Principles of Diagnostic microbio:
Isolation of pure culture from specimen: microorg exist as mixed cultures hence difficult to study.
- **must isolate** the different species **to determine species i.e diagnose infection, quantify species (**amount of microorg) **and characterize it:** (determine proteins/properties/nutrients). allow us to **make vaccines based on characteristics.** allow us to **compare with others of same species to determine mutations**, done by culturing: inoculating and isolating (growing and differentiating)
> **Isolation involves inoculating** the substance: the **transferring and growing of small sample of microorg** ("inoculum: what is being grown") on surface of which we can study it: **must keep alive** to study it, hence **need nutrients called culture media** which **allows growth and isolation of microorg**. type of media used is based on 3 factors:
- **source of sample tested** (from blood, or urine, or skin)
- **species assumed** to be in sample (intracellular diff procedure than noncellular mircoorg)
- **nutritional requirement** of suspect species (aerobic vs. Anarobic (need oxygen), thermostable vs. Thermolabile (heat sensitivity))
> I**solation involves separating microorg from rest of culture / differentiating done by:**
- **streak plate method**: most common to isolate organism from clinical specimen. take a tiny bit of specimen **make streak**, sterilize whatever used to pick up specimen, use to streak same streak, spreading and **diluting allowing isolated colonies to form** - hopefully have the right media, put this into incubator. take out, hopefully grown. study isolated colony: one bacteria that grew and divide to make identical cells. (from there can make diagnostic).
- **spread plates method**: take **sample prepare dilutions:** in each dilution spread with stick one spread will give isolated dilution that **can count amount of bacteria.**
- **pour plate method**: not often, take sample put in middle petri dish **add molten agar mix** and incubate. - isolated colon is embedded in agar. - if melted agar too hot can kill bacteria.
> **Isolation involves Incubation**: once inoculated must be incubated at apropriate temp: must store: norm in freezers that go 37degree allowing best conditions for growth and multiplication. when enough mutliplaction colonies form which re visible to naked eye. - diff species have diff colonial morphologies can be used to distinguish species.
> **Isolation involves Preservation of pure cultures:** want to keep copy to study its characteristics, study it to make vaccine against it, and to compare it to other/new strains of species determining mutations.
- **short-term preservation by storing at appropriate temp**, typically **refrigeration temp (4-10 degree).**
- **longterm preservation: by freezing in liquid nitrogen**, **freezing in special freezer (-80)**, or **lyophilization: (freeze drying** using vacuum, sucking out moisture turning into powder, stable for storege at room temp).
> **once isolated pure culture,** can study culture and isolated microorg/cell for **identification: 2 approaches:**
- **colonial morphology**: study colony based on form (shape), elevation (height), margin (spread/space takes up)
- cellular morphology: study cell. size of most microorg is nanometers so **need magnification i.e need microscope.** microscope magnification **limited by resolving power i.e resolution: ability to distinguish 2 closely located object as separate entities**, **determined by wavelength of light and lenses.**
- **Light microscopy**: lenses used to manipulate the path of light between species and eye.
- **electron microscopy**: beam of electrons controlled by magnetic fields, replacing light source of light microscope. allow for greater resolving power (magnification). 2 approaches:
- **transmission electron microscopy**: stain whole specimen or slicing of microorg into thin sections with heavy metal, allow to see inside.
- **scanning electron microscopy**: election bean moves back and forth, generates 3D image of cell surface which is coated by metal.
- **Flourescne microsopy**: allows reveal/ illuminate only objects of interest, **by specific dye which fluoresces at specific wavelength** visualized using light microscope. **Immuneoflourences** is common and important, antibody produced in blood after exposure to antigen, antibody and antigen bind forming complex. **Flouresent dye attracted to and attaches to antibody "tags antibody" so can easily detect antigen/microorg**.
* i**mportance of studying detailed morphology** of microorg: - **determines if gram-pos or gram-neg which allows determine antibiotics** - **determine absence or presence and characteristics of cell structures aiding classification** - **determines how cells respond/behave in environment** which tells us how to treat patient, diagnosis culprit. ex. Capsules make microorg more pathogenic.

- to view structures/cells of colony easier/isolate specific under microscope must strain:
1) **make smear:** touch colony, put on microscope glass slide, smear, let dry
2) **heat fix:** pass glass slide over flame, allow some proteins to denature allows species to bind to glass

slide. important because going to wash if doesn't bind to glass then wash it off.

3) **stain** with one or more dyes

- **Simple stain:** single dye used, general dye, colours all organisms same colour. allows observation of size, shape, number and arrangement of cells.
- **Differential stain**: 2 or more dyes used. special dyes. allow observe differences between microorg cells or parts of cells. Ex. **acid fast stain:** differential acid fast bacteria from nonacid fast bacteria, based on colour picks up ex. **gram stain:** differentiate bacteria as either gram + or gram - based on colour pick up. - Other staining include: - **endospore staining:** dye applied with heat to penetrate spores followed by contour straining dye. - **capsule staining:** treat with chemical before dying to visualize capfuls as clear zone surrounding cells - **flagella staining:** use mordant to thicken flagella before staining to visualize.

* **Gram stain:** named after Hans Christian Gram: gram stain **has to do with cell wall of bacterium, difference in staining determined by cell wall structures** (cell wall: rigid, gives shape, which is determined by peptidoglycan, essential for growth and division
- **is either gram neg or gram postivie**

- **Gram-pos: have thick cell wall made of peptidoglycan layer. - one cell wall.** - teichoric acids attached to peptidoglycan give neg charge to help transport positive ions into cell. - **produce/liberate EXOotoxin. - alcohol shrinks pores of gram-pos so doesn't take up saferin when stained = appear purple !!**

- Gram-neg: more complex - **thin cell wall made of peptidoglycan, covered by outter membrane anchored to peptidoglycan. Lipopolysaccharide located on outer membrane. Outer memb selective barrier** based on size and charge of molecule. - **small space between 2 membranes**. - **have endotoxins** (liberating not secreting, when cell wall destroyed endotoxin exposed and excreted) which binds to second cell wall
- **alcohol increases permeability so take up sapherin when stained appear pink!!**

Steps to gram staining:
1) flood slide with crystal violent, wash with water
2) **flood with iodine,** wash with water: **cause forming of complex that binds to peptidelycan layer**
3) **add ethanol at right time:** solubilize peptidoglycan wash away layer
4) **add saferin (pink)**, wash with water: **binds to complex** washed away.
5) air dry and put slide in micro: **if looks purple gram pos because dont take up sapherin**, **if pink gram neg because take up sapherin.** so important because depending if gram + or neg certain set of antibiotics to give to patients.

Bacteria: - small - higher surface area (used for more efficient means of nutrient entry - high metabolism - fast growth and reproduction
bacteria hang in groups in certain fashion. how bacteria look in micro: stuck togehter/ individual / move certain way, tell you what the bacteria is.
NOT IMPORTANT > - specific patterns/shapes: 3 basic shapes: some can change shape as grow called pleiomorphic

- spherical cells called coccus
- cylindrical or rod shaped called bacillus
- spiral or helical shape called spirllum: corkscrew shape

- individual bacteria arranged in specific patterns used for idefication:
  - spiral shaped / rod shaped bacteria
  - coccus (spherical) divide in one plane = diciccus (pairs
  - coccus divide in one plane but remain attach to form chain: streptococcual
  - when coccus divide at right angle to first plane of vision = tetrads
  - coccus divide in third plane =cubical packet pop 8 cells called sarcinae

NOT IMPORTANT> - division in 3 plants in irregular pattern = grape like clusters = staphylococcus

size, shape and arrangement of bacteria is referred to as gross morphology.

To isolate microorg need specific media:

- **chemically defined media: control/ know exact composition**/ every ingredient of media, allow alter individual component separately.
- **chemically undefined media: cant control**/ cant know exact composition / ingredient of media, is natural product (blood/urine) added to media for routine lab cultivation: **blood agar plate**: cant control amount blood cells / whats in them, always diff.
- **1.5% agar when need solid support.**

- **“enrichment media”: used to increase # of organism in sample by favouring growth of the interested species. want to grow as fast as can so can identify it to determine what is damaging host.**

- **media for growing bacteria: requirements varies between different bacteria. - demanding nutritional requirements called fastidous, require complex undefined media for cultivation. test with substrate plate: gives identity of bacteria.**

- **growing yeast:** all fungi and heterotrophs require organic substance to get carbon. **higher sugar context, lower pH.**

- **growing anaerobes**: careful to study because as soon as removed from body and exposed to oxygen destroyed, to study must remove oxygen, grown deep in agar in test tubes.

- **selective media: designed to enhance growth of one kind of organism suppress growth of another kind. - something in media that slow growth of one organism and kill other**

- **differential media: differentiate organisms based on unusual nutritional requirements and characters appearance in media. something in media that allows me to tell how bacteria differ / both.**

- **selective differential media: useful in public health microbe, practical 2 answer for price of one: ex.** selective differential agar for girls in class, has chemical inhibit growth of guys (selective) and differential allows to look at only girls and distinguish them. **MacConkey media: inhibit gram positive growth, allowing only gram negative.** gram neg grow also tell differences between gram neg or type of gram neg. (use lactose or not) selective (only want one gram) AND differential: (tell which gram present).

- tissue culture media: used to cultivate viruses done in vitro (in plant or animal cells grown the has been grown in specialized media) since virus can only replicate inside living host cells.

urine test: look for presence of bacteria on dip stick. “bacteriuria": bacteria in urine.

- media test tubes: take colony from plate stab agar, look at ability of organism to react to media. without oxygen.

- tray: test 20 substrate , depending on how colony use or dont use substrate give idnety of bacteria.

- disk diffusion test: put colony on agar plate, add one or multiple antibiotic on top which diffuse out of disc onto agar, incubate, remove look and see if zone of clearance: meaning antibiotic killed organism, no zone of clearance means that antibiotic to not useful against bacteria, can measure zone of clearance to determine organisms sensitivity or resistance to antibiotic. takes 3 days.

- bacteria phage: take colony make line, isntead of antibiotic disc use bacterial phage (virus that can infect bacteria only nothing to human cells), either destroy bacteria or doesnt. Important because addresses bacterias resistance without antibiotic trails.

4 essential conditions for successful cultivation: temp, O2,

<u>temperature:</u>

- hope have right agar plate for believed bacteria to grow. must put into incubator because has right temp for growth called optimum growth temperature: microorg grow over wide temperature range called cardinal temperatures (3):
- minimum temp
- optimum temp - optimum temp usually closer to max temp since enzyme activity increases with temp until limit where enzyme is degraded.
- maximum temp.
- cardinal temp changes depending on nutritional context of growth medium.

Organisms divided into 3 groups based on optimum growth temperature:

- psychrophiles: grow at 15-20oC (found in COLD areas/water), may die if exposed to room temperature. Problem of food because put food in fridge to prevent/kill bacteria except this bacteria can still survive. ONLY PATHOGEN NOT MESOPHILE = Lysteria can grow and survive at refrigeration temp, in food cause no change in colour/ taste or odor, but make sick, high fatality rate, kills unborn baby. not easy to avoid because widespread in environment. Resistant. Economical impact= leads to throwing out food.

- mesophiles: most bacteria and almost all that cause human disease. Grow best between 25 to 40oC, best at 37, which corresponds to body temp.

- Thermoohiles: can grow from 40 to 85oC but grow best between 50 to 60oC. not prob for humans because bod should never get hot. / extreme thermophiles 160 degrees. mostly prokaryotes because eucaryotes cant grow above 60. in volcanic areas/hot springs. - all super hot had to work with.

<u>Gaseous atmosphere / i.e oxygen levels:</u> bacteria require O2 (aerobic) or no O2 (anaerobic).

- obligate aerobics: need O2 for reactions/survival, can be grown on surface plate.
- obligate anaerobe: dont need O2 for reactions, prefer NO oxygen, range of oxygen tolerance, cant survive i air atmosphere. toxicity of oxygen due to production of superoxide radical. - grown in aerobic jars/chamber/glove box/ candle jar to protect against oxygen.
- facultative: can survive in air atmosphere. - facultative aerobes: prefer O2 but can survive without O2. - aerotolerant anaerobes: dont need O2 for reactions, prefer NO oxygen, but can survive with it.
- microaerophiles: SPECIFIC/EXACT amount of O2, too much/too little kills too.

<u>pH</u>
- optimal pH diff for various organism.
- regardless of external Ph microrog must maintain intracellular pH.
- most bacteria grow at min pH of 4, max of 9. Optimum 6 and 8.
- molds and yeast broader pH range. optimum is 5 to 6.
- growing cells release acid and alkaline waste into growth medium without buffering medium can Inhibit growth.

<u>osmotic pressure (water coming into cell): liquid media</u>
- if liquid media is isotonic solution: water in and out of microorg at same rate by osmosis, allow normal growth. if hypotonic solution: less solute in media than bacteria, draw water into bacteria, burst/lyse cell. if hypertonic: too much solute in media than bacteria, draw water out of bacteria, shrink and die.
- if liquid media is NOT isotonic, bacteria wont grow, hence believe that infection is a virus. may have been bacteria but since messed up tonicity of solution bacteria killed.

2 types of test for antibodies: USING ELISA: enzyme linked immune source antibody:
1) detect pathogen/ antigen i.e nitrogen using direct ELISA
2) detect immune response if human made antibody to pathogen using indirect ELISA
want to detect antigen use direct ELISA: use dish, put antibodies specific for antigen, pass blood over antibodies, if antigen in blood will bind. Wash. add secondary antibody which is same as first but is tagged with enzyme. if antigen not present secondary antibody wont join, washed off, if antigen present will bind. add colourless solution, if enzyme is bound then will pick up colour, if no colour antibody didn't bind to antigen. intensity of colour tells amount of antigens per mL of blood. - if do direct ELISA tests neg, may think no antigen or may think body created immune response against antibodies. to test immune response use indirect ELISA. instead of putting antibodys, but antigen in dish add blood which contains antibodies. if antibodies in blood will bind to antigen. wash. add secondary antibody tagged with enzyme which recognizes antigen. add colourless, if colour made then antibody in blood. amount of colour tells amount of antibodies. - Indirect ELISA also tells type of antibody made. ?????????

Gram stain: tells colour which due to cell wall thickness/permeability made of peptidoglycan. Prescribe antibiotics based on if gram pos or gram neg: dangerous to mess up

- **Gram-pos: one thick cell wall of peptidoglycan layer** - **produce/liberate EXOtoxin. - purple**

Gram positive COCCI (single spherical bacteria)
- **STAPHS: STAPHYLOCOCCI** (grape like, multiple, division in many planes, spherical): 2 kinds: staphylococcus aureus, epidermis, all pathogenic staphylococci produce coagulase, non pathogenic dont.

1) **<u>Staphylococcus aureus</u>:**
- normal flora in hands, nose, throat, genitals, anal but pathogenic when gets internal. Hospital acquired alley nosocomial infections because infected surgical wounds
- <u>exotoxins: (5)</u>
1) cytotoxins: toxic to bmany cells and WBC (cyto think circulatory)
2) Haemolysins: affect skin
3) Enterotoxin: exotoxins act in GItract cause food poisoning - are super-antigens activate 20% Tcell population activate = bad! Dont want immune system that active
4) Exfoliative toxins: cause skin to come off look burnt
5) Toxic shock symdrome toxin: cause toxic shock associated with tampons (absorbs blood but also bacteria) but guys can get it too.
<u>- enzymes:</u>
- coagulase: fibrin: forms blood clot
- beta-lactamase (penicillinase): destroys penicillin

other enzymes: hyalronidase, staphylokinase.

location: found in nose, throat, anus, genitals. - most resisitant against penicilin.

- cause variety of infections: - **pustule**: acne, **boils:** affects hair follicles, **stye**: in eye lids, if get to eye lead to **conjunctivitisis** = **pink eye**. - also cause **pneumonia, septicaemia in immunocompromised**. -**food poisoning, toxic smoke syndrome, scalded skin, IMPETIGO:** contagious skin infection

treated by penicillin: but many in norm pop and 85-90% in hospitals are penicillin resistant**.**

- lysed by number of diff viruses (bacteriophages, used as identity markers.

- prevention: wound precaution, education, handwashing.

2) **Staphylococcus epidermis**:

- **normal flora of skin, non pathogenic but opportunistic:** when break skin can cause disease from hospitals: nosocomial: break in skin from surgery: Endocarditis, shunt infections.

Gram positive COCCI

- **STREP: STREPTOCOCCI** (chain, multiple, one place, spherical):

- divided into groups based on **hemolytic properties**: ability to destroy/lyse RBC: alpha-hemolysis: partial RBC destruction which allow oxidation of hemoglobin = change colour, beta-hemolysis: complete RBC destruction. (gamma = cant destroy/lyse RBC)

- carbohydrate C antigen: from cell wall

- M protein on cell wall: prevents compliment. preventing WBC from phagocytess

3 types of steprtox cocci:

1) streptococcus pyogenes: “group A” strep = beta hemolytic

- found in normal flora. Transmitted by contact, nasal carriers, food.

- acute tonsillitis: strep throat: - untreated strep can lead to rheumatic heart disease, because almost causes autoimmune response because strep has antigen on cell wall which is similar to antigen on pericardium, can mistaken antigen and send antibodies to attack heart.

- skin infections: skin rashes **IMPETIGO, Flesh eating disease = necroticous fascinates - due new toxin:** take plasminogen from blood attach to cell wall change into enzyme which acts on something and with hyaleronidase **can spread.** - bacteriophage: gives ability to avoid WBC and make new toxin.

- puerile fever (sepsis), septicaemia (bacteraemia)

exotoxins:

- streptolysins and beta-hemolytic cells: useful in fighting WBC

- streptococcal pyrogenic exotoxins: super-antigens causes scarlet fever

enzymes:

- coagulase: hyaleronidase: found in CT, by destroying CT allows bacteria to spread.

treatment: - almost all resistant to penicillin

prevention: - carefully in procedures because puerperal fever aka doctors plague: doctors didn't clean hands.

- BACTERIOPHAGE: VIRUS THAT INFECT ONLY BACTERIA not human cell. - specific bacteria -instead of giving antibiotics give bacteriophage which can destroy pathogen, do nothing to human - attach to bacteria inject nucleic acid, often killing off bacteria, sometimes bacteriophage gives DNA to bacteria: gives ability to make toxin (can make scarlet fever, or nectroticous fascinates.

2) streptococcus agalactiae: “group B” strep

- found in normal flora of vagina of healthy women but can cause neonatal infection: delayed septicaemia: respitery distress.

- cause early septicaemia = respiratory distress or shock, high fatality,neurologic/menal abilities.

3) streptococcus pneumoniae: pneumoccos: seen as diplococci. (2 cocci, spherical) - has polysaccharide capsule useful in preventing immune cells from attacking, avoid cell mediated immune response and humoral (antibodies) * looks same as neisseria meningitis only way to tell difference is gram stain (neisseria will be pink, strep will be purple)

- found is nasal. Passed by respiratory

- causing pneumonia (lungs) and sometimes meningitis.

- prevention aimed at infancy, elders and alcoholics (lower immune system), crowded living condition (more host)

- Gram-neg: thin cell wall of peptidoglycan, and outter membrane. - have endotoxins - pink

Gram negative Cocci: (spherical bacteria)
1) Neisseria meningitides: diplococci (2 cocci, spherical) with polysaccharide capsule useful in preventing immune cells from attacking, avoid cell medicated immunity and humeral (antibodies) * looks same as strep pneumonia only way to tell difference is gram stain (neisseria will be purple, strep will be pink)

- normal flora of nose. but also respiratory pathogen - only affects humans (crowded place or lower immune system(children, seniors)
- isolated in lab by “chocolate agar” or heated blood which is specific for neisseria used when isolating from nose.
- multiple antigens which are targets for vaccines.
- starts as skin rash can get inside causing bacteremia/ septicaemia (fever, rash)can cause water house - friderichsen syndrome which is most extreme septicaemia (more severe than menigitides) - also cause meningitides (infection of CNS)
- menigitides: looks like bruises, bed bugs, rash, allergic reaction.
- treatment: with penicillin

prevention: vaccine as young as possible, using a conjugated vaccine (because know common antigens and know on polysaccharide capsule BUT not easily recognized by Thelper cells. Instead conjugate antigens to something that is recognized by Thelper turning on immune system and made into vaccines.

2) Neisseria gonorrhoeae: diplococci (2 cocci, spherical)

- sexually transmitted, found in genitals
- grown in damp environment with CO2, sensitive to drying and changes in temperature. must mimic genital atmosphere. - smear discharge
- causes gonorrhoea. - STD not STI because can leave genitals: go somewhere else. - can cause neonatal infections in birth. give newborns antibiotic into eyes. - in US second highest, highest is chlamydia. treated the same way.
- neonatal infections: in eyes (from birth) - disseminated gonococcal infection: cause joint problems, fever, skin infections
- symptoms = puss. - men: infection of uerthra - women: 50% asymptomatic (no puss/ burning) if untreated can cause cervicitis, pelvic inflame disease, infection in fallopian, sterile. (more things can go wrong for women than man).
- testing: men swab uretha. - women: culture.
- treatment: penicillin, may be resistant to antibiotics, both partners need to be treated.

prevention: condoms, no vaccine available.