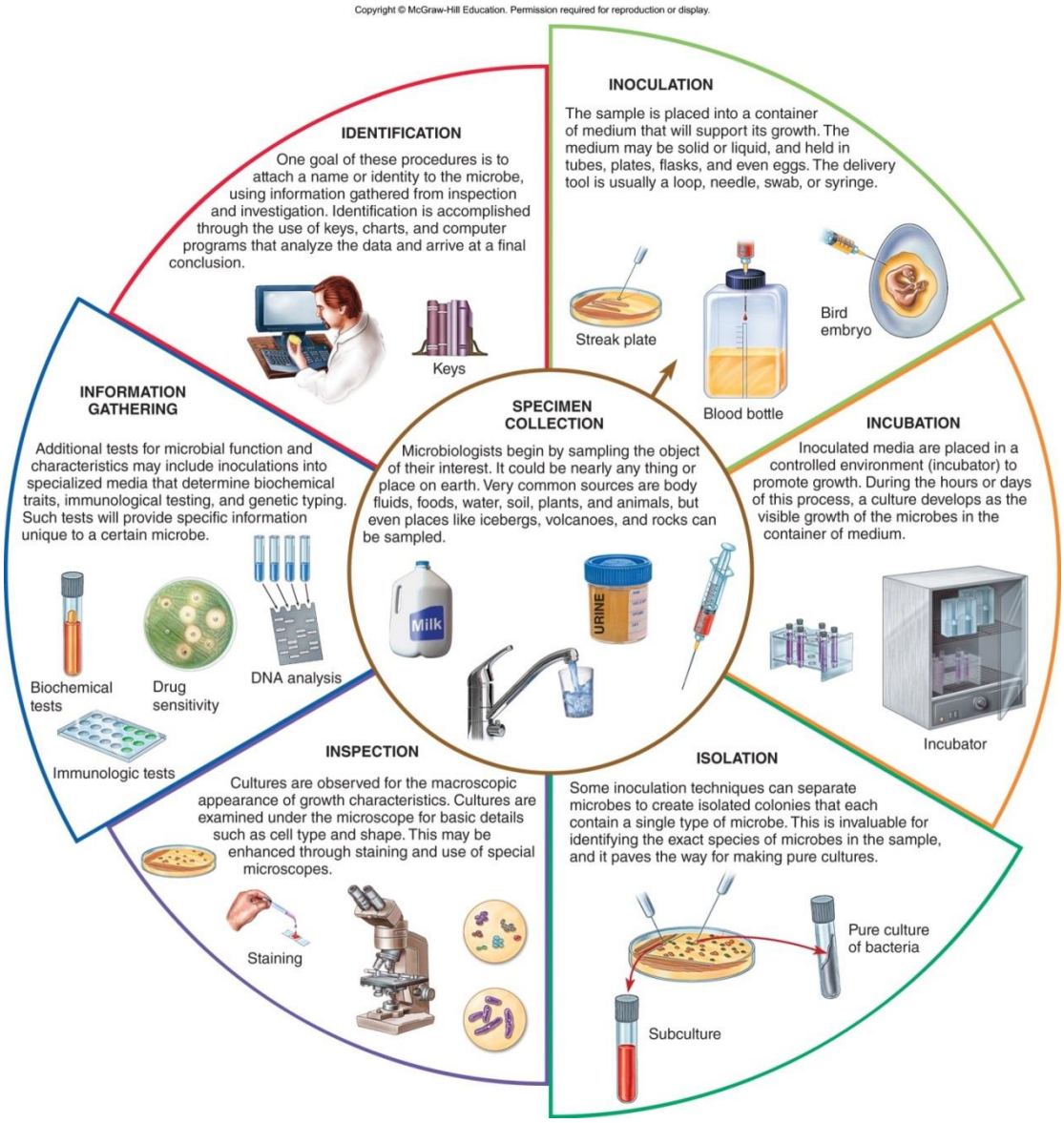
LECTURE 4:

Tools of Laboratory:

The Methods for Studying Microorganisms

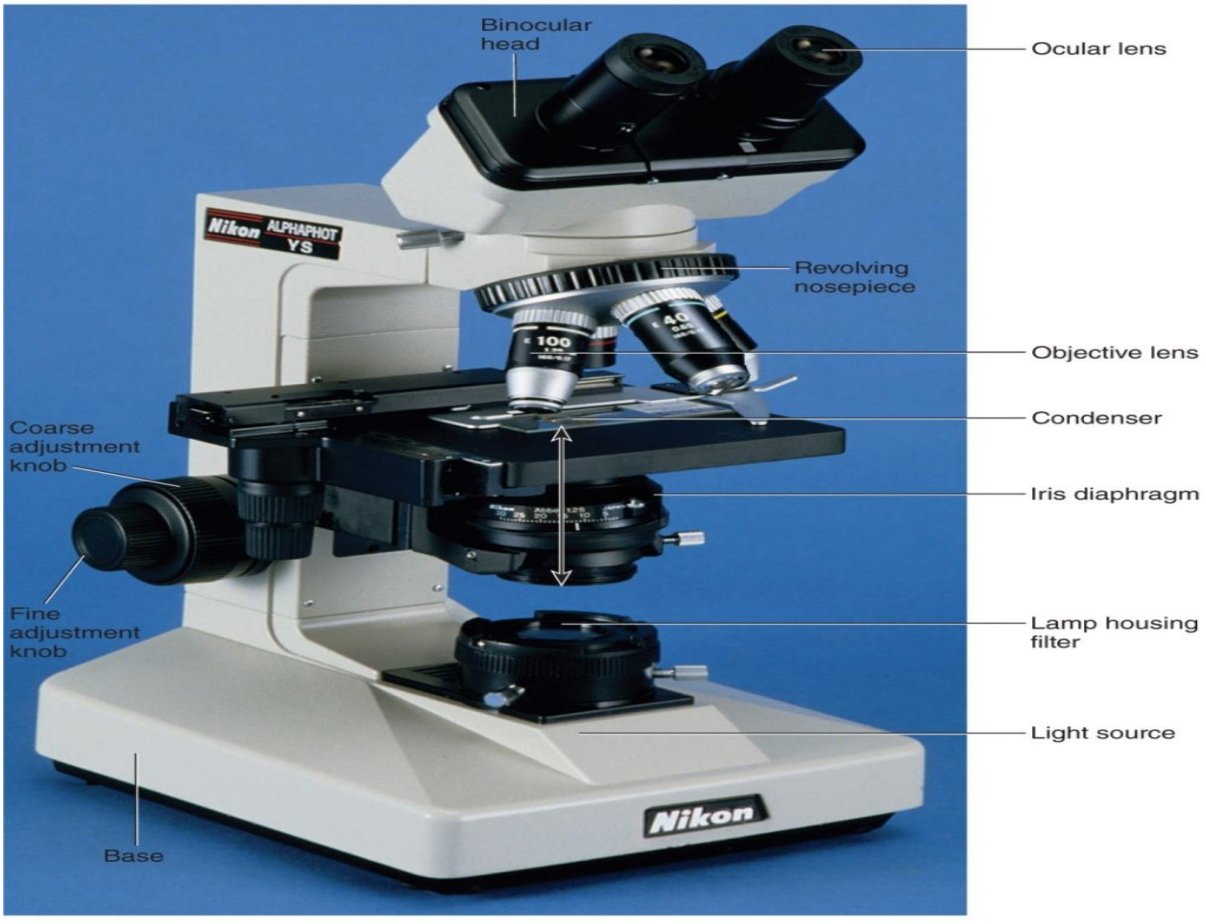


The Microscope

Key characteristics of a reliable microscope are:

Magnification – ability to enlarge objects •

Resolving power – ability to show detail



(a)

Courtesy of Nikon Instruments Inc., Melville, New York, USA, www.nikoninstruments.com

Magnification

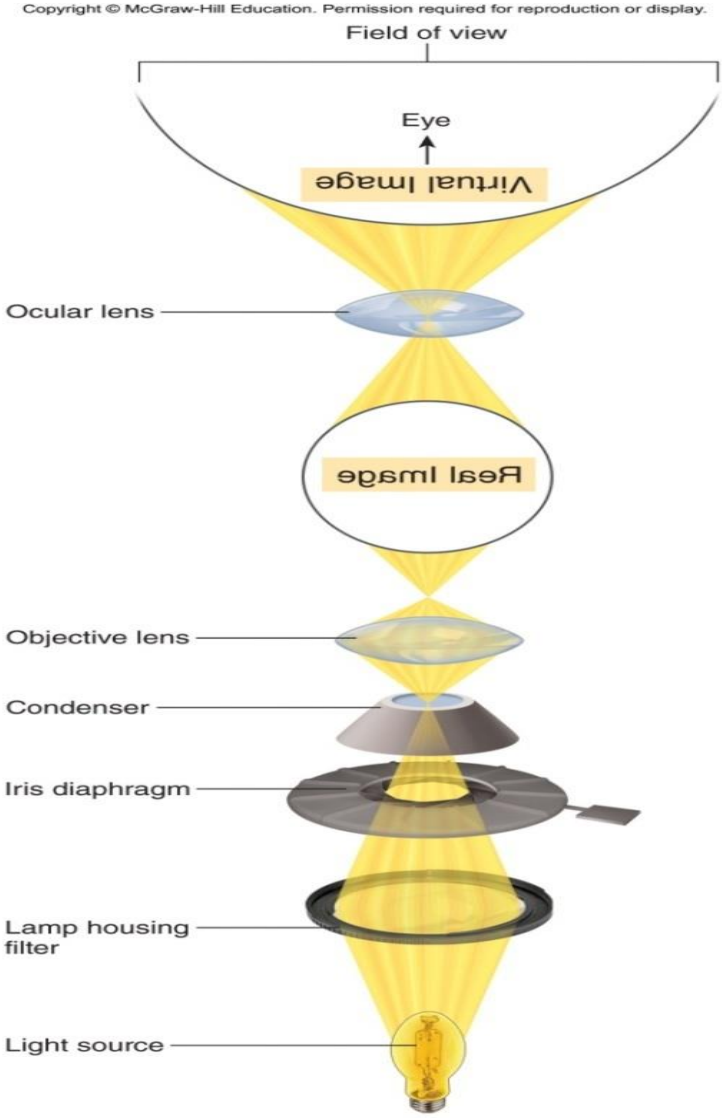
Magnification in most microscopes results from an interaction between visible light waves and the curvature of a lens. The extent of enlargement is the **magnification.**

Magnification in Two Phases

The objective lens forms the magnified **real image.**The real image is projected to the **ocular** where it is magnified again to form the **virtual image**

Total magnification of the final image is a product of the separate magnifying powers of the two lenses

objective power X ocular power= total magnification

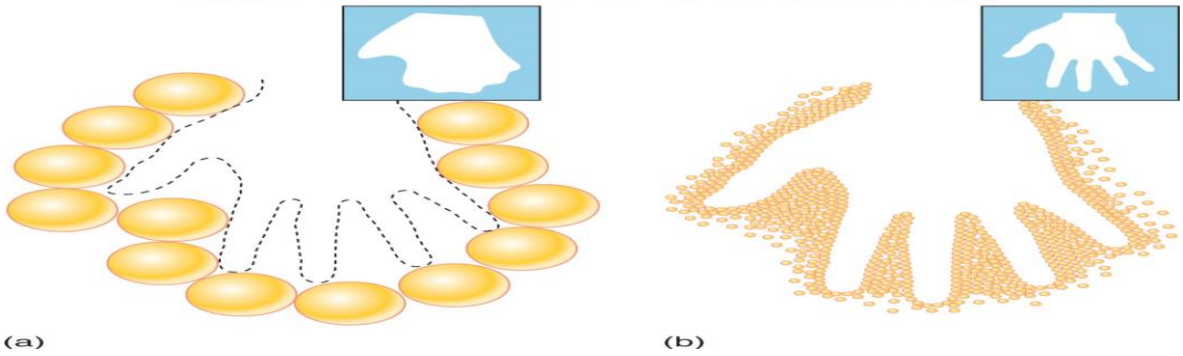


(b)

Courtesy of Nikon Instruments Inc., Melville, New York, USA, www.nikoninstruments.com

Resolution

The capacity to distinguish or separate two adjacent objects and depends on the wavelength of light that forms the image along with characteristics of the objectives



Quantifying Resolution

-Resolving Power (RP)= Wavelength of light in nm/ 2 X Numerical aperture of objective lens

-Visible light wavelength is 400 nm–750 nm

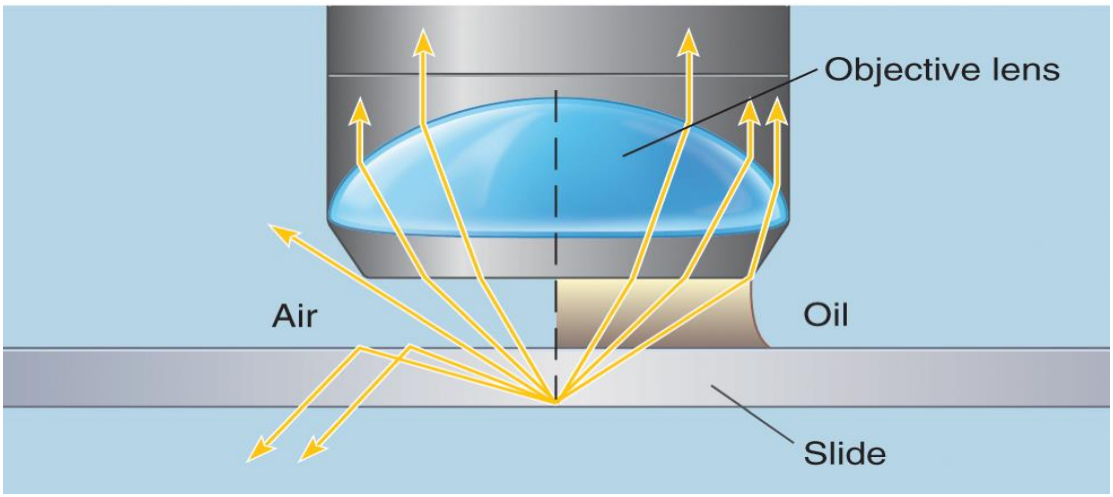
-**Numerical aperture** of lens ranges from 0.1 to 1.25

-Shorter wavelength and larger numerical aperture will provide better resolution

-Oil immersion objectives resolution is 0.2 μm

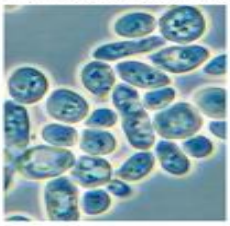
-Magnification between 40X and 2000X

The Purpose of Oil



Variations on the Optical Microscope

- **Phase-contrast** – transforms subtle changes in light waves passing through the specimen into differences in light intensity, best for observing intracellular structures

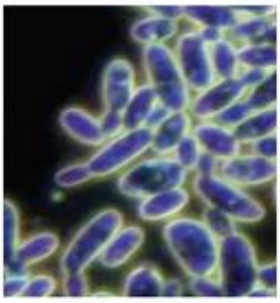


Variations on the Optical Microscope

- **Bright-field** – most widely used; specimen is darker than surrounding field; used for live and preserved stained specimens

Variations on the Optical Microscope

- **Dark-field** – brightly illuminated specimens surrounded by dark field; used for live and unstained specimens



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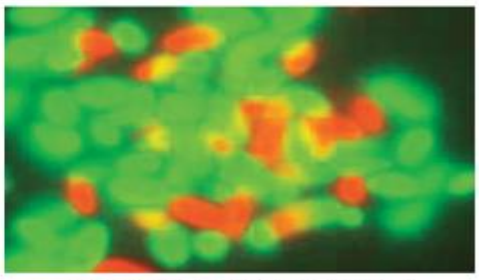
Fluorescence Microscope

- Modified microscope with an ultraviolet radiation source and filter.
- Uses dyes that emit visible light when bombarded with shorter UV rays - fluorescence
- Useful in diagnosing infections



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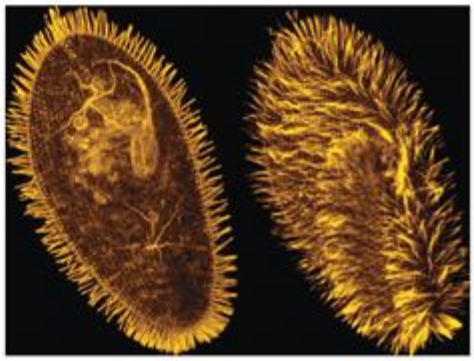
Scanning Confocal Microscope

- Uses a laser beam of light to scan the specimen.
- Integrates images to allow focus on multiple depths or planes.



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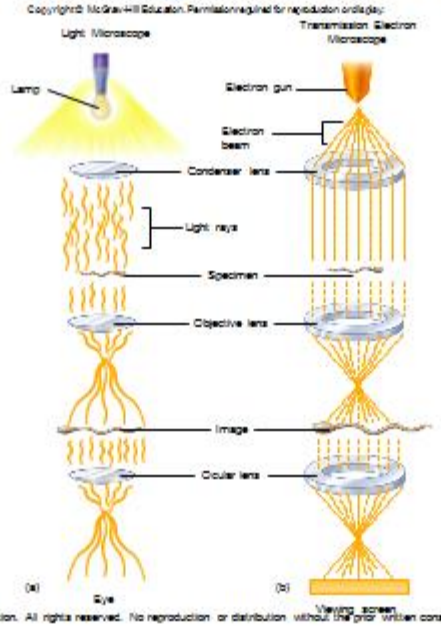
Electron Microscopy

- Forms an image with a beam of electrons that can be made to travel in wavelike patterns when accelerated to high speeds
- Electron waves are 100,000 times shorter than the waves of visible light
- Electrons have tremendous power to resolve minute structures because resolving power is a function of wavelength
- Magnification between 5,000X and 1,000,000X

Comparing Microscopes:



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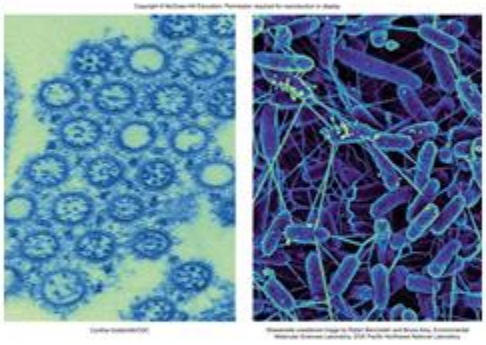
2 Types of Electron Microscopes

- **Transmission electron microscopes** (TEM) – transmit electrons through the specimen. Darker areas represent thicker, denser parts and lighter areas indicate more transparent, less dense parts.



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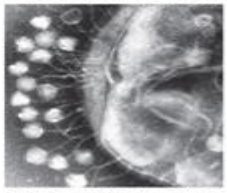
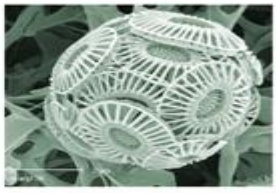
2 Types of Electron Microscopes

- **Scanning electron microscopes** (SEM) – provide detailed three-dimensional view. SEM bombards surface of a whole, metal-coated specimen with electrons while scanning back and forth over it.



Dr. Graham Beards - Wikipedia: http://en.wikipedia.org/wiki/File:Phage.jpg



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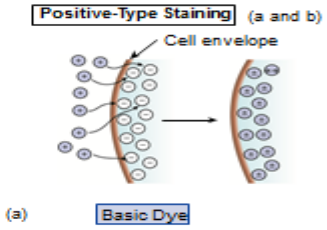
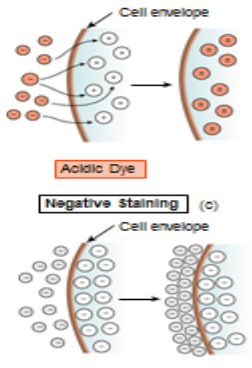
Staining

- Dyes are used to create contrast by imparting color
- **Basic dyes** – cationic, positively charged chromophore
- **Positive staining** – surfaces of microbes are negatively charged and attract basic dyes



Staining

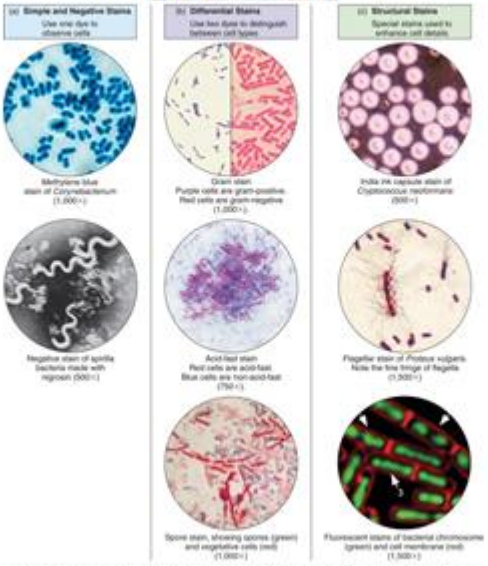
- **Acidic dyes** – anionic, negatively charged chromophore
- **Negative staining** – microbe repels dye, the dye stains the background



Staining

- **Simple stains** – one dye is used; reveals shape, size, and arrangement
- **Differential stains** – use a primary stain and a counterstain to distinguish cell types or parts (examples: Gram stain, acid-fast stain, and endospore stain)
- **Structural stains** – reveal certain cell parts not revealed by conventional methods: capsule and flagellar stains

Staining Examples



The 6 I's of Culturing Microbes

Inoculation – introduction of a sample into a container of media to produce a **culture** of observable growth

Isolation – separating one species from another

Incubation – under conditions that allow growth

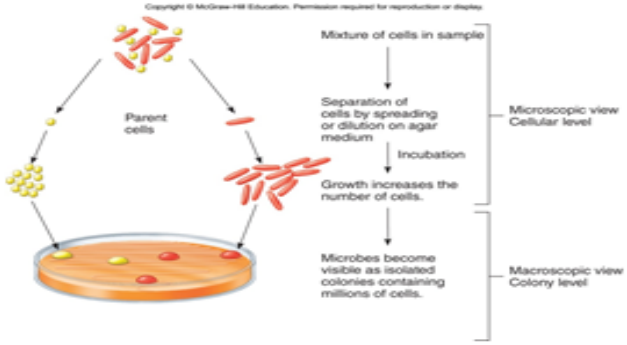
Inspection

Information gathering

Identification

Isolation

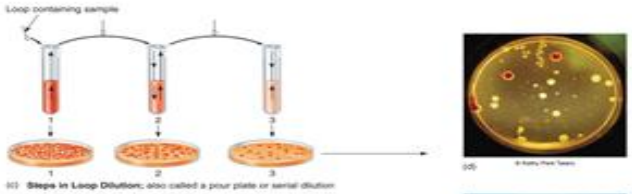
- If an individual bacterial cell is separated from other cells and has space on a nutrient surface, it will grow into a mound of cells— a **colony**. A colony consists of one species.



Isolation Techniques

– Streak plate technique

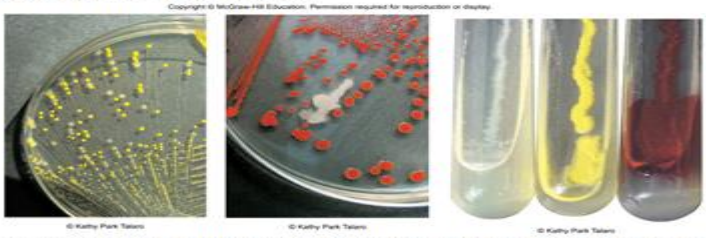
– Pour plate technique



– Spread plate technique

Inspection

- If a single species is growing in the container, you have a **pure culture** but if there are multiple species than you have a **mixed culture**.
- Check for **contaminants** (unknown or unwanted microbes) in the culture.



Ways to Identify a Microbe:

- Cell and colony morphology or staining characteristics
- DNA sequence
- Biochemical tests to determine an organism's chemical and metabolic characteristics
- Immunological tests

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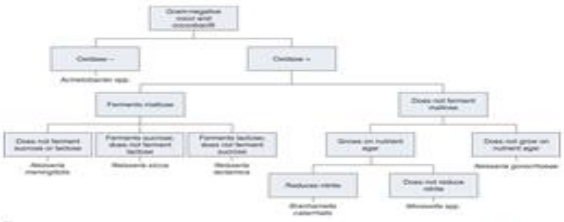


Media: Providing Nutrients in the Laboratory

Media can be classified according to three properties:

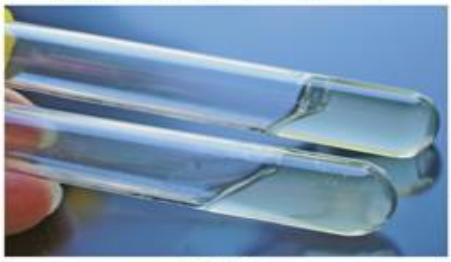
1. Physical state – liquid, semisolid, and solid
2. Chemical composition – **synthetic** (chemically defined) and **complex**
3. Functional type – general purpose, enriched, selective, differential, anaerobic, transport, assay, enumeration

Physical States of Media

Liquid – **broth**; does not solidify

Semisolid – contains solidifying agent

Solid – firm surface for colony formation
- Contains solidifying agent
- Liquefiable and nonliquefiable



Agar

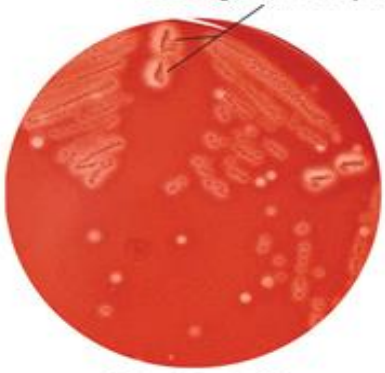
- The most commonly used solidifying agent
- Solid at room temperature, liquefies at boiling (100°C), does not re-solidify until it cools to 42°C
- Provides framework to hold moisture and nutrients
- Not digestible for most microbes

Chemical Content of Media

- **Synthetic** – contains pure organic and inorganic compounds in an exact chemical formula
- **Complex or nonsynthetic** – contains at least one ingredient that is not chemically definable
- **General purpose media** – grows a broad range of microbes, usually nonsynthetic
- **Enriched media** – contains complex organic substances such as blood, serum, hemoglobin, or special growth factors required by fastidious microbes

Examples of Enriched Media

Growth of *Streptococcus pyogenes* showing beta hemolysis



Dr. Richard R. Facklam/CDC

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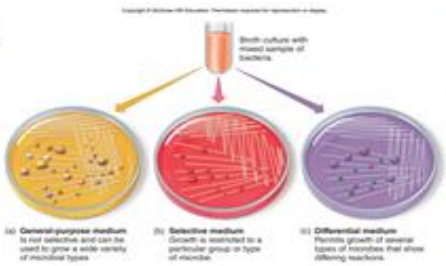
Selective & Differential Media

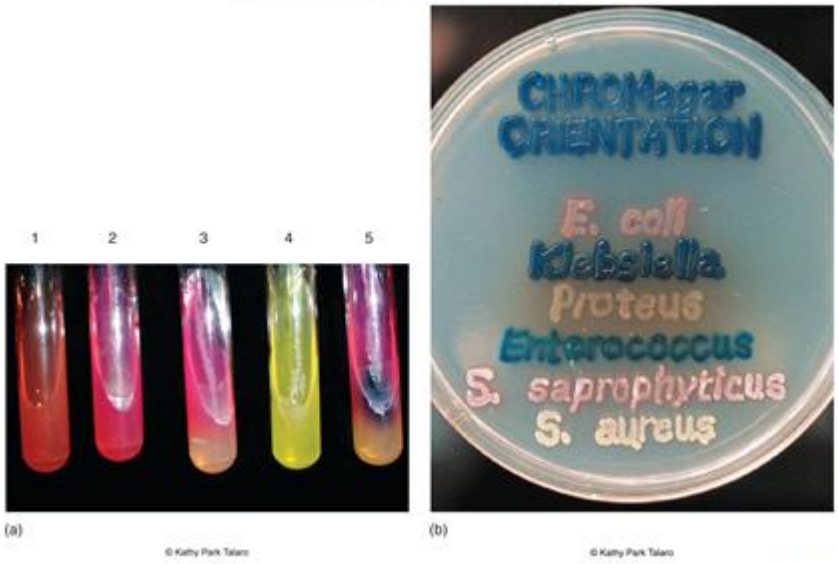
Selective media: contains one or more agents that inhibit growth of some microbes and encourage growth of the desired microbes



Differential media: allows growth of several types of microbes and displays visible differences among those microbes

Some media can be both Selective & Differential

(a) (b)



(a) (b)