MICROSCOPY, STAINING, AND CLASSIFICATION

Ch. 4
Microbiology
CCV

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TABLE 4.1 Metric Units of Length

Metric Unit (abbreviation)	Meaning of Prefix	Metric Equivalent	U.S. Equivalent	Representative Microbiological Application of the Unit
Meter (m)	—[a]	1 m	39.37 in (about a yard)	Length of pork tapeworm, *Taenia solium* (e.g., 1.8–8.0 m)
Decimeter (dm)	1/10	0.1 m = 10^{-1} m	3.94 in	—[b]
Centimeter (cm)	1/100	0.01 m = 10^{-2} m	0.39 in; 1 in = 2.54 cm	Diameter of a mushroom cap (e.g., 12 cm)
Millimeter (mm)	1/1000	0.001 m = 10^{-3} m	—	Diameter of a bacterial colony (e.g., 2.3 mm); length of a tick (e.g., 5.7 mm)
Micrometer (μm)	1/1,000,000	0.000001 m = 10^{-6} m	—	Diameter of white blood cells (e.g., 5–25 μm)
Nanometer (nm)	1/1,000,000,000	0.000000001 m = 10^{-9} m	—	Diameter of a poliovirus (e.g., 25 nm)

[a]The meter is the standard metric unit of length.
[b]Decimeters are rarely used.

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Figure 4.3 The limits of resolution (and some representative objects within those ranges) of the human eye and of various types of microscopes.

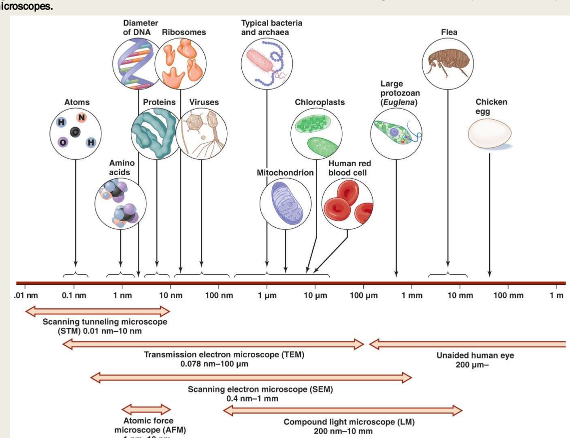

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Figure 4.4 A bright-field, compound light microscope.

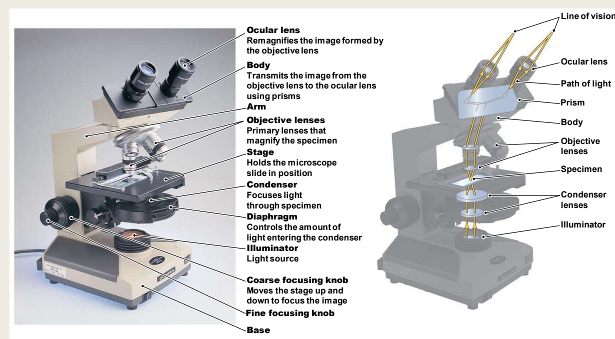

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Figure 4.5 The effect of immersion oil on resolution.

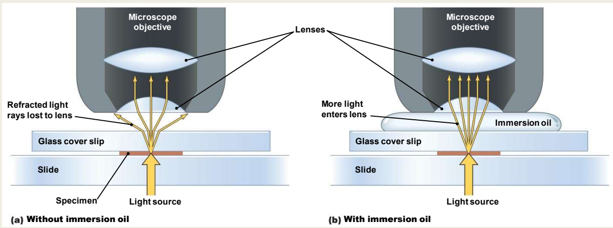

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TABLE 4.2 Comparison of Types of Microscopes

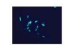
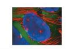
Type of Microscope	Typical Image	Description of Image	Special Features	Typical Uses
Light Microscopes		Useful magnification 1× to 2000×; resolution to 200 nm	Use visible light; shorter, blue wavelengths provide better resolution	
Bright field		Colored or clear specimen against bright background	Simple to use; relatively inexpensive; stained specimens often required	To observe killed stained specimens and naturally colored live ones; also used to count microorganisms
Dark field		Bright specimen against dark background	Uses a special filter in the condenser that prevents light from directly passing through a specimen; only light scattered by the specimen is visible	To observe living, colorless, unstained organisms
Phase contrast		Specimen has light and dark areas	Uses a special condenser that splits a polarized light beam into two beams, one of which passes through the specimen, and one of which bypasses the specimen; the beams are then rejoined before entering the oculars; contrast in the image results from the interactions of the two beams	To observe internal structures of living microbes
Differential interference contrast (Nomarski)		Image appears three-dimensional	Uses two separate beams instead of a split beam; false color and a three-dimensional effect result from interactions of light beams and lenses; no staining required	To observe internal structures of living microbes
Fluorescence		Brightly colored fluorescent structures against dark background	An ultraviolet light source causes fluorescent natural chemicals or dyes to emit visible light	To localize specific chemicals or structures; used as an accurate and quick diagnostic tool for detection of pathogens
Confocal		Single plane of structures or cells that have been specifically stained with fluorescent dyes	Uses a laser to fluoresce only one plane of the specimen at a time	Detailed observation of structures of cells within communities

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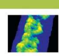
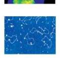
TABLE 4.2 Comparison of Types of Microscopes *(Continued)*

Type of Microscope	Typical Image	Description of Image	Special Features	Typical Uses
Electron Microscopes		Typical magnification 1000× to 100,000×; resolution to 0.001 nm	Use electrons traveling as waves with short wavelengths; require specimens to be in a vacuum, so cannot be used to examine living microbes	
Transmission		Monotone, two-dimensional, highly magnified images; may be color enhanced	Produces two-dimensional image of ultrastructure of cells	To observe internal ultrastructural detail of cells and observation of viruses and small bacteria
Scanning		Monotone, three-dimensional, surface images; may be color enhanced	Produces three-dimensional view of the surface of microbes and cellular structures	To observe the surface details of structures
Probe Microscopes		Magnification greater than 100,000,000× with resolving power greater than that of electron microscopes	Uses microscopic probes that move over the surface of a specimen	
Scanning tunneling		Individual molecules and atoms visible	Measures the flow of electrical current between the tip of a probe and the specimen to produce an image of the surface at atomic level	To observe the surface of objects; provide extremely fine detail, high magnification, and great resolution
Atomic force		Individual molecules and atoms visible	Measures the deflection of a laser beam aimed at the tip of a probe that travels across the surface of the specimen	To observe living specimens at the molecular and atomic levels

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Staining

- Most microorganisms are difficult to view by bright-field microscopy
- Coloring specimen with stain increases contrast and resolution
- Specimens must be prepared for staining

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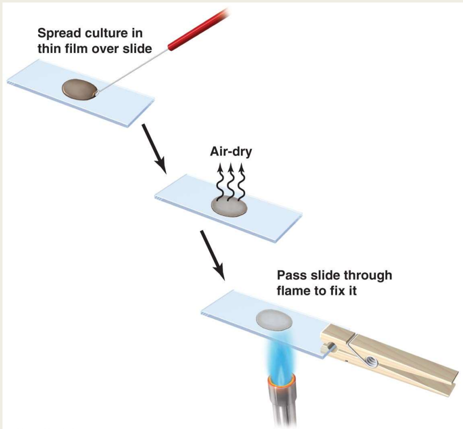
Figure 4.15 Preparing a specimen for staining.



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Staining

- **Principles of Staining**
  - *Dyes used as stains are usually salts*
  - *Chromophore is the colored portion of the dye*
  - *Acidic dyes stain alkaline structures*
  - *Basic dyes stain acidic structures*
    - More common because most cells are negatively charged

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Staining

- Simple stains—composed of single dye
- Differential stains—use more than one dye
  - *Gram stain*
  - *Acid-fast stain*
  - *Endospore stain*
  - *Histological stains*
- Special stains—reveal specific structures
  - *Negative (capsule) stain*
  - *Flagellar stain*

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Figure 4.16 Simple stains.

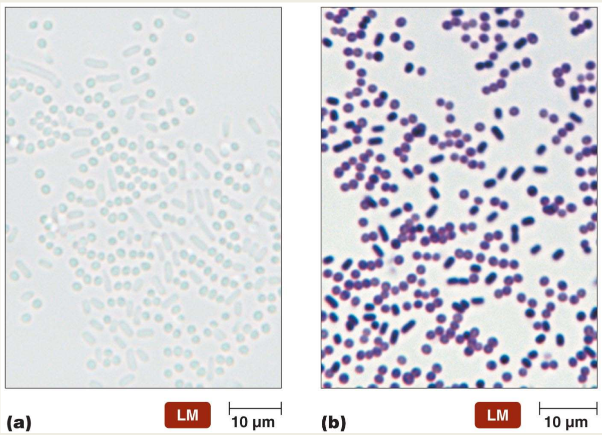

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Figure 4.17 The Gram staining procedure.

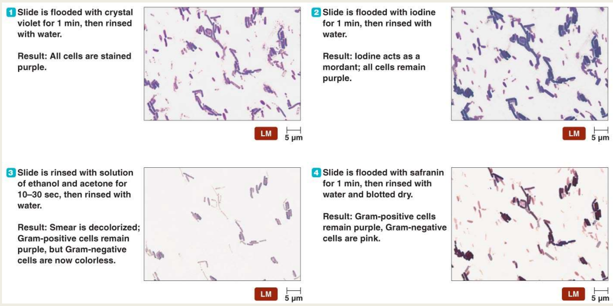

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Figure 4.18 Ziehl-Neelsen acid-fast stain.

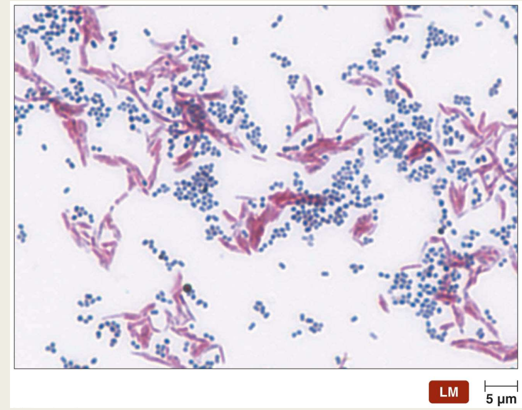

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Figure 4.19 Schaeffer-Fulton endospore stain of *Bacillus anthracis*.

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Staining

- Differential Stains
  - *Histological stains*
    - Two common stains used for histological specimens
      - *Gomori methenamine silver (GMS) stain*
      - *Hematoxylin and eosin (HE) stain*

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Figure 4.20 Negative (capsule) stain of *Klebsiella pneumoniae*.

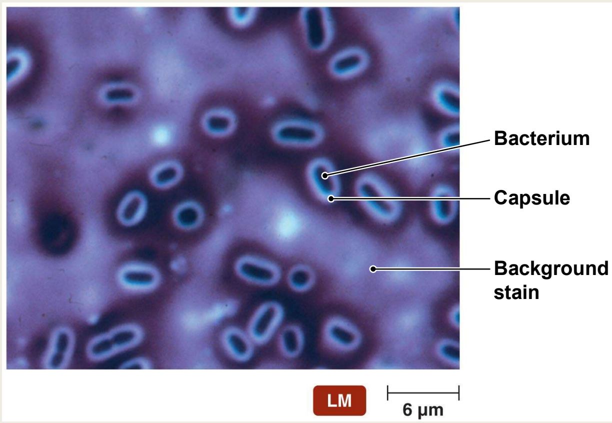

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Figure 4.21 Flagellar stain of *Proteus vulgaris*.

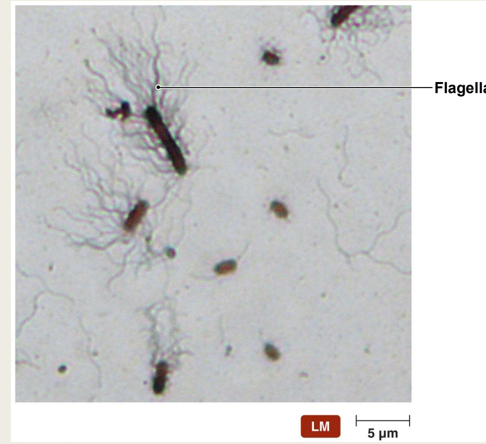

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TABLE 4.3 Some Stains Used for Light Microscopy

Type of Stain	Examples	Results	Typical Images	Representative Uses
Simple stains (use a single dye)	Crystal violet Methylene blue	Uniform purple stain Uniform blue stain		Reveals size, morphology, and arrangement of cells
Differential stains (use two or more dyes to differentiate between cells or structures)	Gram stain	Gram-positive cells are purple; Gram-negative cells are pink		Differentiates Gram-positive and Gram-negative bacteria, which is typically the first step in their identification
	Ziehl-Neelsen acid-fast stain	Pink to red acid-fast cells and blue non-acid-fast cells		Distinguishes the genera *Mycobacterium* and *Nocardia* from other bacteria
	Schaeffer-Fulton endospore stain	Green endospores and pink to red vegetative cells		Highlights the presence of endospores produced by species in the genera *Bacillus* and *Clostridium*
Special stains	Negative stain for capsules	Background is dark, cells unstained or stained with simple stain		Reveals bacterial capsules
	Flagellar stain	Bacterial flagella become visible		Allows determination of number and location of bacterial flagella

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Classification and Identification of Microorganisms

- Taxonomy consists of classification, nomenclature, and identification
- Organize large amounts of information about organisms
- Make predictions based on knowledge of similar organisms

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Classification and Identification of Microorganisms

- **Linnaeus and Taxonomic Categories**
  - *Current taxonomy system began with Carolus Linnaeus*
    - His system classified organisms based on characteristics in common
    - Grouped organisms that can successfully interbreed into categories called species
    - Used binomial nomenclature

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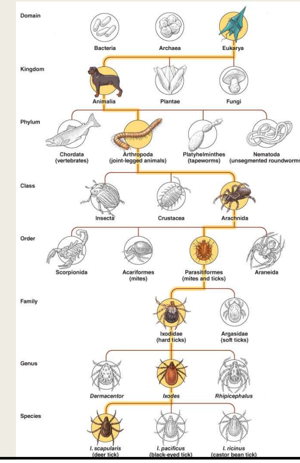
Figure 4.22 Levels in a Linnaean taxonomic scheme.



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Classification and Identification of Microorganisms

- **Linnaeus and Taxonomic Categories**
  - *Linnaeus proposed only two kingdoms*
  - *Later taxonomic approach based on five kingdoms*
    - Animalia, Plantae, Fungi, Protista, and Prokaryotae
  - *Linnaeus's goal was to classify organisms in order to catalog them*
  - *Modern goal is to understand relationships among organisms*
  - *Goal of modern taxonomy is to reflect phylogenetic hierarchy*
  - *Greater emphasis on comparisons of organisms' genetic material led to proposal to add domain*

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Classification and Identification of Microorganisms

- **Domains**
  - *Carl Woese compared nucleotide sequences of rRNA subunits*
  - *Proposal of three domains as determined by ribosomal nucleotide sequences*
    - Eukarya, Bacteria, and Archaea
  - *Cells in the three domains also differ with respect to many other characteristics*

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Classification and Identification of Microorganisms

- Taxonomic and Identifying Characteristics
  - *Physical characteristics*
  - *Biochemical tests*
  - *Serological tests*
  - *Phage typing*
  - *Analysis of nucleic acids*

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Classification and Identification of Microorganisms

- Taxonomic and Identifying Characteristics
  - *Physical characteristics*
    - Can often be used to identify microorganisms
      - *Protozoa, fungi, algae, and parasitic worms can often be identified based only on their morphology*
      - *Some bacterial colonies have distinct appearance used for identification*

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Classification and Identification of Microorganisms

- Taxonomic and Identifying Characteristics
  - *Serological tests*
    - Serology—study of serum (liquid portion of blood after clotting factors removed)
    - Many microorganisms are antigenic
      - *Trigger immune response that produces antibodies*
    - Serum is an important source of antibodies
    - Antibodies can be isolated and bind to the antigens that triggered their production

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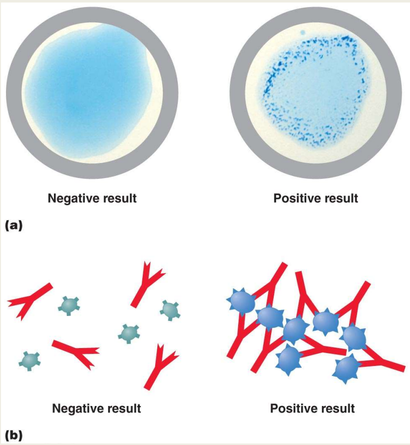
Figure 4.25 An agglutination test, one type of serological test.



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Classification and Identification of Microorganisms

- Taxonomic and Identifying Characteristics
  - *Phage typing*
    - Bacteriophage (phage)—virus that infects bacteria
    - Phages are specific for the host they infect
      - *Phage typing is based on this specificity*

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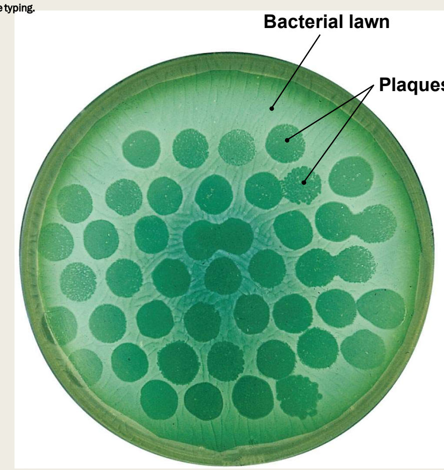
Figure 4.26 Phage typing.



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Classification and Identification of Microorganisms

- Taxonomic and Identifying Characteristics
  - *Analysis of nucleic acids*
    - Nucleic acid sequence can be used to classify and identify microbes

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Classification and Identification of Microorganisms

- Taxonomic Keys
  - *Dichotomous keys*
    - Series of paired statements where only one of two "either/or" choices applies to any particular organism
    - Key directs user to another pair of statements or provides name of organism

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Figure 4.27 Use of a dichotomous taxonomic key.

1a. Gram-positive cells.............................. Gram-positive bacteria
1b. Gram-negative cells.............................. 2

2a. Rod-shaped cells.............................. 3
2b. Non-rod-shaped cells.............................. Cocci and pleomorphic bacteria

3a. Can tolerate oxygen.............................. 4
3b. Cannot tolerate oxygen.............................. Obligate anaerobes

4a. Ferments lactose.............................. 5
4b. Cannot ferment lactose.............................. Non-lactose-fermenters

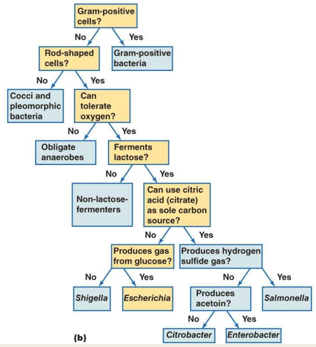
5a. Can use citric acid as a sole carbon source..............................6
5b. Cannot use citric acid alone..............................8

6a. Produces hydrogen sulfide gas.............. *Salmonella*
6b. Does not produce hydrogen sulfide gas...7

7a. Produces acetoin.............................. *Enterobacter*
7b. Does not produce acetoin.............................. *Citrobacter*

8a. Produces gas from glucose.................... *Escherichia*
8b. Does not produce gas from glucose........ *Shigella*

(a)



(b)

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