Bacterial Stains

Simple Stain	-Single basic dye e.g. Methylene blue - All bacteria take the color of the dye
Differential Stain	
Primary Stain	_ First dye used in the staining process -Will initially stain all cells and then be removed from a subset
Mordant	Improves the ability of the primary stain to bind cells
Decolorizer	Removes the primary stain from a subset of cells
Counterstain	Second dye that stains decolorized cells
Positive stain = purple color, negative charge	
Negative stain = pink stain, negative charge	

Types of Microscopes

Light Microscope (Bright-field)	*Unstained* Passes light directly through specimen unless cell is naturally pigmented or artificially stained, image has little contrast *Stained* Staining with various dyes enhances contrast, but most staining procedures require that cells be fixed (preserved)	1.Iris diaphragm 2.Condenser 3.Specimen 4.Objective lense 5.Eye
Dark-field Microscope	-Light is projected at an angle to the surface, causing any variations to deflect light up into the camera -Nothing is seen by the vision system if there are no aberrations on the surface -dark background makes the organism glow	1.Light source 2.Dark Field Patch Stop 3.Condenser Lens 4.Sample 5.Direct Illumination Block 6.Objective Lens 7.Eyes

Types of Microscopes (cont)

Phase-contrast microscope	Enhances contrast in unstained cells by amplifying variations in refractive index within specimen -especially useful for examining living, unpigmented cells	1.Light from source 2.Annular ring 3.Condenser 4.Specimen 5.Objective 6.Deflected Light 7.Phase Ring 8.Eye
Fluorescent microscope (UV light)	-Shows the location of specific molecules in the cell -Fluorescent substances absorb UV radiation and emit visible light -the fluorescing molecules may occur naturally in the specimen but more often are made by tagging the molecules of interest with fluorescent dyes or antibodies *Fluoresceins=Apple Green* *Rhodamines=Orange red*	1.HBO lamp house 2.Excitation filter 3.Objective 4.Specimen 5.Dichroic beam splitter 6.Barrier Filter 7.Eyepiece
Electron microscope	-Uses electrons to scan specimens to create and magnify images -Produces clear/detailed 3D images -Specimen has to be held in place -Unable to view living specimen -up to 50,0000x magnification	1.Electron 2.Condenser 3.Specimen 4.Objective lens 5.First image 6.Final image 7.Fluorescent screen 8.Eye

Bacterial Cell Structures

Outer Layers	
1. Glycocalyx (capsule,slime layer)	
2. Cell Wall	*Gram Positive Cell Wall* -Peptidoglycan -teichoic acid and lipoteichoic acid *Gram Negative Cell Wall* -Outer membrane --LPS, Lipoprotein, Porin protein -Peptidoglycan -Periplasmic space
Cell Membrane	
Phospholipid Bilayer	-fatty acids -glycerol
Cytoplasm	
Chromatin	
Ribosomes	
Cell Shapes	
Cocci	-Circles
Bacilli	-Rods
Spirilla	-Spirals

Types of Differential Stains

Stain Type	*Specific Dyes*	*Purpose*	*Outcome*
Gram Stain	Uses crystal violet, Gram's iodine, ethanol (decolorizer) and safranin	Used to distinguish cells by cell-wall type (gram-positive, gram-negative)	Gram-positive cells stain purple/violet. Gram-negative cells stain pink.
Acid-fast Stain	After staining with basic fuchsin, acid-fast bacteria resist decolorization by acid-alcohol. Non acid-fast bacteria are counterstained with methylene blue.	Used to distinguish acid-fast bacteria such as M. tuberculosis, from non-acid fast cells	Acid-fast bacteria are red; non-acid fast cells are blue.

Types of Differential Stains (cont)

Endospore Stain	Uses heat to stain endospores with malachite green, then cell is washed and counterstained with safranin	Used to distinguish organisms with endospores from those without; used to study the endospore.	Endospores appear bluish-green; other structures appear pink to red.
Flagella Stain	Flagella are coated with tannic acid or potassium alum mordant, then stained using either pararosaline or basic fuchsin	Used to view and study flagella in bacteria that have them	Flagella are visible if present
Capsule Stain	Negatie staining with India ink or nigrosin is used to stain the background, leaving a clear area of the cell and the capsule. Counterstaining can be used to stain the cell while leaving the capsule clear	Used to distinguish cells with capsules from those without	Capsules appear clear or as halos if present