonella* Typhimurium, *Bacillus subtilis* cells
bulk aliquots of fixed cells
LB + Chemicon
PBS + Chemicon
PBS + 4% paraformaldehyde
PBS + 10% methanol
fixation in microfluidic flow cell
single-cell imaging
viability plating
unfixed control
fixed cells, day 0
fixed cells, day 4
LB+Chem
PBS+Chem
PBS+4% para
PBS+10% MeOH
fluorescence + length quantification