Introductory Principles of Microbiology

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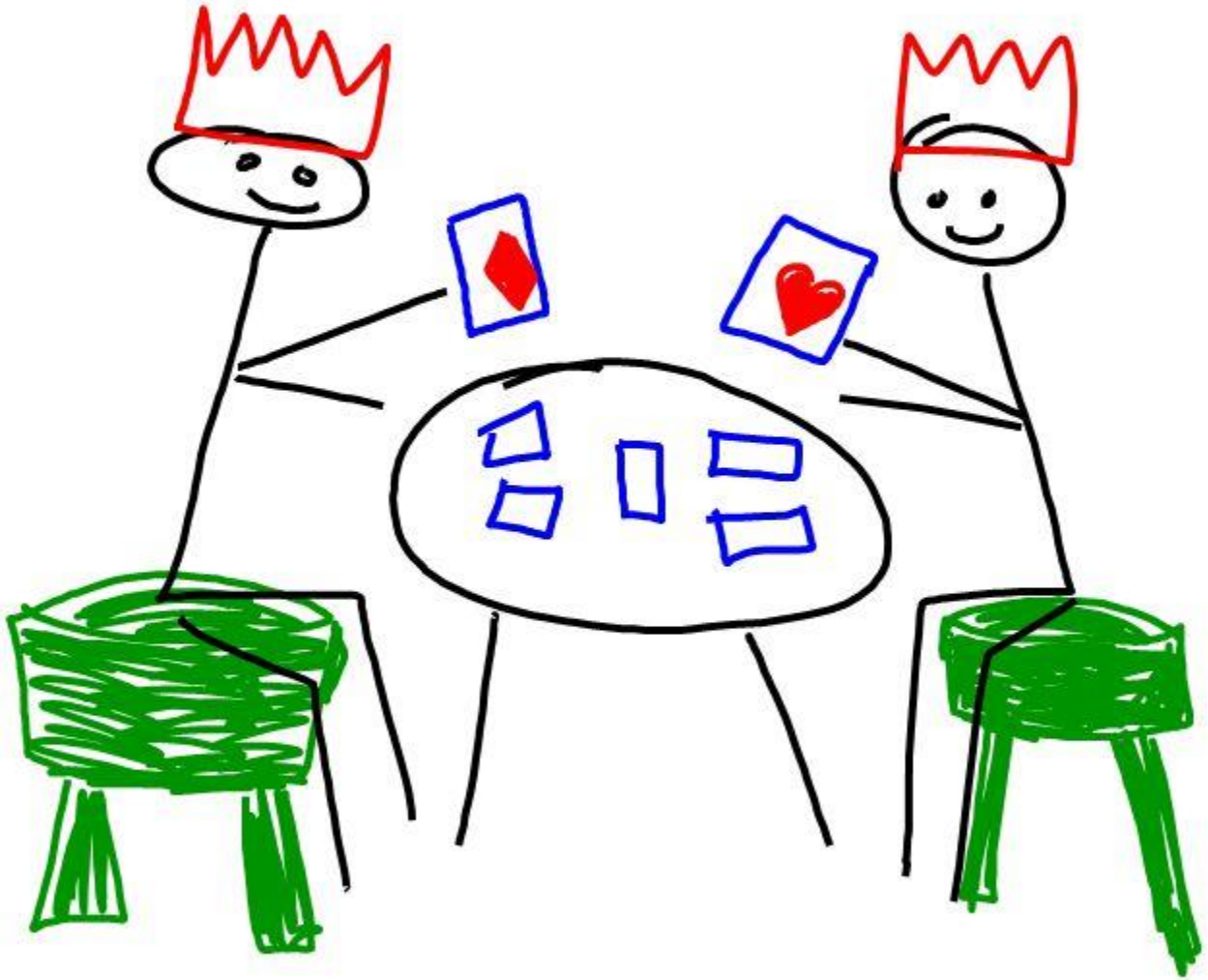
Today's Discussion

- Taxonomy
- Infectious agents
- Nomenclature
- Humans and infectious agents
- Microscopy
- Stains
- Media
- Parameters for growth
- Bacterial biochemistry and metabolism
- Disinfection and Sterilization
- Safety

Taxonomy

- Taxonomy
  - Orderly classification and grouping of organisms into categories
- Genotypic
  - Genetic make up of an organism
  - Ex. nucleic acid base sequencing, nucleic acid base composition ratio
- Phenotypic
  - Observable physical features expressed by the genotype of an organism
  - Ex. morphology, biochemical characteristics, antigenic markers

Taxonomic Classification



Do
Kings
Play
Cards
On
Fat
Green
Stools

3 Domains and 6 kingdoms in tree of life

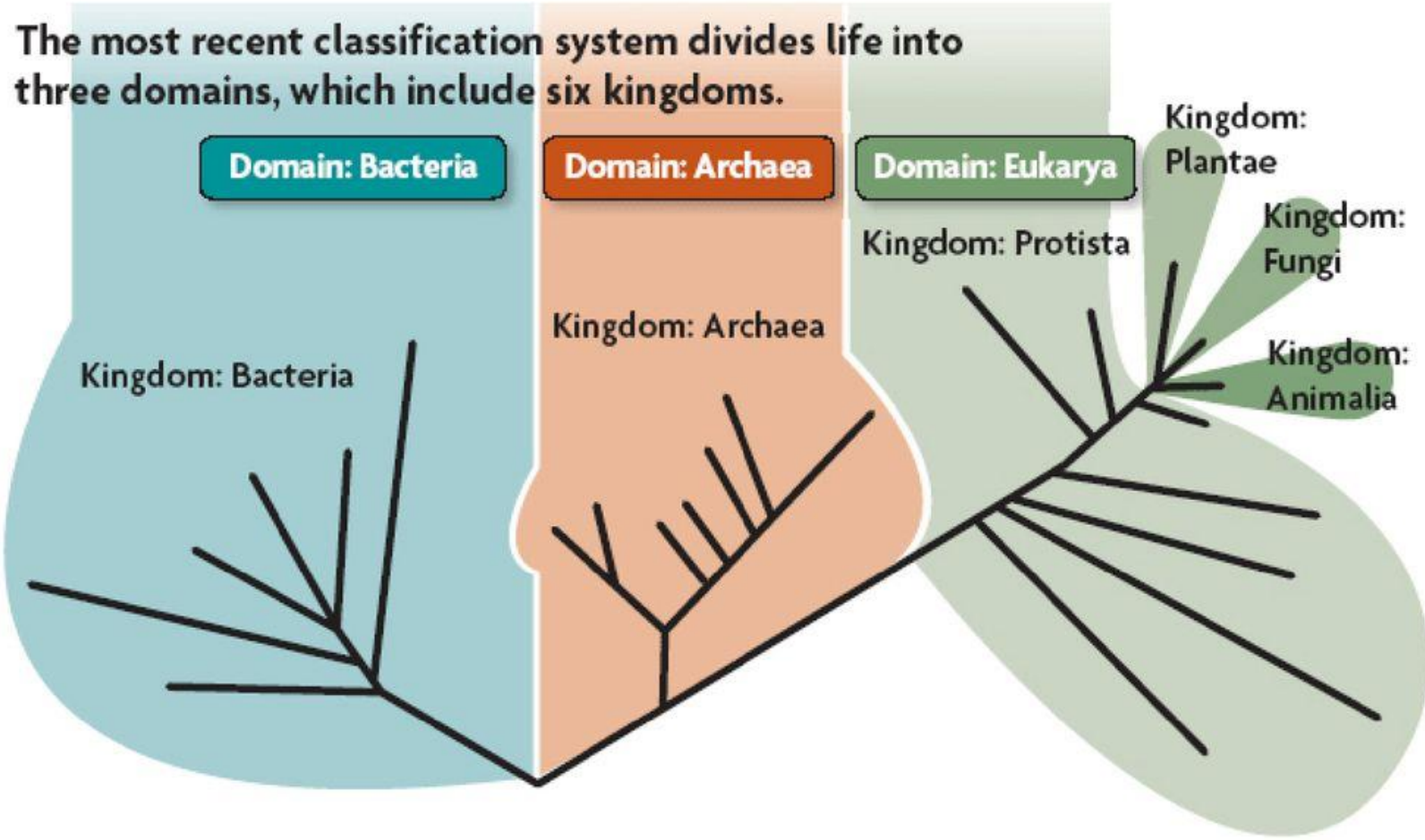


Photo credit: https://www.youtube.com/watch?v=YWu8_KJvx_I:

Infectious Agents

Prokaryote

- Organisms without a true nucleus
- Size: 0.5-5 µm

Eukaryote

- Organisms with a true nucleus
- Size: >10 µm

Unicellular

- Consisting of a single cell

Multicellular

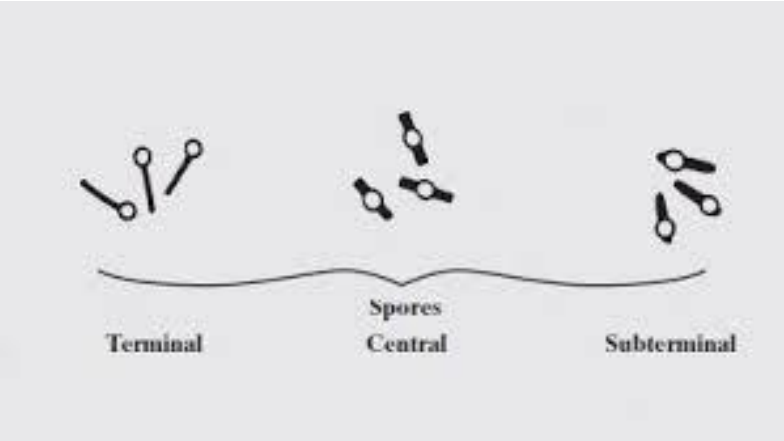
- Consisting of many cells

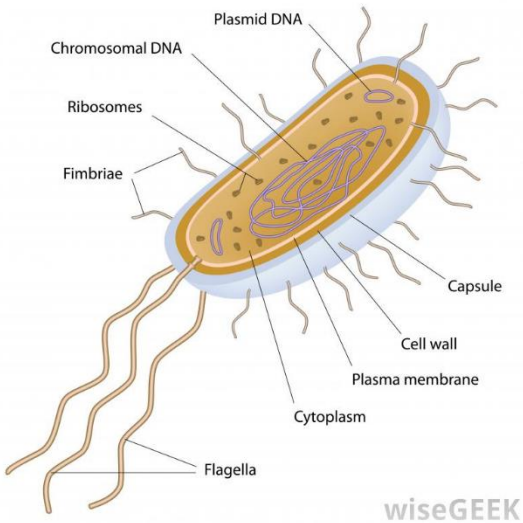
Infectious Agents

	Prokaryote	Eukaryote	
Microorganism	Bacteria	Fungi	Parasites
Unicellular	Bacteria	Yeast	Protozoa
Multicellular	None	Molds	Worms & Arthropods

Prokaryote: cytoplasmic structures

- Present in all
  - **Genome:** single circular chromosome with dsDNA
  - **Ribosome:** site of protein synthesis
- Present in some
  - **Endospore:** internal structure formed during poor environmental conditions





Prokaryote: cell envelope structures

- Present in all
  - **Plasma membrane:** osmotic barrier and location of the electron transport chain
    - Made of phospholipids and proteins. Does not contain sterols
- Present in most
  - **Cell wall:** maintains shape of the cell and prevents bursting due to high osmotic pressure

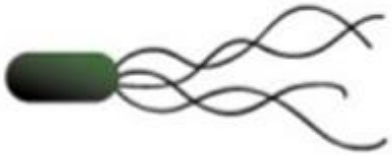
EXCEPTION:
Mycoplasma* and *Ureaplasma
- ***Lacks cell wall***
- ***Plasma membrane with sterols***

Prokaryote: surface polymers

- Present in some
  - **Capsule:** inhibit phagocytosis
  - **Glycocalyx:** inhibit phagocytosis and aids in adherence to host tissue
  - **Cell appendages**
    - **Flagella:** exterior protein filaments that rotate
      - Polar
        - Monotrichous
        - Amphitrichous
        - Lophotrichous
      - Peritrichous
    - **Pili/fimbriae:** proteinaceous appendages used for adhering to other cells
      - **Conjugation pili:** Use to exchange DNA between two cells

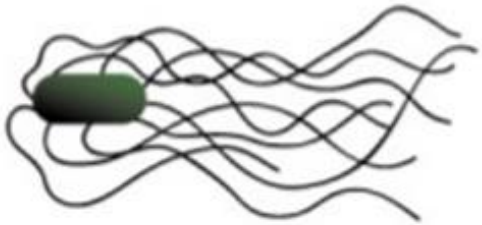
Types of flagellar arrangement

Polar/ Monotrichous – single flagellum at one pole

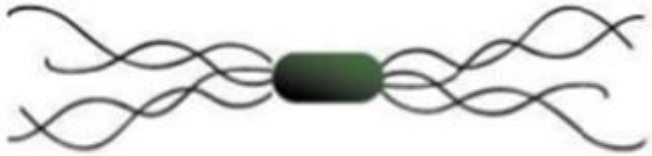


Lophotrichous – tuft of flagella at one pole

Amphitrichous – flagella at both poles



Peritrichous – flagella all over



Amphilophotrichous – tuft of flagella at both ends

Eukaryote: cytoplasmic structures

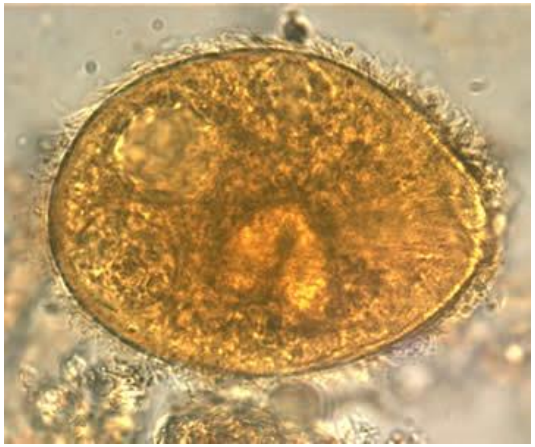
- Present in all
  - **Genome:** dsDNA packaged into chromosomes inside a membrane bound nucleus
  - **Endoplasmic reticulum**
    - Smooth: phospholipid synthesis
    - Rough: covered in ribosomes
- Present in most
  - **Mitochondria:** site of the electron transport chain
- Present in some
  - **Golgi apparatus:** package proteins
  - **Lysosomes:** break down macromolecules and microorganisms
  - **Peroxisomes:** breaks down peroxides
  - **Chloroplasts:** site of photosynthesis in plants and algae

Eukaryote: cell envelope structures

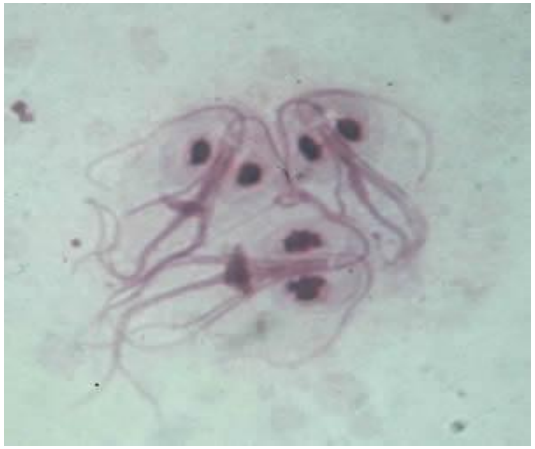
- Present in all
  - **Plasma membrane:** regulates transport of macromolecules into and out of cell.
- Present in some
  - **Cell wall:** provides rigidity and strength to the exterior of the cell

Eukaryote: surface polymers

- Present in some
  - Cell appendages
    - Flagella: long projections used for locomotion
    - Cilia: short projections used for locomotion



Neobalantidium coli



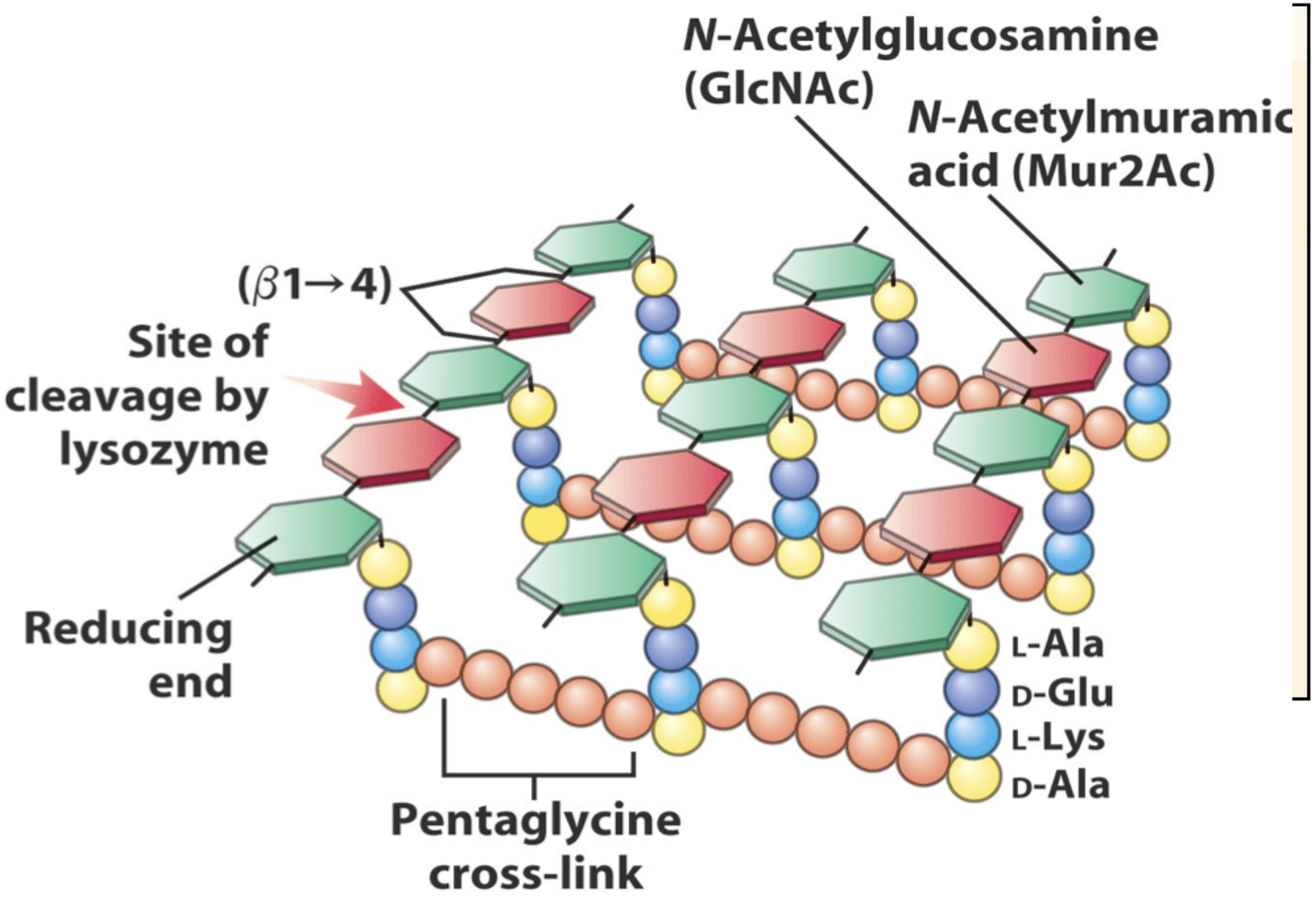
Giardia duodenalis

Photo credit: https://www.cdc.gov/dpdx/az.html#g:

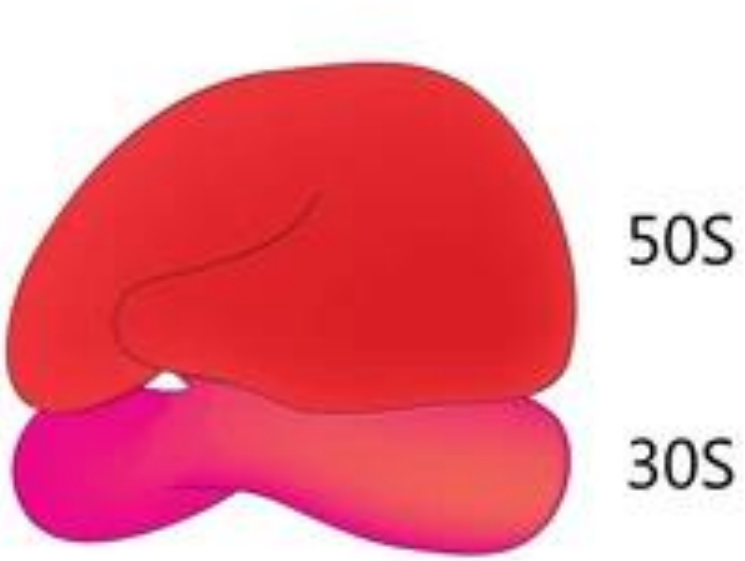
Key Differences

- Pharmaceutical companies can target differences between the cells of bacteria (prokaryotes) and humans (eukaryotes) to treat infections.
- Two common antibiotic targets:
  - Cell walls
  - Ribosomes

Bacterial Cell Walls



Prokaryotic Ribosome

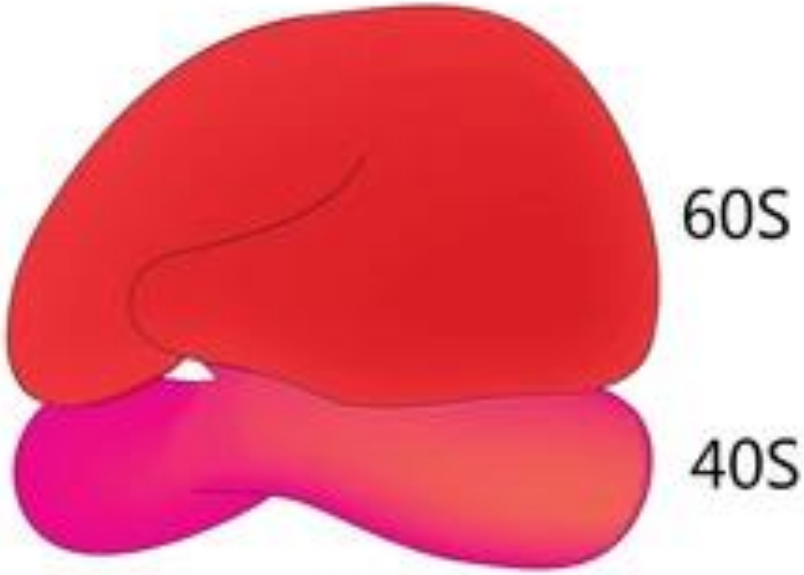


70S

(50S Subunit-5S rRNA,23S rRNA, 34 Proteins)

(30S Subunit-16S rRNA, 21 Proteins)

Eukaryotic Ribosome



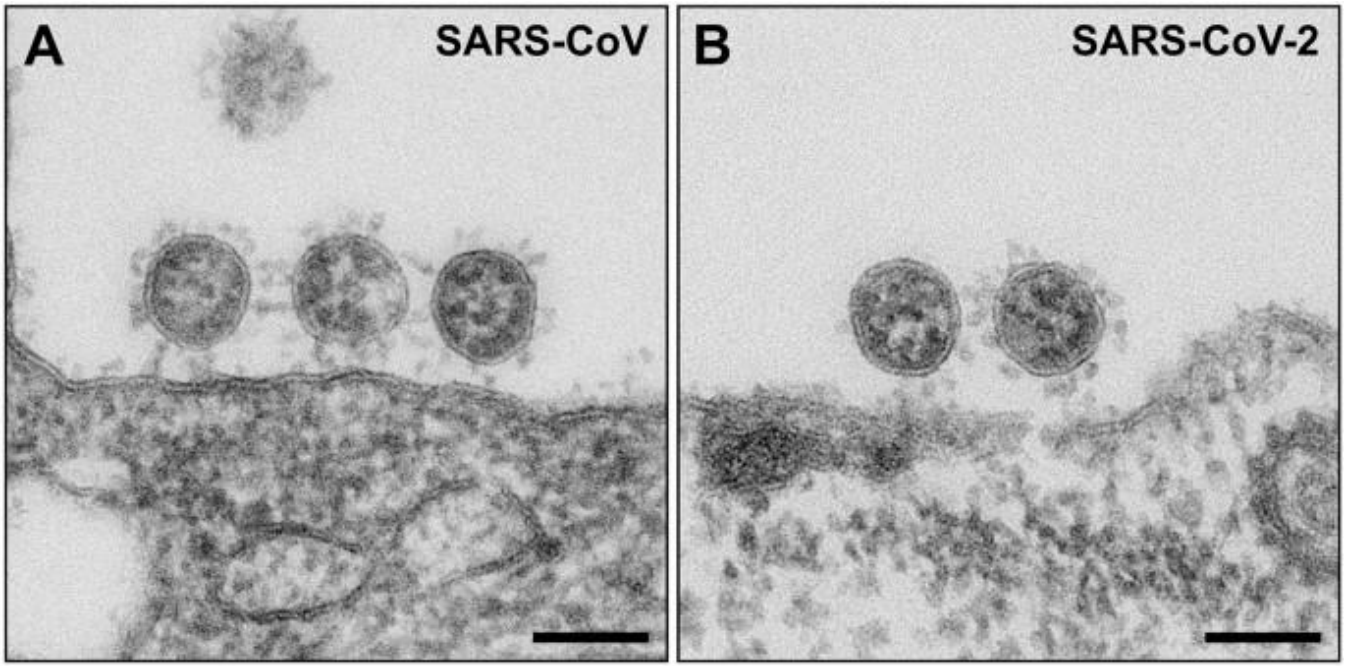
80S

(60S Subunit-5S rRNA, 5.8S rRNA, 28S rRNA, 50 Proteins)

(40S Subunit-18S rRNA, 33 Proteins)

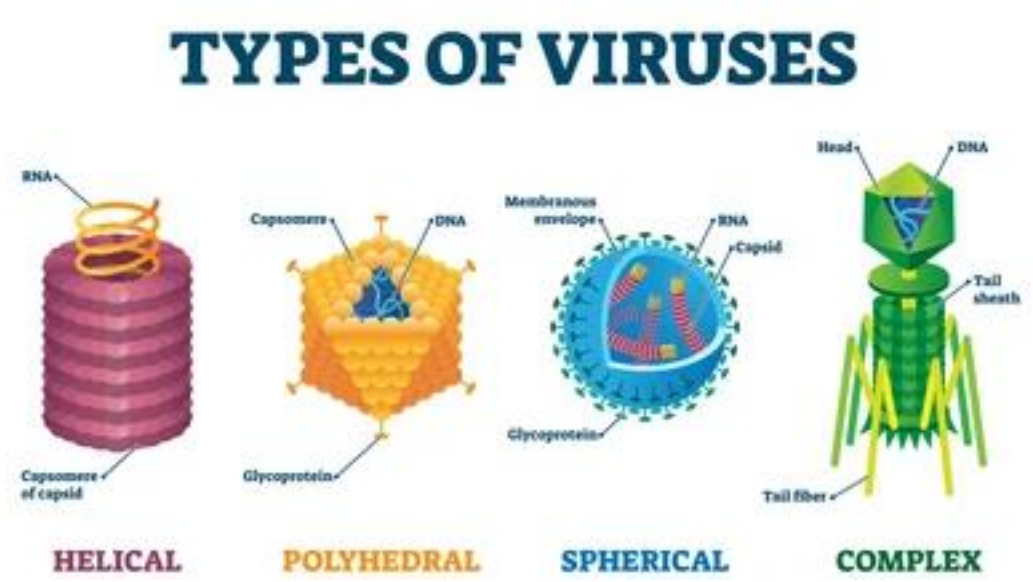
Viruses

- Neither prokaryotic or eukaryotic
- Acellular
- Obligate intracellular agents



Viruses

- Two or three parts:
  - DNA or RNA
    - Single stranded or double stranded
  - Capsid
  - Enveloped or non-enveloped



Nomenclature

- *Genus* and *species italicized*
  - *Staphylococcus aureus*
- Fungi, parasites follow same conventions:
  - *Candida albicans*
  - *Plasmodium falciparum*
- Viruses:
  - influenza virus (not generally italicized or capitalized; if capitalized then must italicize; Italicize species, genus and family of a virus when used in a taxonomic sense.)
- Be specific when necessary
  - *Escherichia coli (E. coli)*
  - *Entamoeba coli (E. coli)*

Host-microbe relationship

- Symbiosis
  - **Mutualism:** benefits both
  - **Commensalism:** one benefits while the other neither benefits nor is harmed
  - **Parasitism:** one benefits at the expense of the other

Mutualism & Commensalism

- **Colonization:** growth of a microorganism in or on a body site without the production of damage or notable symptoms
  - **Resident microbiota :** colonize an area for months or years
  - **Transient microbiota:** colonize microorganisms present temporarily
  - **Carrier state**: pathogenic organisms establish themselves in a host without manifesting symptoms

Parasitism

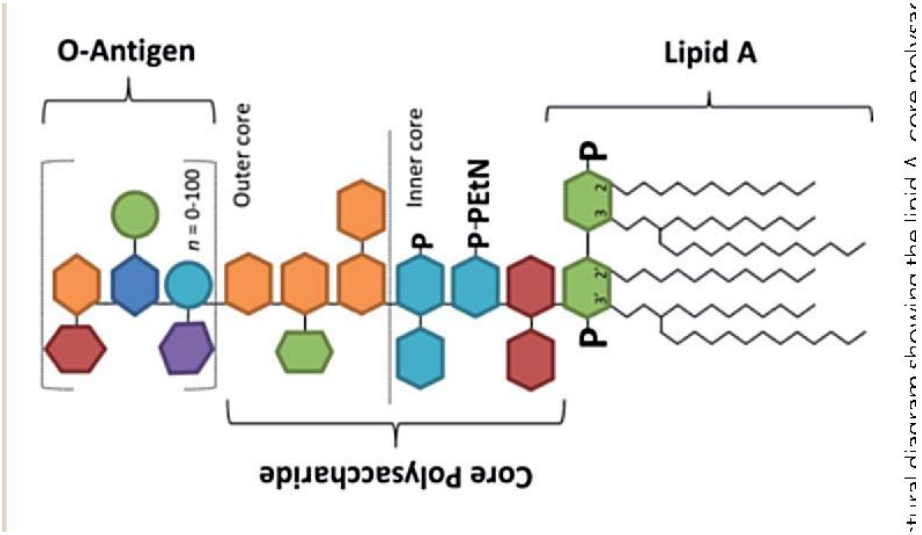
- **Pathogen:** microorganism that causes disease
  - **True pathogen:** cause disease in immunocompetent and immunocompromised individuals
  - **Opportunistic pathogen:** Microorganisms that do not normally cause disease or damage in a host, but under specific conditions or opportunities cause disease

Parasitism

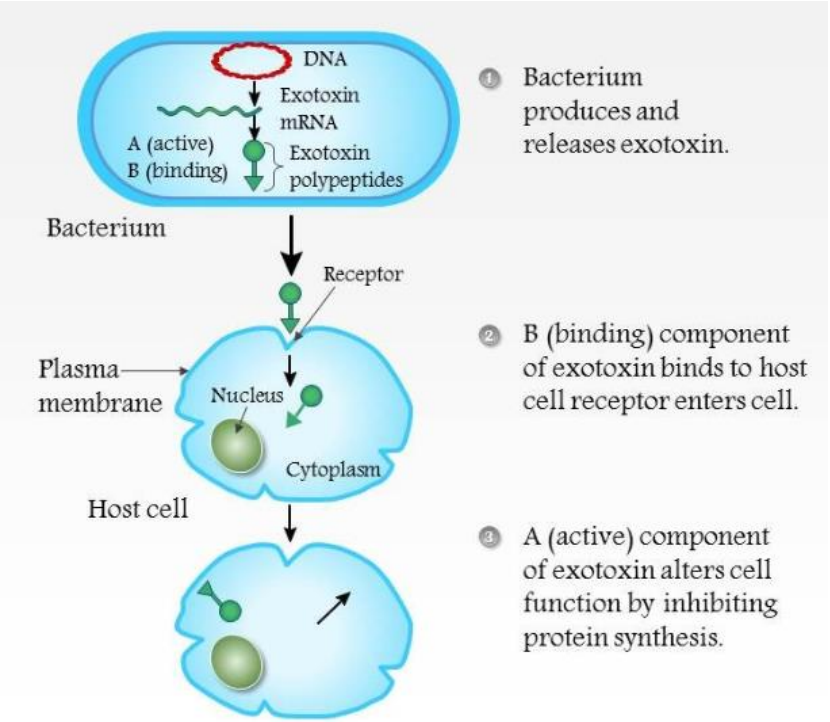
- Virulence factors
  - Resist phagocytosis
  - Adhesion
  - Survive intracellularly
  - Toxin production

Endotoxin vs. Exotoxin

Endotoxin = lipid A portion of LPS.
Elicits immune response



Exotoxin = produced inside and secreted from bacterial cell; effects host cells



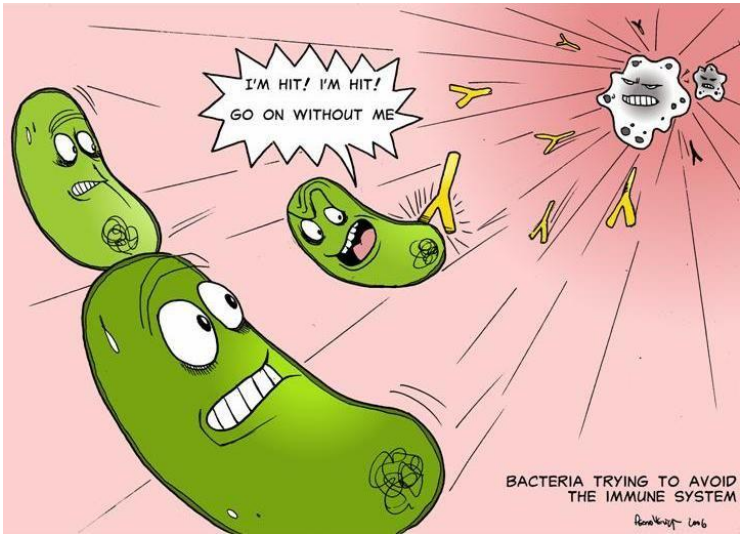
https://www.slideteam.net/catalog/product/gallery/id/38193/image/282409/

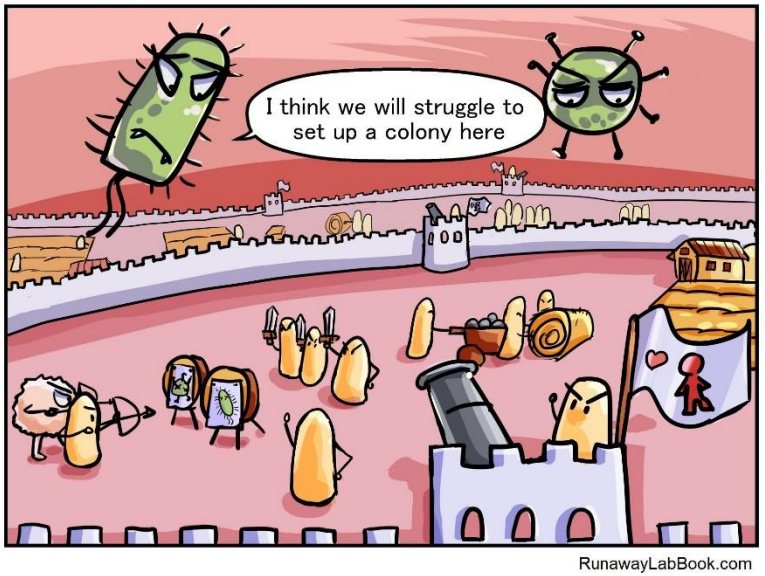
Parasitism

- Routes of transmission
  - Airborne
  - Food and water
  - Close contact
  - Cuts
  - Arthropods
  - Zoonoses

Parasitism

- Host resistance factors
  - Physical barriers
  - Cleaning mechanisms
  - Indigenous microbial biota
  - Phagocytosis
  - Inflammation
  - Immune reponses





Microscopy: Brightfield/Light

- Visualization via transillumination; light passes through condenser to specimen
- Condenser for focusing on specimen
- Two lens system (objective and ocular)
- Magnification at objective lens (10X, 40X, 100X) and at ocular lens (10X)

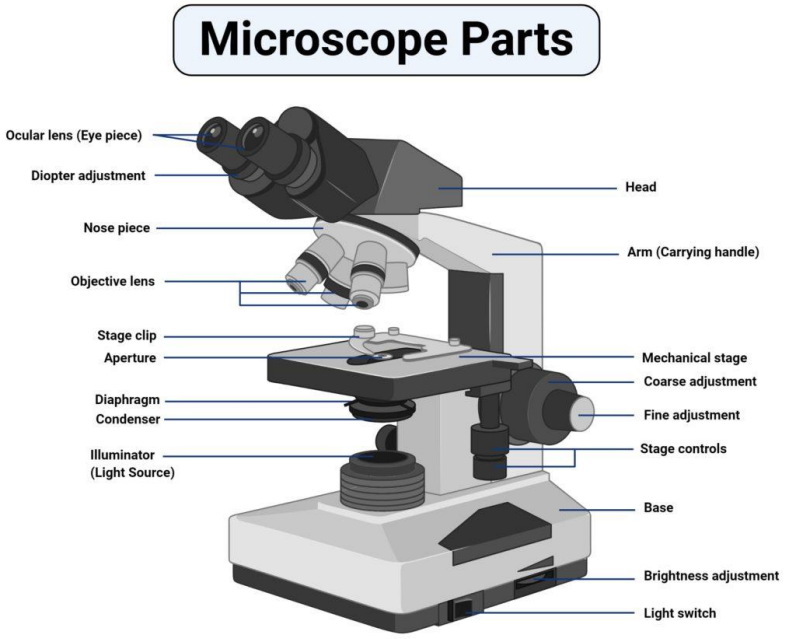
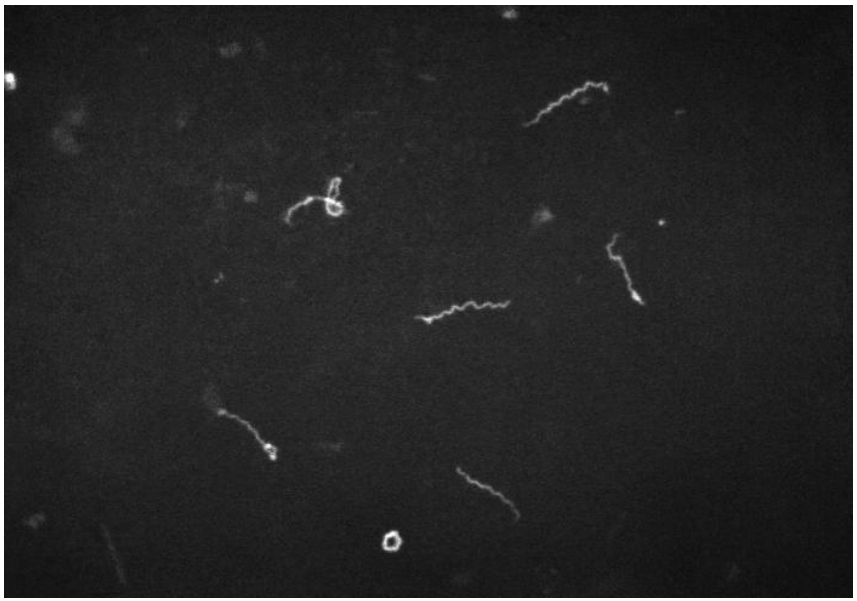


Figure: Parts of a microscope, Image Copyright © Sagar Aryal, www.microbenotes.com

Microscopy: Darkfield

A different condenser is used to prevent transmitted light from illuminating the specimen directly.

- Can see spirochetes and other microbes that are thinner
- Limitation: light passes around, hence no internal structures can be seen.



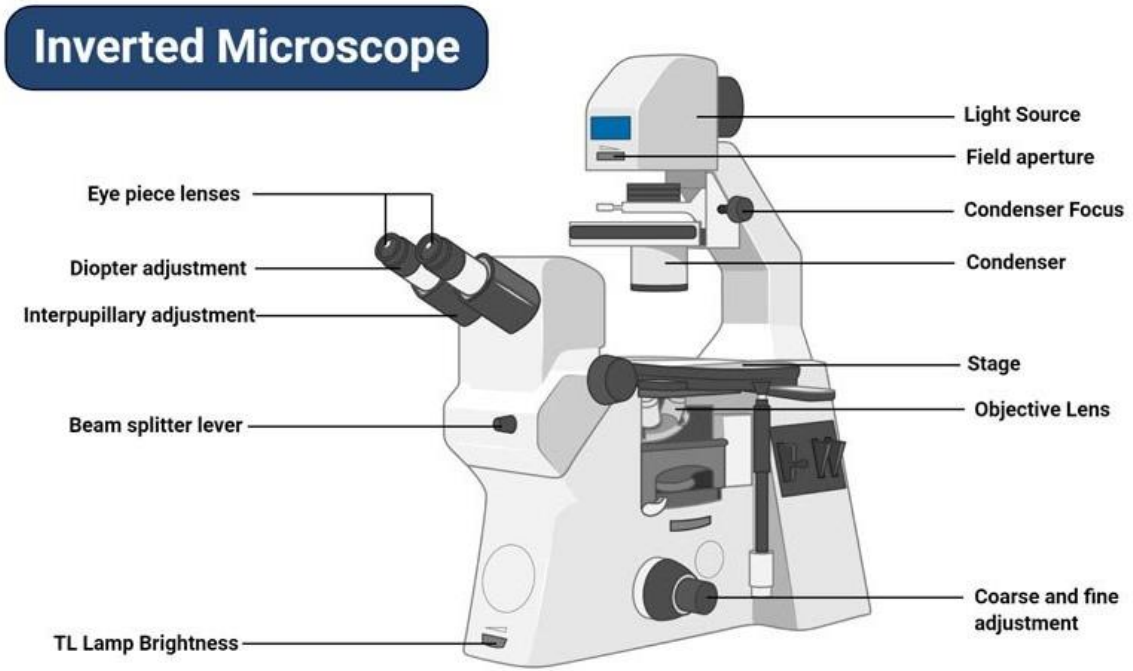
Treponema pallidum

Microscopy: Dissecting

- Low magnification used to study the surfaces of solid specimen
- Uses reflected light rather than transmitted

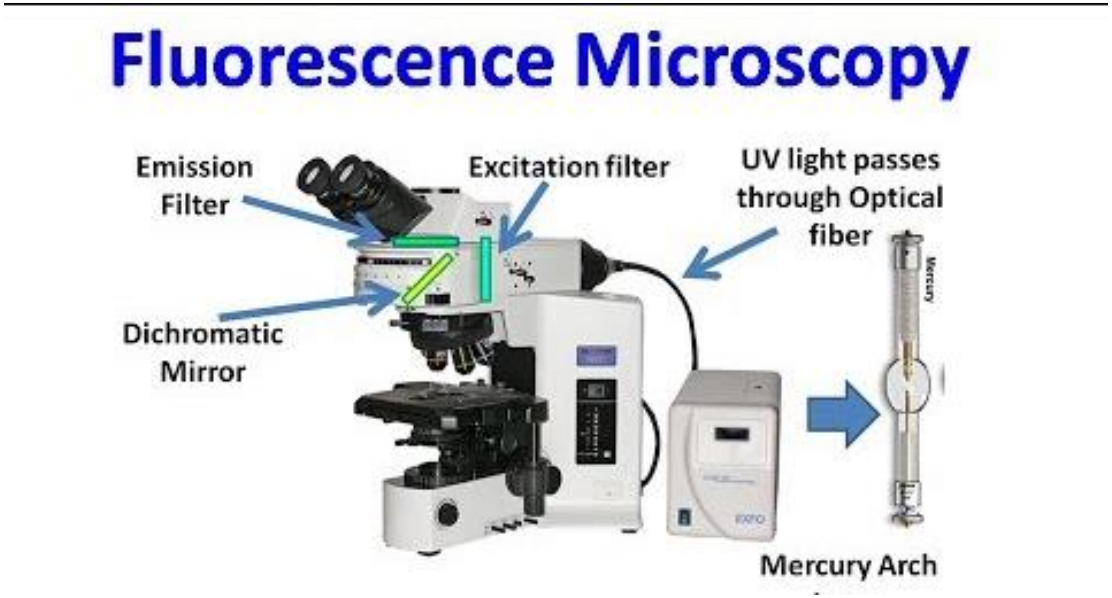
Microscopy : Inverted

- Light source and condenser are above the stage pointing down and objective are below the stage pointing up.
- Useful for observing living cell organisms at the bottom of a large container



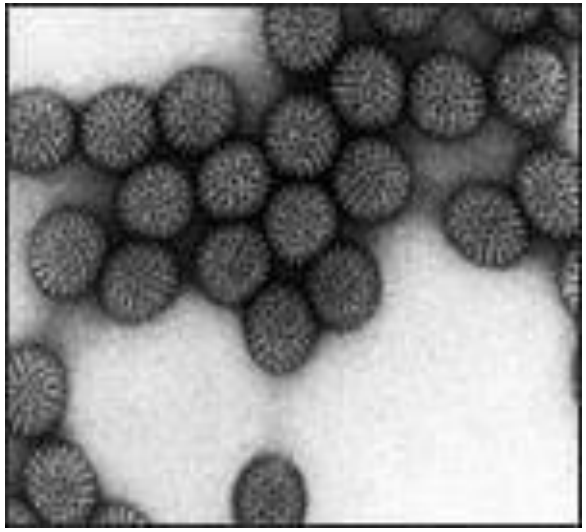
Microscopy : Fluorescence

- Uses a high pressure mercury, halogen, or xenon vapor lamp that emit shorter wavelength light
- Organisms are “stained” with specific fluorescent dyes
    - Advantage: Increases sensitivity, reduces examination time



Microscopy: Electron

- Magnetic coils (not lenses) direct a beam of electrons at the specimen.
  - **Transmission EM**: electrons pass through the specimen
  - **Scanning EM**: electrons bounce off of the specimen surface at an angle and create a 3-D image
  - Application: Viruses

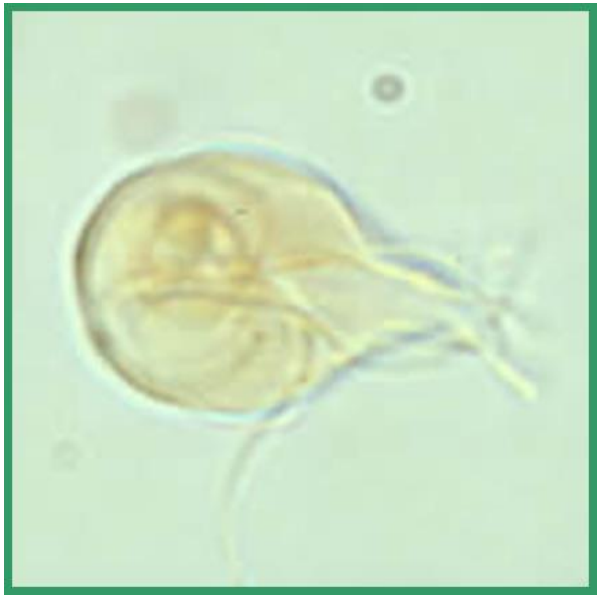


Microscopic Visualization

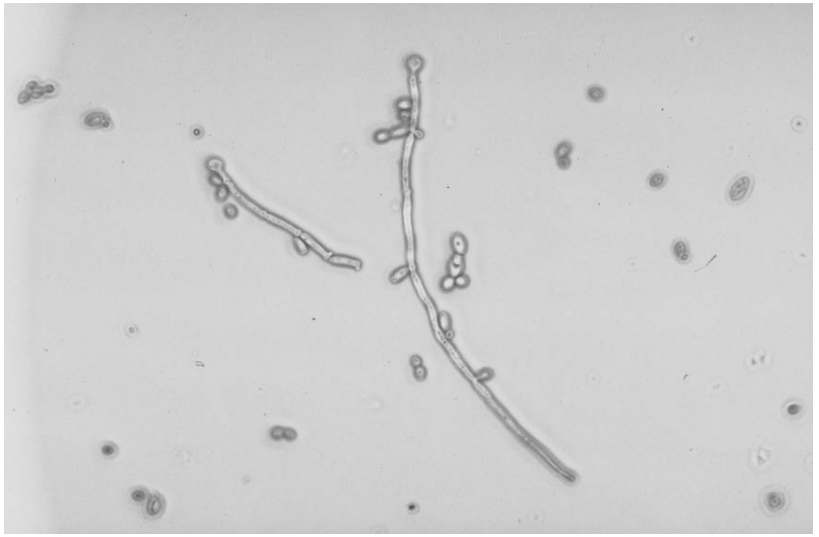
- Wet mount
  - Motility
- Permanent stains
  - Fixation
    - Heating
    - Chemical
      - Methanol
  - Dyes
    - **Simple stains:** one dye
    - **Differential stains:** multiple dyes
    - **Positive stain:** dye absorbed by cells
    - **Negative stain:** dye absorbed by background
    - **Chromophore:** colored ion
      - **Basic dye:** positive charged chromophore
      - **Acidic dye:** negative charged chromophore
    - **Fluorochrome:** chemical compound that can re-emit light upon excitation

Wet mount

- Motility of bacteria and parasites; budding yeast cells
- Provide contrast by adding Lugol's iodine to observe internal structures for parasites
- Remove debris with alkali (**10% KOH**) to observe nails/hair for fungi

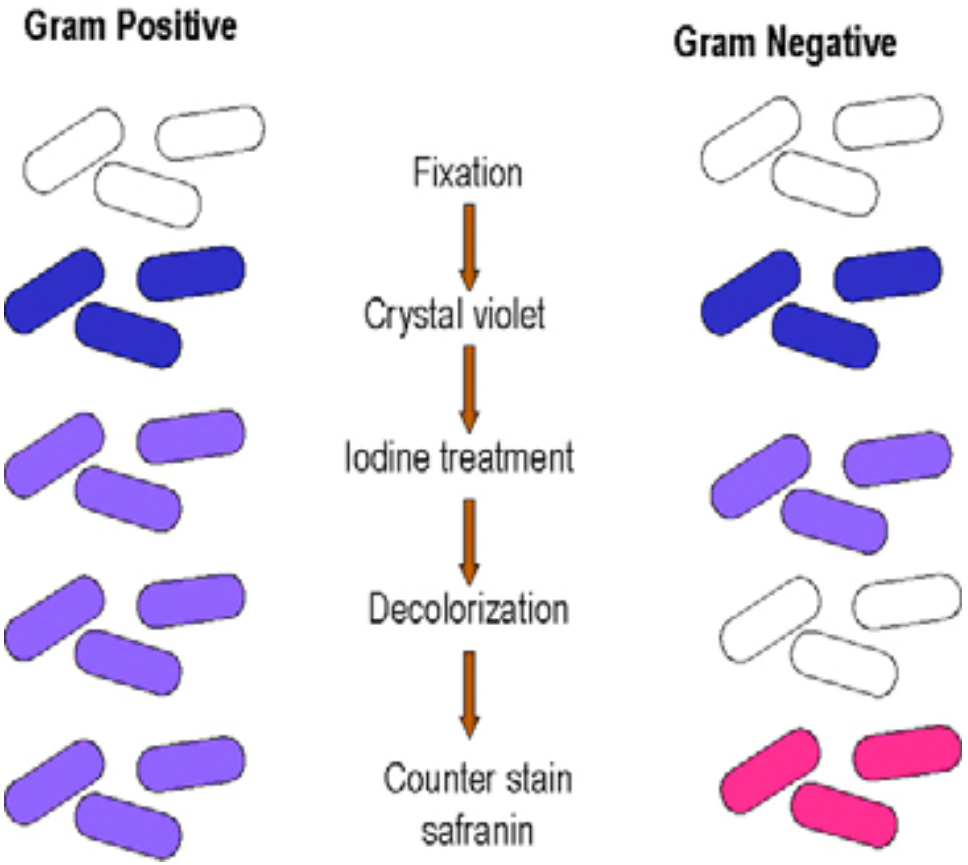


Giardia w/iodine counterstain



Yeast with pseudohyphae (KOH)

Bacteriology stains- Gram stain



http://hardydiagnostics.com/articles/Hans-Christian-Gram.pdf

Gram Stain Interpretation

1 GRAM REACTION

GP

2 CELLULAR SHAPE

COCCI BACILLI

3 CELLULAR ARRANGMENT

CLUSTERS CHAINS PAIRS

1 GRAM REACTION

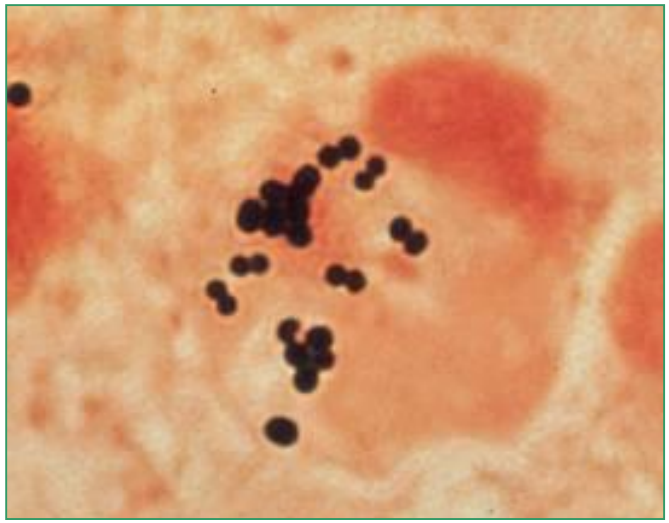
GN

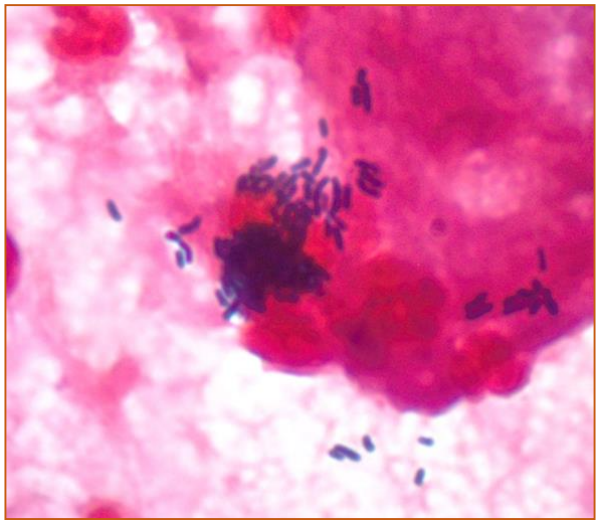
2 CELLULAR SHAPE

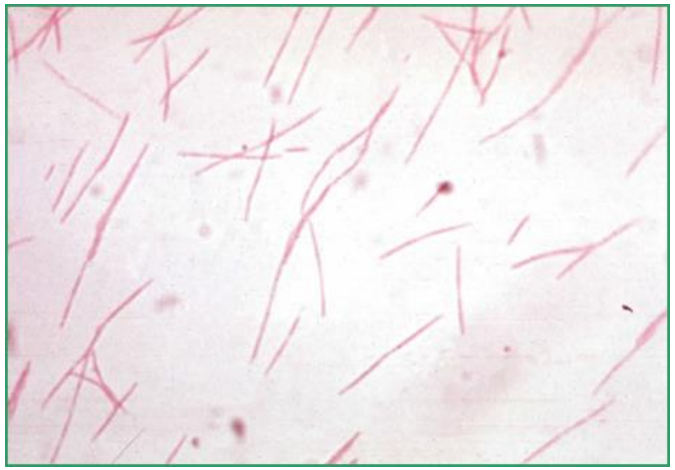
COCCI BACILLI COCCOBACILLI

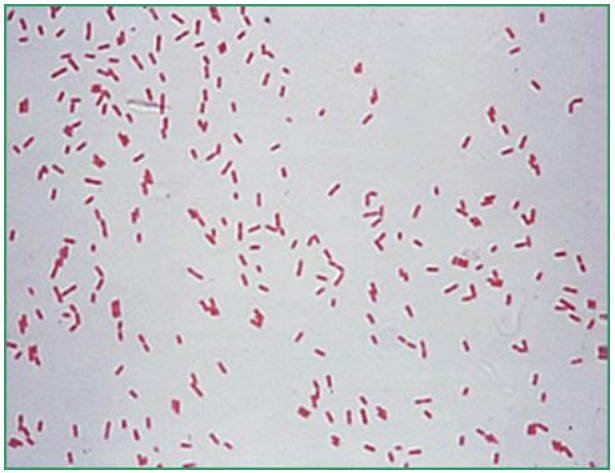
3 CELLULAR ARRANGMENT

DIPLOCOCCI



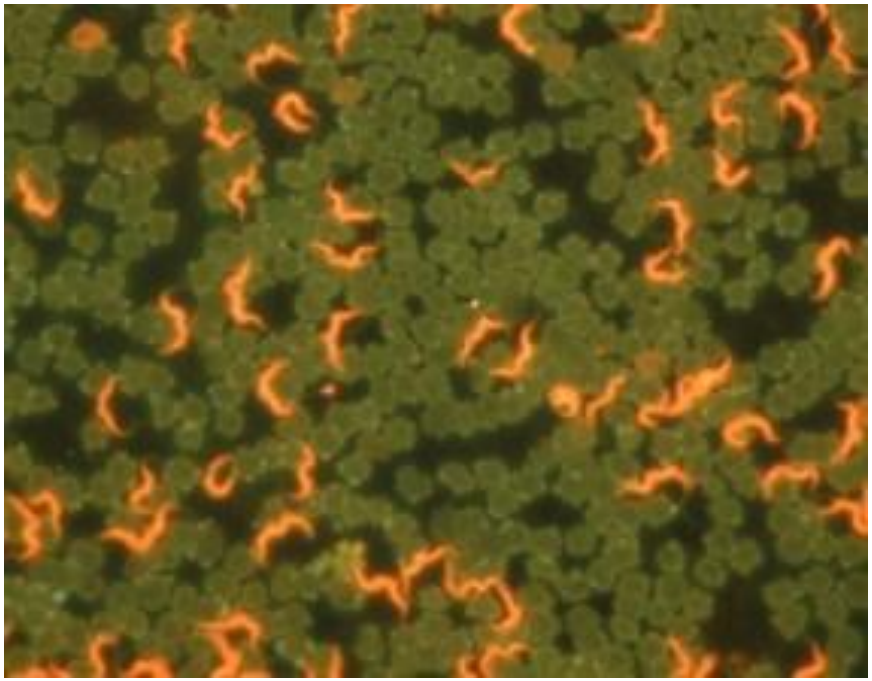




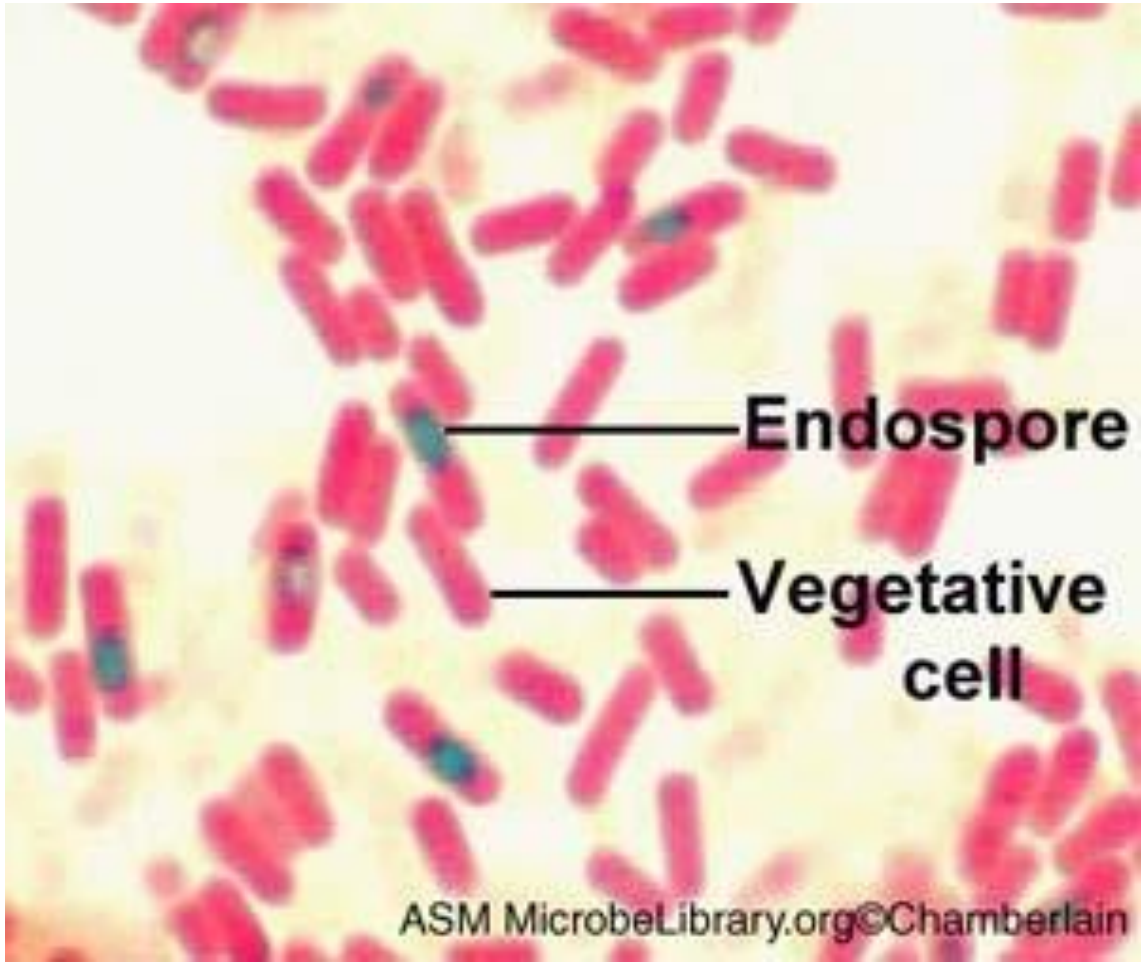


Bacteriology stains: Acridine Orange

- Fluorochrome dye that stains nucleic acids in gram-pos & gram-neg



Bacteriology stains: Schaefer-Fulton Stain

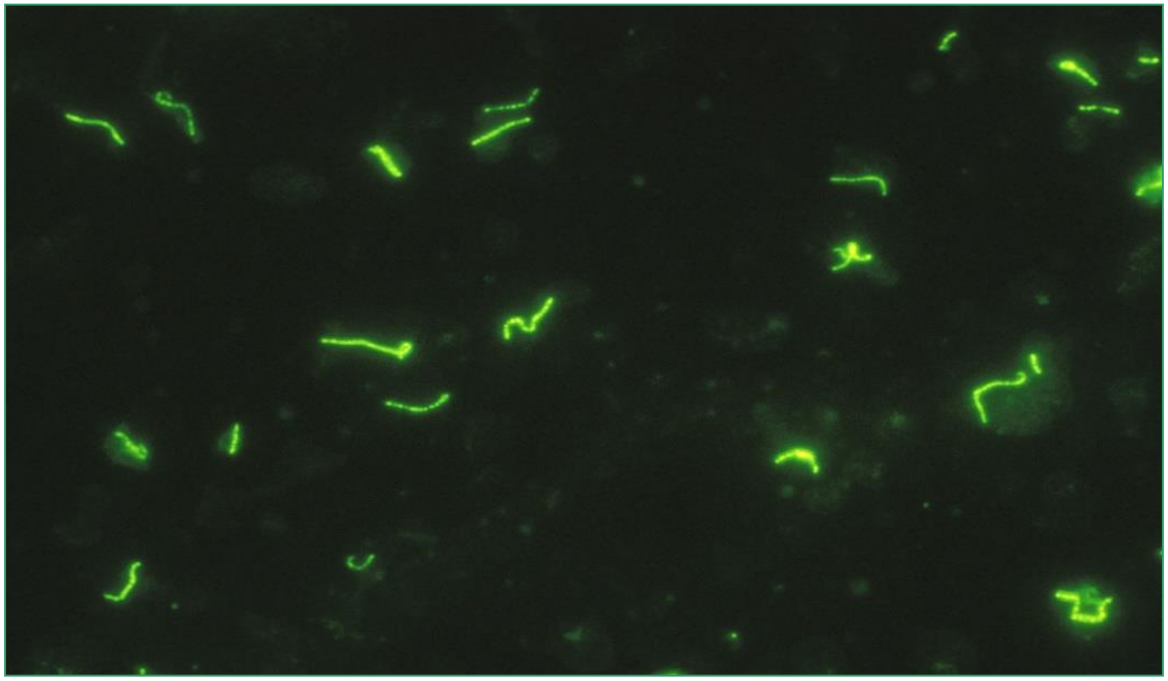


Bacteriology stains: Acid-fast bacilli

- Rely on ability of bacteria, like *Mycobacterium tuberculosis* to retain a primary stain when decolorized with strong acids (HCl).
- Screening
  - Auramine Rhodamine: fluorochrome stain
- Confirmation
  - Ziehl-Neelsen (ZN) Stain or Kinyoun stain
  - Modified acid-fast for partially acid-fast organisms

Auramine-Rhodamine

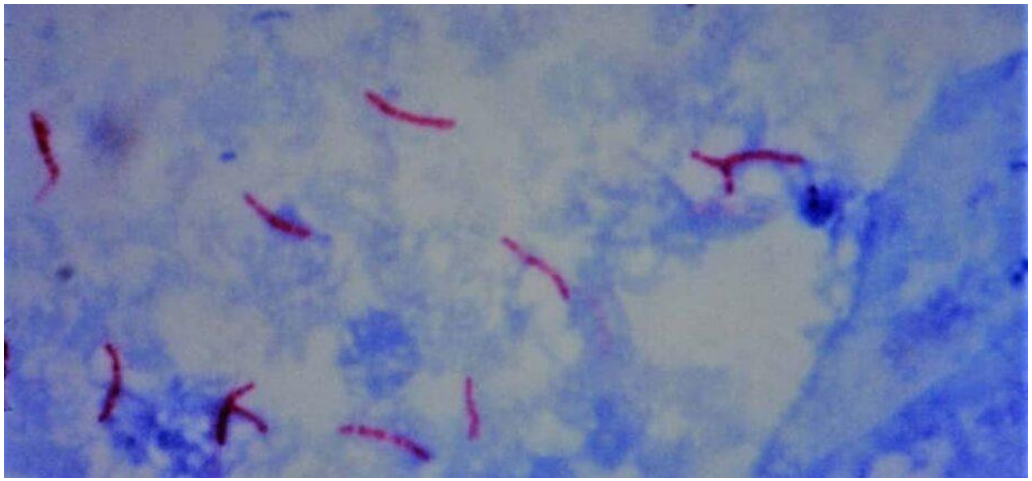
- Fluorescent dyes used
- Yellow-green organisms against a black background; require a fluorescent microscope



Auramine-rhodamine stain

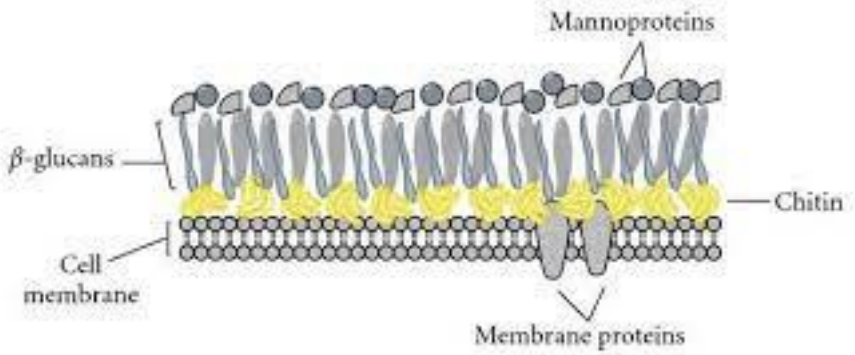
Ziehl-Neelsen or Kinyoun

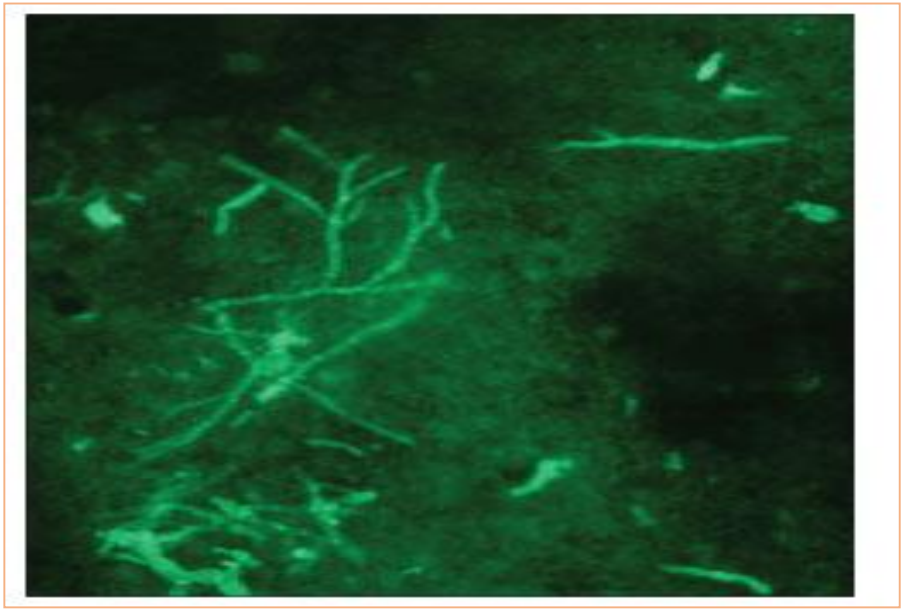
- **Ziehl-Neelsen (ZN) Stain**
  - Carbolfuchsin (red) primary stain; methylene blue counterstain
  - Heating required to allow penetration of primary stain through lipids.
- **Kinyoun**
  - Cold (no-heating required) ZN; higher phenol conc used to help stain penetrate lipids in wall.



Fungal stains: Calcofluor White

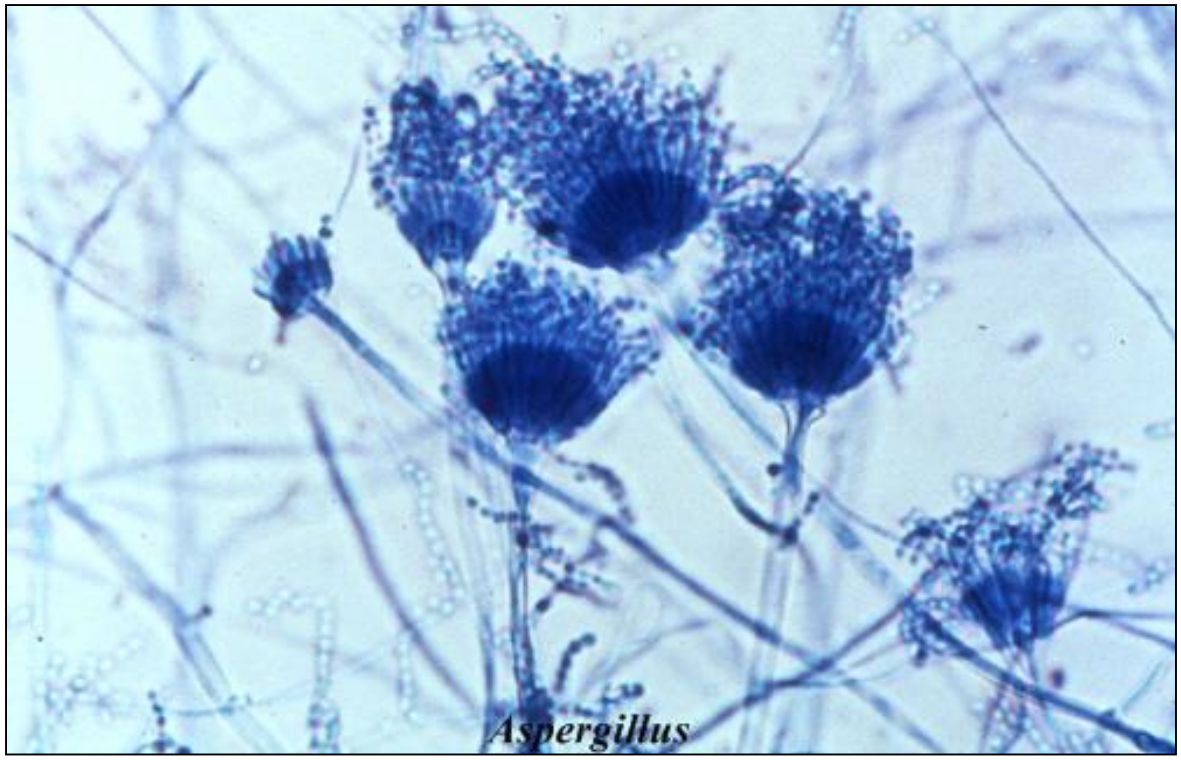
- Fluorochrome stains chitin in fungal cell walls





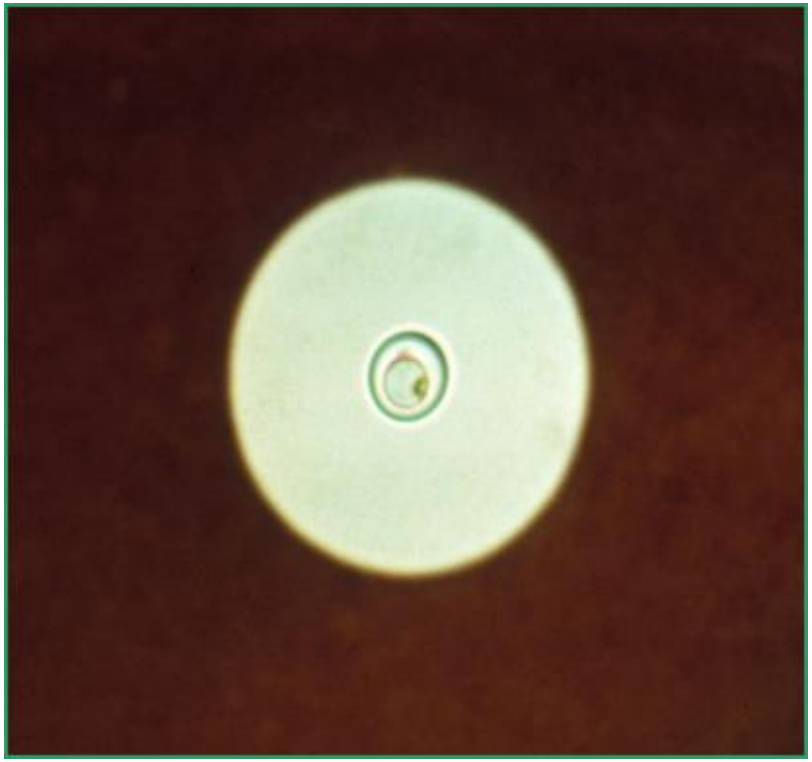
Fungal stains: Lactophenol cotton blue

- Stains for fungi growing in culture



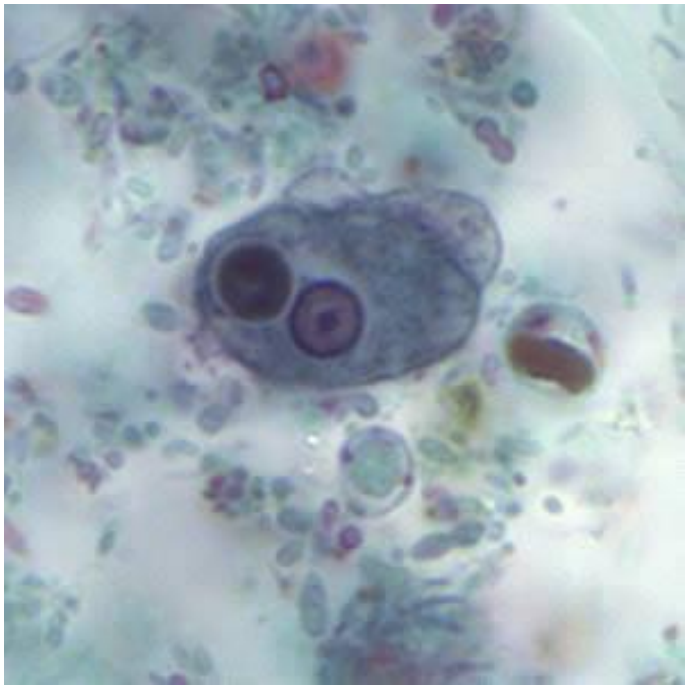
Fungal Stains: India Ink

- **India Ink** can be added as contrast to see the capsules of *C. neoformans.*



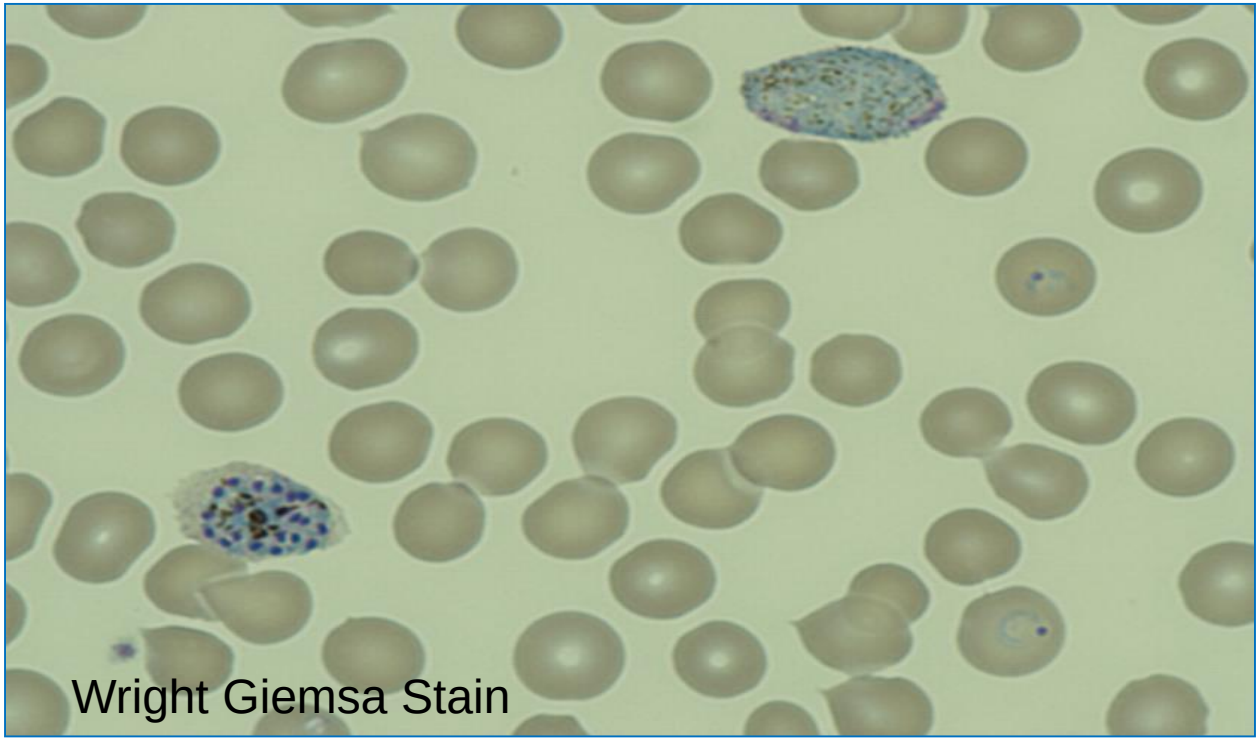
Parasite stain: Trichrome Stain

- Used in Parasitology for staining protozoan trophozoites and cysts
- Dyes that stain cellular structures red-purple and greenish



Parasite stain: Giemsa

- For blood parasites
- Combines methylene blue, azure blue and eosin



Media

- Solid media
  - Provides colonies
  - Differential and selective characteristics can provide preliminary information rapidly

- Liquid media
  - Culture more volume
  - Improve sensitivity
  - Longer length of incubation

Types of Growth Media: Nonselective

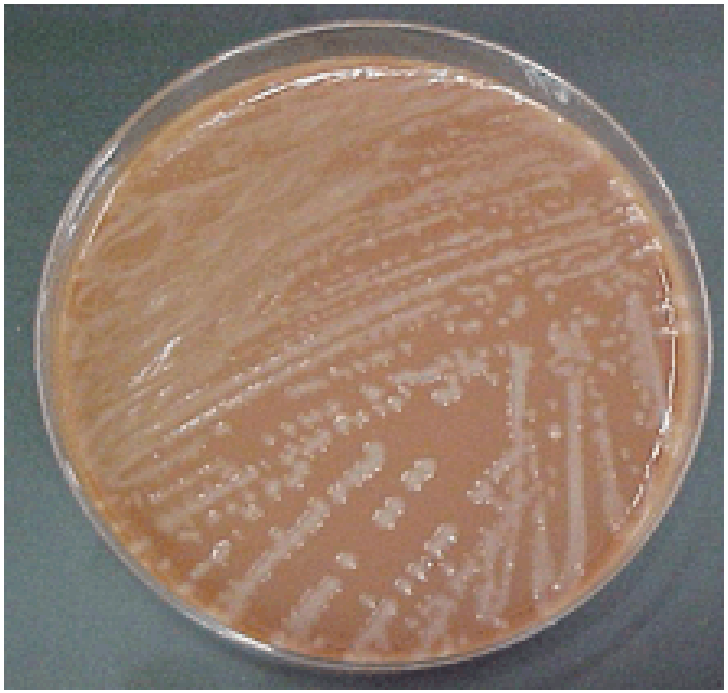
- **Nutrient media:** contain extracts of meat or soybean
  - Nutrient agar
  - Trypticase soy broth
- **Enriched**: added blood, vitamins, or growth factors
  - Blood agar (BAP)
  - Chocolate agar

Chocolate agar contains hemin (X) & NAD (V) or can be made by heating blood prior to pouring the plates.

- Fastidious organisms usually grow best on choc agar

BAP – blood agar plate

Chocolate agar plate



Types of Growth Media- Selective

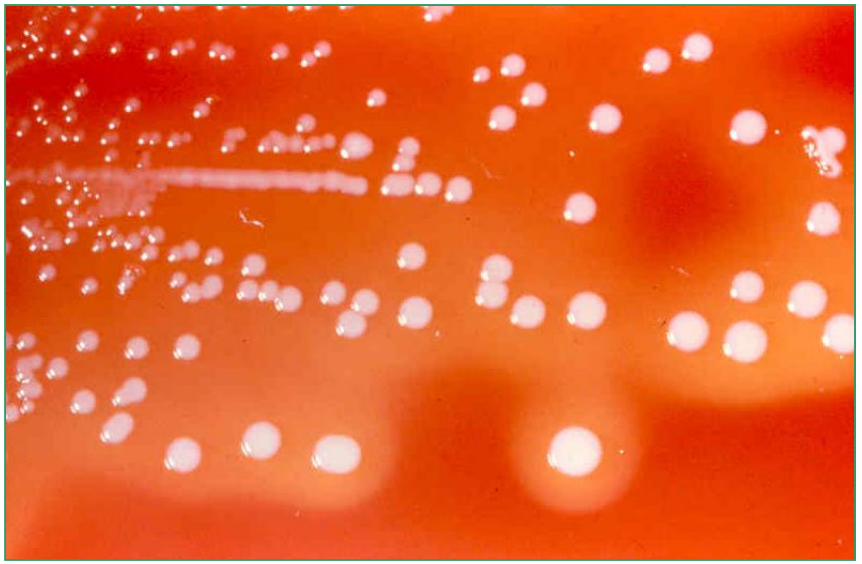
- Contains additives that inhibits some bacteria & allows others to grow
  - MacConkey agar selects for gram-negative bacilli
  - Colistin Nalidixic Acid (CNA) & Phenylethyl alcohol (PEA) select for gram-positive
  - Thayer Martin Agar w/abx for *N. gonorrhoeae*
  - Potato Dextrose agar (PDA) with chloramphenicol for fungi

Types of Growth Media - Differential

- Ingredients in media that allow visualization of metabolic differences.
  - MacConkey agar – lactose fermenter vs. non-lactose fermenter
  - BAP – hemolytic vs. non-hemolytic

Blood Agar Plate:

What do you see with respect to color of the colony and area around it?



Media/Reagents QC

- **Homemade or Purchased, but not listed as exempt in M22-A2:**
  - QC with organisms selected to give appropriate reactions or growth on media, verify sterility
- **Purchased media: If listed in CLSI M22-A2 as exempt, assess & document each lot or shipment**:
  - Moisture
  - Sterility
  - Breakage
  - Appearance

Media/Reagents QC

- **Media not listed in CLSI M22-A2**
  - All media must be tested before use.
  - Each medium must be tested with organisms expected to grow as well as organisms expected not to grow
  - The organisms selected should represent the most fastidious organisms for which the medium was designed.
  - The medium should be checked for sterility.
  - Test with dilute suspensions of organisms

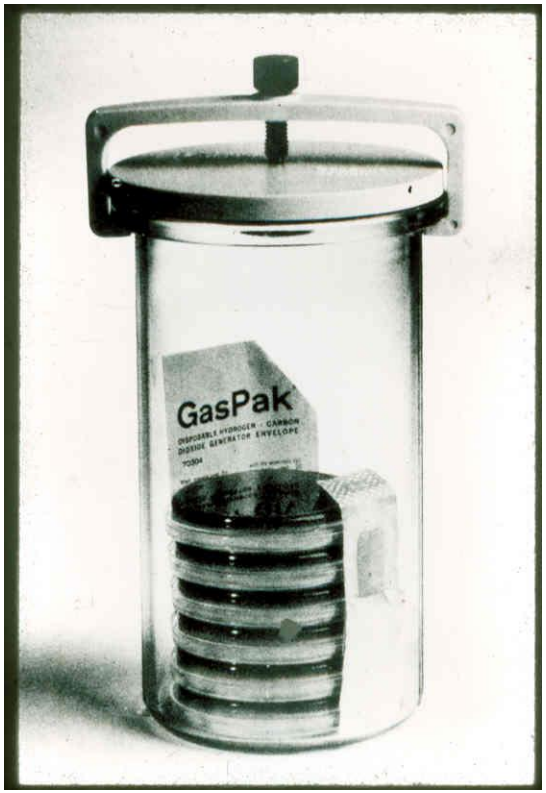
Growth Conditions for Bacteria

- **Psychrophiles:** 10 to 20°C
- **Mesophiles:** 20 to 40°C
  - Most pathogenic bacteria grow optimally at 35°C.
  - Some can grow at 42°C and this is used as differentiating tool
  - Molds grow better at 20 to 22°C.
  - Yeast grow better at 35°C i.e. body temperature.
- **Thermophiles**: 50 to 60°C

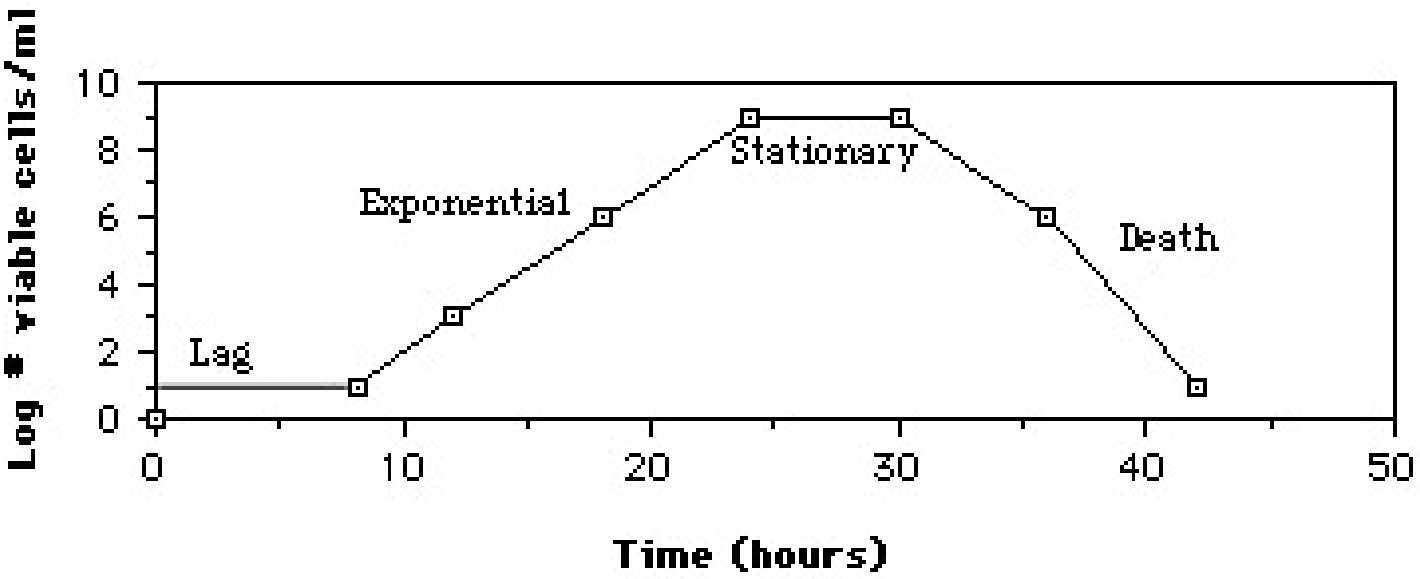
Growth Conditions for Bacteria

- **Obligate aerobes**: require oxygen
- **Facultative anaerobes:** can grow either with or without oxygen
- **Obligate anaerobes:** cannot grow in presence of oxygen
- **Aerotolerant anaerobes:** can survive in the presence of oxygen (also called facultative aerobes)
- **Microaerophilic:** Require reduced oxygen to grow (~5%)
- **Capnophilic:** Require increased CO_2 (5 -10%)

Anaerobe Chamber/GasPak Jar



Bacterial Growth Curve



Bacterial Biochemistry and Metabolism

- **Glucose to Pyruvic acid**
    - Embdem-Meyerhof-Parnas glycolytic pathway
    - Pentose phosphate pathway
    - Entner-Doudoroff pathway
- **Pyruvic acid is broken down further**
    - Anaerobic fermentation
        - Alcoholic (yeasts)
            - Ethanol
        - Homolactic (streptococci and lactobacilli)
            - Lactic acid
        - Heterolactic (lactobacilli)
            - Lactic acid, formic acid, and acetic acid
        - Propionic acid (*Cutibacterium acnes*)
            - Propionic acid
        - Mixed acid (*E.coli* , *Salmonella* sp., *Shigella* sp.)
            - Latic acid, acetic acid, succinic acid, and formic acid
        - Butanediol (*Klebsiella* sp., enterococci, *Serratia* sp.)
            - Acetoin and 2,3-butanediol
        - Butyric acid (obligate anaerobes)
            - Butyric acid
    - Aerobic respiration
        - Krebs cycle/tricarboxylic acid cycle

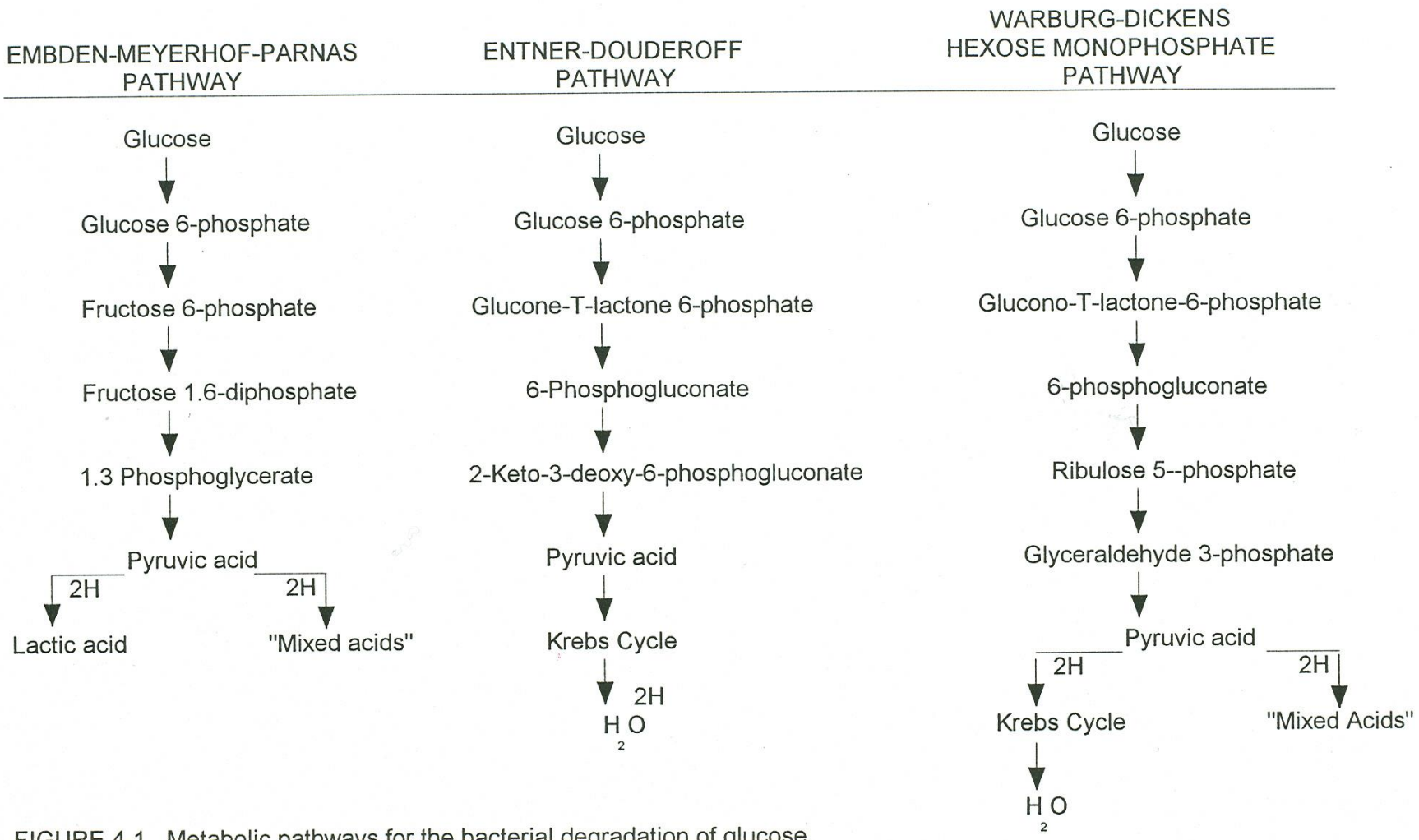


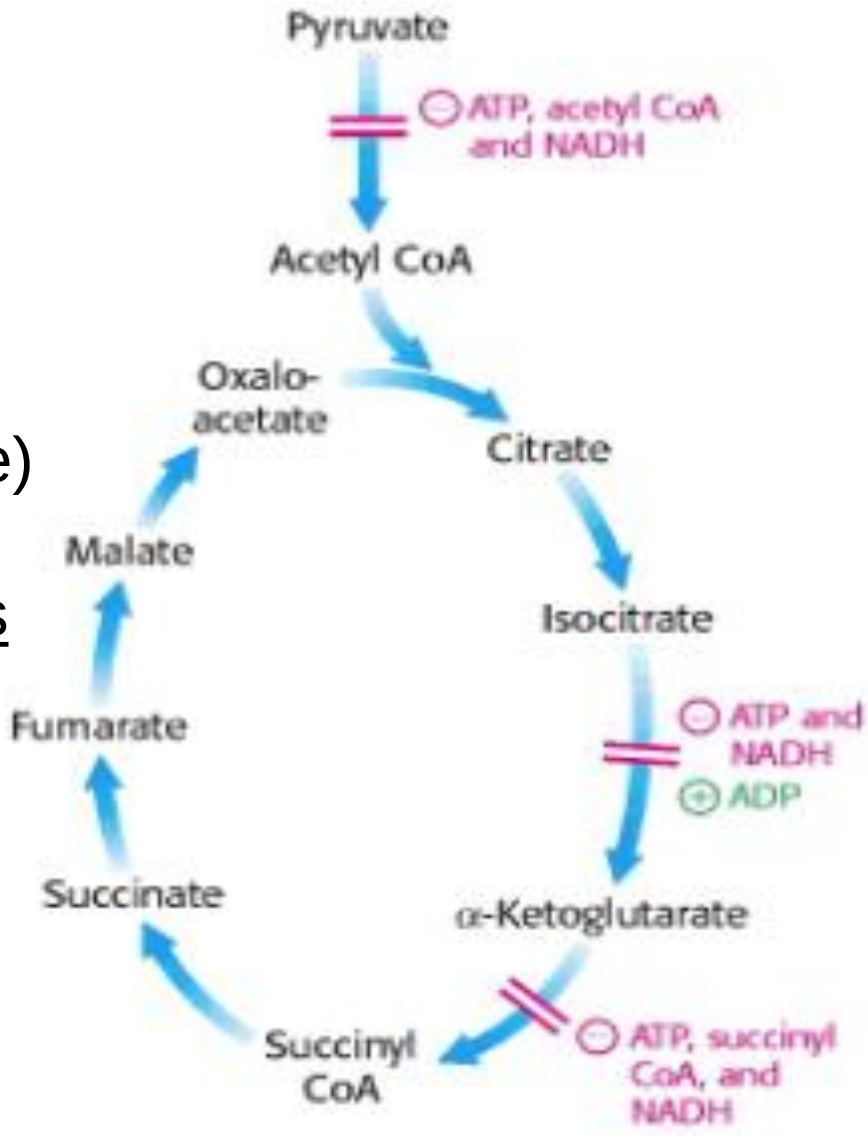
FIGURE 4-1. Metabolic pathways for the bacterial degradation of glucose.

Kreb's (TCA) Cycle

Pyruvate enters the TCA cycle, generating ATP, CO_2 and NADH

NADH enters the electron transport chain (in membrane) where $\underline{O_2}$ <u>is the terminal electron acceptor for aerobes</u> and more ATP is generated.

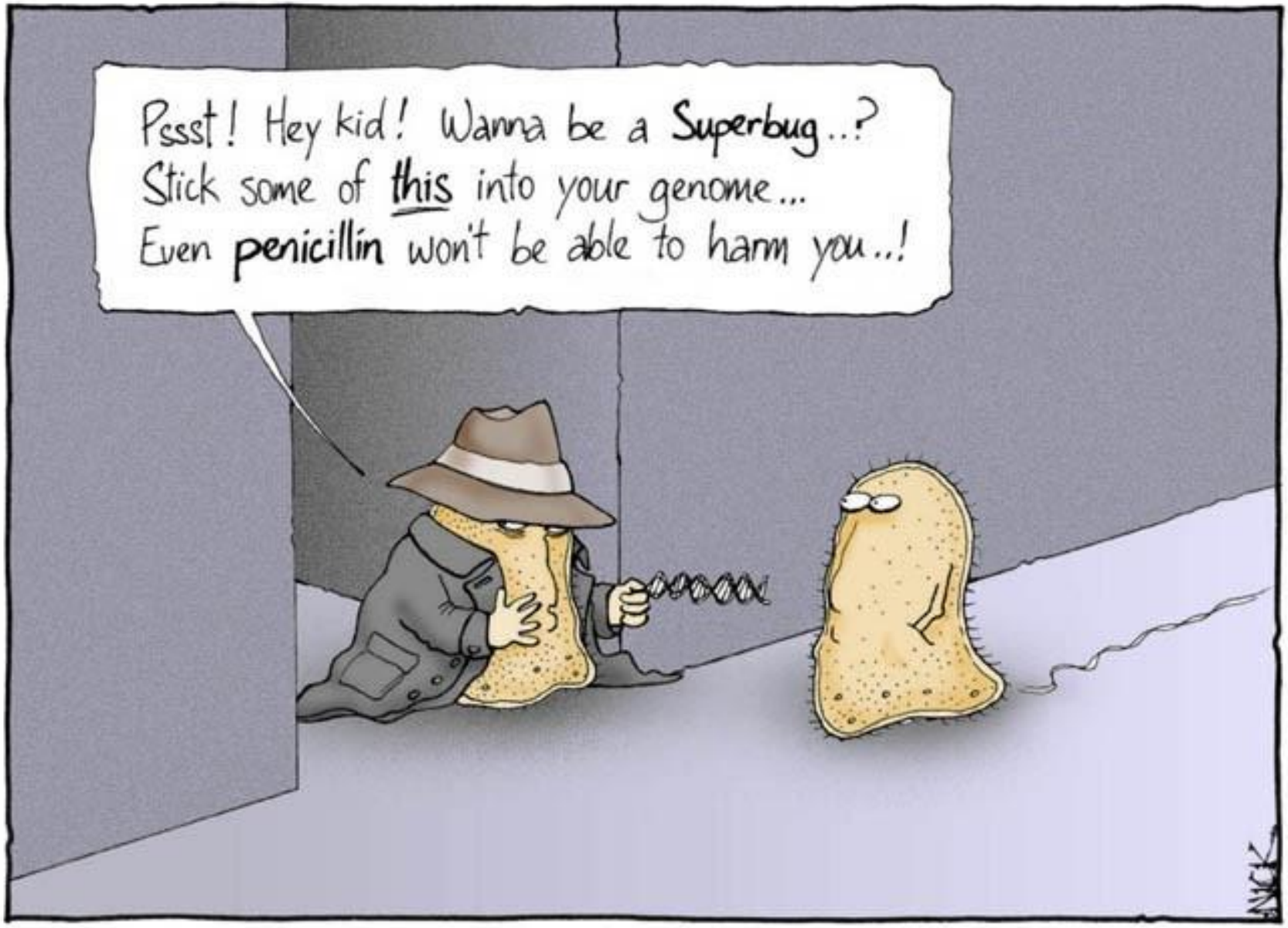
Aerobes or facultative anaerobes produce more energy in the presence of oxygen because oxidative respiration is used.



From Biochemaholic site

Bacterial Genetics

- **Constitutive:** genes that are always expressed
- **Inducible:** expressed under certain conditions
- **Transcription:** synthesis of mRNA by RNA polymerase using one strand of DNA as template
- **Translation:** synthesis of proteins from mRNA
- Extrachromosomal DNA
  - **Plasmids:** extra pieces of dsDNA that are not essential for bacterial growth
- Gene transfer
  - **Transformation:** uptake of free DNA from the environment
  - **Transduction:** transfer of genes by a bacteriophage
  - **Conjugation:** transfer of genetic material from the F+ cell to the F- cell through a conjugation pilus



It was on a short-cut through the hospital kitchens that Albert was first approached by a member of the Antibiotic Resistance.

Disinfection vs. Sterilization

- **Sterilization:** process that destroys all forms of life including bacterial spores
- **Disinfection:** Process that eliminates a defined scope of microorganisms
  - **Disinfectants:** physical or chemical methods usually used on surfaces (fomites)
  - **Antiseptics:** chemicals applied to the skin to reduce the number of bacteria present

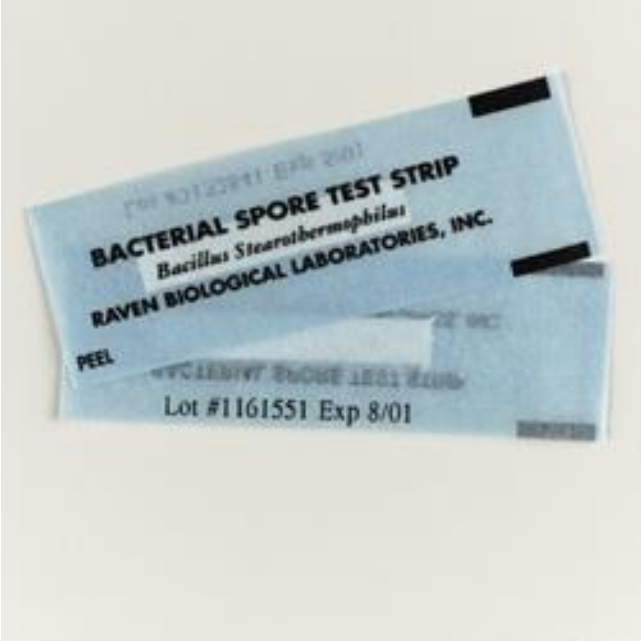
What factors effect disinfecting process?

- Amount of organisms
- Wet/dry surface & presence of organic material
- Temperature & pH
- Contact time
- Nature of surface
- Type of infectious agent

  [enveloped viruses<bacteria<fungi<non-env viruses<mycobacteria<spores<prions]

Sterilization methods:

- **Heat**
  - Autoclaving (121°C, 15 psi, usually 15 min,
    - Use *Geobacillus stearothermophilus (Bacillus stearophilus)* spore test to verify
- **Filtration**
  - 0.22 µm filter eliminates most bacteria
- **Radiation**
- **Gases**
  - Ethylene oxide, hydrogen peroxide



Disinfection Methods

- Chemicals
  - **Quaternary ammonium detergent compounds**
  - **Phenolics**
    - Chlorhexidine gluconate (skin)
    - Lophene, Vesphene (lab surfaces)
  - **Alcohols**
    - 70% ethanol, isopropanol
    - not sporicidal
    - not recommended for lab disinfection

Disinfection Methods

- **Aldehydes**
  - Formaldahyde gas is used to disinfect BSCs
  - Glutarldehyde used on medical equipment
- **Halogens**
  - Tincture of iodine - plus alcohol
  - Iodophores - plus detergent (Betadine)
- **Chlorine compounds** – used in drinking water; surfaces
- **Heavy metals**
  - Silver nitrate – rarely used; toxic

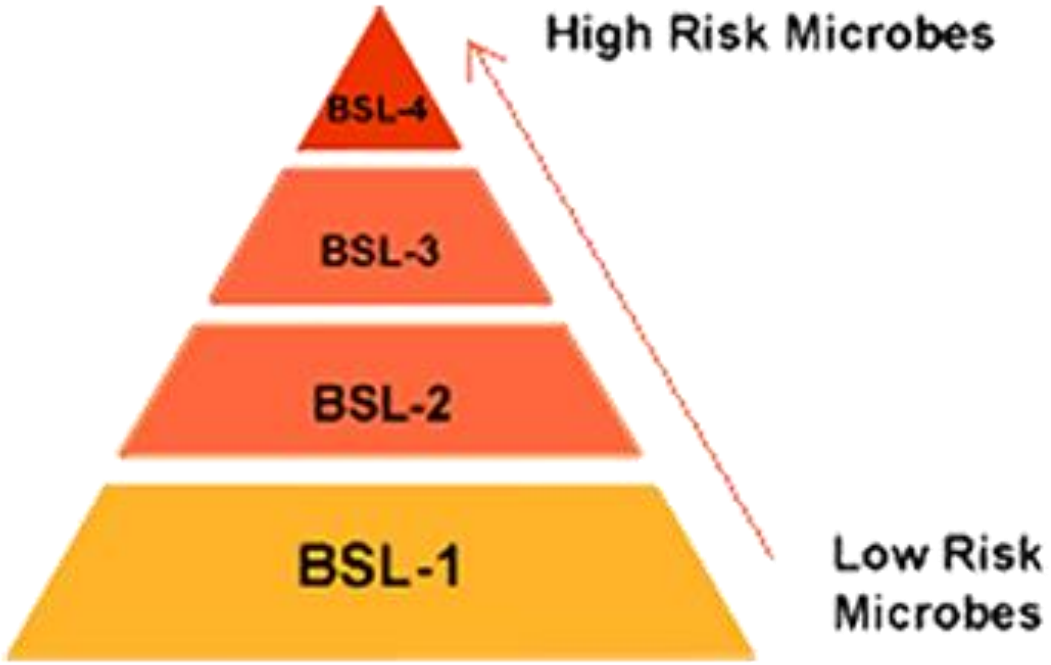
Hang in there….

BioSafety

- Standard precautions
  - Engineering controls
  - Work practice control
  - PPE
- Biological hazards
  - Processing of the patient specimen
  - Handling of the actively growing cultures
- Major infection routes for LAI
  - Parenteral inoculations
  - Spills and splashes onto skin
  - Ingestion
  - Inhalation

Risk level of organism

- Mode of transmission
- Risk to human
- Lab procedures/aerosol production risk



Reference: BMBL – Biosafety in Microbiological and Biomedical Laboratories

Classification of Microorganisms by Risk

- **Risk Group 1:** agents that are not known to cause disease consistently in healthy adults
- **Risk Group 2:** agents that pose moderate potential hazard for the employees and the environment
- **Risk Group 3:** agents with potential for aerosol transmission, and diseases that may be lethal
- **Risk Group 4:** agents have high risk of causing life-threatening infections, transmitted by aerosols, unknown risk of transmission

Clinical Microbiology – BSL-2

- Limited access
- Posted biohazard sign
- Hand washing
- Safe disposal of needles & sharps
- Decontaminate work surfaces daily & after spills
- Lab coats; gloves & eye protection, as needed for infection risk
- Class II BSC for procedures that generate aerosols or splashes
- No eating, drinking, smoking, applying cosmetics
- Decontaminate cultures/infectious materials or dispose in durable leak-proof containers prior to decontamination
- Safety Training
- Biosafety manual
- Rodent & insect control plan
- Provision of immunizations/tests for exposure
- Eyewash station available

Clinical Microbiology – BSL-3
BSL-2 practices plus:

- Safety training
- Handling of cultures must be in Class II BSC
- Gloves, impermeable, solid-front lab coat/gown
- N-95 respirator or PAPR
- Separated from rest of building by BSL-2 or anteroom
- Sealed windows, walls, floors & ceiling
- Design for easy-cleaning; non-porous materials
- Ducted ventilation w/negative air flow; exhaust outside or HEPA filtered.
- Hands-free sink
- Waste decontamination within facility.
- Facility design & procedures verified annually.

Example of BSL-3 pathogens sometimes encountered in clinical labs

- *Mycobacterium tuberculosis*
- *Histoplasma capsulatum, Coccidioides immitis, Blastomyces dermatitidis*
- *Brucella* spp.
- *Francisella tularensis*

Bioterrorism Preparedness

- Laboratory Response Network
  - Sentinel Laboratory
    - Most hospital labs
    - Rapid recognition of possible BT agent
    - Basic testing within BSC; NOT definitive identification
  - Reference Laboratory
    - Receive isolates or specimens from sentinel labs or clinics
    - Identify agents
  - National Laboratory
    - CDC, USAMRID, NMRC

Select Agents (BT agents) are biological agents and toxins with potential for severe harm to public health.

Pathogen Risk Categories

- Category A – easily transmitted & highly infectious
  - Smallpox virus, *Bacillus anthracis* (anthrax), *Francisella tularensis* (tularemia)
- Category B – moderate morbidity & low mortality
  - *Coxiella burn*etii (Q fever), *Burkholderia pseudomallei* (melioidosis)
- Category C – emerging pathogens
  - Nipah virus, Hantavirus

(Not the same as category A & B for shipping)

Guidelines for Sentinel Labs

- Handle any suspect BT agent in biosafety cabinet
- Minimize procedures that create aerosols
  - flaming loops, pipetting, centrifugation
- When BT/select agent is suspected perform all work with BSL-3 practices
- Refer to Reference Lab when organism is identified as possible select agent.

Shipping Categories for Infectious Substances

- Category A – an infectious substance which is transported in a form that, when exposure occurs is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals.
    - *Bacillus anthracis cultures*
    - *M. tuberculosis* cultures
    - *Brucella* spp. cultures
    - *Francisella tularensis* cultures

Shipping Categories

- Category B – an infectious substance that is not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposed.
    - *Staphylococcus aureus*
    - *Pseudomonas aeruginosa*
    - *E. coli*

Shipping Infectious Substances

- Guidelines issued by
  - U.S. Department of Transportation
  - U.S. Postal Service
  - International Civil Aviation Organization
- Patient specimen or culture isolates must be triple packaged
- Every employee who packages specimen and infectious materials for shipment must be appropriately trained

Philosophy of Clinical Microbiology Laboratory: Our Job

- Rapidly & accurately identify microbes that could be pathogenic
- Suggest antimicrobial agents that may be beneficial
- Guide care providers on specimen collection and transport
- Guide test choices
- Support patient needs

- Maintain flexibility; be ready to accept change
- Educate yourselves and others

Acknowledgments

- Dr. Susan Harrington
- Jessica Alban