Microbiology 101:

What the Infection Preventionist Needs to Know

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Disclosures

None related to this presentation.

All information discussions unless otherwise referenced is from the APIC Text.

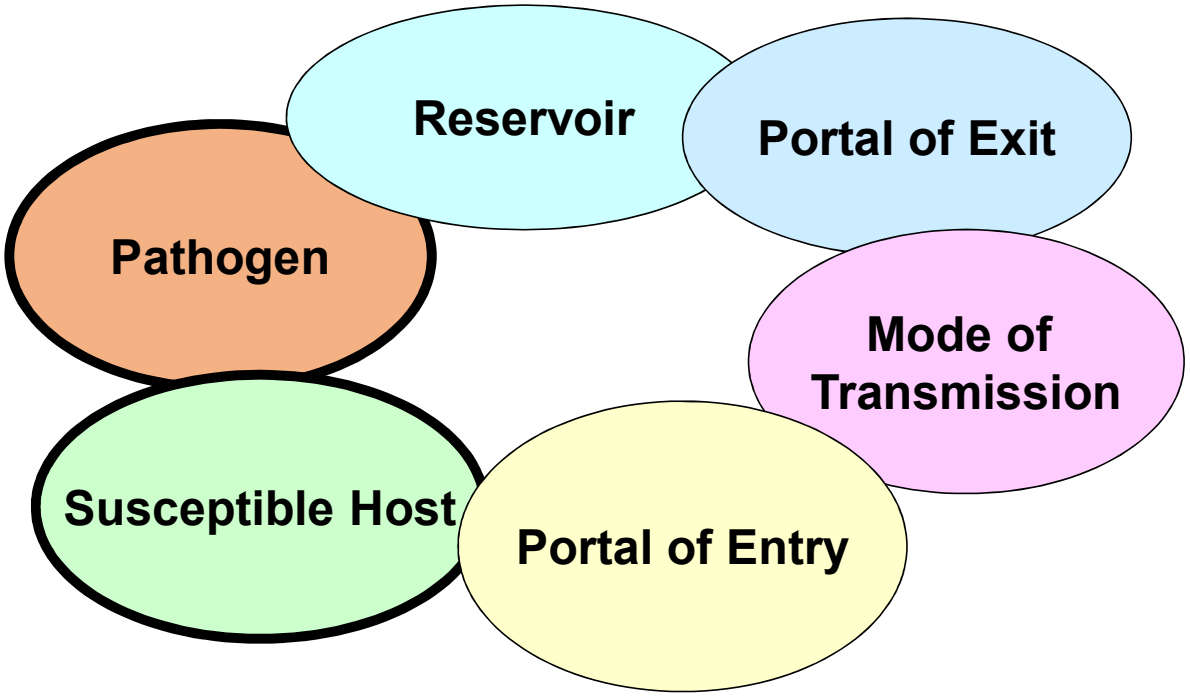
- Chapter 24 – Microbiology Basics
- Chapter 25 – Laboratory Testing and Diagnostics

Learning Objectives

- Identify the morphology of the various types of bacteria
- List two staining methods used to identify microorganisms
- Discuss the importance of proper specimen collection to infection identification
- Discuss laboratory tests can be used to identify bacteria, fungi and viruses to detect the presence of infections
- List two methods used for antimicrobial susceptibility testing

The Chain of Infection

- Components of the Infectious Disease Process



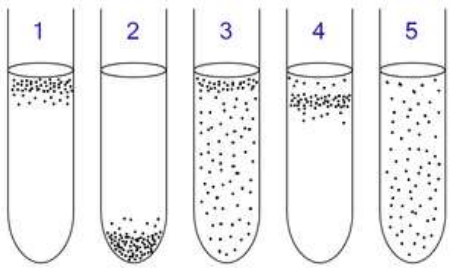
Scope of Microbiology

Study of bacteria, fungi (molds and yeasts), protozoa, viruses, and algae

- Eukaryote vs Prokaryote
- Normal flora (commensals) vs pathogens
- Gram negative vs Gram positive
  - Cell membrane vs Cell wall (peptidoglycan)
- Sporeforming vs non-sporeforming
- Anaerobes vs Aerobes vs Microaerophiles
  - Obligate anaerobes vs facultative anaerobes
- Lactose fermenting vs Non-lactose fermenting
- Hemolytic vs Non-hemolytic
- Thermophilic vs mesophilic
- Intracellular parasites
- Virus / Fungi / Parasites

Diagnostic testing

- Microbiological - Direct Examination
  - Gram Stain
  - Histology/Cytology
  - Wet Mount
- Serology - Detection of Antigen/Antibody Reaction
  - Antigen detection
  - Antibody detection
- Molecular Diagnostic Testing
  - Nonamplified nucleic acid probes
  - Target amplification methods
  - Probe Amplification Methods
  - Signal Amplification Methods



Thioglycollate – chemical reducing agent put into broth media to eliminate free O_2

Anaerobic bacteria can be identified by growing them in test tubes of thioglycollate broth:

1. Obligate aerobes need oxygen because they cannot ferment or respire anaerobically. They gather at the top of the tube where the oxygen concentration is highest. (e.g., *M. tb., Nocardia asteroides*, most fungi (except yeast)

2. Obligate anaerobes are poisoned by oxygen, so they gather at the bottom of the tube where the oxygen concentration is lowest. (e.g., *Clostridiodes spp., Bacteroides spp., Fusobacterium spp., Peptostreptococcus spp.)*

3. Facultative anaerobes can grow with or without oxygen because they can metabolize energy aerobically or anaerobically. They gather mostly at the top because aerobic respiration generates more ATP than either fermentation or anaerobic respiration. (e.g., *E. coli. Staph spp., Strep spp*, *Salmonella spp. Listeria spp.)*

4. Microaerophiles need oxygen because they cannot ferment or respire anaerobically. However, they are poisoned by high concentrations of oxygen. They gather in the upper part of the test tube but not the very top. (e.g., *Campylobacter spp., Helicobacter spp., H. influenza, Strep pneumoniae)*

5. Aerotolerant organisms do not require oxygen as they metabolize energy anaerobically. Unlike obligate anaerobes however, they are not poisoned by oxygen. They can be found evenly spread throughout the test tube. (e.g., *Strep mutans)*

https://en.wikipedia.org/wiki/Microaerophile

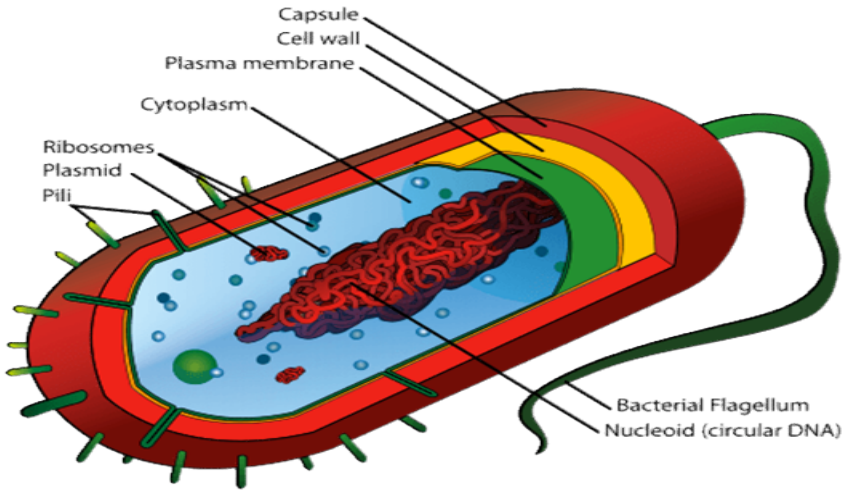
Microbiology 101

Overview of Microorganisms

Bacteria Classification

Prokaryote

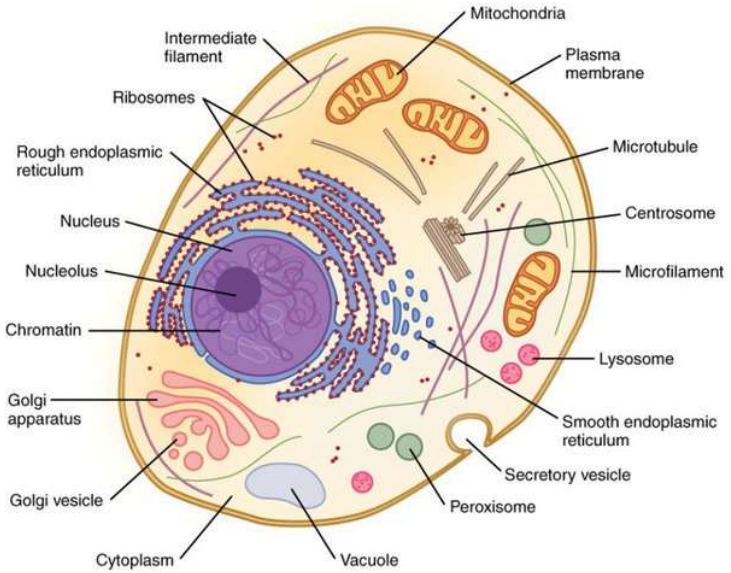
- Bacteria and blue-green algae
- Lack a visible nucleus, a membranous nuclear envelope, and membrane-bound internal structures such as mitochondria
- Single loop of chromosomal DNA
- Smaller (1-10 um) than eukaryotic cells (10-100 um)
- Have a cell wall with peptidoglycan
- Some carry plasmids



Graphics from: https://www.diffen.com/difference/Image:Prokaryotic-Cell.png

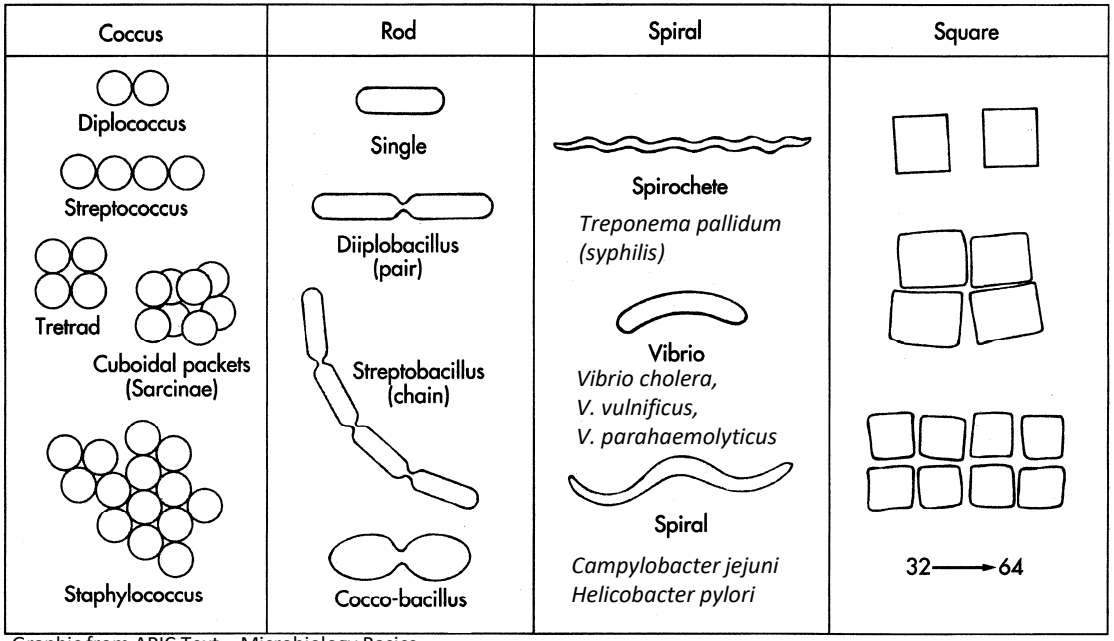
Eukaryote

- Fungi, protozoa, algae, humans
- Nucleus (contains DNA) is membrane bound (Nuclear envelope) and nucleolus
- Mitochondria, lysosomes



Google Image: Accessed 02/04/2019

Overview of Microorganisms - Bacterial Morphology



Graphic from APIC Text – Microbiology Basics

- 17 groups
- Nomenclature – genus and species
  - e.g., *Staphylococcus aureus*
- Gram positive – cell wall (peptidoglycan)
- Gram negative - no cell wall but cell membrane (lipoproteins, lipopolysaccharides, phospholipids)
- Outer membrane – some organisms evade phagocytosis (glycocalyx - capsule, barrier to certain antibiotics and virulence (endotoxin) properties
- Many have flagella – peritrichous/single (motility) and cilia
- Some have fimbriae – allows bacteria to adhere to surfaces; some have pili – join bacterial cells
- Some Gram positive bacteria form endospores
  - e.g., *Clostridium spp., Bacillus spp.*
  - *Geobacillus stearothermophilus* – steam sterilization
  - *Bacillus atrophaeus* – ethylene oxide sterilization
- Survive extreme in temperature, drying and exposure to some toxic chemicals

Reproduction

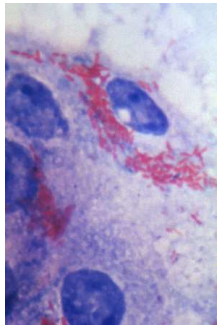
- Binary fission
  - e.g., *E. coli* – every 20 mins; *M. tb*. – every 12 to 24 hours
- Diversity
  - Transformation – naked DNA picked up by bacteria and incorporated into existing DNA or becomes part of plasmid pool
  - Conjugation – plasmid transfer from donor to recipient via pilus (e.g., antibiotic resistance)
  - Transduction – DNA transferred from donor cell to recipient cell via a virus
    - E.g., Bacteriophage

Overview of Microorganisms - Bacterial Morphology

- Mycoplasma (*M. pneumoniae, Ureaplasma urealyticum, M. hominis)*
  - 70 species
  - Small pleomorphic bacteria
  - No cell wall
  - Do not Gram stain – dark field / phase contrast microscopy
  - Fastidious- enriched medium
  - Resistant to cell wall-active antibiotics
- Obligate intracellular parasites
  - Chlamydia – *C. trachomatis* (LGV), *C. pneumonia, C. psittaci* (psittacosis or parrot fever)
    - Gram negative coccoid; non-motile
    - RNA and DNA; cell membrane similar to Gram negative bacteria
    - Replicates in cytoplasm of host cell – intracellular inclusions
    - Susceptible to broad spectrum antibiotics
  - Rickettsia – *R. rickettsia, R. prowazekii, Coxiella brunetti* (Q fever – infectious aerosols)
    - Gram negative rod shape or coccobacilli
    - Multiply in an arthropod (tick, fleas, mite) as part of life cycle
    - Grow in tissue cultures or embryonated eggs
  - Viruses
    - Not eukaryotes or prokaryotes
    - Contain either DNA or RNA; double or single stranded
    - Presence or absence of an envelope



3D computer generated image
CDC Public Health Image Library Accessed 02/04/2019

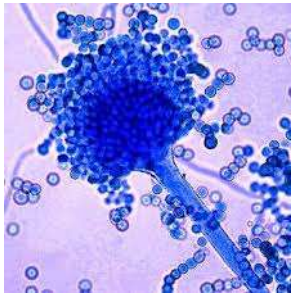


Photomicrograph reveals the presence of *R. prowazekii* bacteria in a yolk sac smear specimen

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Overview of Microorganisms- Fungi

Google Images: Accessed 02/04/2019



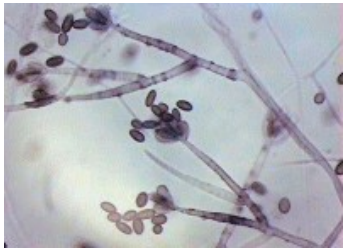
Aspergillus fumigatus



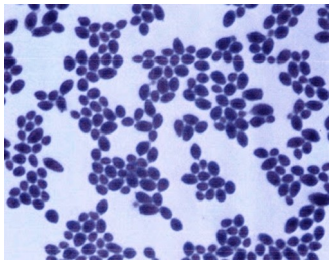
Penicillium chrysogenum



Fusarium spp.



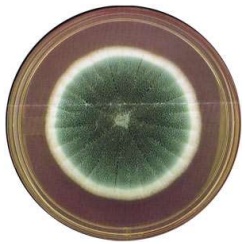
Stachbotrys chartarum



Candida albicans



Rhizopus nigricans



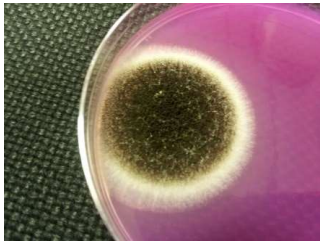


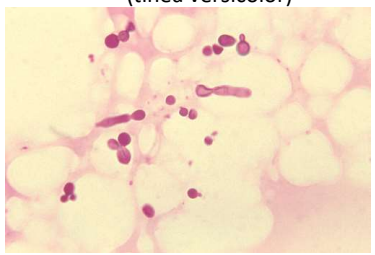
Malassezia furfur
(tinea versicolor)











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- Eukaryotic
- Cell wall contain chitin
- Most are accidental pathogens
- Yeast – single cell (*Candida spp., C. neoformans*)
- Molds – long branching filaments (hyphae)
  - Reproduce sexual and asexual
  - Opportunistic pathogen (Aspergillus, Rhizopus, Mucor)

Overview of Microorganisms- Fungi

Organism	Source	Image	Disease Spectrum
Candida spp.[1]	Commensal of the skin, GI tract, and vagina.		•Hematogenously disseminated infections •Mucocutaneous infections: Oropharyngeal infections (thrush) Skin/nail infections Vaginitis
Pneumocystis spp.[2]	Species are host specific, with no known environmental reservoir.		•Pneumonia
Histoplasma capsulatum[3]	Found in the environment in soil contaminated with bird or bat guano. Humans infected by inhaling conidia (spores).		•Self-limited flu-like syndrome •Acute pneumonia •Chronic pulmonary infection •Disseminated disease
Blastomyces dermatitidis[4]	Found in the environment in soil / decaying wood. Humans infected by inhaling conidia (spores) or direct inoculation of the skin.		•Acute/chronic pulmonary infections •Skin lesions/ subcutaneous nodules •Disseminated disease
Malassezia spp.[5]	Commensal of the skin.		•Primarily cutaneous infections Pityriasis versicolor Seborrheic dermatitis •Allergic atopic eczema
Cryptococcus spp.[6]	Found in the environment in association with soil, pigeon guano, and trees. Infection occurs through inhalation of spores or small yeast.		•Pneumonia •Meningitis

Dimorphic fungi
- Grow either as mold or yeast

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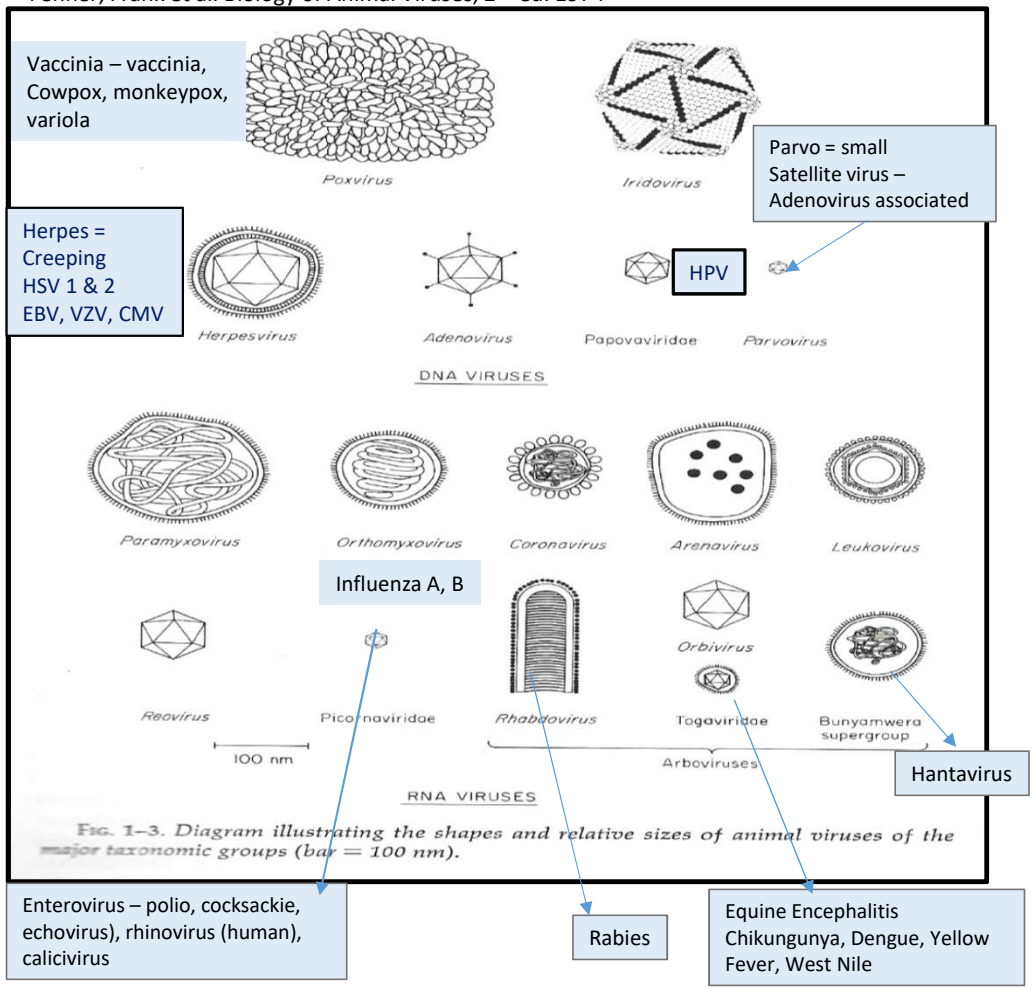


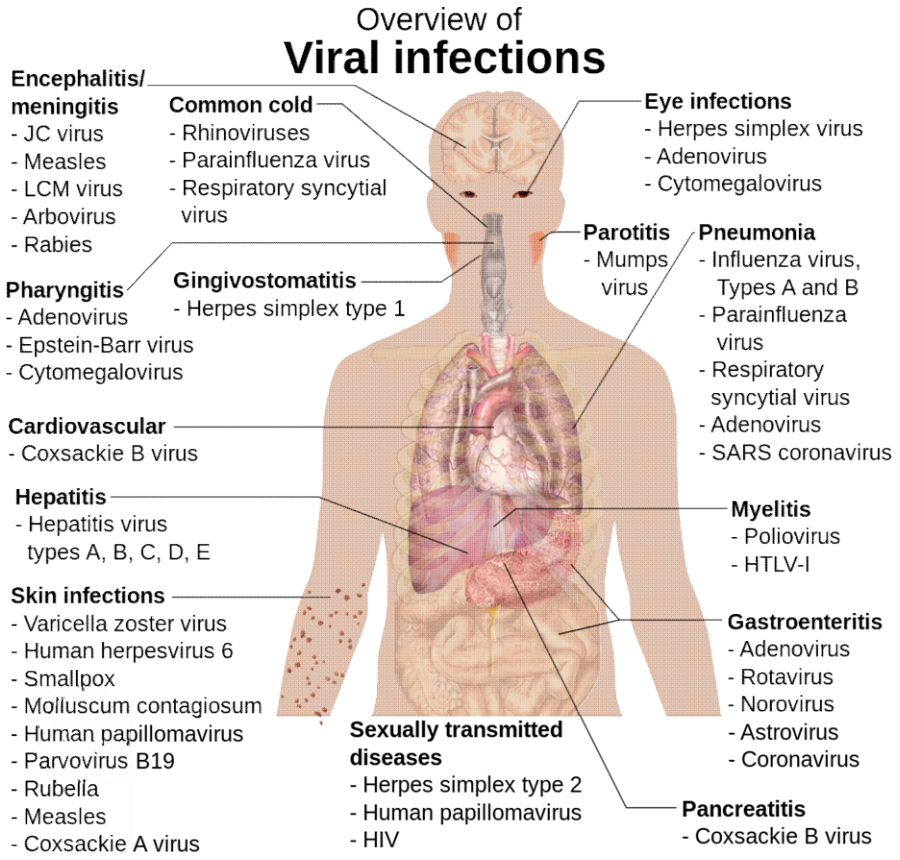
Be a virus, see the world.

The Far Side Gary Larson

Overview of Microorganisms - Viral Morphology

Fenner, Frank et al. Biology of Animal Viruses, 2nd ed. 1974





https://commons.wikimedia.org/wiki/File:Viral_infections_and_involved_species.svg
Accessed 02/04/2019

Microbiology 101

Staining Techniques

Staining Methods

- Stains – basic (nuclear cell components or acidic (cytoplasm and granules)
  - Colouring organism with a dye to enhance visualization of structures
  - Simple stain (one dye) or differential (2 or more dyes) for shape and biochemical colour reaction
- Gram Stain
  - Differential stain
  - Primary microscopic exam
  - Organism classified as Gram positive or Gram negative
- Acid-fast stain Ziehl-Neelsen (ZN) non-fluorescent
  - Differential stain
  - Direct smear
  - Acid fast organisms – red under microscope
    - Mycobacteria, Nocardia, Actinomyces
- Kinyoun non-fluorescent
  - Direct smear
  - Detection of m
  - Mycobacteria, Cryptosporidia, and Cyclospora parasites in stool
- Fluorochromes (fluorescent stains)
  - Detection of cell wall–deficient bacteria such as mycoplasmas
    - Acridine orange – stain cells with DNA and RNA
      - Detect mycobacteria
      - Bacteria nucleic acid fluoresces orange; mammalian nucleic acid fluoresces green
    - Auramine-rhodamine
      - Uses fluorescent stain
      - Under UV light, fluoresces bright yellow-orange
      - More sensitive than ZN stain (carbol fuchsin)
    - Calcofluor white
      - Rapid screening for fungal elements and pneumocystis cysts
      - Binds to cellulose and chitin in cell wall
      - Fluoresces under UV light
    - Immunofluorescent
      - Antibody directed/probe which binds organism and fluoresces under microscope
      - *Chlamydia, Legionella pneumophila, Bordetella pertussis*, HSV, VZV, CMV, adenovirus, RSV
    - Trichome
      - fecal parasites (protozoa, cysts and ova)

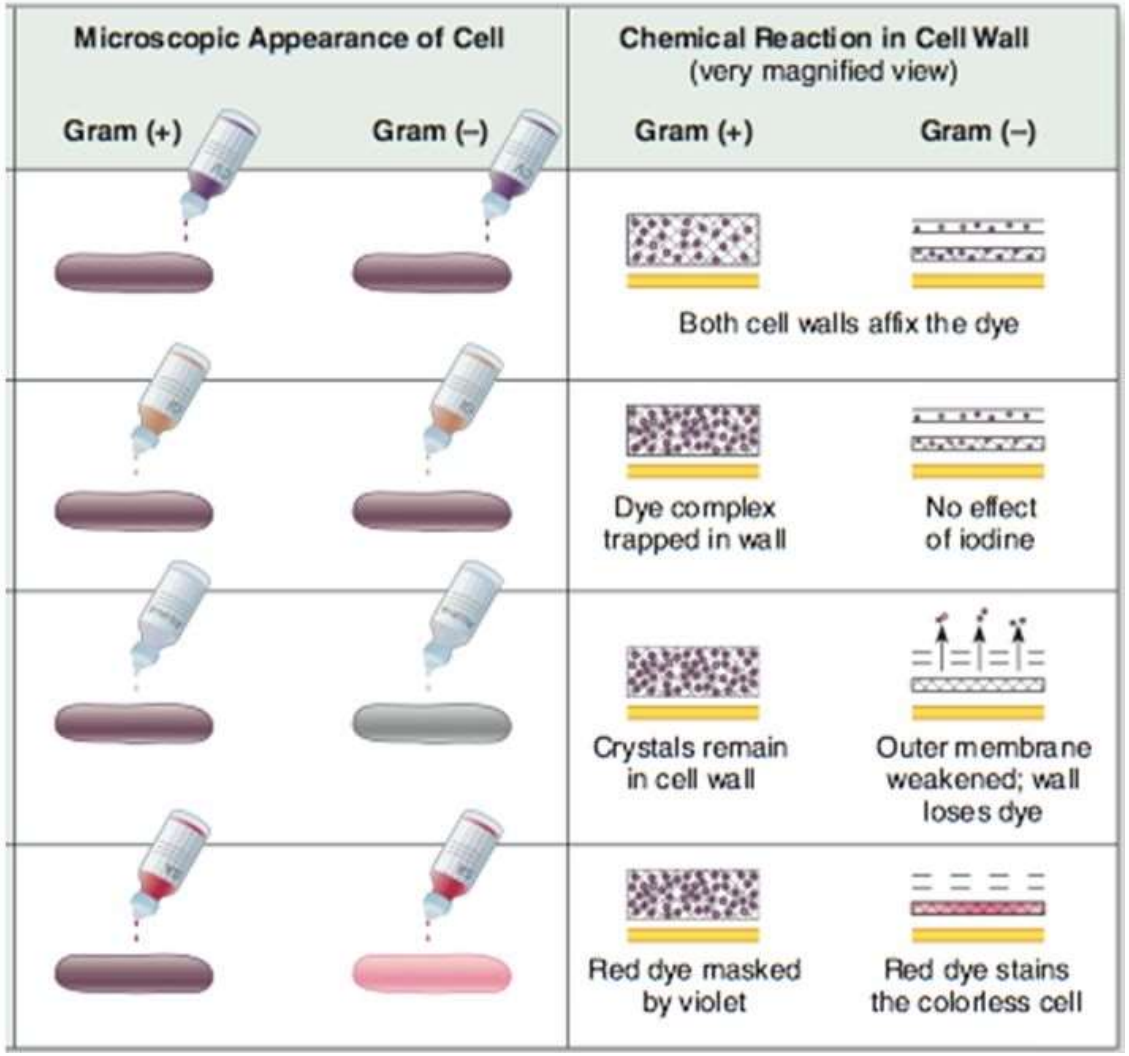
GRAM STAIN PROCEDURE

1. Flood with crystal violet for 1 minute. Rinse

2. Flood with iodine for 1 minute. Rinse

3. Decolorize with alcohol until no more purple comes off the slide. Rinse

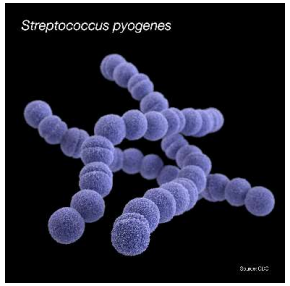
4. Flood with safranin. Rinse and dry.



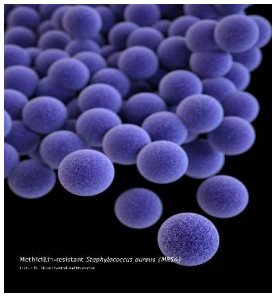
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Bacterial Morphology and Staining Methods

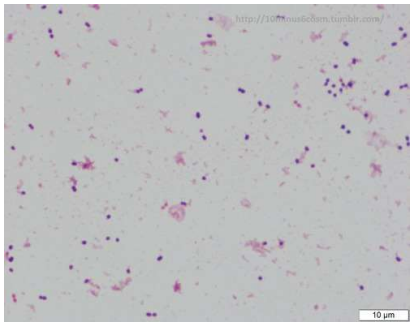
Streptococcus – Gram +ve cocci in chains



Staphylococcus – Gram +ve cocci in clusters

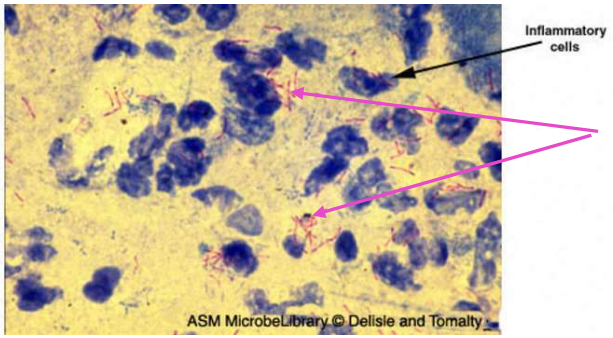


Neisseria - Gram -ve diplococci

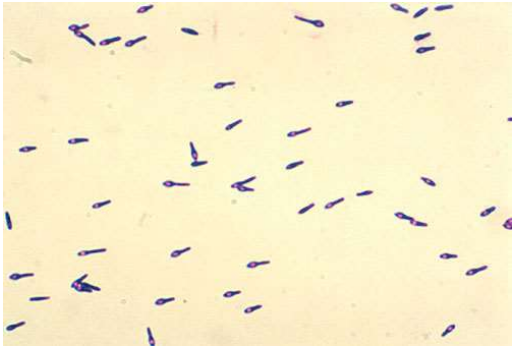


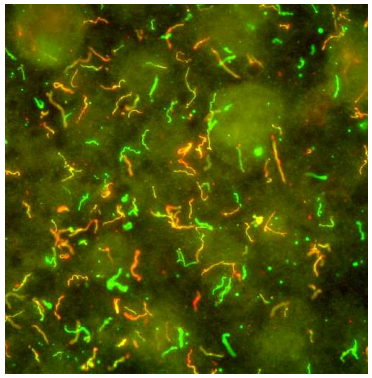
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Acid Fast Stain – *Mycobacterium tuberculosis*

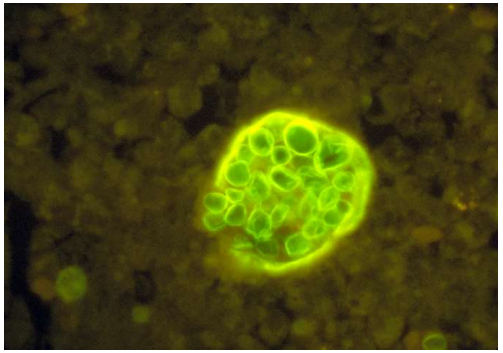


Gram positive sporeforming rod – *C. botulinum*





Immuno fluorescent antibody (IFA) – Lyme borreliosis
CDC Public Health Image Library: Accessed 02/05/2019



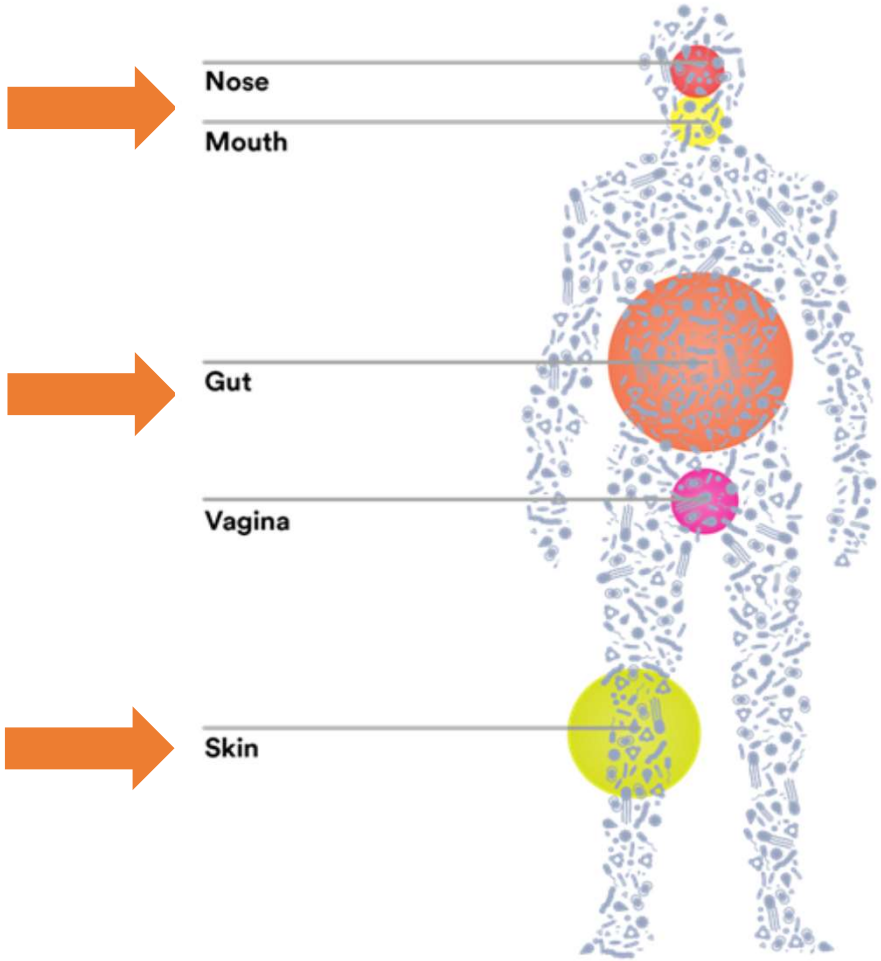
Calcofluor staining – *Coccidioides imitis* spherule with endospores
CDC Public Health Image Library: Accessed 02/05/2019

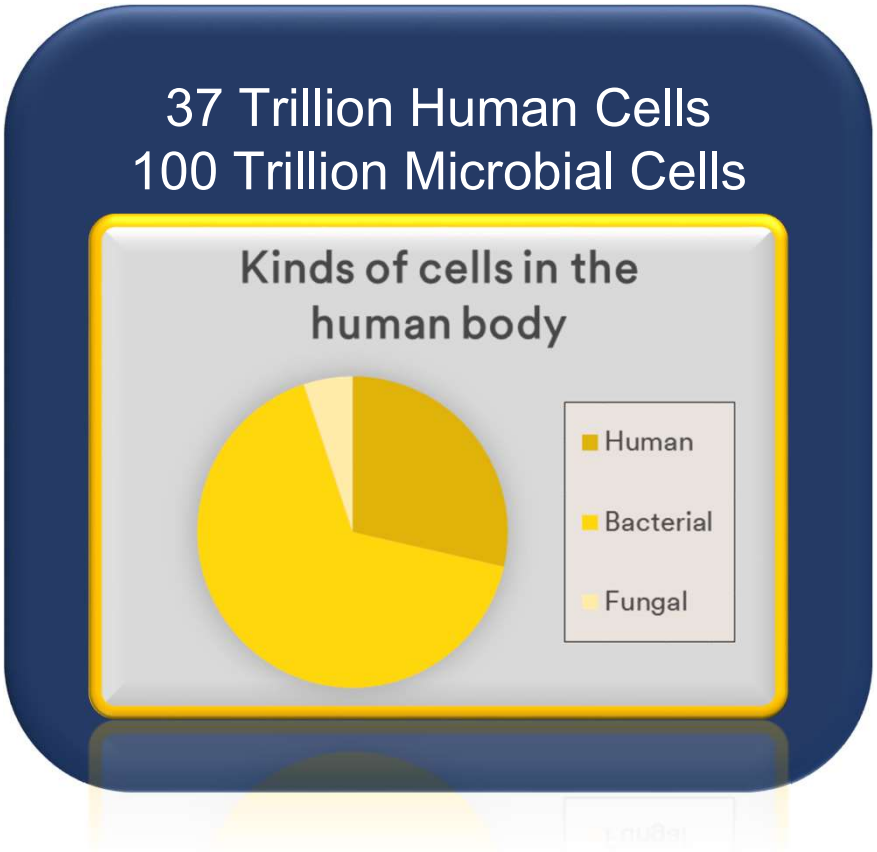
http://www.asmscience.org/content/education/imagegallery/

Microbiology 101

Normal Flora (Commensals)

Human Microbiome





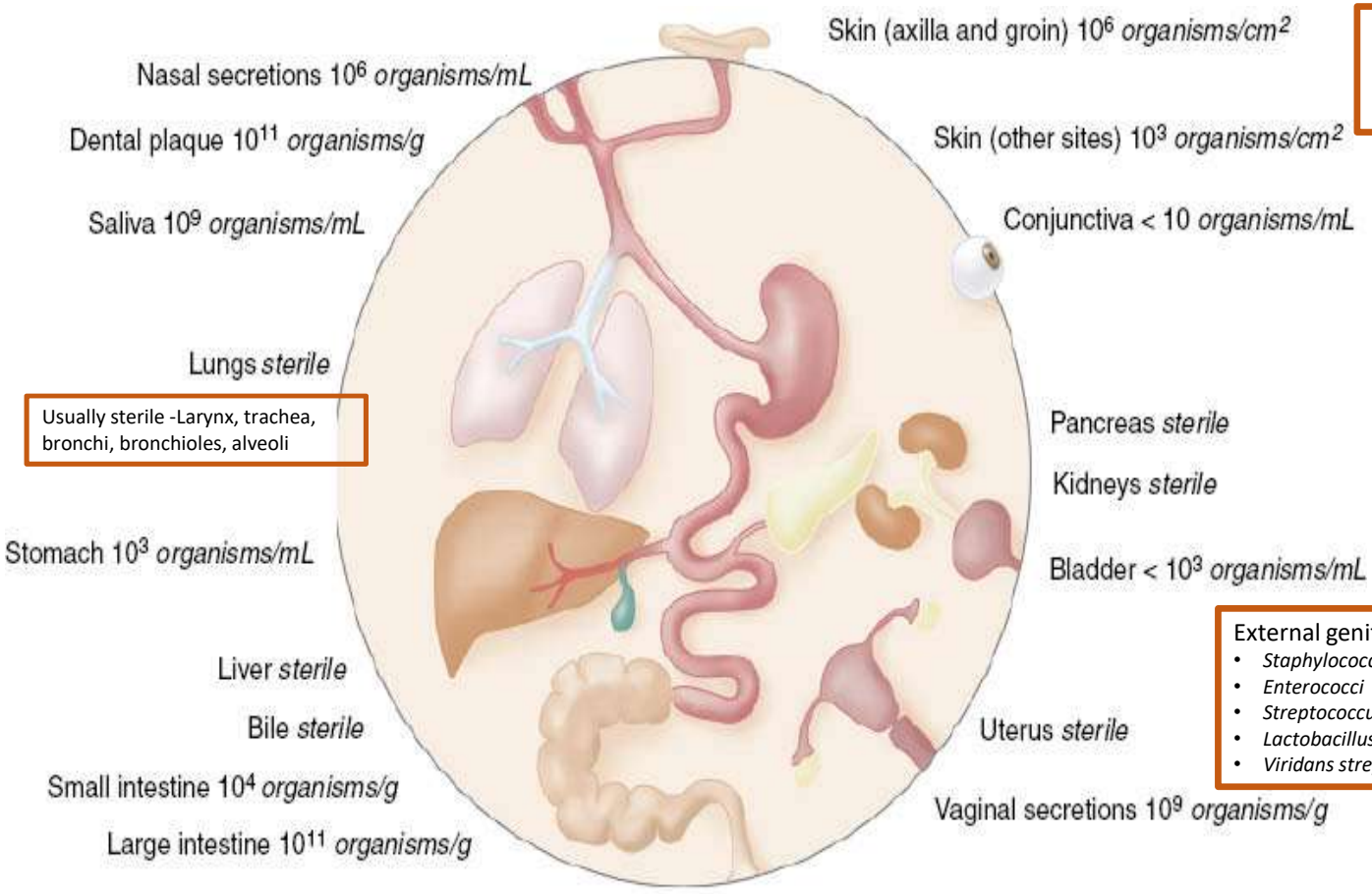
American Society for Microbiology Academy. FAQ: Human Microbiome 2014.Retrieved December 08, 2016, from http://academy.asm.org/index.php/faq-series/5122-humanmicrobiome

Normal Flora

- Microorganisms are naturally present on and in humans
  - Endogenous (e.g., *Staph aureus, E. coli)* or exogenous (e.g., HSV, *M. tb, Legionella spp*)
- Healthy surfaces - normal flora or common commensals
  - Vary by body site
- Colonization
  - presence of a microorganism in the absence of symptoms or deep tissue invasion
  - Colonizing organisms - may facilitate transmission to others or may lead to disease in the colonized individual during a disruptive situation (e.g., normal flora out-of-balance from antimicrobial treatment, invasive device, or wound
    - *N. gonorrhoeae* - pharynx, *Salmonella* spp. in faeces
    - MRSA – nares
    - Yeast such as *C. albicans* in genital tract)
- Infection
  - Symptomatic – e.g., meningitis, osteomyelitis, septicemia, endocarditis
  - Asymptomatic – latent TB

Breakdown of the Microbiome

Respiratory Tract
- *Fusobacterium spp*
- *Neisseria spp.*
- *Staphylococcus spp.*
- *Micrococcus spp.*
- *Haemophilus spp.*
- *Veillonella spp.*



Usually sterile -Larynx, trachea, bronchi, bronchioles, alveoli

Skin
- *Staphylococcus spp.*
- *Propionibacterium spp.*
- *Corynebacterium spp.*

External genitalia and Vagina
- *Staphylococcus spp.*
- *Enterococci*
- *Streptococcus spp. (Gp B strep)*
- *Lactobacillus spp.*
- *Viridans streptococci*

GI Tract
- *Enterobacteriaceae*
- *Bacteroides spp.*
- *Clostridium spp.*
- *Enterococcus spp.*

(From Noskin GA, ed. Management of Infectious Complications in Cancer Patients. Boston: Kluwer Academic; 1998.)

Microbiology 101

Specimen Collection

What makes a good specimen?

- Blood culture – 1/3 growing CNS?
- Sputum culture - +++ squamous epithelial cells +++ normal respiratory tract flora?
- Swab abdominal incision – No wbc ++ *E. coli* + *Ps. aeruginosa*?
- Pressure ulcer – +/- wbc ++ normal skin flora

Proper Specimen Collection

- Improperly selected, collected, or transported specimens can generate misleading data resulting in inappropriate patient management
  - Deep cough quality sputa vs. oral secretions vs. saliva
- Collected aseptically and placed in a sterile container
- Specimens may be placed directly into culture media (e.g., blood cultures, genital cultures)
- Special handling of anaerobes
- Prompt delivery to the laboratory is essential to prevent the death of pathogenic organisms and/or the overgrowth of commensal organisms
- Delayed transport, some specimens may be refrigerated (e.g., urine, stool, sputum), whereas others should be maintained at room temperature

Microbiology 101

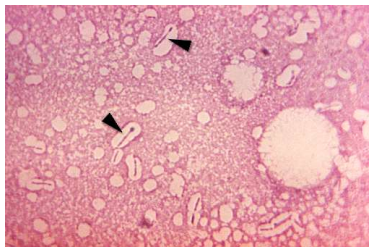
Diagnostic Tests

Microorganisms Identification - Diagnostic Tests

- Definitive diagnosis of infectious diseases or processes
  - Determination of the presence of an infectious agent
  - Detecting in clinical specimens those specific product(s) resulting from a specific infectious agent (e.g., *Clostridioides difficile* toxin in feces)
  - Detecting an immunological response (Ag-Ab reaction) specific to the infecting agent in the patient's serum (e.g., HBsAb)
  - Detecting the presence of an infectious agent through nucleic acid hybridization and amplification techniques
  - Requires accuracy (sensitivity and specificity) and precision (repeatability)
    - Sensitivity = TP / #TP + FN
    - Specificity = #TN / #TN + FP

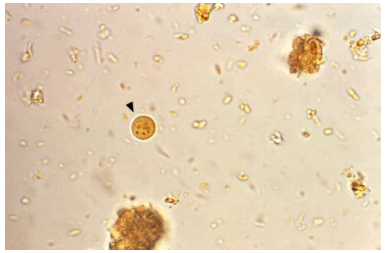
Microorganisms Identification - Diagnostic Tests

- Microbiological - Direct Examination
  - Gram Stain – distinguish Gram positive from Gram negative bacteria
    - Cell shape and arrangement
  - Histology (study of the microscopic structure of tissues) /Cytology – study of the formation, structure, and function of cells
    - gross and microscopic evaluation of a specimen or tissue by a qualified pathologist
    - useful in the diagnosis of actinomycosis, chlamydia, cytomegalovirus, genital herpes, giardiasis, histoplasmosis, leprosy (Hansen's disease), lymphogranuloma venereum, and rubeola
    - Special fixing or staining techniques may be used
    - Histology - diagnosing infections with agents that are difficult or impossible to culture



Histology – spore stain – *C. ramosum*

  - Wet Mount – fresh clinical specimen
    - sputum viewed for fungal elements; stool examined for larvae, adult worms, ovum, cysts, or parasites; cerebral spinal fluid for *Cryptococcus neoformans*; vaginal secretions for *T. vaginalis*; and urine sediment for WBC, bacteria, yeast, and parasites (e.g., T. vaginalis)
    - 10 percent potassium hydroxide (KOH), Lugol's iodine, and simple stains (e.g., calcofluor-white, India ink, Loeffler's methylene blue) are often added to the wet mount to increase visibility of the infectious agent or to highlight cellular features of suspected infectious agents.



Iodine stained wet mount - *E. nana* amoeba

Public Health Image Library (PHIL): Accessed 02/05/2019

Microorganisms Identification - Diagnostic Tests

- Serology
  - Antigen detection – focus
    - Proteins or polysaccharides
    - Infectious agents, tumor cells, transplanted tissue
    - Agglutination, immunofluorescence, ELISA
    - Clinical specimen – serum, body fluids, etc.
    - adenovirus, bacterial meningitis (*Haemophilus influenzae, Streptococcus pneumoniae, Neisseria meningitidis*, group B streptococcus), *Brucella spp., Cryptococcus spp*., etc.
  - Antibody – Immunoglobulins
    - Facilitate the body's removal of the agent
    - Circulate in the blood, secretions, or lymphatic fluid
    - Five classes
    - Reported qualitatively (positive or negative) or quantitatively (titers)
    - Positive antibody titer does not necessarily indicate active infection but may represent a previous infection
    - Agglutination tests, complement fixation, indirect immunofluorescent-antibody (IFA), immunoblot, indirect hemagglutination (IHA), bentonite flocculation (BF), and radioimmunoassay (RIA)
    - Serum is the specimen typically tested
    - Adenovirus, chlamydia group, coxsackievirus, *Cryptococcus spp.*, cytomegalovirus, Echinococcus, encephalitis viruses, etc

Microorganisms Identification – Molecular Testing

- Nonamplified nucleic acid probes
  - Nucleic acid (NA) probes (i.e., segments of DNA or RNA)
  - Labeled with reporter molecules (i.e., radioisotopes, enzymes, or chemiluminescent)
  - Probes bind to complementary NA sequences
  - Used to identify pathogens directly from specimen samples or culture isolates
  - Detection of gp A streptococcal pharyngitis; *N.* gonorrhoeae and *C. trachomatis* genital tract infections; and organisms associated with vaginitis including *Gardnerella vaginalis, Trichomonas vaginalis, and Candida albicans*
  - Most common application - culture confirmation of slow-growing mycobacteria and dimorphic fungi
- Target amplification methods
  - PCR, transcription-based amplification, and strand displacement amplification
  - use enzyme synthesis to create copies of the target nucleic acid
  - NA - labeled with a chemiluminescent marker, a radioactive marker, or an enzyme marker that can then be quantified
- Probe Amplification Methods
  - ligase amplification reaction, cycling probe technology, cleavase-invader technology
  - enzyme produces a ligated product that serves as a template for amplification
  - labeled with fluorogenic substrate that can be quantified.
- Signal Amplification Methods
  - signal amplification methods (i.e., branched DNA and hybrid capture assay
  - concentration of the target NA is not increased
  - based on increasing the concentration of label molecules attached to the NA
  - reduced false-positive results from cross contamination because the number of target molecules is not altered
  - Signal is directly proportional to the amount of target sequence present in the original specimen

Microorganisms Identification - Microscopy

- Microscopy – direct exam of clinical and culture materials
  - Light
    - Compound – 2 types of lens (ocular and objective)
    - Scanning lenses – Low power (10X); high-power (40X); oil immersion (100X)
  - Bright Field – most common
    - Commonly used for Gram stains
  - Dark field microscopy
    - Fresh material / motile organisms that cannot be stained by common methods
    - *Treponema pallidum* (syphilis)
    - *Borrelia burgdorferi*
  - Phase Contrast Direct observation of unstained material
    - Used for tissue culture for viral isolation and identification
  - Fluorescent
    - Uses UV light source
    - Ease of interpretation of stained materials
    - Coupled with antibodies rapid diagnosis
      - Direct (DFA) or indirect (IFA)
  - Electron
    - Uses electron beam of short wavelength to increase magnification
    - Gold or palladium stains used
    - Viruses and large molecules
    - Transmission electron microscopy (TEM)
    - Scanning electron microscopy (SEM)

Bacterial Culture / Growth

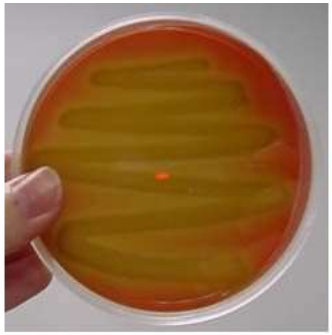
Blood agar plate - Hemolysis

Google Images



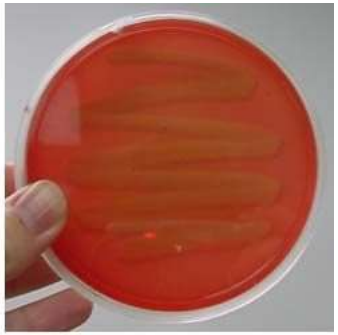
Beta Hemolysis

Strep pyogenes



Alpha Hemolysis

Viridans strep



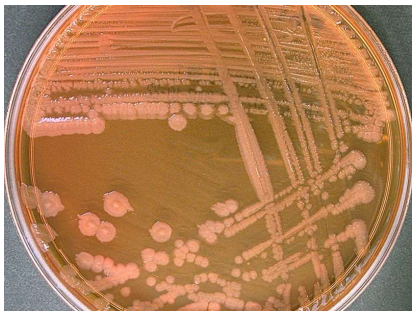
Gamma Hemolysis

Staph epid

Selective / Differential Agar



MRSA on mannitol salt agar
Public Health Image Library (PHIL)



Proteus mirabilis (swarming motility) – non lactose fermenting
Google Images: Accessed 02/04/2019)

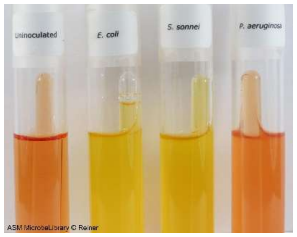
Selective / Differential Agar



E. coli on MacConkey agar – Lactose fermenting



Salmonella spp., Proteus spp., Yersinia sp, Pseudomonas aeruginosa, and *Shigella spp.*



Escherichia coli and Shigella sonnei are Members of the Enterobacteriaceae family and ferment glucose to acid

Bacterial Culture

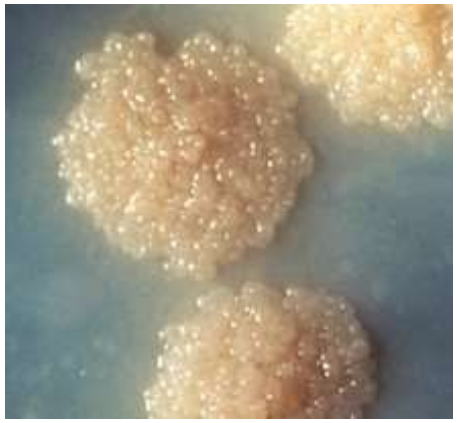
- Specimen streaked onto agar plates
- Incubated in moist environment at the proper temperature to allow growth (may be mixed or pure)
- Media used depends on site being cultured and growth requirements of the pathogen
  - Nutrient (blood agar), enrichment (chocolate agar), selective (MacConkey), differential agar
  - Incubation atmosphere – aerobic / anaerobic / microaerophilic (increased CO2)
  - Incubation temperature – body (37°C); thermophilic (>45°C); mesophilic (20°C-45°C)

Bacterial Culture / Growth

Google Images: Accessed 02/05/2019



Sabourad agar growing yeast



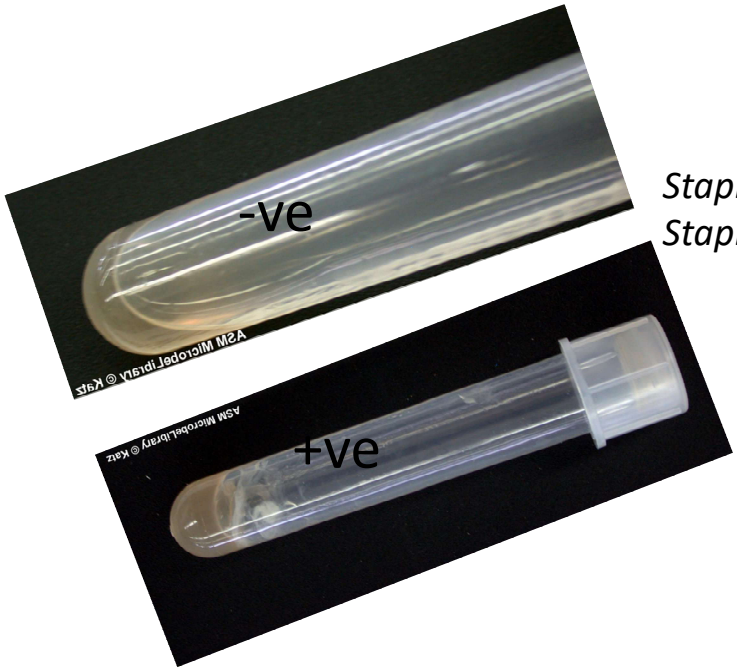
M. tb. grown on Lowenstein- Jensen agar
Incubation – 4-6 weeks



Bacillus spp. on blood agar plate

http://www.asmscience.org/content/education/imagegallery/

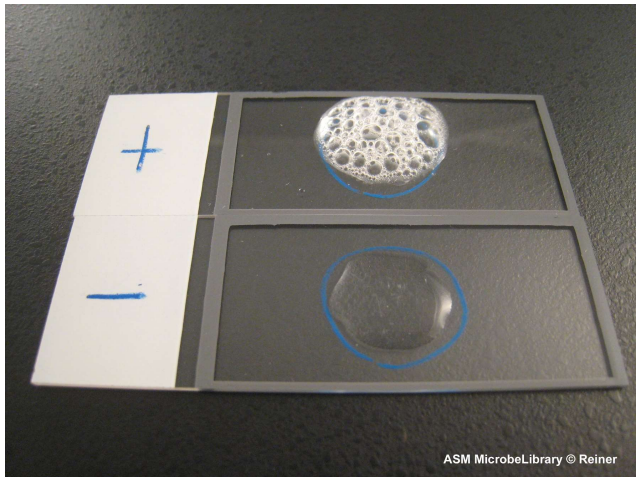
Coagulase Test



Staph aureus – positive
Staph epid - negative

A positive tube coagulase test result for *Staph aureus* after a 24-hour incubation in coagulase plasma. A positive result is indicated by clotting in the tilted tube.

Catalase Test



Slide catalase test results. Hydrogen peroxide was added directly to the culture on a microscope slide. Positive reaction - bubbling produced by *Staph aureus*;
Negative reaction – no bubbling - *Strep pyogenes*

http://www.asmscience.org/content/education/imagegallery/

Microorganisms Identification - Diagnostic Tests

- Virus Identification
  - 3 methods
    - Direct detection
      - Electron microscopy
      - ELISA
      - Latex agglutination
      - DNA probes
      - PCR
      - Optical immunoassay
      - Light microscopy
      - Pap smear
      - Negri bodies

- Virus Identification
  - Serological testing - Antibody assay
    - Presence or absence of immunoglobulin (IgM - early, IgG - late)
    - VZV, adenovirus
    - Non-culturable organisms (HBV); immune status (rubella, measles, varicella)
  - Viral culture
    - Specialized media
    - Cell culture – organ, tissue, cell

Cerebrospinal Fluid Analysis - Findings in Cerebrospinal Fluid Analysis for Meningitis

Component	Adult	Neonate	Bacterial Infection	Viral Infection	Fungal Infection
Color/clarity	Clear/colorless		Cloudy	Clear/hazy	Clear/hazy
Protein (mg/dL)	15 to 45	115 to 170	↑	Normal to ↑	Normal to ↑
Glucose (mg/dL)	50 to 80	60 percent of plasma value	↓	Normal to ↓	Normal to ↓
WBC count					
Agglutinating capacity	0 to 5	0 to 30	↑	Normal to ↑	↑
WBC differential					
Lymphocytes	62 to 34	20 to 18	↓	Normal to ↑	Normal to ↑
Monocytes	36 to 20	72 to 22	↓	Normal to ↑	Normal to ↑
Neutrophils	2 to 5	3 to 5	↑	Normal to ↓	Normal to ↓
Eosinophils	Rare	Rare	Rare	Rare	Rare

Fluid Analysis - Urine

- Used as a screening test
- Total urinalysis - assessment of color and clarity; presence of proteins, glucose, ketones, blood, nitrite, and leukocyte esterase
- Microscopic - presence of RBCs, WBCs, casts, crystals, bacteria, or yeast
- Dipsticks - presence of leukocyte esterase (LE)
  - positive LE - leukocytes (WBCs) or debris from ruptured WBCs are present in the urine
  - True infection - leukocytes (>10 WBC/mm3of unspun urine or >5 WBC/hpf of spun urine) - pyuria
  - Very high association between infection and pyuria - helpful in differentiating between colonization and true infection in the presence of bacteriuria
- Nitrite in the urine - used as a screening tool for the presence of infection
  - positive nitrite test - screen for the presence of bacteria in the urine because many bacteria produce an enzyme that can reduce urinary nitrates to nitrites
  - If nitrites are present in the urine, it may indicate that bacteria are present
- Microscopic evaluation - specimen examined for the presence of bacteria or yeast

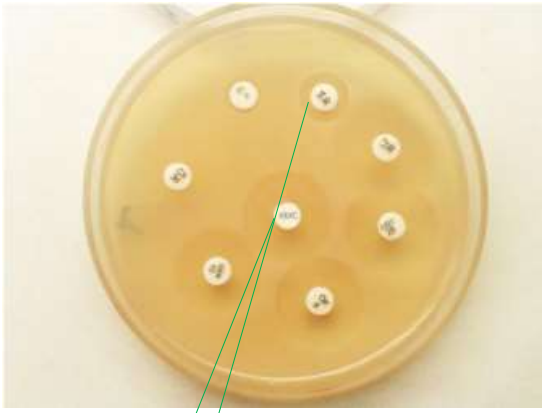
Microbiology 101

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing

- Antibiotics – bacteriostatic/bactericidal
- Type of testing performed depends on the organism, source of culture, available antimicrobial agent and susceptibility pattern
- Test Methods
  - Disc diffusion – Kirby-Bauer
    - Standardized suspension of bacteria forms lawn on agar
    - Paper discs impregnated with standard amount of antibiotic is placed on the plate and incubated
    - Antibiotic diffuses from the disc and inhibits growth
    - Results – susceptible / intermediate / resistant
  - Broth Dilution
    - Determines the least amount of antibiotic to inhibit growth of organism – MIC
    - Incubated overnight and interpreted
    - Newer automated systems , photometric, provide results in 3-10 hours
- E-Test
  - Used when resistance is clinically important
  - Limited to penicillin, ampicillin and vancomycin
  - Standardized suspension spread on agar to form lawn
  - One or more non-porous plastic strips impregnated with serial dilution of select antimicrobial agent, marked with MIC reading scale
  - MIC is where the growth inhibition intersects the strip
- Other Tests
  - Beta-lactamase (e.g., *H. influenza, N. gonorrhoeae*)
  - Disc Approximation – D Test
    - Inducible resistance in *Staph spp.* to clindamycin
  - Synergy testing
    - Combination of antibiotics
  - Hodge test
    - Beta lactamase testing in Gram negative (Enterobacteriaceae - *K. pneumoniae, E. coli*)

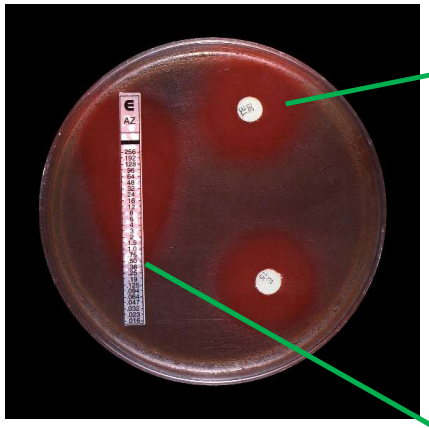
Antimicrobial Susceptibility Testing



Kirby-Bauer - Mueller-Hinton agar with antibiotic discs

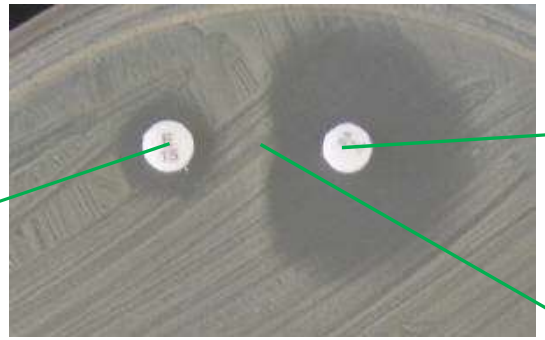
Zones of inhibition

Google Images: Accessed 02/05/2019



E-test and disc diffusion

Public Health Image Library (PHIL): Accessed 02/05/2019



D test – positive

Google Image: Accessed 02/05/2019



Modified Hodge Test – bacteria resistant to carbapenem, clover leaf pattern

Public Health Image Library (PHIL): Accessed 02/05/2019

Mechanism of resistance – Beta lactam

- Enzyme alters antibiotic
- Target altered
- Decreased uptake

Summary

- Microbiology is the study of bacteria, fungi (molds and yeasts), protozoa, viruses, and algae
- Microorganisms come in various sizes, shapes and configurations
- Multiple staining and culture methods available to identify microorganisms
- Improperly selected, collected, or transported specimens can generate misleading data resulting in inappropriate patient management
- Diagnostic tests range from Gram stains, cultures, serology, and amplification methods
- Antimicrobial susceptibility testing is provides relevant information to determine patient treatment options

Thank you!



Q&A

Q: What anaerobic Gram negative bacteria would you expect to grow in a perirectal abscess?

A: *Bacteroides fragilis*

Q: What organism would you use to monitor the steam sterilization process? Ethylene oxide sterilization process?

A: Steam – *Geobacillus stearothermophilus;* EO – *Bacillus atrophaeus*

Q: Sputum sample grows a black fuzzy mold on differential agar. What is it?

A. Aspergillus niger

Q. What organism would grow best in IV fluid containing dextrose?

A. Enterobacter spp.

Q. Common cause of meningitis in neonates? Adults?

A. Gp B strep; Strep pneumoniae

Q. How do you swab a pressure ulcer?

A. Culture the aspirate

Q. Which population of patients are you most likely to isolate Aspergillus fumigatus? Cause or Why?
A. Bone marrow, transplant; Construction

Q. Which organism is likely to have resistance to 3rd generation cephalosporin due to ESBL isolated?

A. K. pneumoniae

Q. Patient has a secondary bacteremia with Enterococcus faecalis, what is the source?

A. Pyelonephritis, GI

Q. Patient admitted with community acquired pneumonia, Gram stain shows +++ G+C in chains with +/- normal flora. What is the likely pathogen?

A. Streptococcus pneumoniae