

Microbiology Lab Procedures Supplement : Fixation and Staining

Specimens must be fixed and stained to

1. increase visibility
2. accentuate morphology
3. preserve for future study

Fixation :

defined as the process to preserve and fix into position.

- * toughens cell wall structure so they do not change
- * microorganisms killed and attached firmly to the slide

Two types of fixation techniques :

1. Heat Fixation -> flame heating a dry film
preserves overall morphology, but not the structures within the organism

2. Chemical fixation -> penetrates to render microorganisms
inactive
insoluble
immobile

preserves morphology and internal structure

examples :: ethanol, acetic acid, formaldehyde

Stain :

dye in chromophore groups (double bonds give the dye its color)

bind to cells using ionic, covalent, or hydrophobic means (+ dye binds to - cell structure)

Two groups of dyes ->

1. Basic : methylene blue, basic fuchsin, crystal violet, safranin, & malachite green
basic dyes bind to (-) molecules like nucleic acids and protein
2. Acidic : eosin, rose bengal, acid fuchsin
acidic dyes bind to (+) cell structures

** Remember :: age of culture, thickness of smear and pH can alter the stains effectiveness

Types of Staining Techniques

A. Simple Staining

single staining agent is used on a fixed smear
agent is left on for the appropriate length of time

Basic dyes are used : crystal violet, methylene blue, carbol fuschin

B. Differential Staining

1. Gram stain

differences due to cell wall

g(+) have thick peptidoglycan layer

g(−) have single thin peptidoglycan layer

Basic dye with mordant to increase interaction

Decolorized and then counter stained with basic dye

g(+) retain the crystal violet --> purple

g(−) are decolorized and counter stained to pink/red

2. Acid Fast

harsher treatment due to heating of stain

ZN method uses basic fuschin and phenol

helps to i.d. *Mycobacterium*

due to the high lipid content of the acid fast wall, these cells are not easily decolorized by the acid alcohol, whereas non-acid fast cells can be decolorized and counter stained.

3. Special Stains

a. Negative Staining :

bacteria mixed with India Ink or Nigrosin
helps to identify capsule

b. Spore Staining :

malachite green and safranin
helps to identify endospores

c. Capsule Stain :

crystal violet and copper sulfate
helps to identify capsule

d. Flagella Staining :

formalin and pararosaniline
helps to identify flagella of motile bacteria