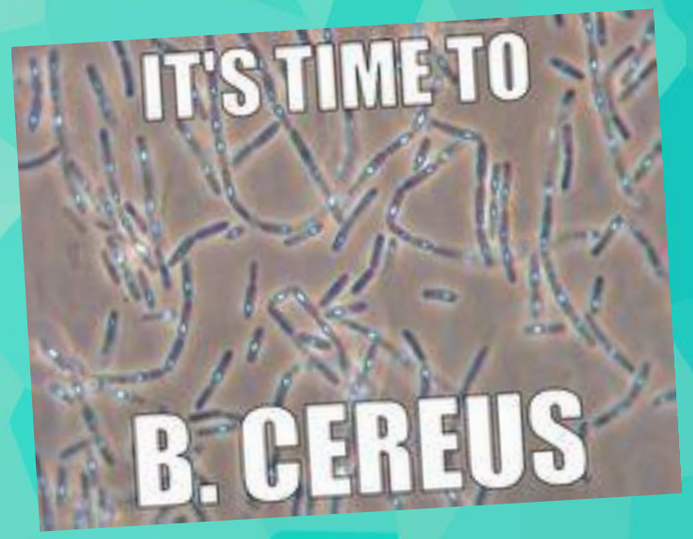
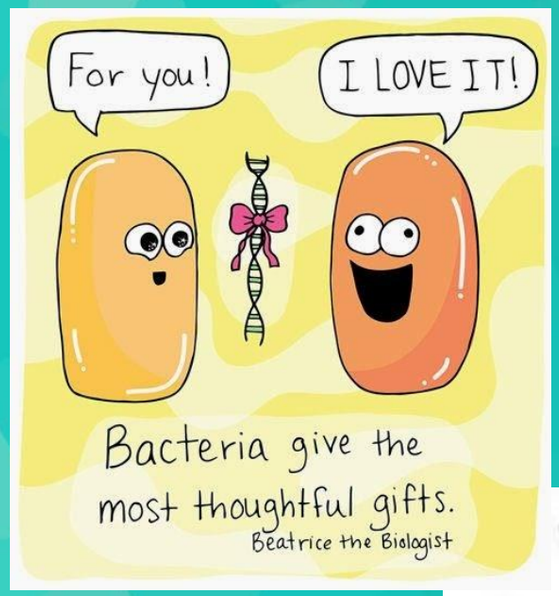
Microbiology Revision

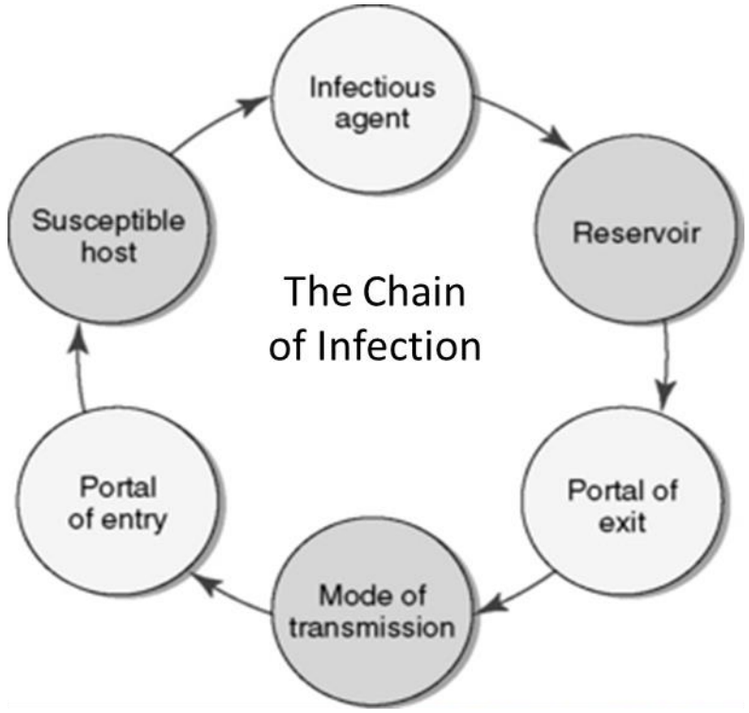
By Emily Beswick (Phase 2b)





Objectives of Session

We will be covering bacteria, mycobacteria, fungi, viruses and protozoa.



- A few examples but don't have time to fully cover pathogenesis of infections unfortunately.
- Cover each microorganism and related clinical/laboratory topics.
- Mock questions at the end

Bonus slides at the end (not presented unless time!). If you want to stay the end I can talk more about these slides on:

- Tips how to study Micro
- Tips on antibiotic choice
- Resources for studying Micro
- Worms
- Clinical tables of bacteria with antibiotics

Microbiological Terms to Know

Pathogen – any type of microorganism that causes disease.

Colonisation – the microorganism can be cultured from the host but it is not causing disease. Also referred to as a "commensal" or "asymptomatic carriage".

Infection – the microorganism can be cultured from the host and it is causing disease.

Opportunistic pathogen/infection – either term is used to refer to microorganisms that only cause disease when the host defence is somehow compromised.

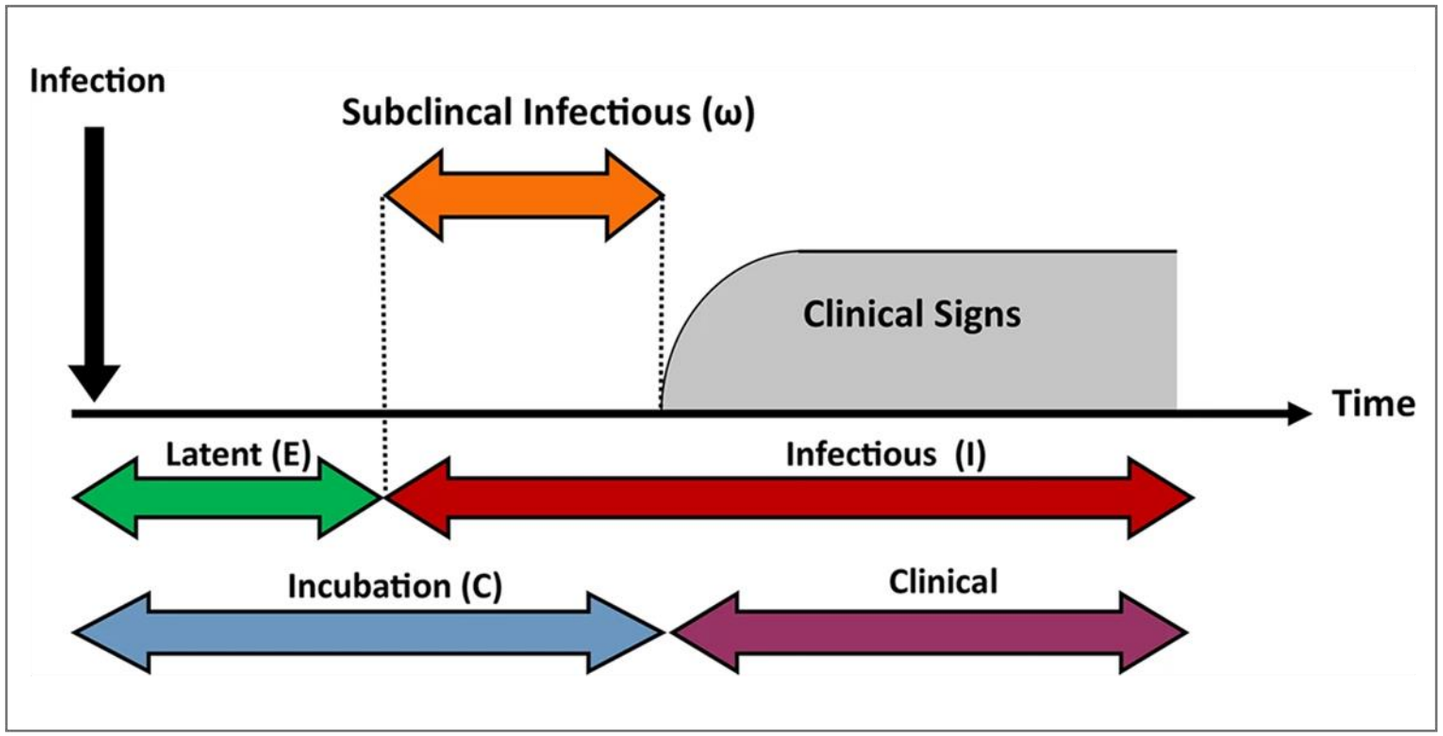
Virulence/Pathogenicity - the degree to which a given microorganism is pathogenic

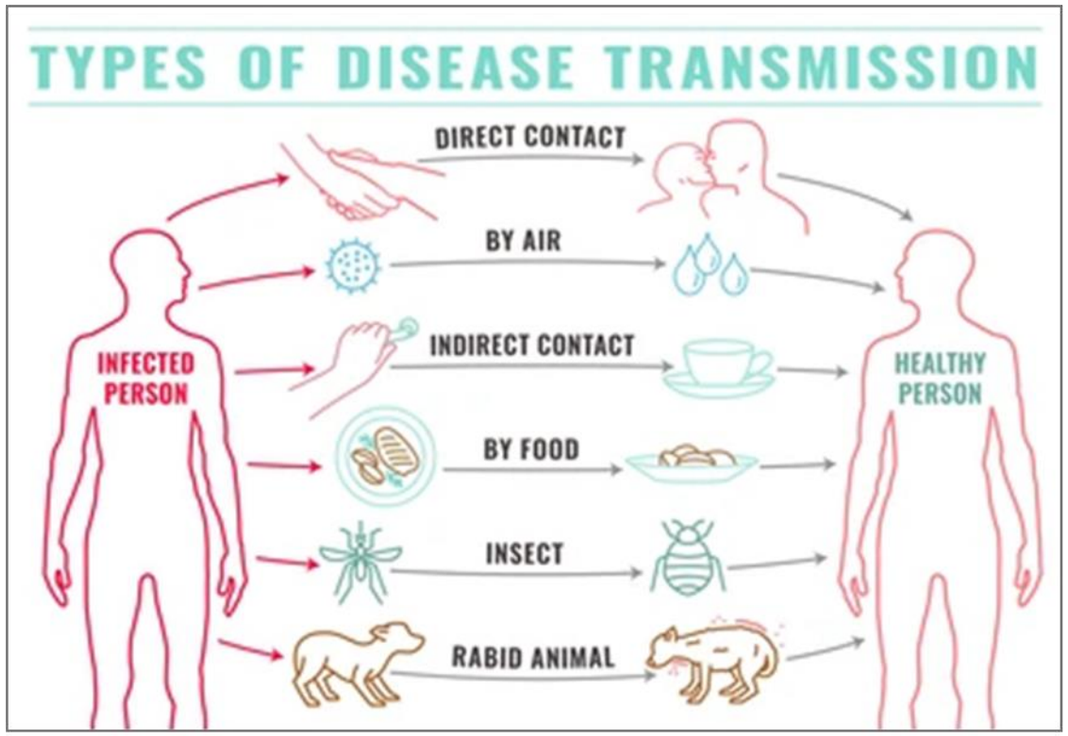
Which patients might get opportunistic infections?

Those on immunosuppressant drugs e.g. transplant patients, rheumatoid, IBD etc..

Those with immunocompromise e.g.
HIV/AIDS
genetic immunodeficiency e.g. IgA deficiency
Premature babies
Pregnant women
Chemotherapy

Those with breakdown of host defence
Skin e.g. Intravenous drugs users, eczema
Lungs e.g. Cystic fibrosis, TB, COPD



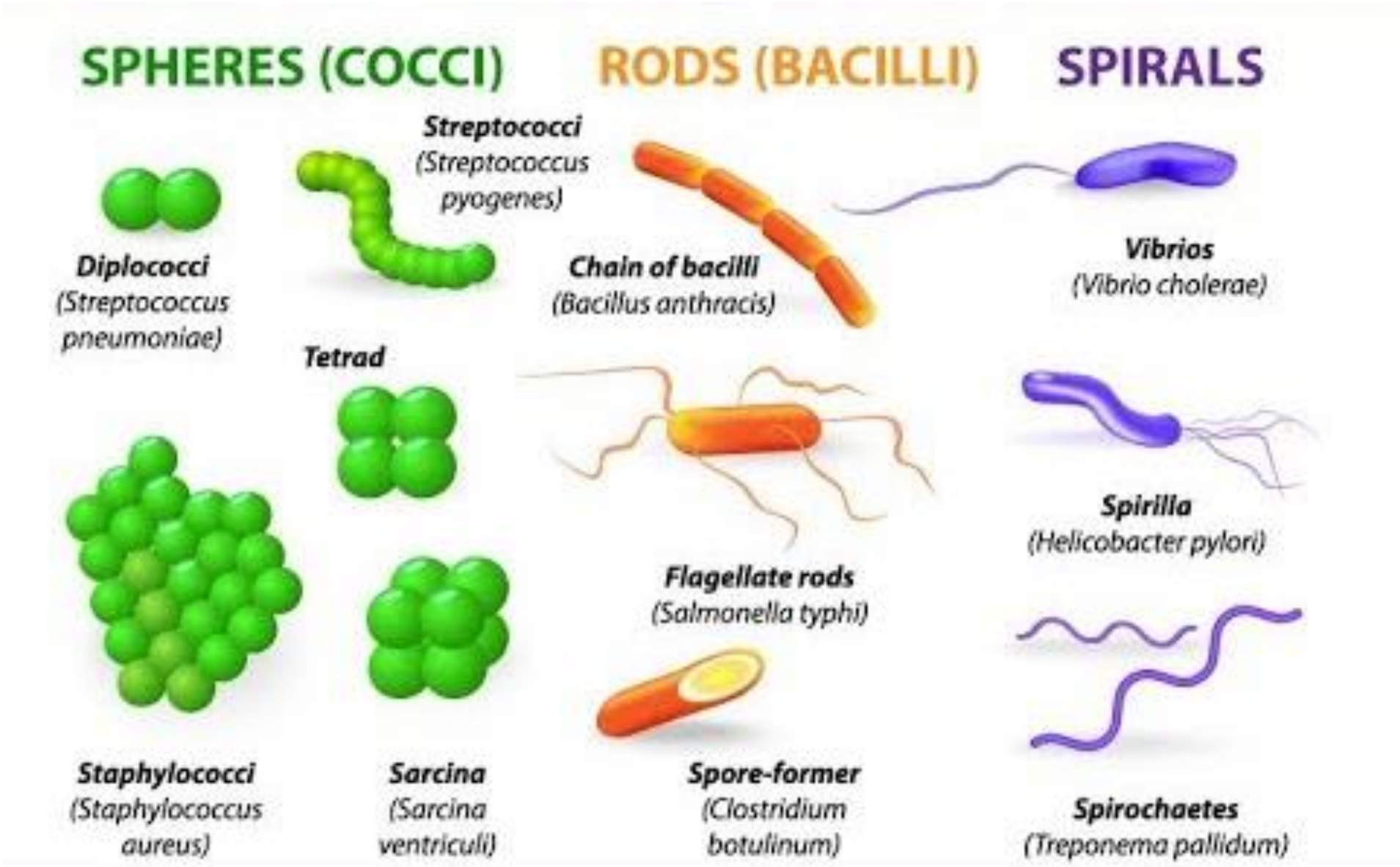


BACTERIA

Bacterial Nomenclature and Microscopic Morphology

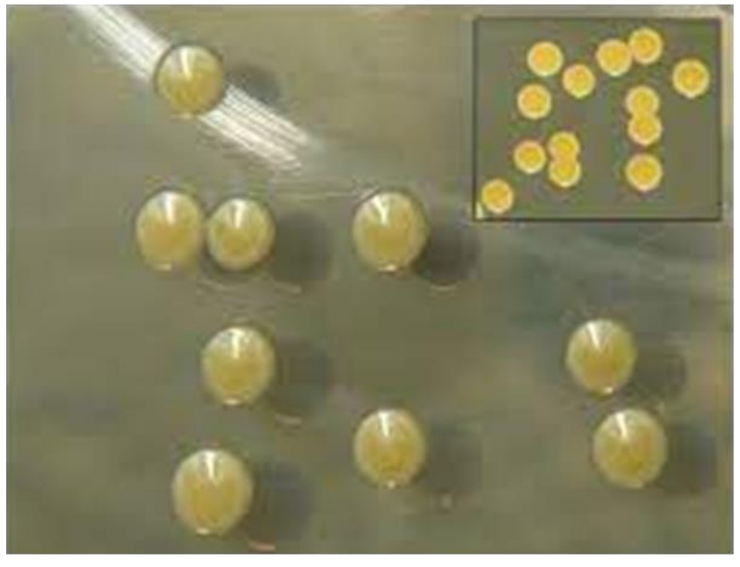
Nomenclature: *Staphylococcus* **(genus)** *aureus* **(species)**

***Name commonly reflects shape.**



Colonial Morphology

Staph aureus – gold and round

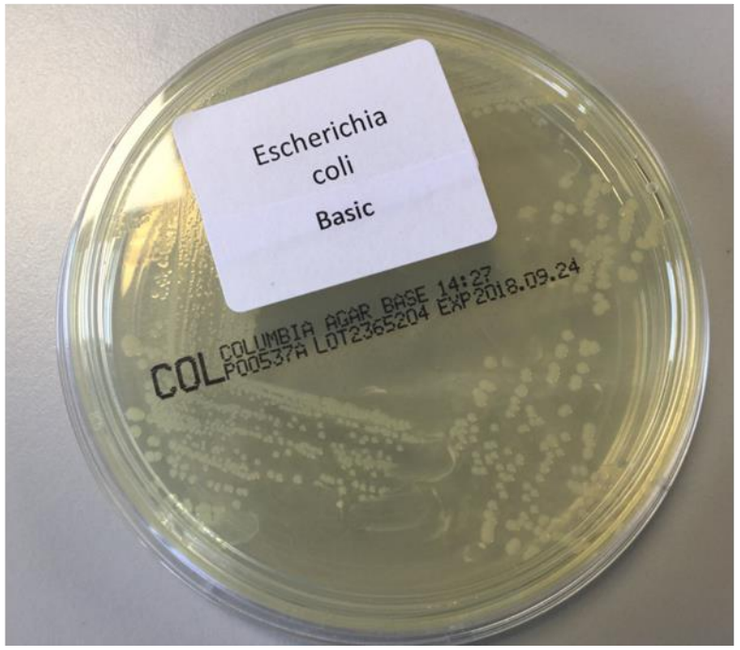


Staph epi and sapro – white and round

Strep pyo – white, round, beta haem

Pseud aeru – green, irregular, mucoid, smells like swimming pool

E. Coli – greyish, round, faecal smell



Neisseria – greyish, round, only grows on chocolate agar

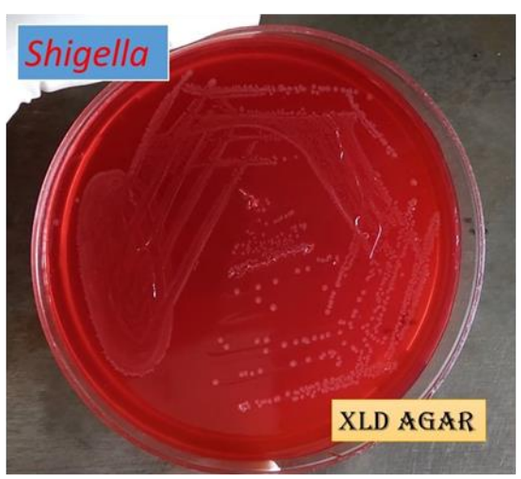
How do we grow bacteria?

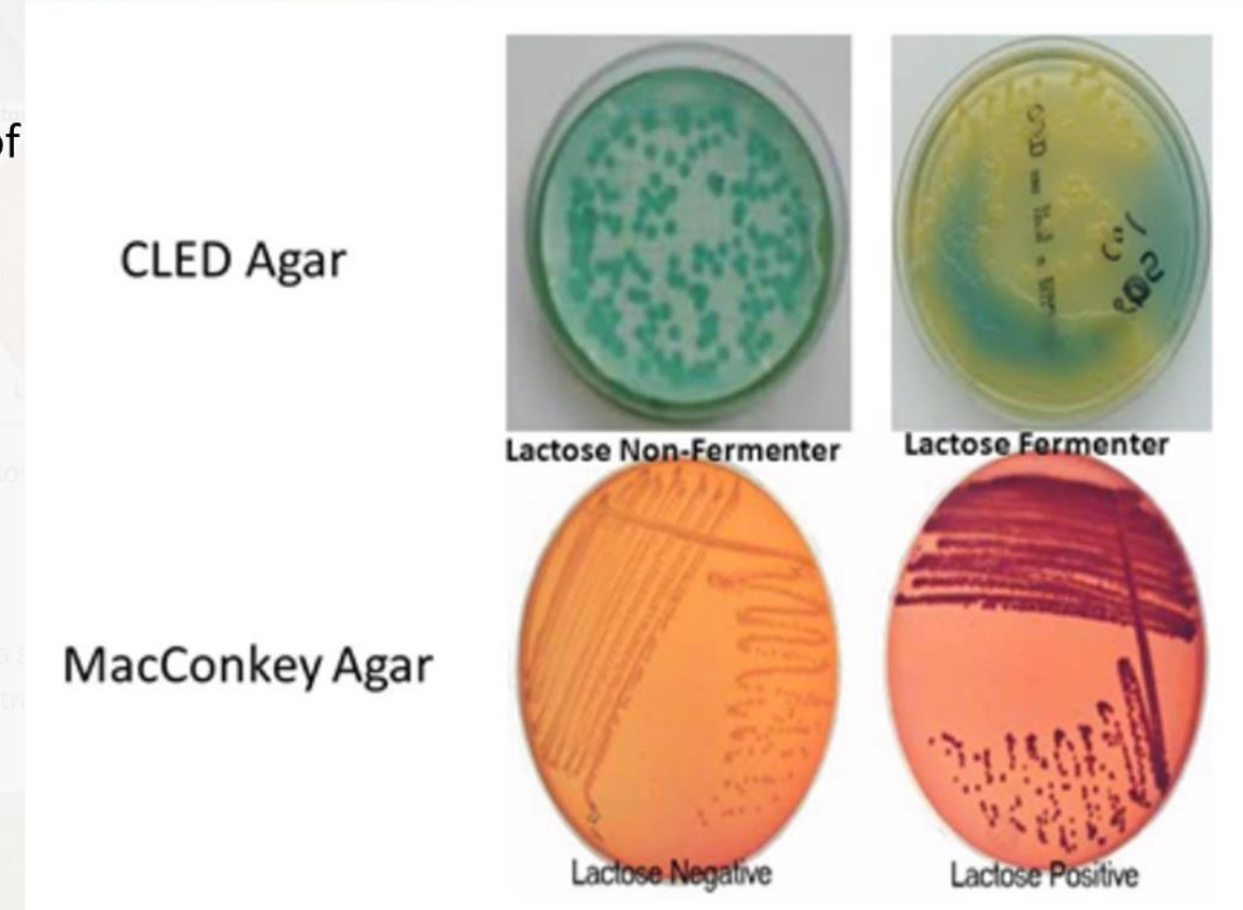
- Solid media = agar plate, agar slope
- OR Liquid media = test tubes

Can be selective or non-selective.

2a need-to-know agars:

1. **Blood** – for Streptococcus and others
2. **Chocolate** – (cooked blood) for fastidious Neisseria
3. **MacConkey** – for lactose status
4. CLED –stops motile Proteus swarming and also for lactose status
5. **Xylose Lysine Deoxycholate agar (XLD agar)** is a selective growth medium used in the isolation of Salmonella and Shigella species from clinical samples and from food. Salmonella and Shigella both ferment the lactose in XLD and go red, but Salmonella then also turns black dot due to hydrogen sulfide production.



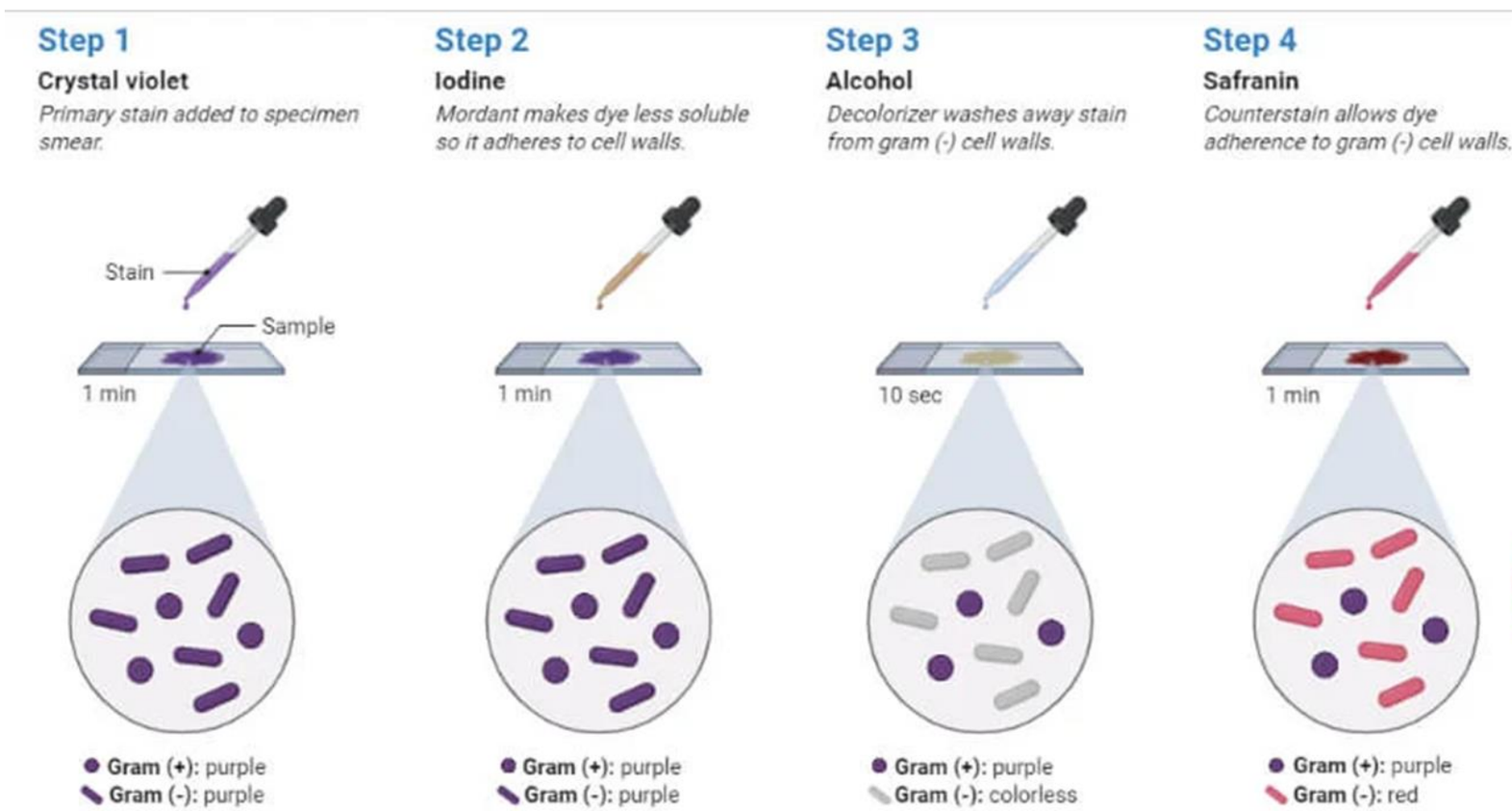


Why is bacterial structure important? = The Gram Stain

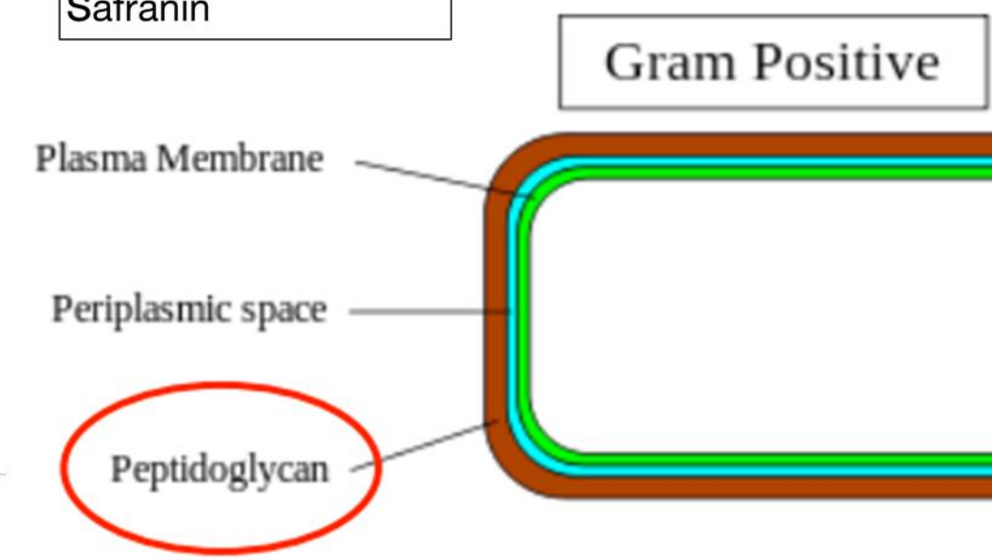
The peptidoglycan layer soaks up crystal violet, if it is thin then it will be decolourised by washing with alcohol (Gram-negative). If it is thick then the Gram stain cannot be washed away and will remain (Gram-positive).

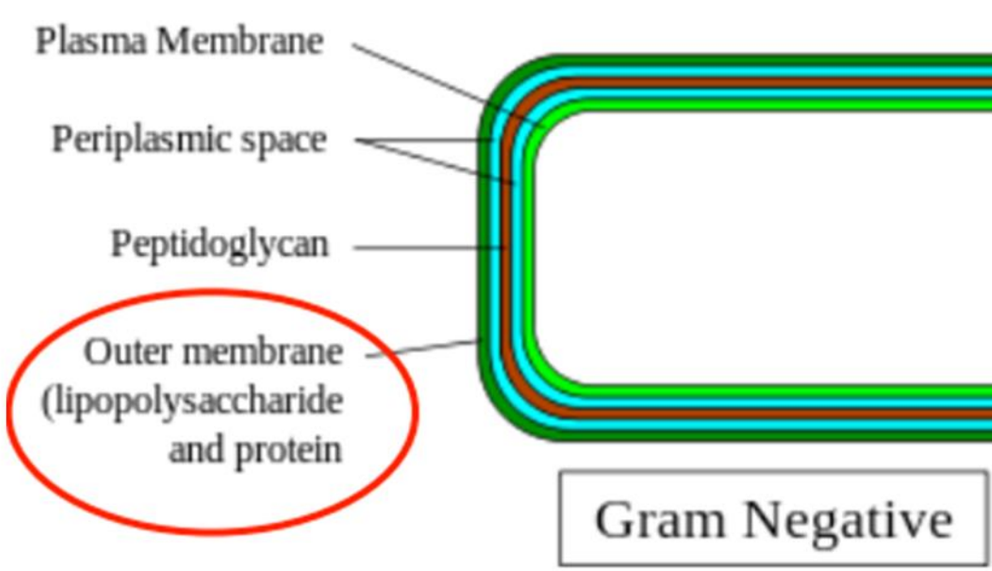
Gram + = purple, prefer dry and dusty environments: they are great skin colonisers and can be spread by breathing in shed skin scales.

Gram - = pink, prefer wet and damp environments: majority prefer to colonise mucous membranes.



Mnemonic:
"Come In and Stain"
Crystal violet
Iodine
Alcohol
Safranin

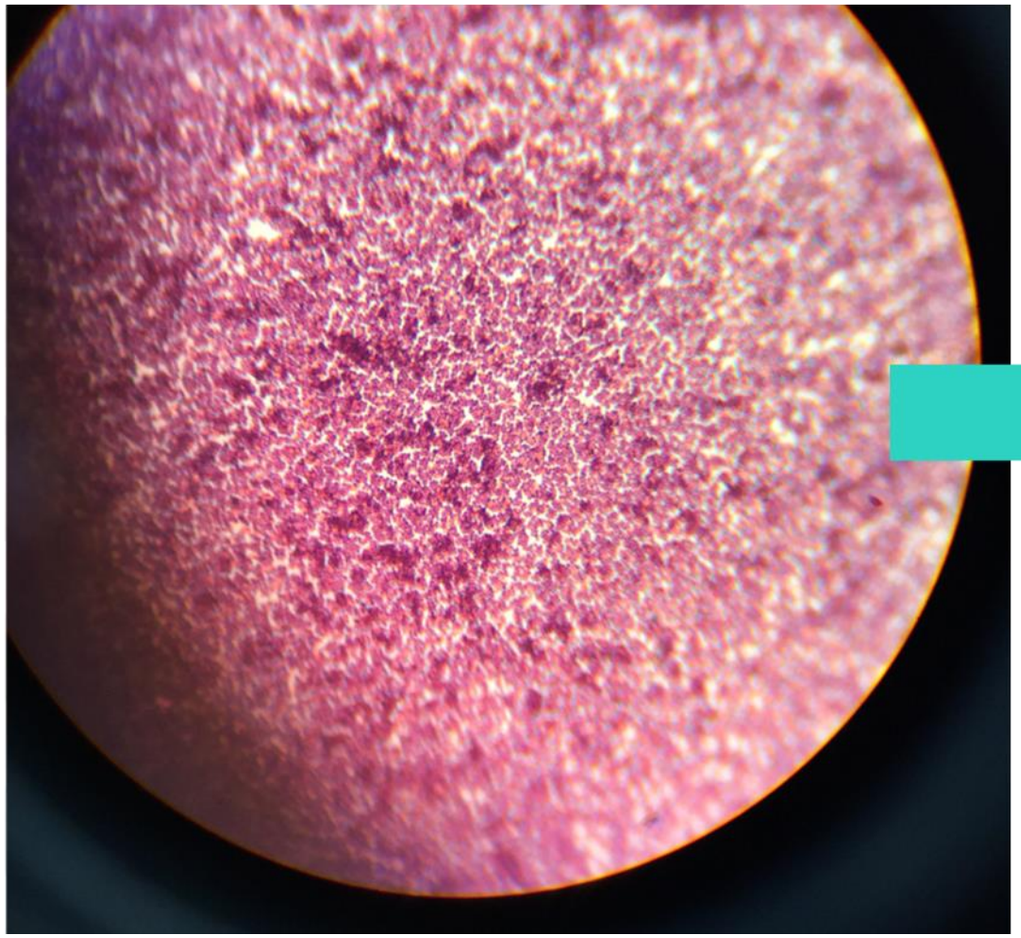




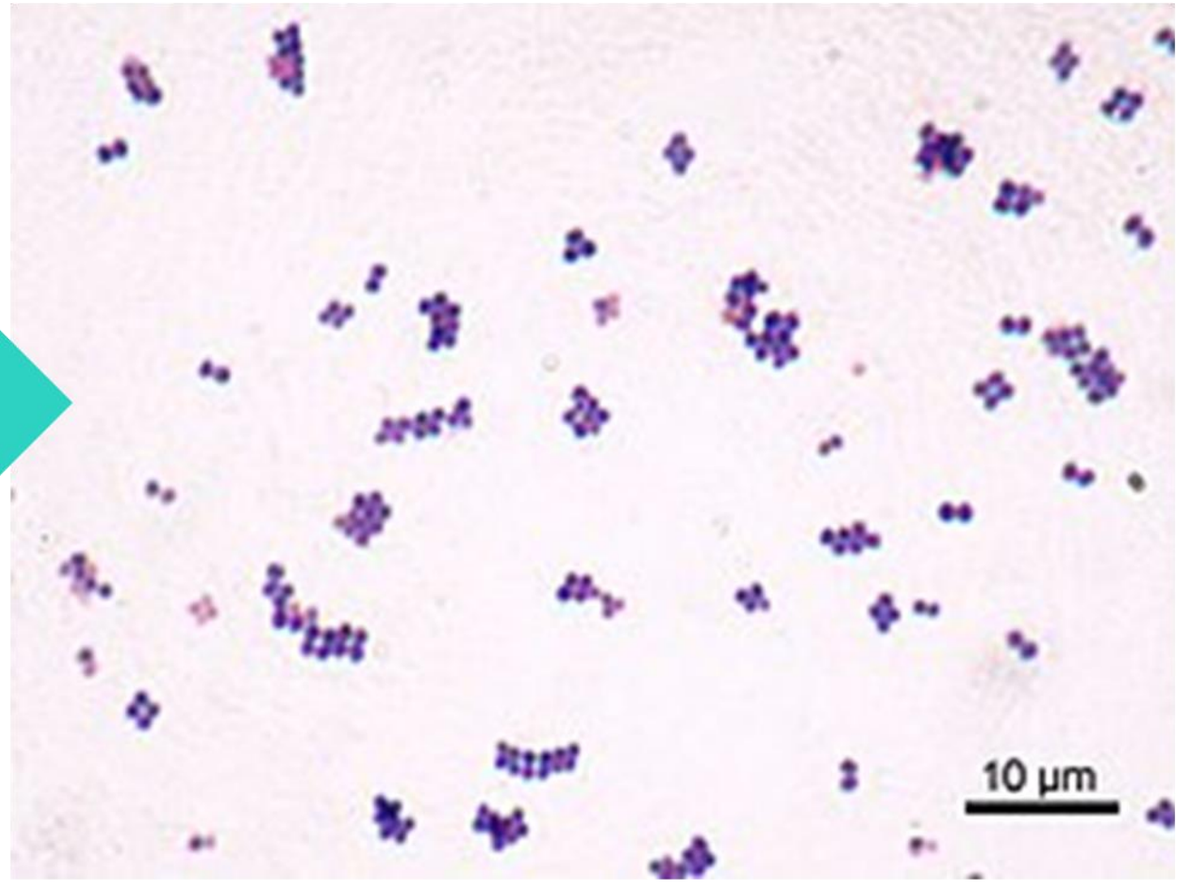
Gram Stain examples

Staphlycoccus aureus - Gram positive cocci, bunch of grapes

My first stain: way too thick!

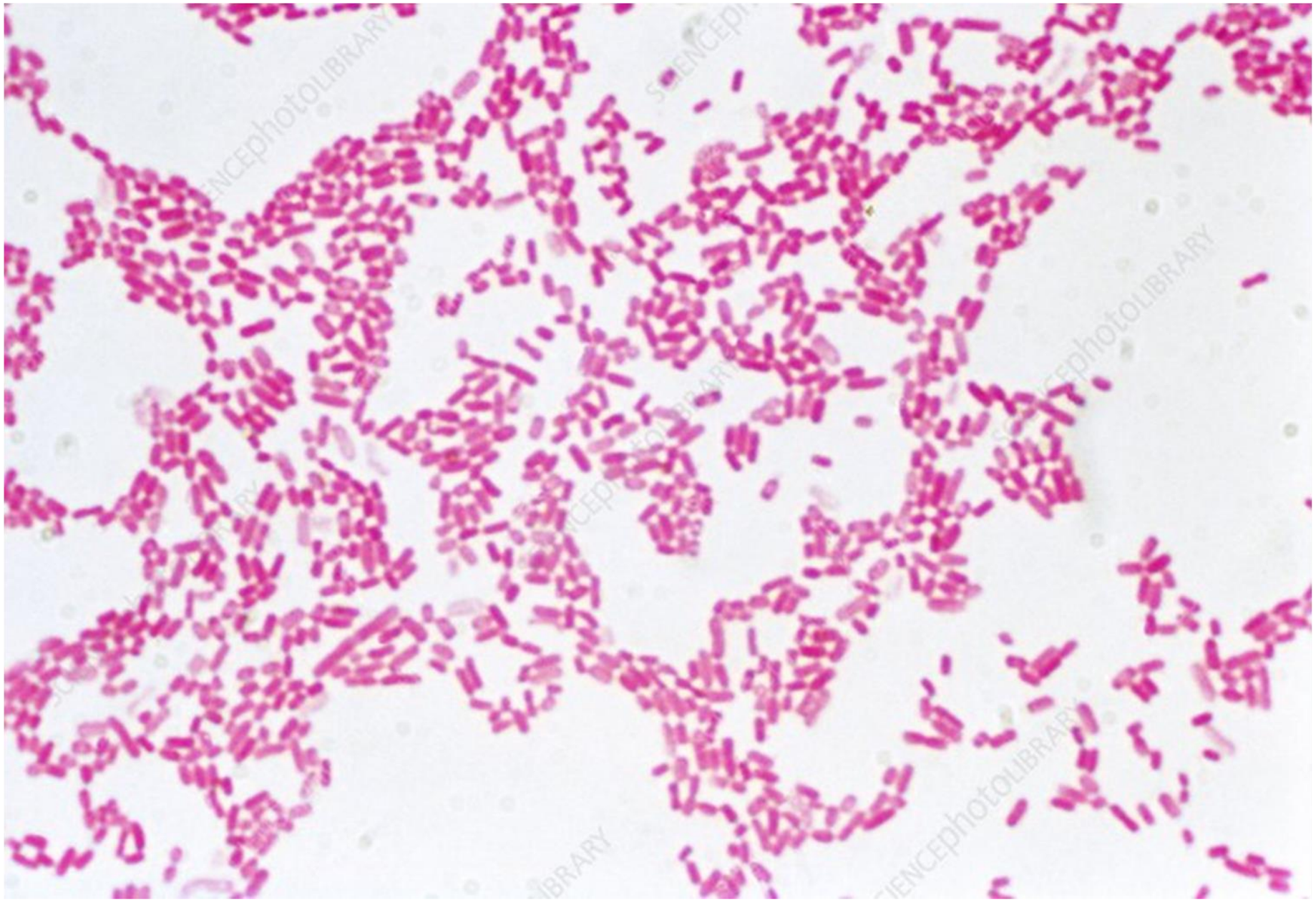


proper stain ↓



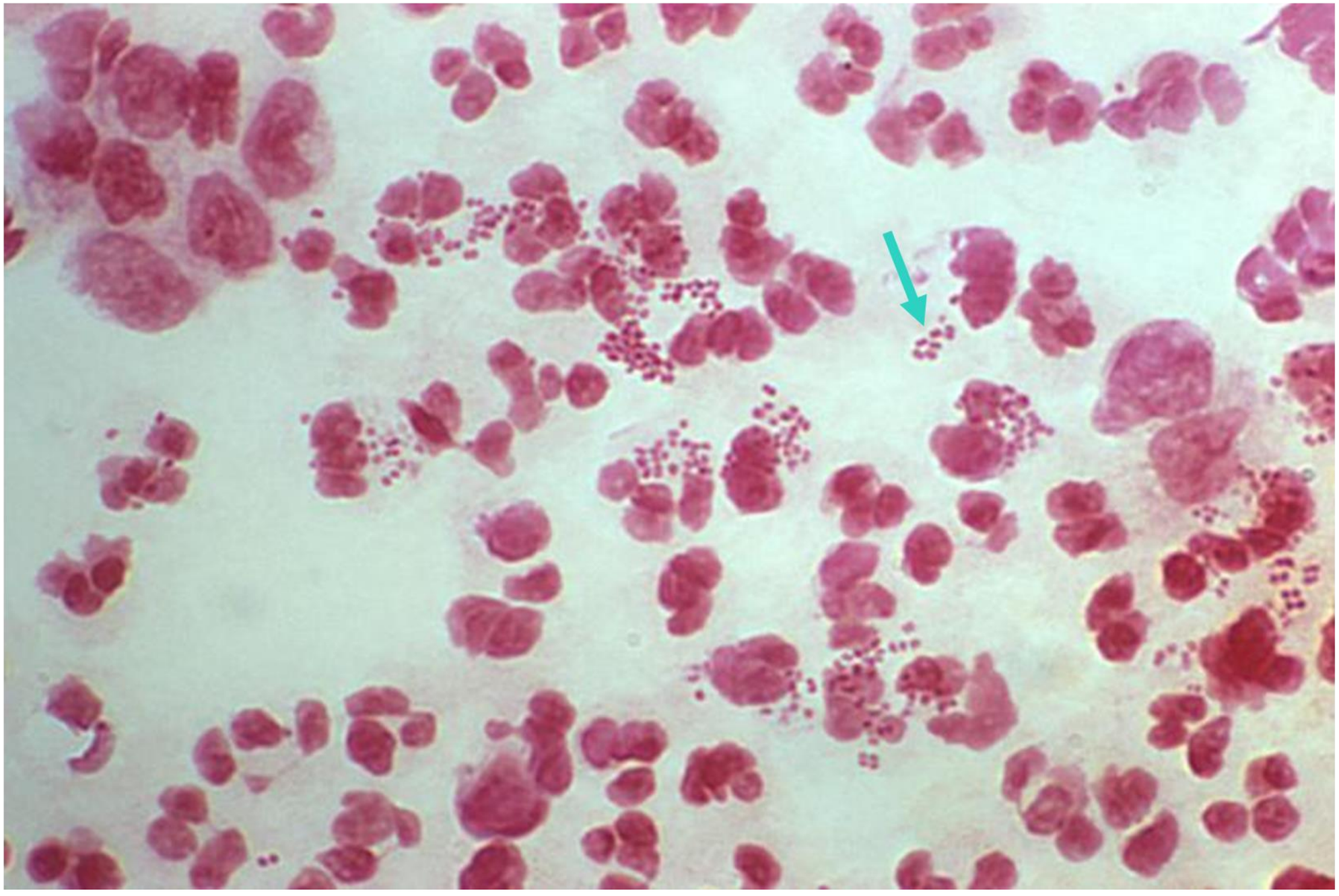
Gram Stain examples

E. Coli - Gram negative rod, short and stubby



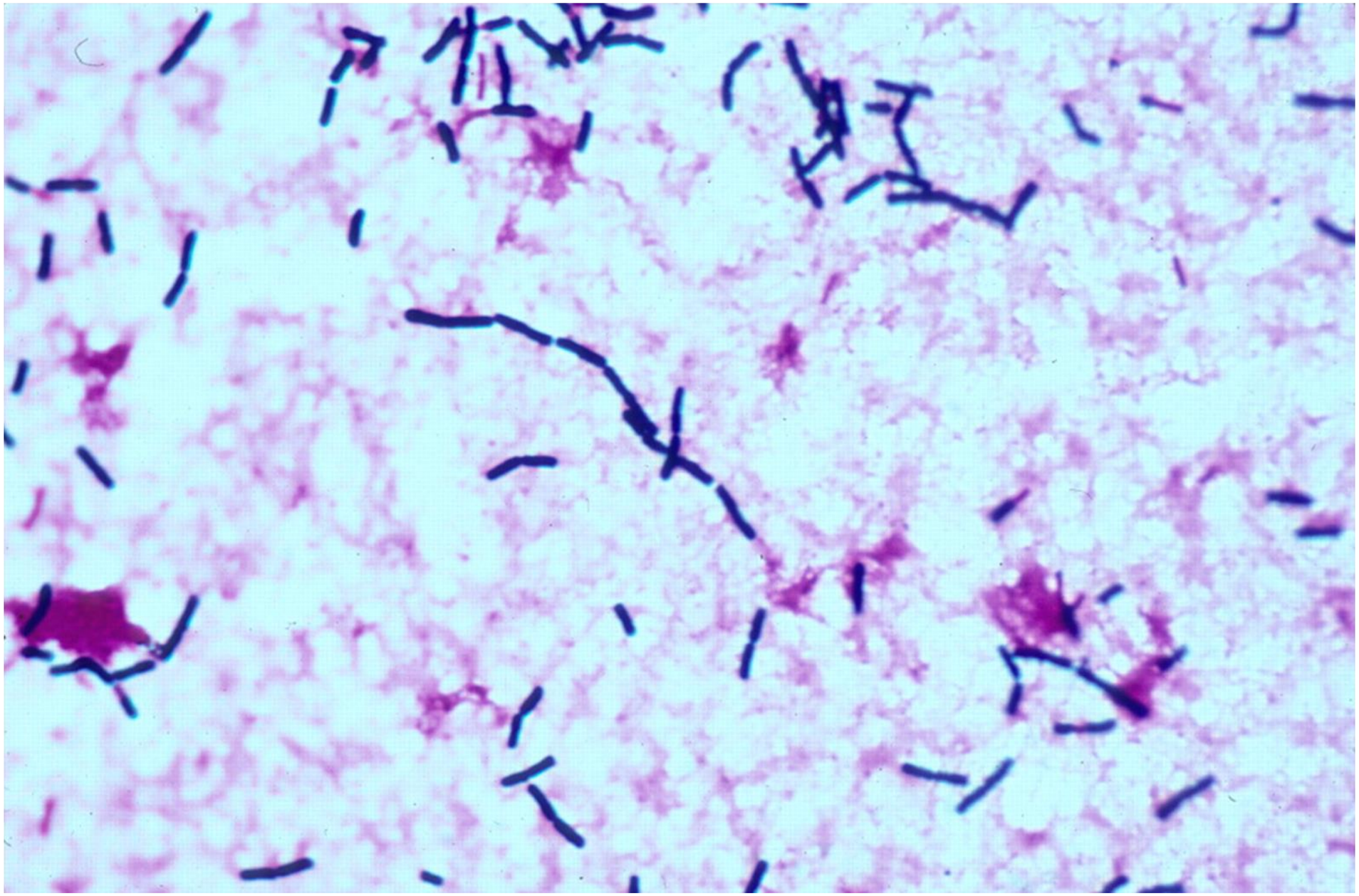
Gram Stain examples

Neisseria meningitidis – Gram negative diplocci



Gram Stain examples

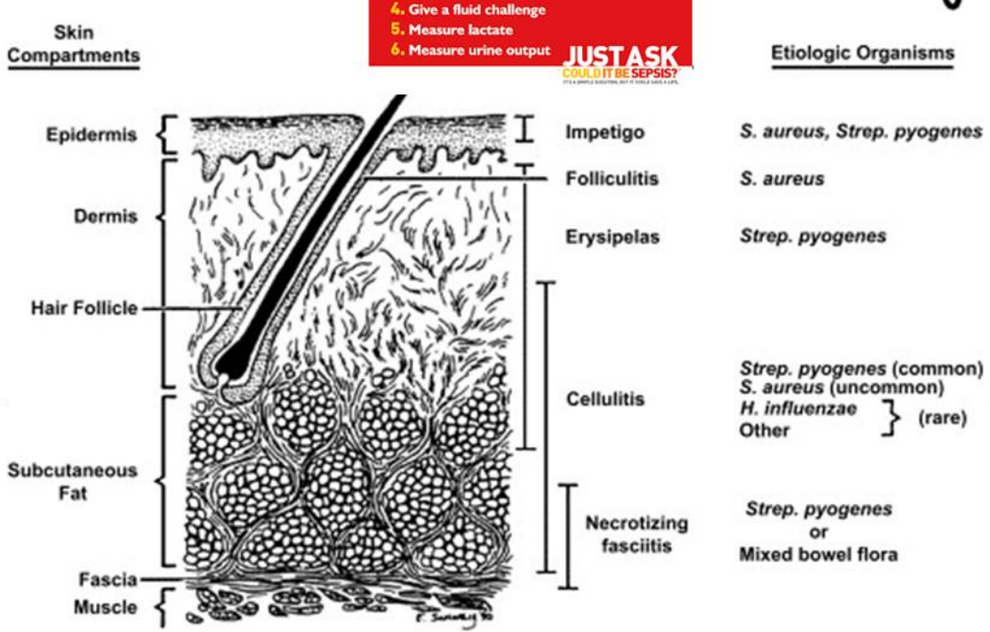
Bacillus cereus – Gram negative rod, long rod, some occurring in chains



Gram +ve Cocci

- **Catalase +ve**
  - **Coagulase +ve** → *S. aureus*
    - 
    - 

  - **Coagulase −ve** → Coag −ve Staph spp.
    - *S. epidermidis*
    - *S. saprophyticus*
- **Catalase −ve**
  - **α-haemolytic on blood agar**
    - **Optochin resistant** → *S. mitis*
    - **Optochin sensitive** → *S. pneumoniae*
    - 
  - **β-haemolytic on blood agar** → Perform Lancefield Grouping:
    - ↳ A = *S. pyogenes*
    - ↳ B = *S. agalactiae*
    - ↳ C = *S. dysgalactiae*
    - ↳ D = *S. bovis* or *Enterococcus faecalis*.
    - 

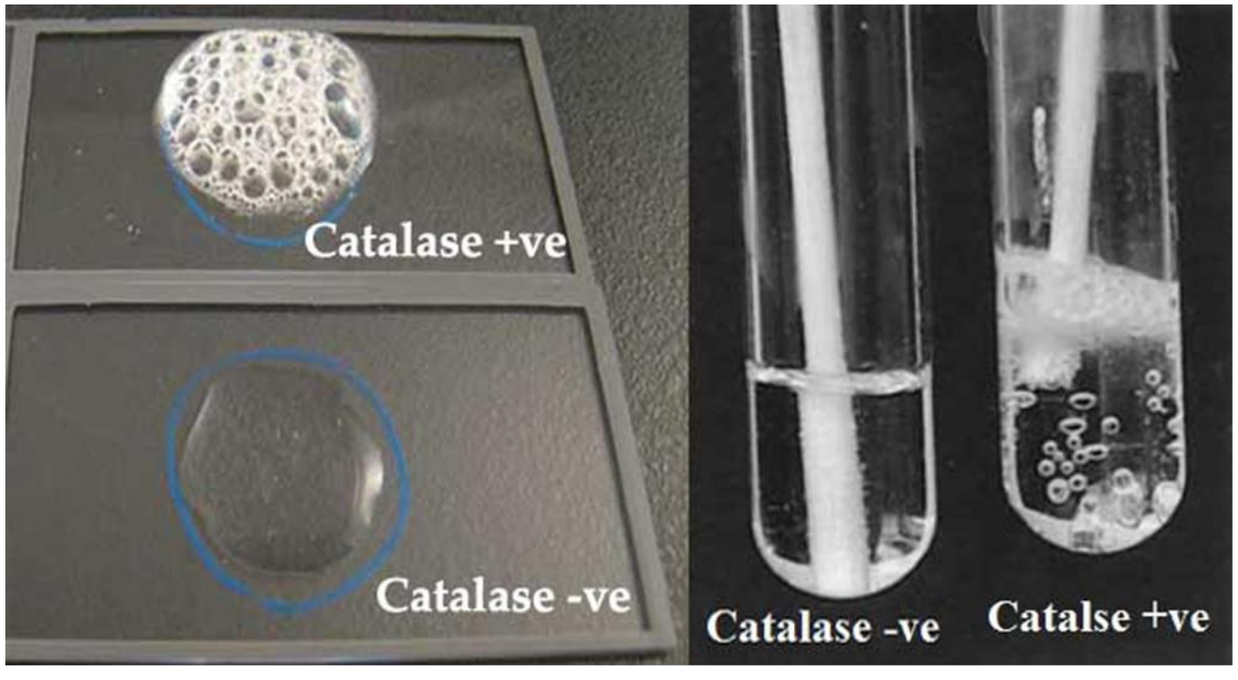


Catalase = is it Staph?

The catalase test differentiates between *Staphylococcus* and *Streptococcus* genus.

Staph = Catalase +ve
Strep = Catalase –ve

Method: apply hydrogen peroxide 3% to a small sample of pure colony. Observe for bubbling. Any weak bubbling = positive.



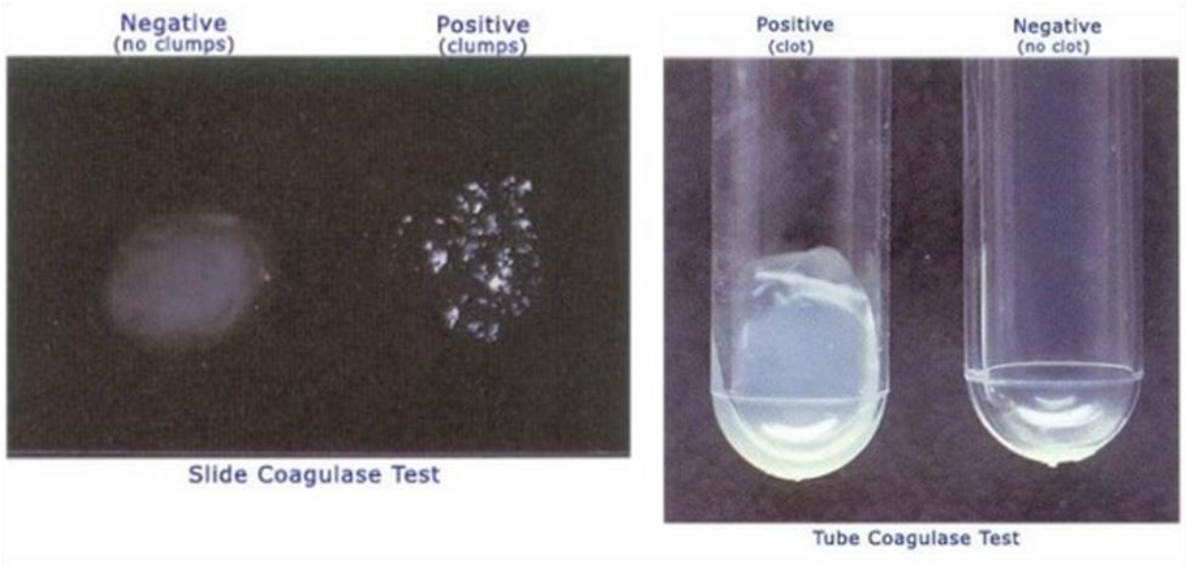
$$2H_2O_2 \xrightarrow[\text{catalase}]{} 2H_2O + O_2 \text{ (gas bubbles)}$$

Coagulase = is it S. aureus?

The coagulase test differentiates *Staph aureus*, which produces the enzyme coagulase, from *S. epidermis* and *S. saprophyticus*, which do not produce coagulase. i.e Coagulase Negative Staphylococcus (CONS).

Staph aureus = Coagulase +ve
Coag neg staph (epi and sapro) = Coagulase –ve

Method: apply rabbit plasma to a small sample of pure colony. Observe for fibrin clot clumps.

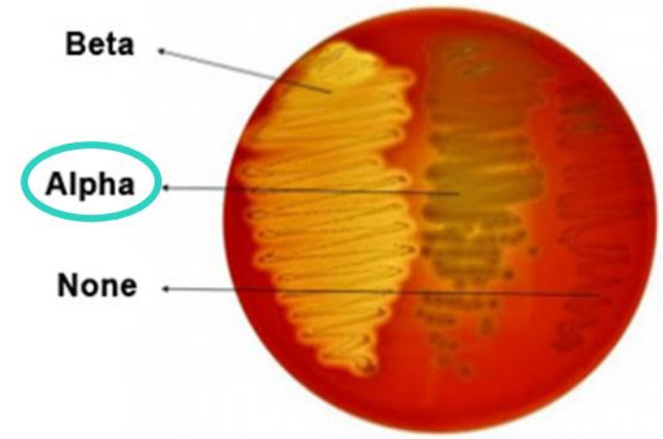


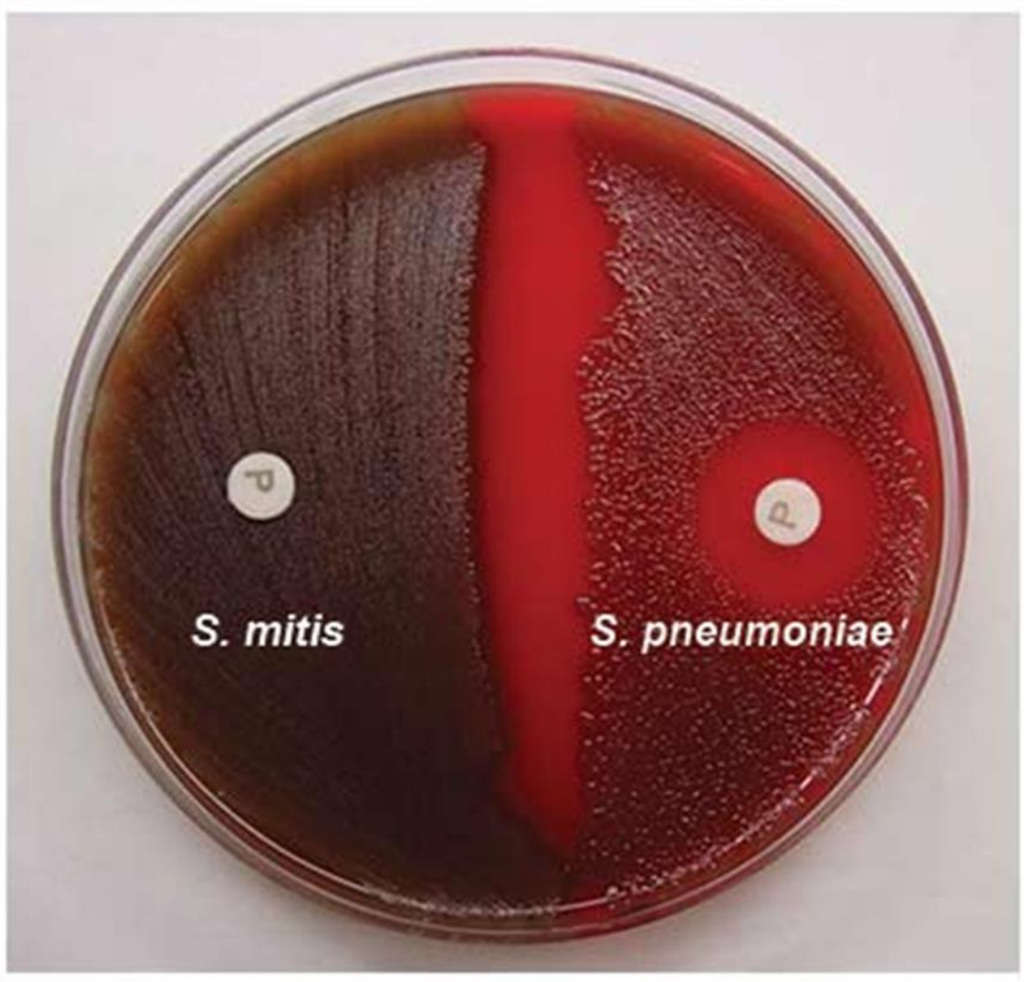
Differentiating alpha-haemolytic Streps - Optochin

Optochin is a chemical (ethylhydrocupreine hydrochloride). Filter paper disc contains optochin.

S. pneumoniae aka pneumococcus, the major human pathogen, is SUSCEPTIBLE to optochin.

All other alpha Streps, also termed *viridans* Streps, are RESISTANT.





Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

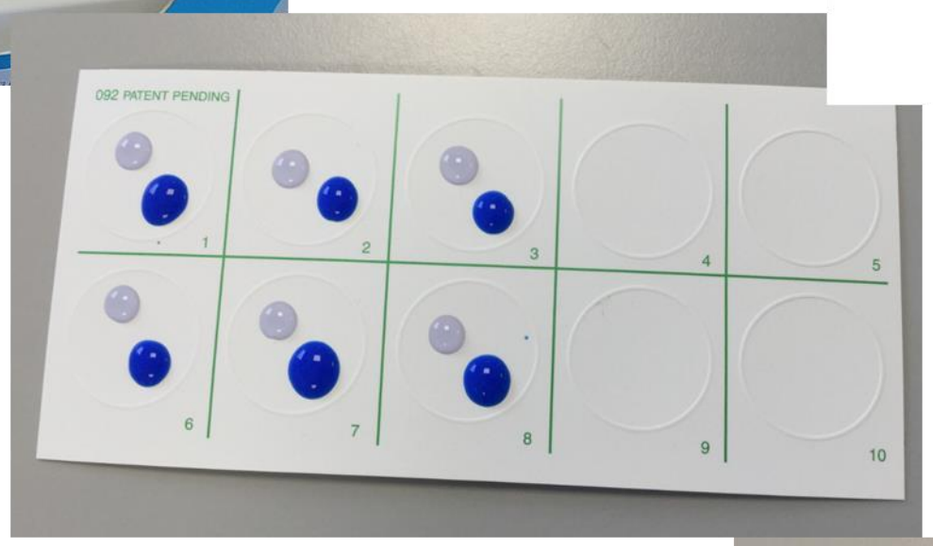
Streptococcal Classification is Complex!

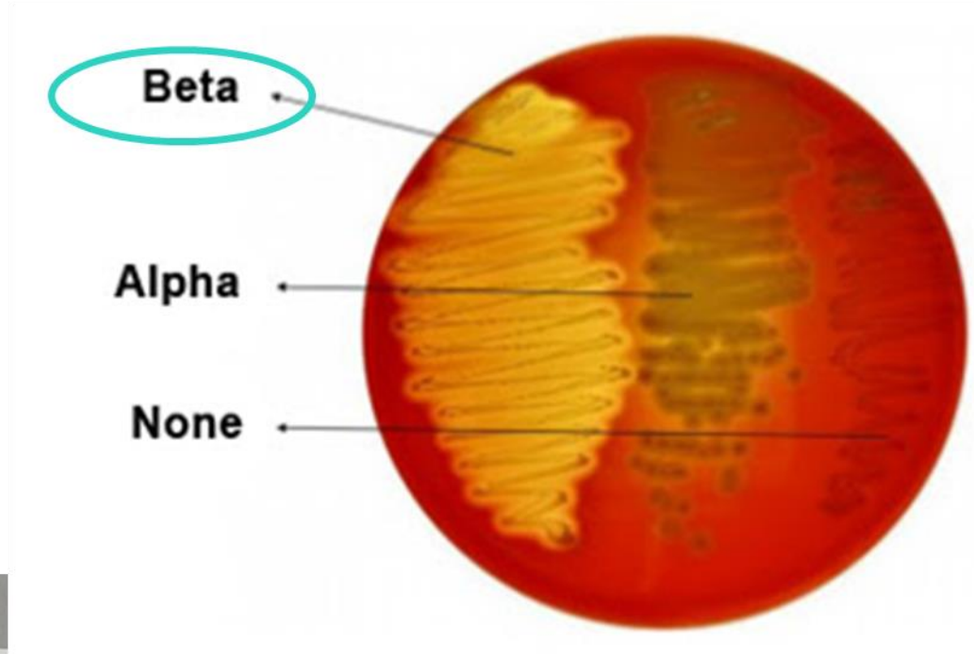
- Classification is pretty messed up. Bugs change groups all the time as we learn more about them.
- Don't get confused: alpha haem Strep are also known as viridans group, which means green due to the green agar haemolysis effect.

Need-to-know basis for 2a, you just need to understand how we identify pneumococcus (alpha) and S. pyo (beta) by following these steps:

1) Haemoloysis on BLOOD agar.
2) If alpha haemolysis do Optochin.
3) If beta haemolysis do Lancefield.

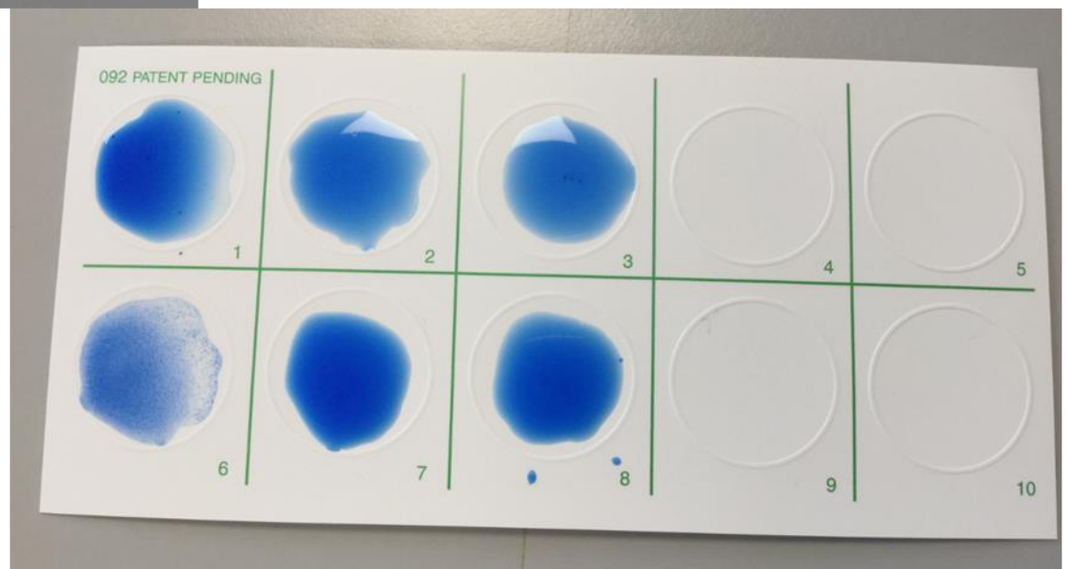
Differentiating beta haem Streps - Lancefield Grouping





Lancefield serotyping, relies on the different, group-specific carbohydrates antigen present on the bacterial cell wall. Groups labelled alphabetically.

<u>2a need-to-know</u> Group A (*S. pyo*). In later years others become more relevant e.g. paeds module Group B colonises vaginal tract and can cause newborn sepsis.



Clinical Picture: Pneumonia

Community-acquired Pneumonia (CAP)

CAP = symptoms first occur after LESS than 48 hours in hospital.

Commonest culprit = *S. pneumoniae* – know this!

Typical: *H influenzae, Moraxella catarrhalis*

Atypical*: Mycoplasma pneumoniae, Chlamydophila pneumoniae*, and *Legionella pneumophila.*

Diagnosis – CXR w/ new consolidation, sputum or blood culture.

Treatment – amoxicillin first line (CURB-65 score), co-amoxiclav if unwell. Otherwise guided by micro.

Hospital-acquired Pneumonia (HAP)

HAP = symptoms first occur after MORE than 48 hours in hospital.

Usually in people on ventilators, stroke, long stays, infectious contact.

Many culprits = *E. coli, Klebsiella pneumoniae, Enterobacter spp., Pseudomonas aeruginosa, Acinetobacter baumannii, Stenotrophomonas maltophilia, MRSA, Streptococci*

Diagnosis – CXR w/ new consolidation, sputum or blood culture.

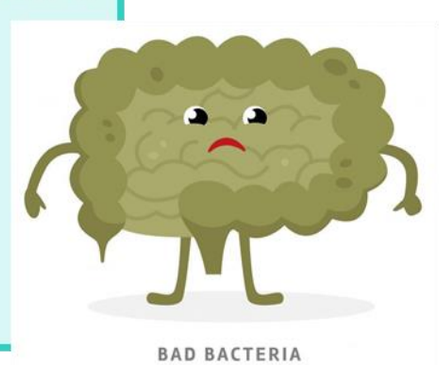
Treatment –guided by micro.
Often IV co-amoxiclav and gentamicin.

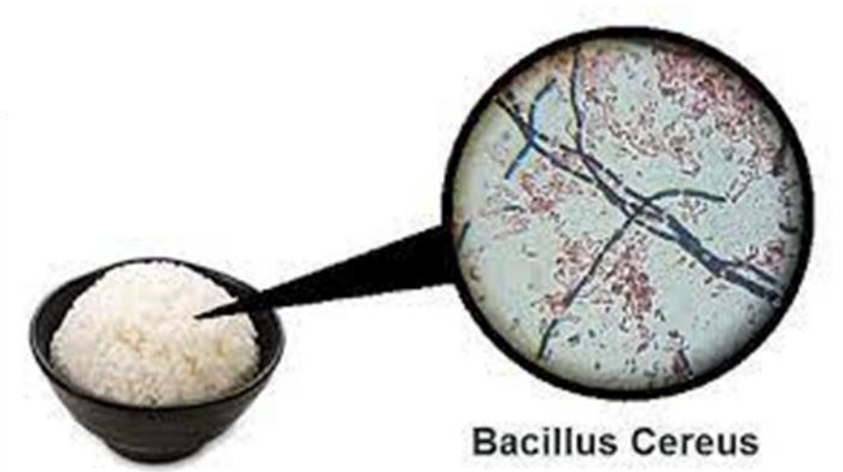
Gram +ve Rods

- Strict anaesobe
  - ↓
  - Clostridium spp
    - e.g. C. difficile
    - C. perfringens
    - (actually classed as Gram variable due to tendency to easily decolourise)
- Aerobic or Facultative
  - Bacillus spp. if sporing
    - e.g B. cereus (food poisoning)
    - B. subtilis (opportunistic)
  - Corynebacterium
    - e.g. C. diptheriae (diptheria)

The 4 C's associated with C difficile infection:

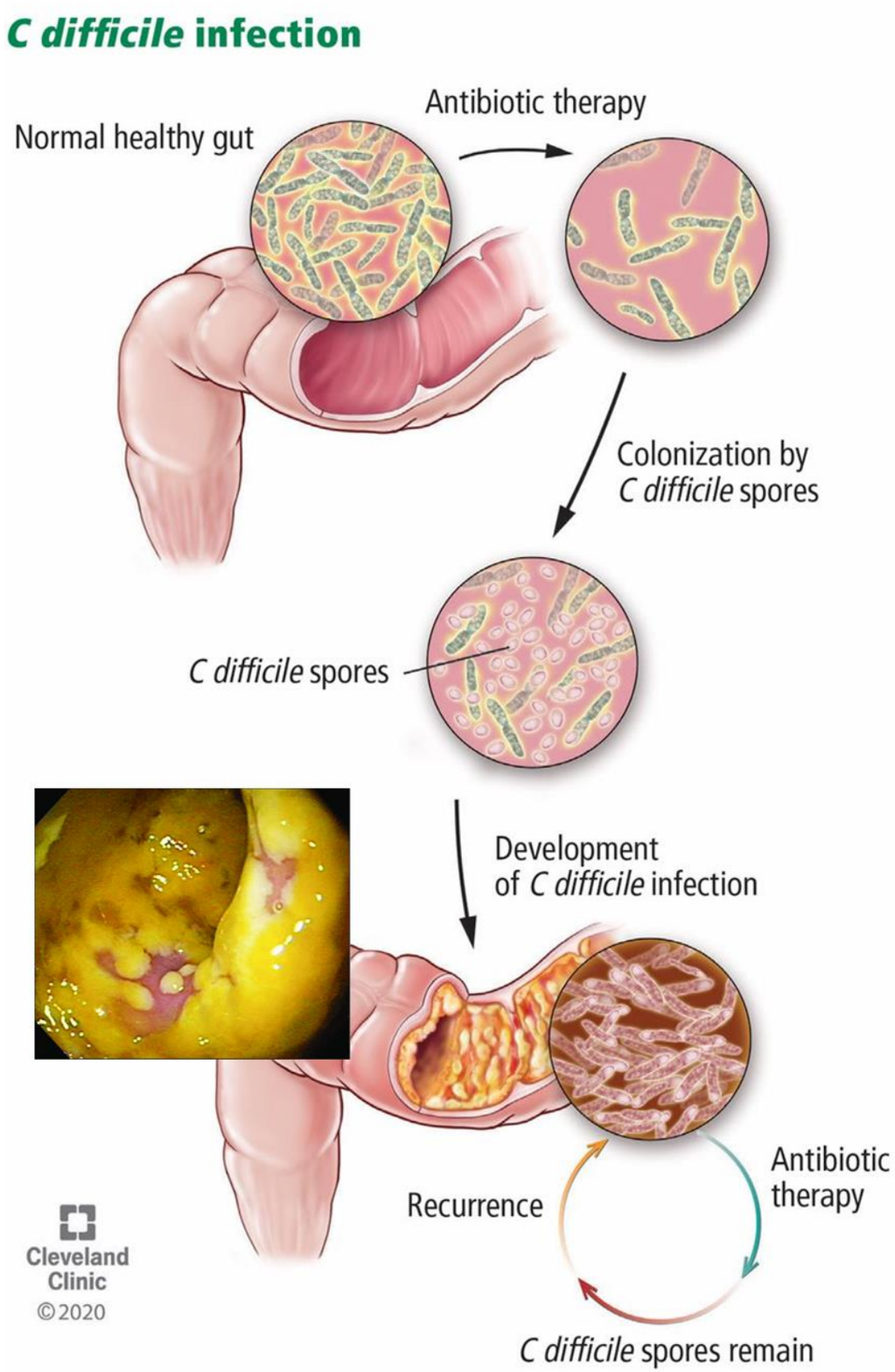
clindamycin, cephalosporins, co-amoxiclav and ciprofloxacin





Clinical Picture: C. Difficile associated disease

Sx = diarrhoea, fever, abdo pain, Hx of Ab



Antibiotic therapy

↓

Disruption of colonic microflora

↓

C. difficile exposure and colonisation

↓

C. diff toxin A and toxin B cause mucosal injury and inflammation.

↓

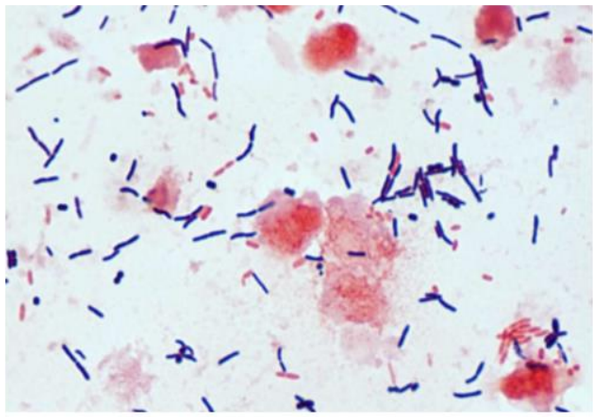
Pseudomembranous colitis

↓

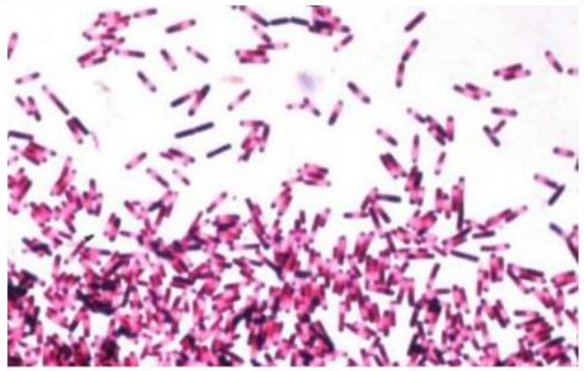
Toxic megacolon

↓

Perforation and death



Officially Gram +ve (variable)



Treatment is to discontinue the inciting antimicrobial agent and start therapy with 10 days **oral fidaxomicin or vancomycin** (metronidazole may be used in some locations).

Gram negative cocci

Neisseria spp.

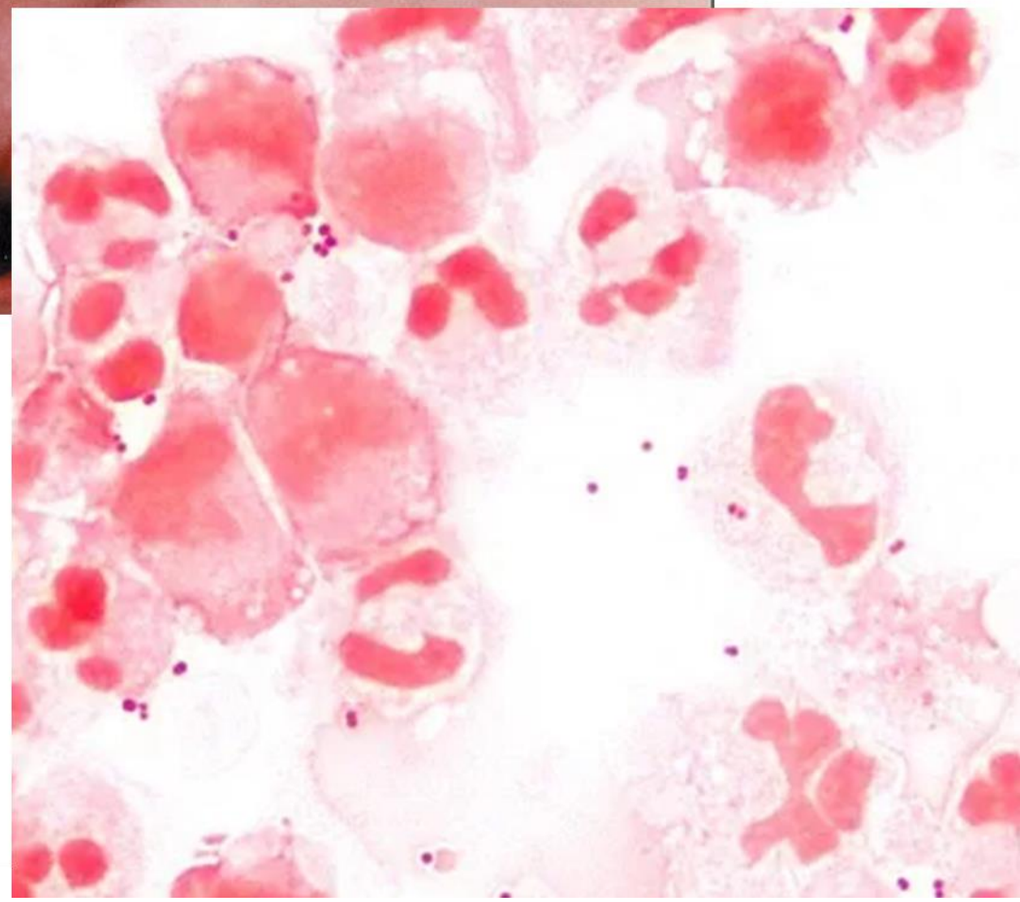
both diplococci

N. meningitidis

(meningitis
sepsis)

N. gonorrhoeae

(urethritis
proctitis
salpingitis / PID
conjunctivitis
↳ reactive arthritis)



Clinical Picture: Meningitis

Pathological Basis:
Inflammation of the pia + arachnoid mater - microorganisms infect the CSF
Not always an infective cause - can have chemical/ post-surgical etc

Symptoms:
Stiffness of the neck, photophobia + severe headache
Infective: fever, malaise
Petechial rash associated with meningococcal meningitis

Causes:

In neonates	In infants	In young adults	In the elderly
E.coli, Group B Strep, Listeria monocytogenes	Neisseria meningitidis, Haemophilus influenzae, S.pneumoniae	Neisseria meningitidis, S.pneumoniae	Neisseria meningitidis, S.pneumoniae, Listeria monocytogenes

Treatment:
Bacterial: start antibiotics before tests come back if suspected

- Cephalosporins: IV cefotaxime/ IV ceftriaxone
- If over 50/immunocompromised add IV amoxicillin to cover listeria
- One dose oral ciprofloxacin - prophylaxis for contacts

Meningococcal septicaemia: immediate IM benzylpenicillin in community/ IV cefotaxime in hospital

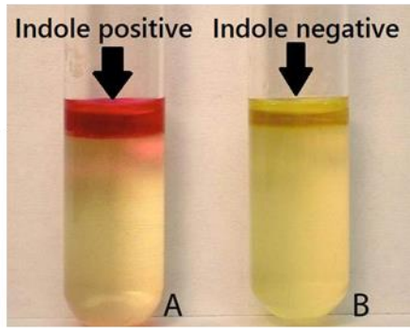
Viral: supportive treatment, self-limiting in 4-10 days, acyclovir for HSV meningitis

CSF Findings

	Appearance	Opening Pressure	WBC cell/ul	Protein mg/dL	Glucose mg/dL
Normal	Clear	90-180	<8	15-45	50 - 80
Bacterial	Turbid	High	>1000	>200	<40
Viral	Clear	90-180	<300 (lymphocytes)	<200	Normal

Oxidase test is used to determine if a bacterium produces certain cytochrome c oxidases

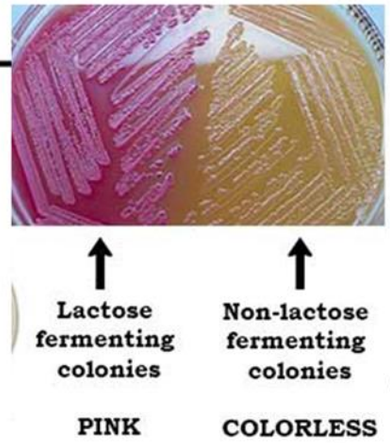
Indole test - ability of certain bacteria to decompose the aa tryptophane to indole



Gram -ve Rods

- **Oxidase +ve**
  - **Indole -ve**
    - motile
      - ↓ Pseudomonas aeruginosa
        - (CF lung infection, Diabetic foot, Burn infections, UTI esp. catheters)
  - **Indole +ve**
    - ↓ Factor XV
      - Haemophilus influenzae
        - (· septic arthritis · COPD exacerbations · pneumonia)

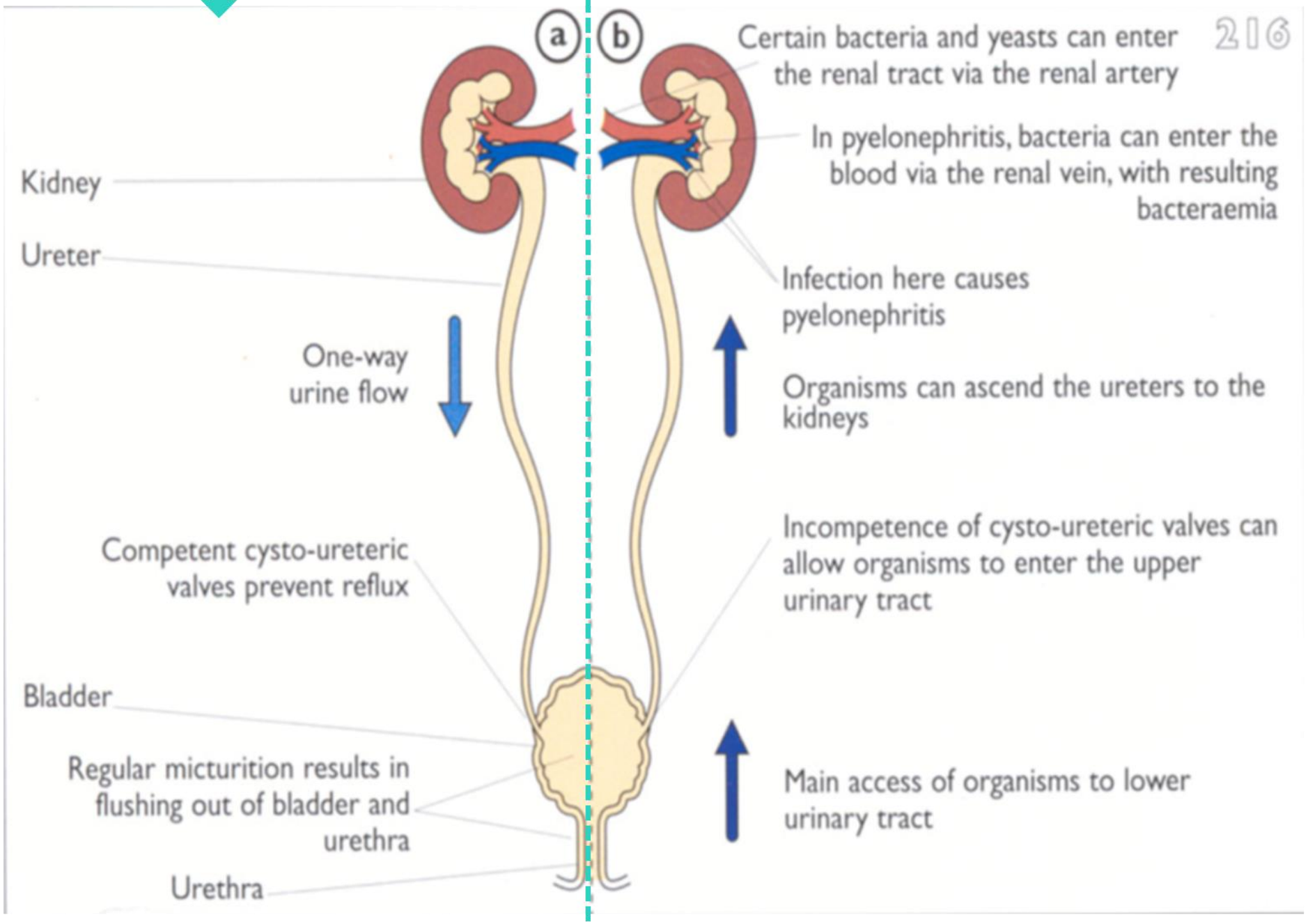
- **Oxidase -ve** → Bordetella pertussis (whooping cough)
- **Enterobacteria**
  - Non-lactose fermenter (transparent on MacConkey)
    - ↳ Proteus mirabilis → UTIs
    - ↳ Shigella spp. → gastroenteritis
    - ↳ Salmonella spp. — S. typhi = typhoid fever
  - Ferments lactose (pink on MacConkey)
    - Indole +ve → ↓ E. coli (UTI, gastroenteritis, abdo infections)
    - Indole -ve → ↓ Klebsiella pneumoniae (UTI, abdo infections, CF lungs)



Clinical Picture: UTIs

Host defences of this usually sterile tract

How infection can occur



Clinical Picture: UTI Symptoms, Diagnosis & Treatment

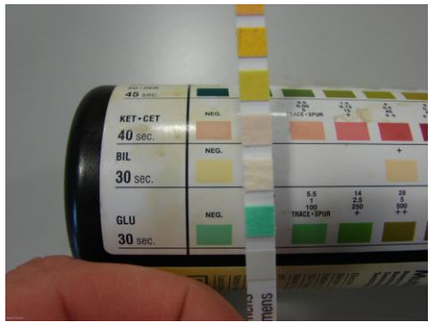
Symptoms

Lower UTI:

- Dysuria
- Urgency
- Frequency
- Cloudy or foul urine
- Haematuria (micro or macro)
- Suprapubic tenderness

Upper UTI:

- Fever
- Confusion
- Loin tenderness
- Prior Hx of above Sx



Dipstick (under 65s) – nitrates reduced to **nitrites** = E. coli and Proteus.

Common organisms:

85-90% of UTIs are caused by *E. coli* (endogenous infection).
S. saprophyticus is also common in young women.

Less common organisms:
Pseudomonas aeruginosa
Klebsiella spp.
Proteus spp.
Enterobacter spp

Treatment (KNOW):

Oral Nitrofurantoin or Trimethoprim.

Pregnancy – cefalexin.

Simple = 3 days.
Complicated = 7 days.

Common Micro-organisms Responsible for UTI/Pyelonephritis

KEEPS
***K**lebsiella*
***E**. coli*
***E**nterococcus*
***P**roteus*
***S**. saprophyticus*

More Modern Methods of Clinical Identification

API Strip

Uses a slide with many small capsules on it to perform many different biochemical tests at the same time.

API 20 E after incubation...Positive results for all tests :

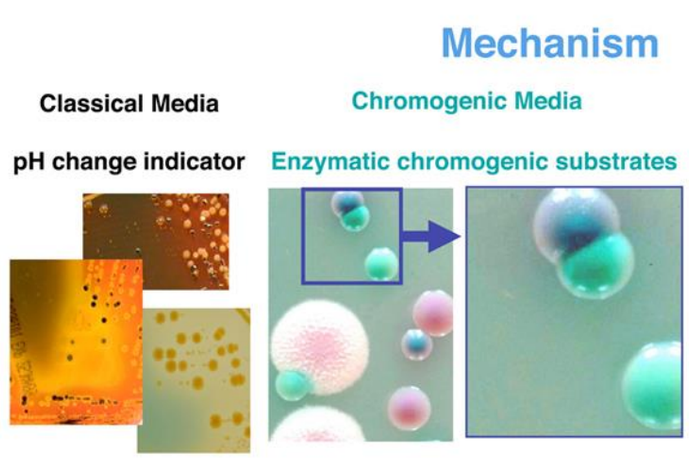
Microbiology Info.com

API 20 E after incubation...Negative results for all tests :

16S rRNA gene sequencing is used as a tool

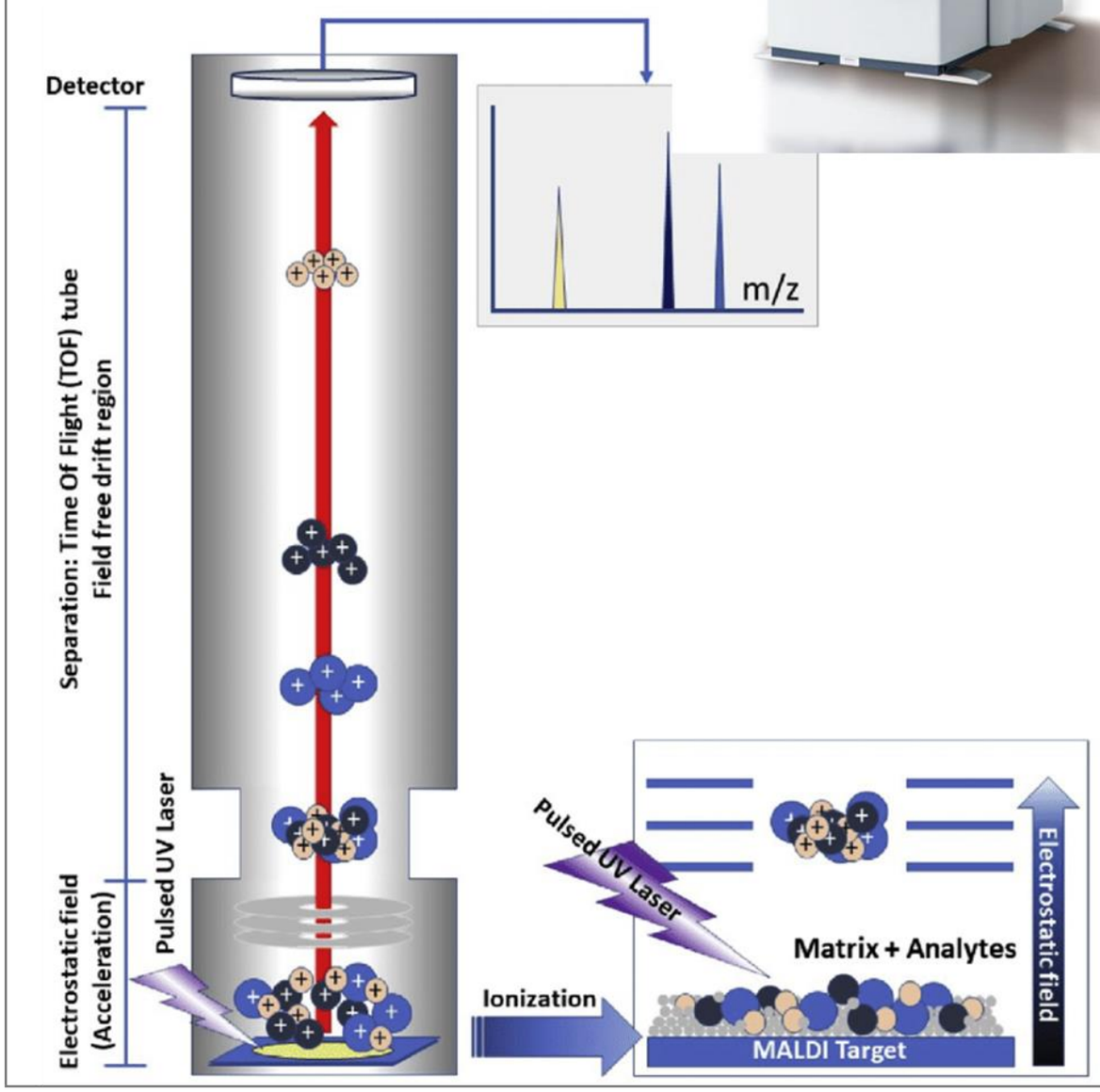
to identify bacteria at the species level and assist with differentiating between closely related bacterial species. Useful for complex mixtures like environmental or gut samples.

Chromogenic Media

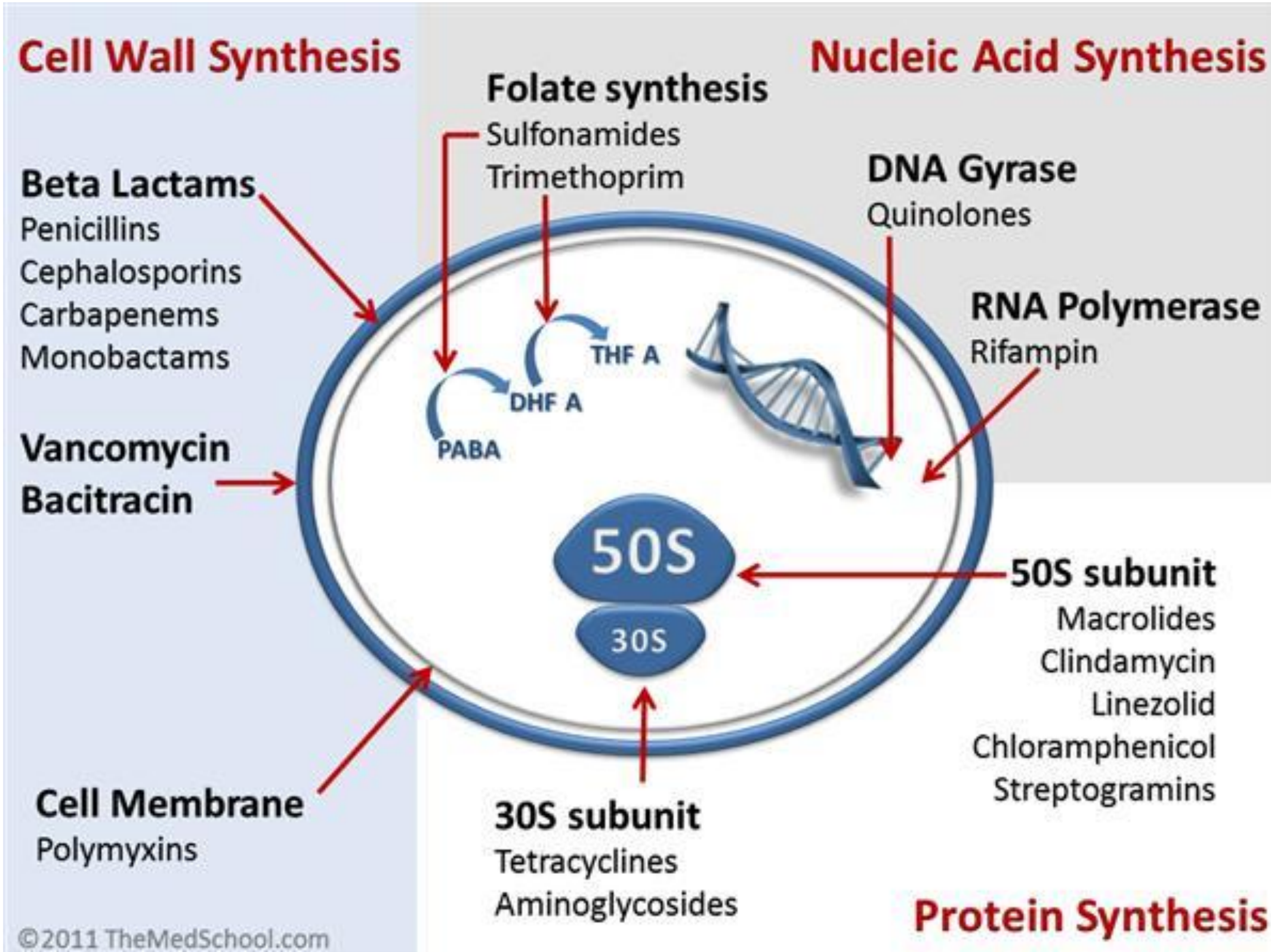


MALDI-TOF Mass Spectrometry

- Rapid
- Accurate
- EXPENSIVE



Antibiotics

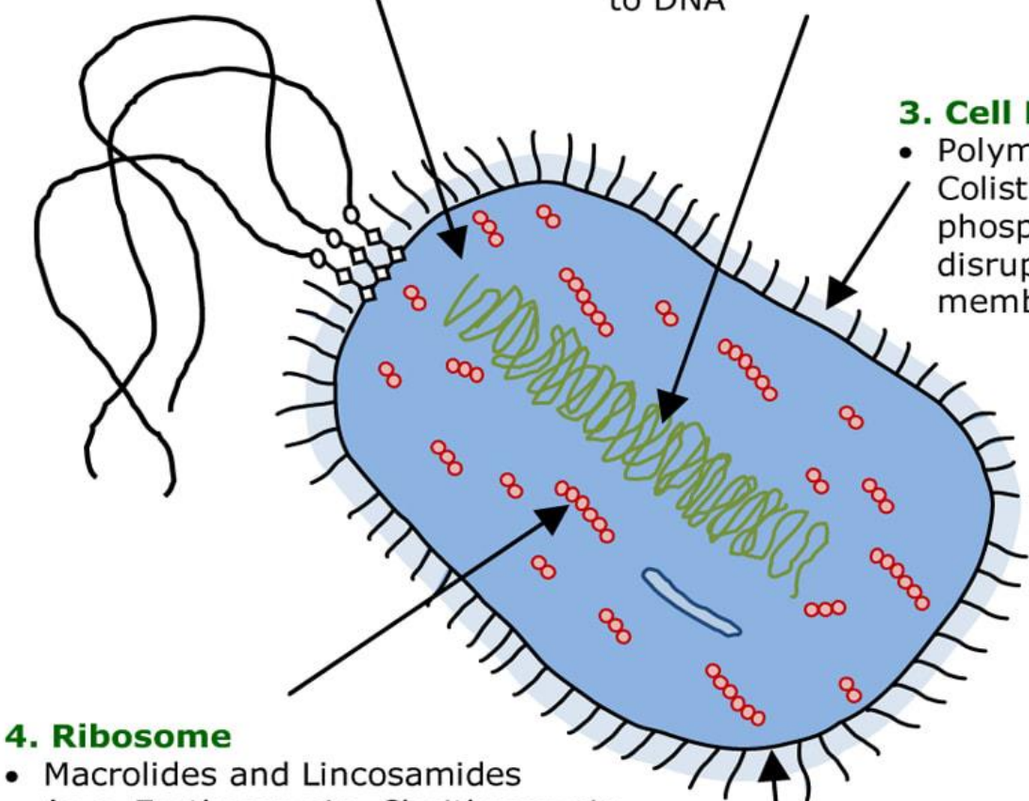

Cell Wall Synthesis
Nucleic Acid Synthesis
Folate synthesis
Sulfonamides
Trimethoprim
Beta Lactams
Penicillins
Cephalosporins
Carbapenems
Monobactams
DNA Gyrase
Quinolones
RNA Polymerase
Rifampin
THF A
DHF A
PABA
Vancomycin
Bacitracin
50S
30S
50S subunit
Macrolides
Clindamycin
Linezolid
Chloramphenicol
Streptogramins
Cell Membrane
Polymyxins
30S subunit
Tetracyclines
Aminoglycosides
Protein Synthesis
©2011 TheMedSchool.com

1. Cytoplasm

- Nitroimidazoles (e.g. Metronidazole) produce oxygen free radicals which damage proteins and DNA
- Lipopeptides (e.g. Daptomycin) depolarise cell membranes inside the cell

2. Chromosome

- Diaminopyramidines (e.g. Trimethoprim) interfere with folic acid synthesis
- Quinolones (e.g. Ciprofloxacin, Levofloxacin) inhibit DNA coiling
- Rifampicin and Fidaxomicin inhibit RNA polymerase
- Nitrofurantoin's actual mechanism is unknown but it causes direct damage to DNA



3. Cell Membrane

- Polymyxin (e.g. Colistin) binds to phospholipids disrupting the cell membrane

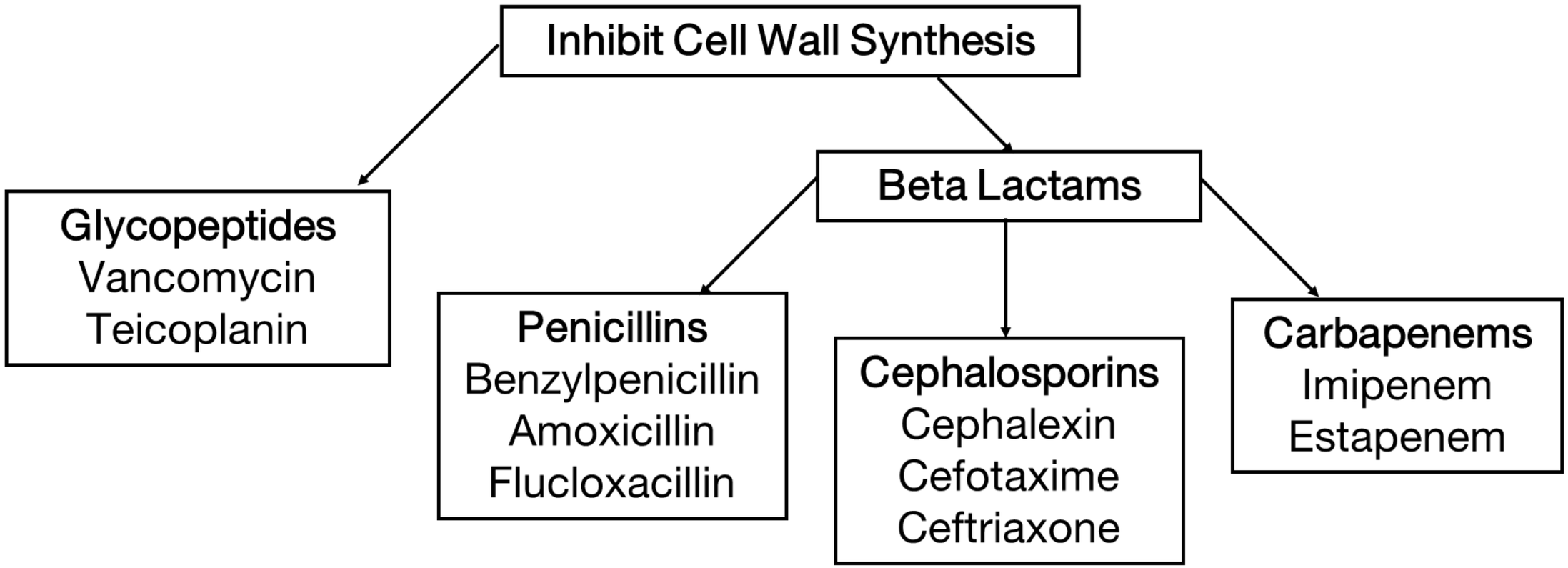
4. Ribosome

- Macrolides and Lincosamides (e.g. Erythromycin, Clarithromycin, Azithromycin, Clindamycin) prevent protein elongation and inhibit ribosome formation
- Aminoglycosides (e.g. Gentamicin, Amikacin, Tobramycin) interfere with translation and protein formation
- Tetracyclines and Glycylcyclines (e.g. Doxycycline, Tigecycline) prevent protein synthesis
- Oxazolidinones (e.g. Linezolid) prevent ribosome formation
- Fusidic Acid blocks elongation factor G, preventing protein formation
- Chloramphenicol inhibits protein synthesis
- Nitrofurantoin's actual mechanism is unknown but it interferes with translation

5. Cell Wall

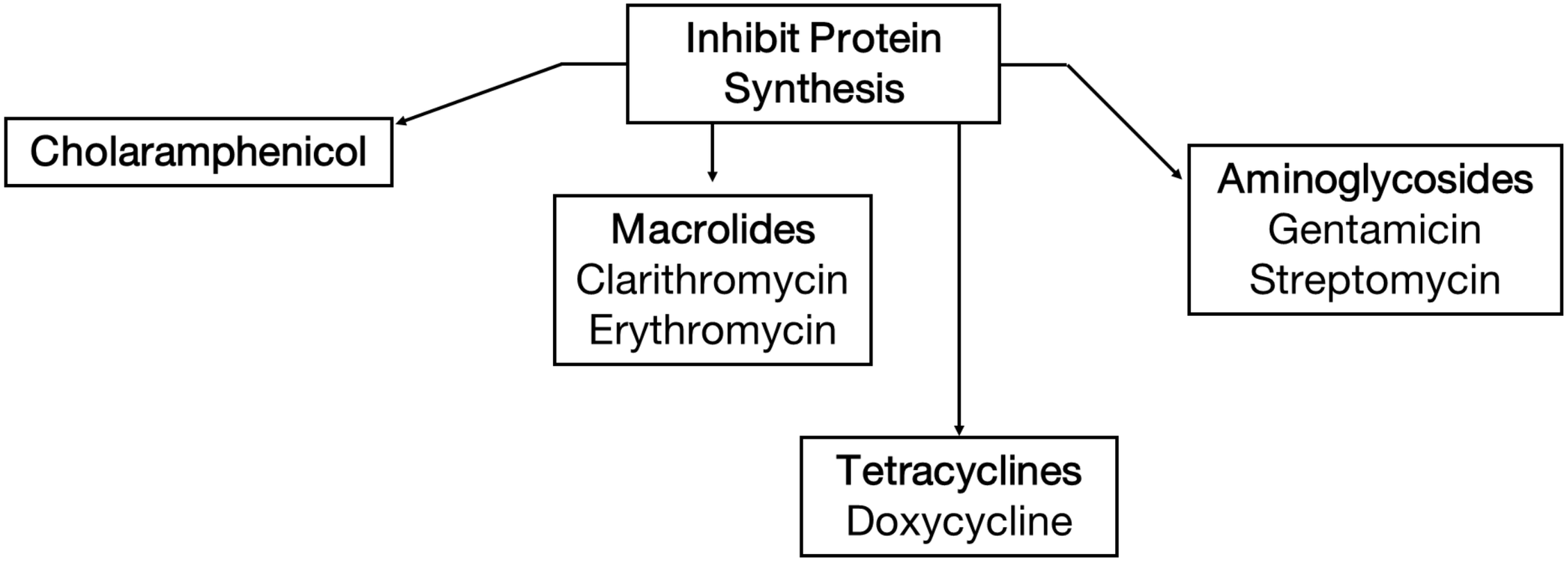
- Beta-Lactams (e.g. Penicillins, Cephalosporins, Carbapenems) inhibit cell wall formation
- Glycopeptides (Vancomycin, Teicoplanin) prevent peptidoglycan cross-linkage
- Fosfomycin blocks peptidoglycan synthesis

Antibiotic Mechanism of Action: Cell Wall



Handy Hint
Remember gram positive bacteria have thick cell walls - make them vulnerable by attacking this with one of the above antibiotics!

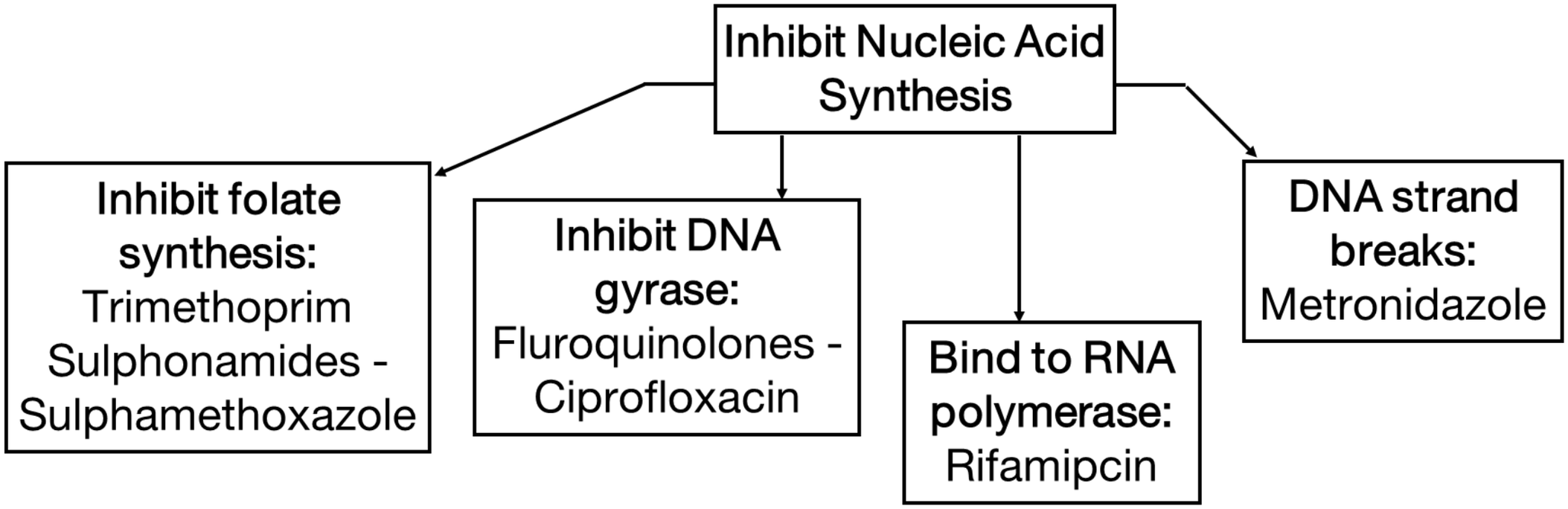
Antibiotic Mechanism of Action: Protein Synthesis



Handy Hint
We generally use macrolides instead of penicillin for those with a penicillin allergy

The aminoglycoside vancomycin is used to treat severe MRSA infections.

Antibiotic Mechanism of Action: Nucleic Acid Synthesis

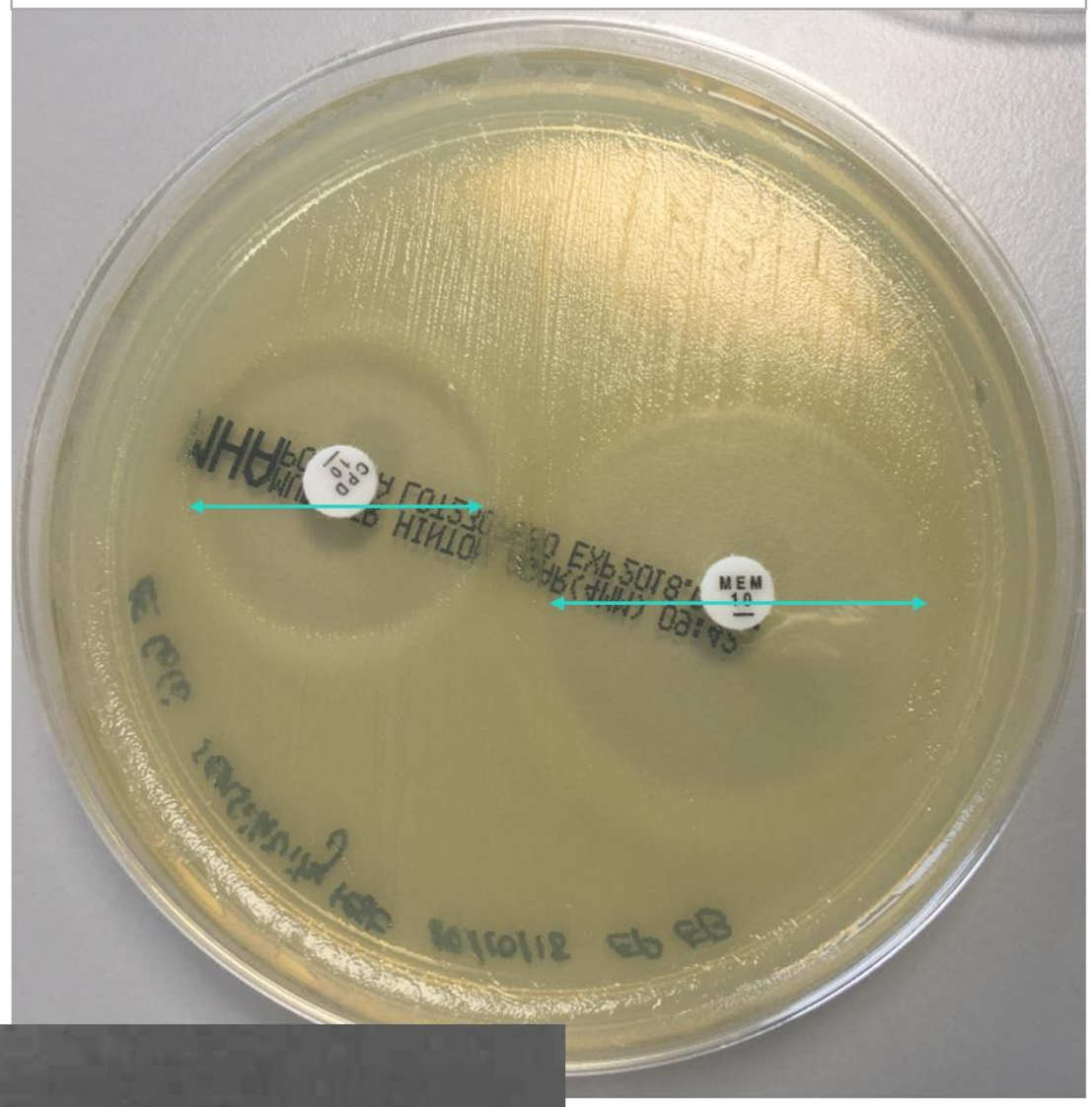


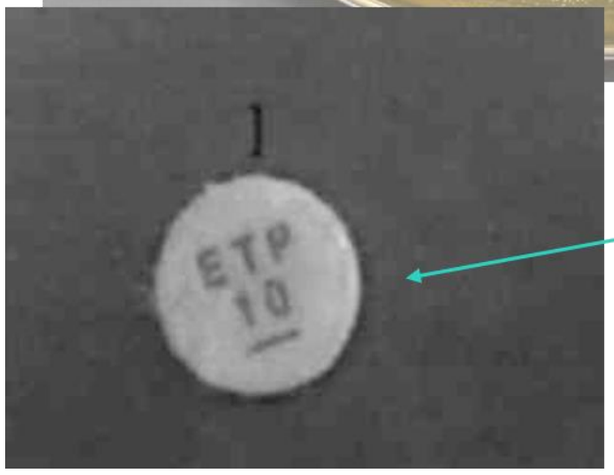
Handy Hint

Never give trimethoprim to a pregnant woman – it inhibits folate metabolism, which is important during pregnancy to prevent spina bifida.

Antibiotic Sensitivity Testing

E. coli lawn tested with Cefpodoxime 10 ug and Meropenem 10 ug. Looking susceptible.





A resistant result

Simply inoculate a lawn of the test microorganism onto Mueller-Hinton agar. Place antibiotic discs onto lawn. Incubate.

Mueller-Hinton agar allows standardised diffusion rate of antibiotics away from the disc and through the agar. Reliable and repeatable.

Measure diameter of the zone of inhibition. Use international data set to convert the diameter into “susceptible”, “intermediate” or “resistant”.

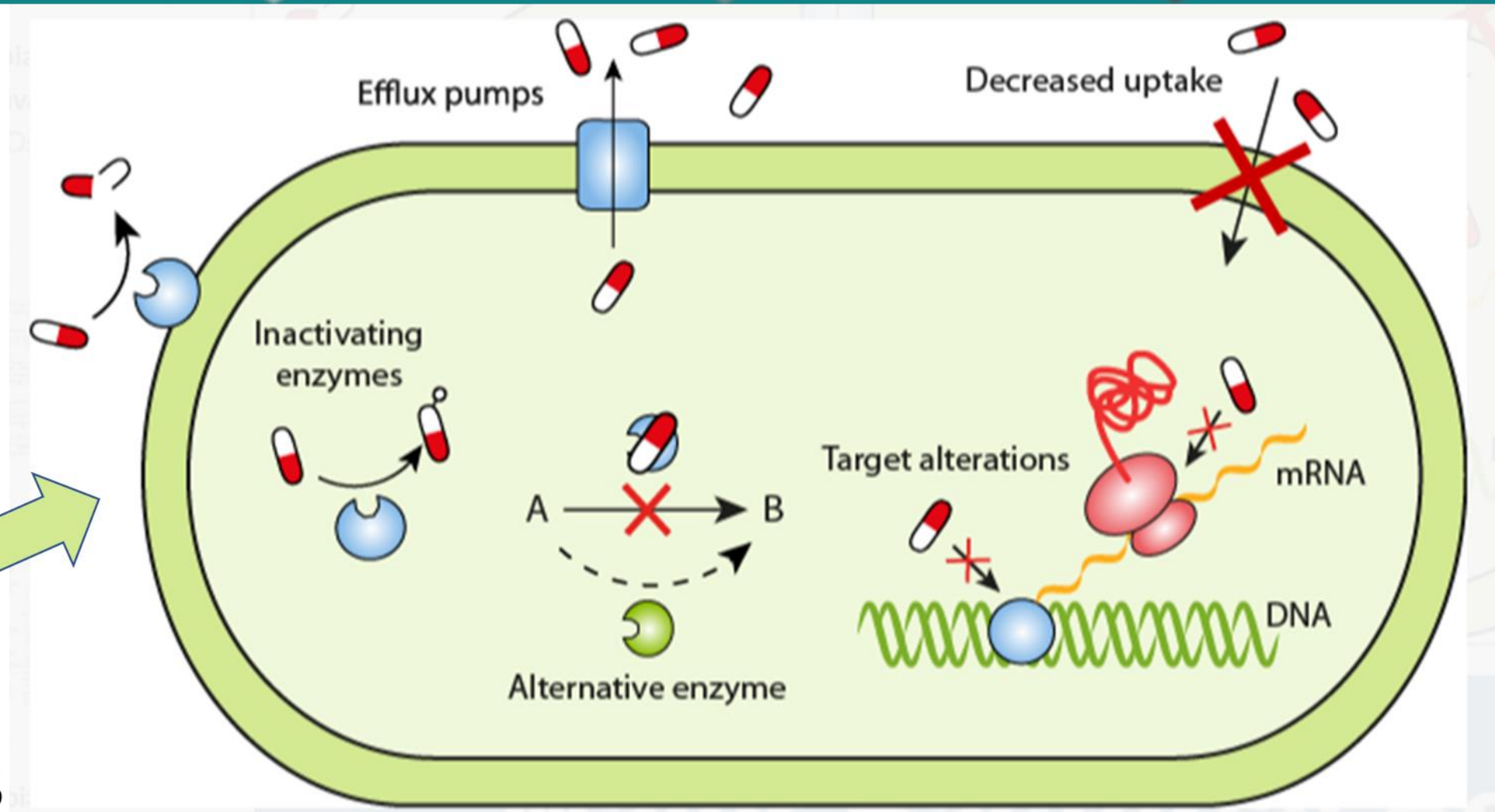
Reported via ICE as below, so you can make an informed choice of antibiotic.

MICROSCOPY
Verified Date/Time: 08-Mar-18 02:07
Gram positive coccus resembling staphylococci

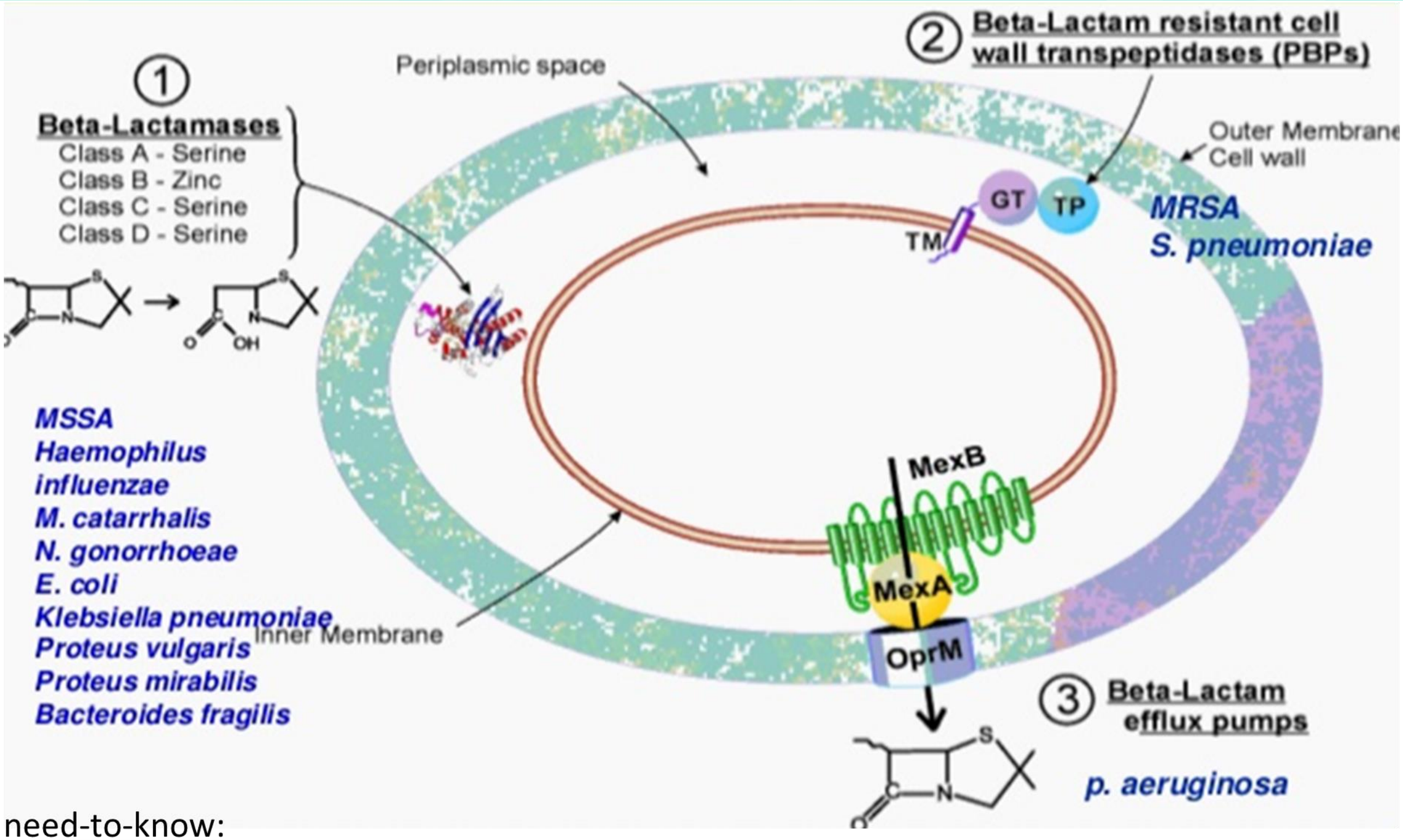
SUSCEPTIBILITY RESULTS

	Methicillin-resistant Staphylococcus aureus		
Antibiotic	MIC Interp	E Test MIC(mg/L)	E Test Interp
Ampicillin	R		
Ceftaroline			S
Cephazolin	R		
Daptomycin	S		
Flucloxacillin	R		
Linezolid	S		
Penicillin	R		
Vancomycin	S	1.5	S

Various Antibiotic Resistance Mechanisms



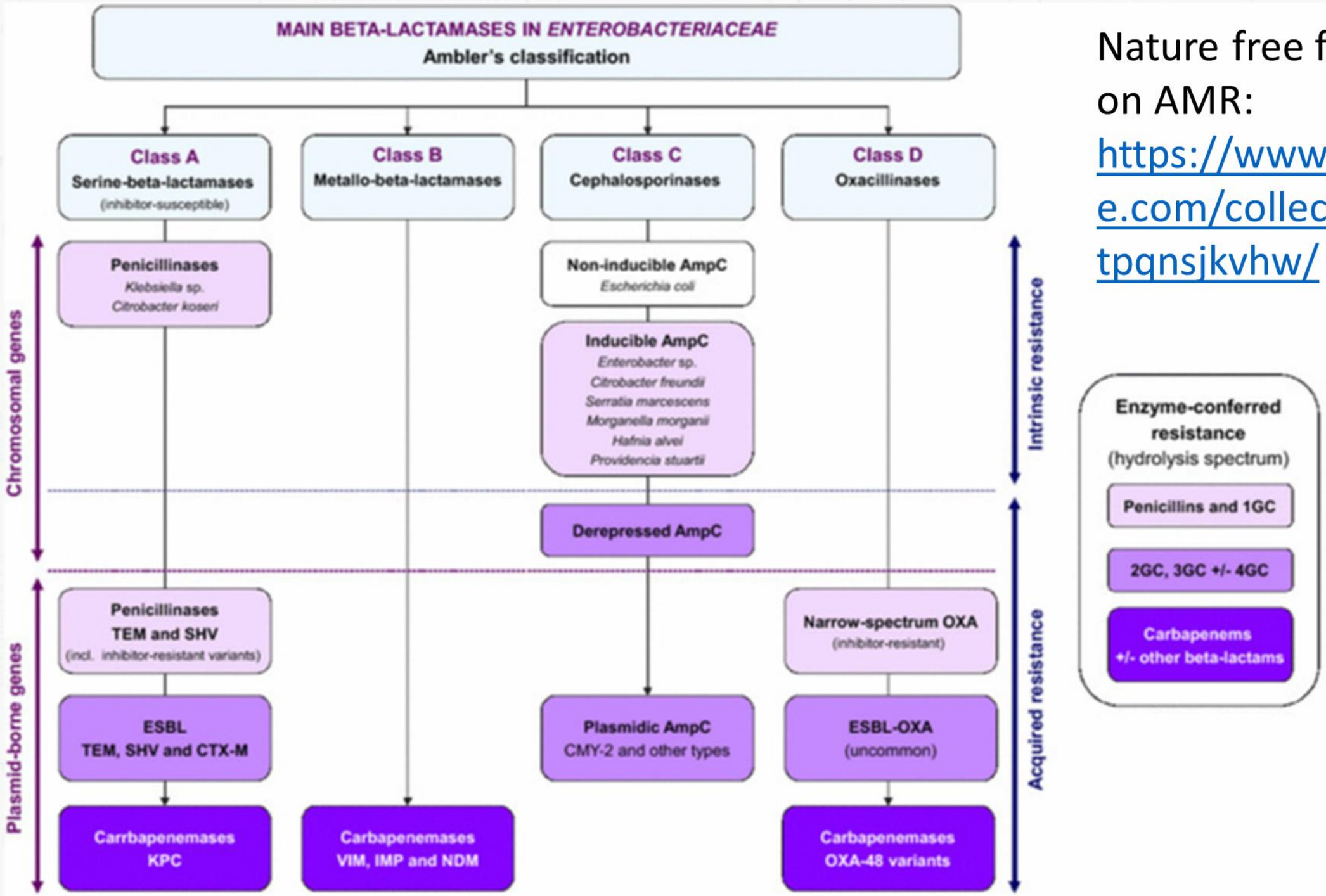
Three Specific Examples of Resistance



2a need-to-know:

1. beta-lactamase producing bacteria = resistant to penicillin-derived Ab.
2. MRSA carries a gene called *mecA*, which a unique transpeptidase that is not inhibited by β-lactam antibiotics. Hence, MRSA is able to continue peptidoglycan crosslinking (PBP2a) and resist.
3. Efflux pumps are also a common resistance mechanism.

hundreds of resistance genes out there…



Nature free feature on AMR: https://www.nature.com/collections/tpqnsjkvhw/

MYCOBACTERIA

Mycobacteria

Mycobacteria are atypical bacillus, slow-growing and cause gradual onset diseases.

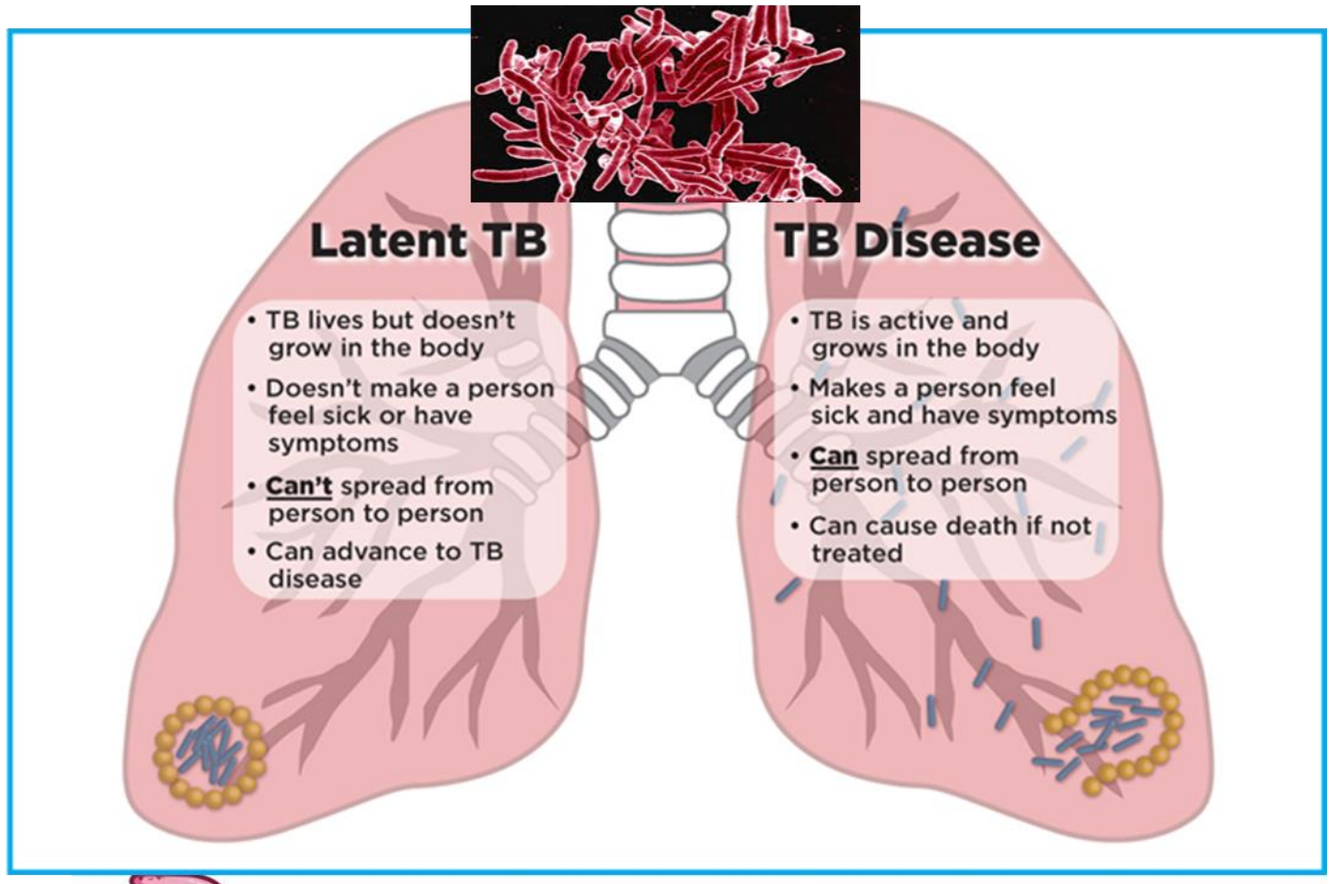
2 examples: *Mycobacterium tuberculosis* (TB) and *Mycobacterium leprae* (leprosy). **Only learn TB.**

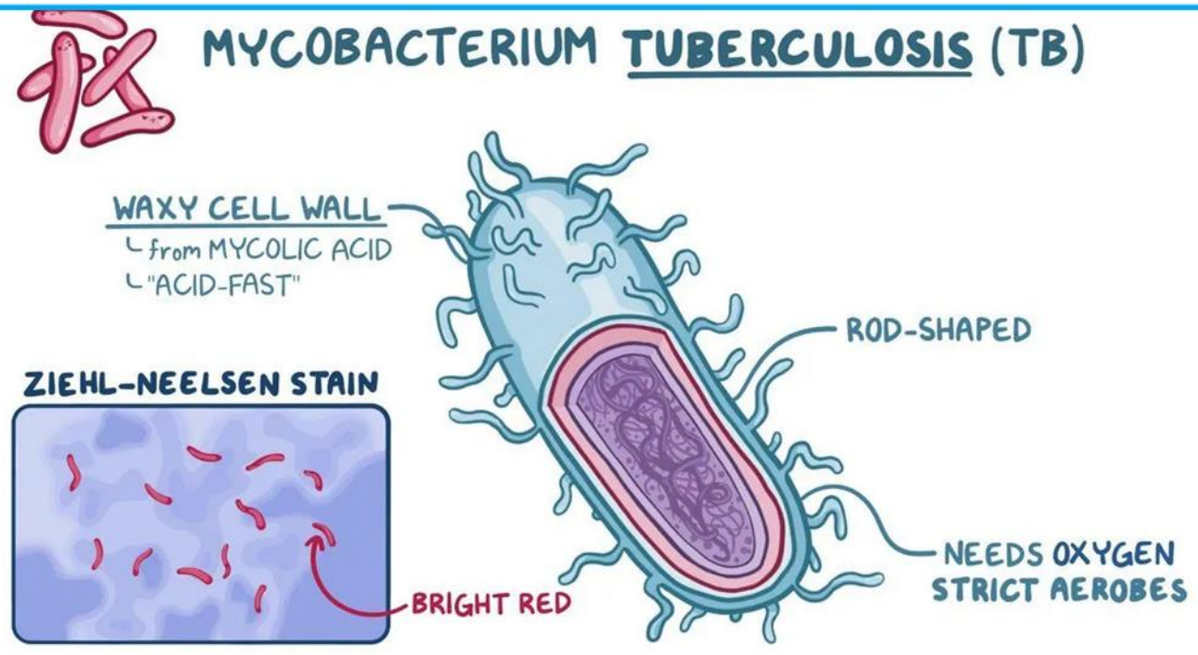
Sx of TB = night sweats, cough, haemoptysis, wt loss, malaise.

Treatments and side effects:

Isoniazid – numb/tingly extremities
Ethambutamol – ocular side effects
Rifampicin – orange/red urine
Pyrazinamide – arthralgia

Ison- I can't feel my hands
EYEthambutamol
Red and orange picin





Staining ?TB Specimens - Top exam q!

- Key point - Gram stain doesn't work for every bacteria! Other stains include Ziehl-Neelson, Loeffler's Blue, Capsule stain (India ink), flagellar stain and spore stain.

- *Mycobacterium, Actinomycetes* and the parasite *Cryptosporidium* all have waxy, lipid cell walls and do not stain well with traditional Gram method.

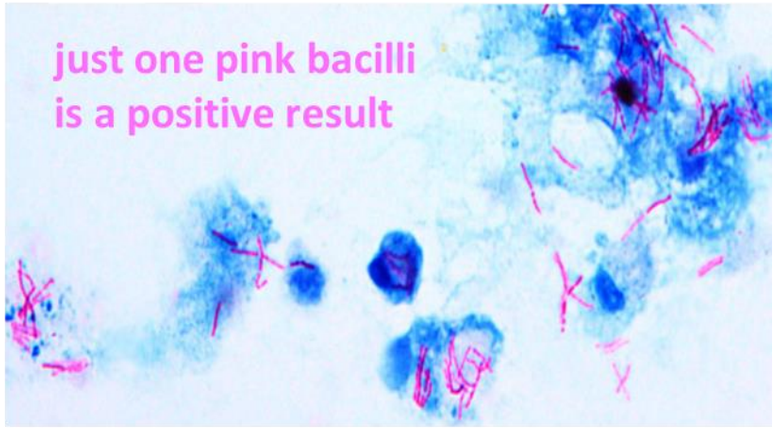
- Ziehl-Neelson stain is used instead for these microbes.

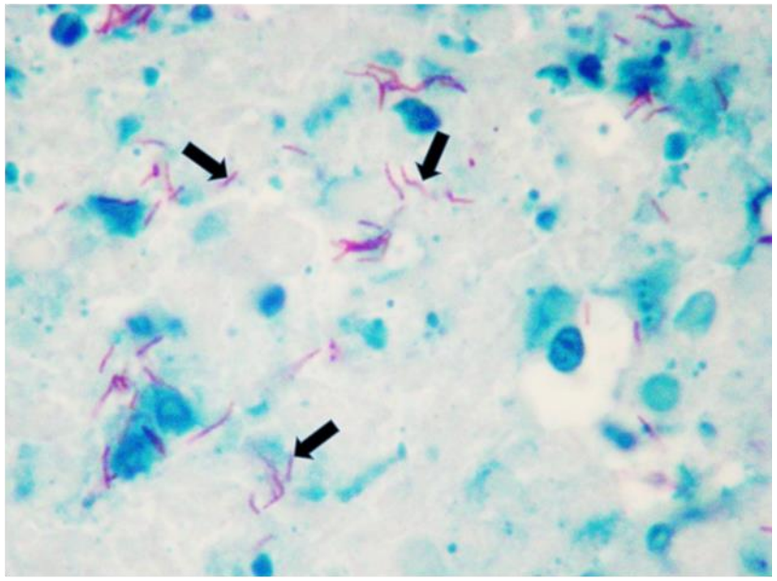
Z-N involves heating the sample with a strong dye, carbol fuchsin.

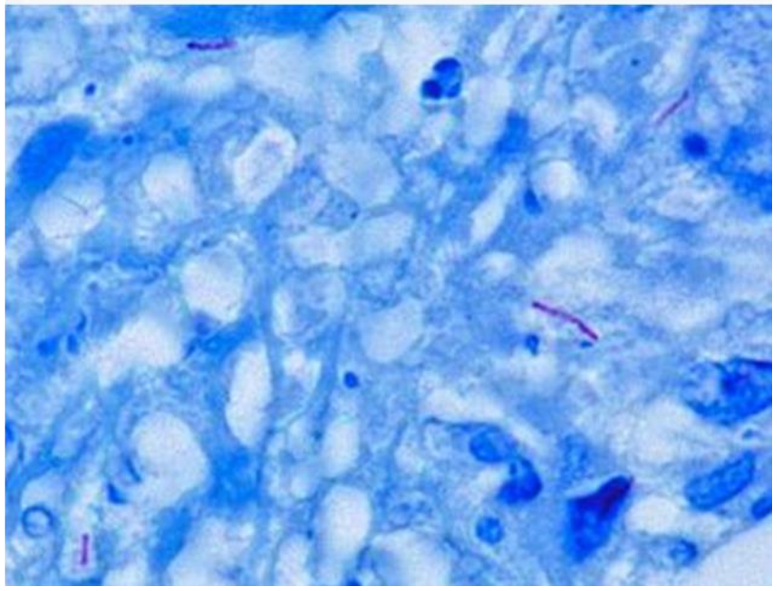
Heat makes the bacteria resistant to decolourisation by acid = acid-fast.

So when the slide is then flooded with acid, *Mycobacteria* retain the pink dye. Meanwhile everything else is decolourised to blue.

Acid-fastness is a characteristic property of all *Mycobacteria.*

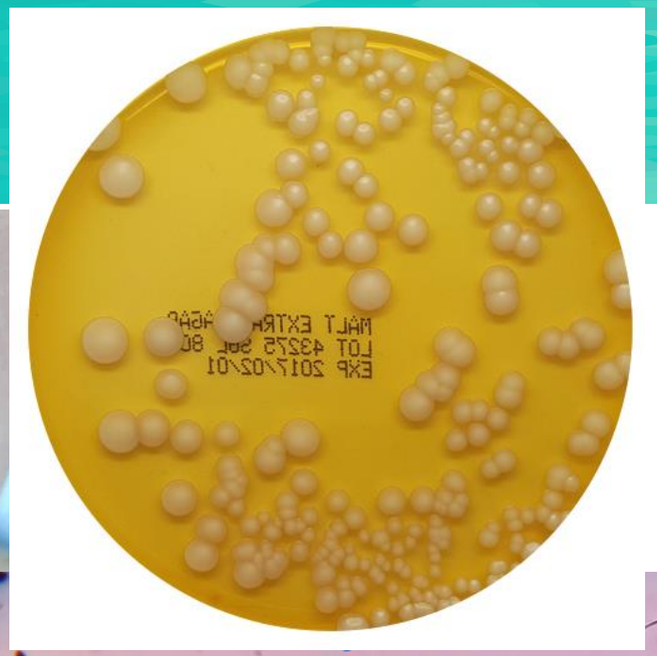






FUNGI

Fungi: Two to Know



- Eukaryotes.
- Cell wall is made of chitin and glucan.
- Clinically usually opportunistic

Two forms of fungi:

1. Yeast - single cell that divide via budding
2. Moulds - form multicellular hyphae or spores

Treatment:

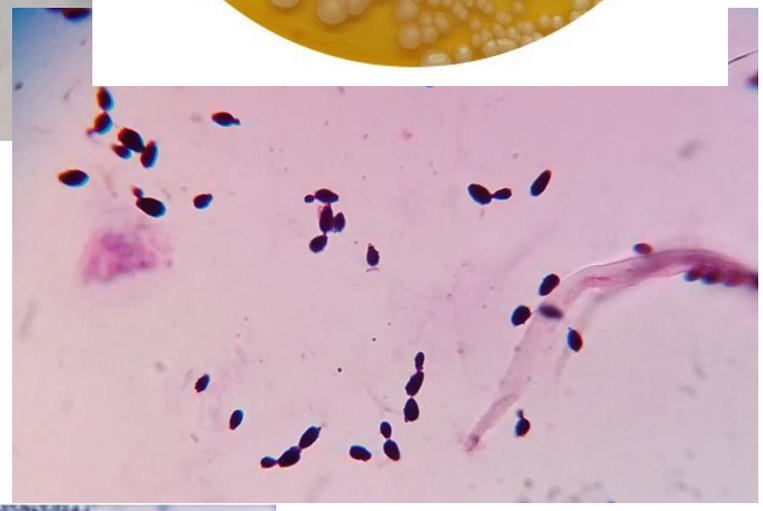
- Antifungal drugs target cell wall/ plasma membrane, don't work that well
- '-azole' drugs
- generally difficult to treat

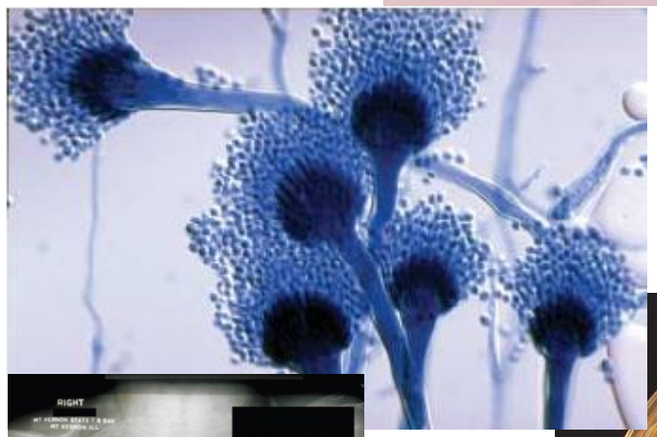
Candida albicans – most pathogenic Candida spp.

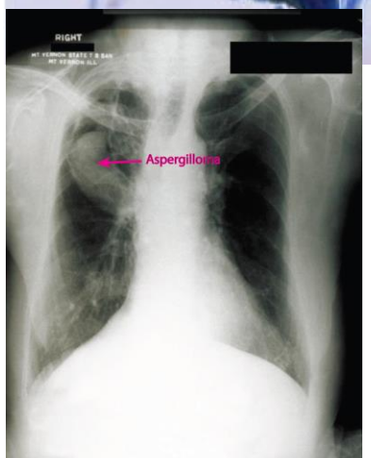
- Vaginal and oral infections, sepsis (candidiasis), line/catheter infections.
- Can kill rapidly.

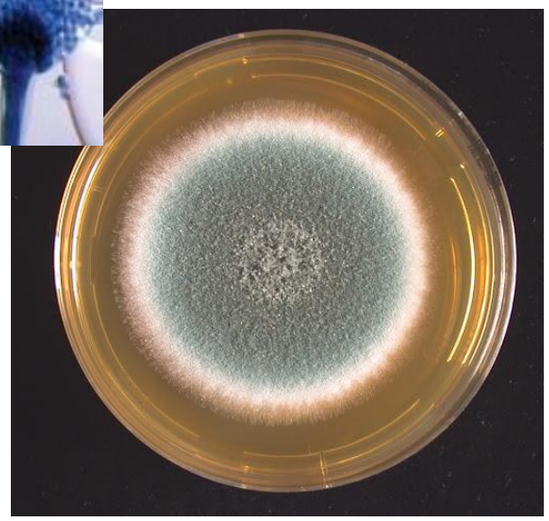
Aspergillus fumigatus – most pathogenic Asp. spp.

- Predominantly lung infections, allergic disease.
- Poor prognosis but kills slowly.





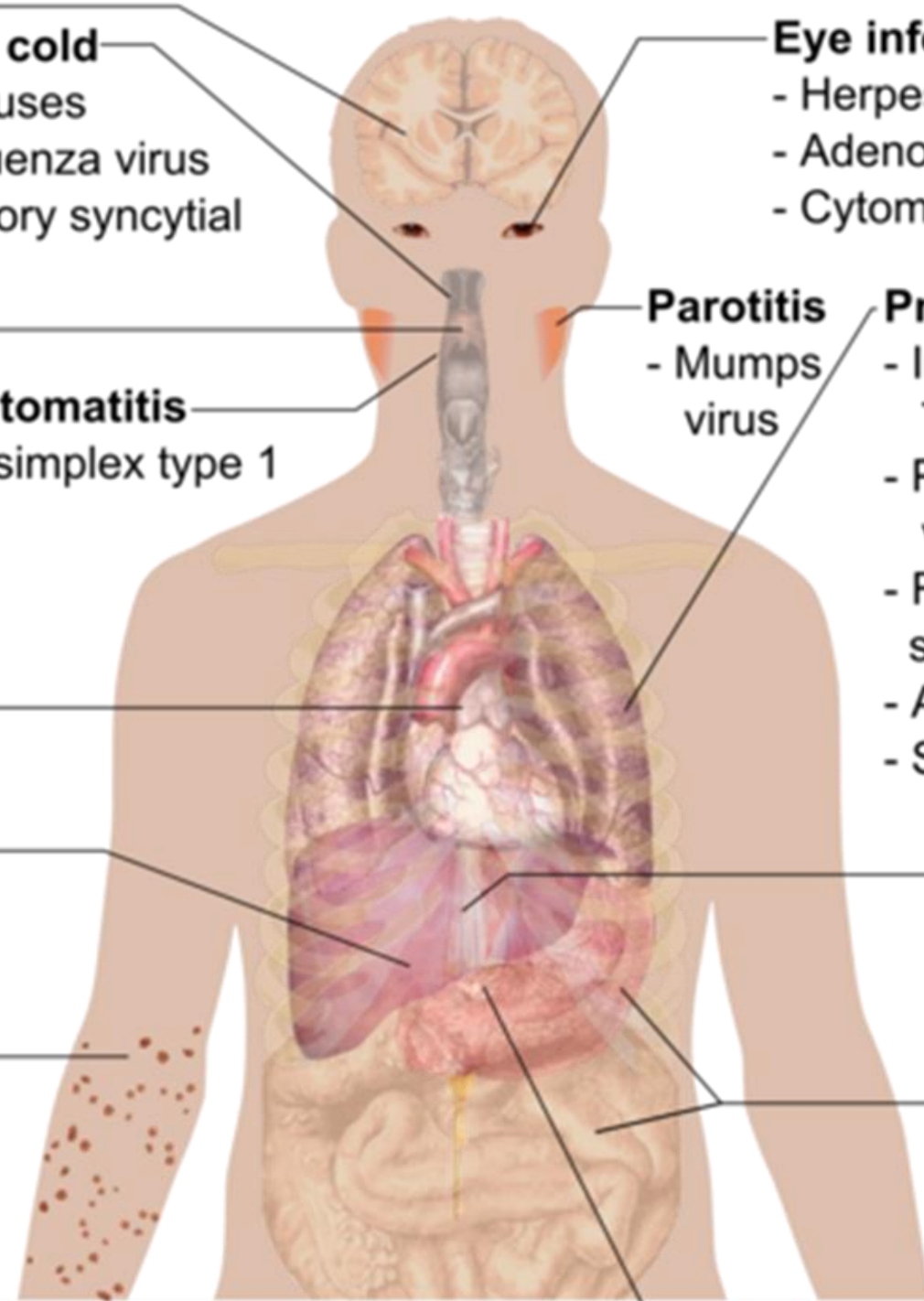




VIRUSES

Overview of

Viral infections



Encephalitis/ meningitis
- JC virus
- Measles
- LCM virus
- Arbovirus
- Rabies

Common cold
- Rhinoviruses
- Parainfluenza virus
- Respiratory syncytial virus

Eye infections
- Herpes simplex virus
- Adenovirus
- Cytomegalovirus

Pharyngitis
- Adenovirus
- Epstein-Barr virus
- Cytomegalovirus

Gingivostomatitis
- Herpes simplex type 1

Parotitis
- Mumps virus

Pneumonia
- Influenza virus, Types A and B
- Parainfluenza virus
- Respiratory syncytial virus
- Adenovirus
- SARS coronavirus

Cardiovascular
- Coxsackie B virus

Hepatitis
- Hepatitis virus types A, B, C, D, E

Myelitis
- Poliovirus
- HTLV-I

Skin infections
- Varicella zoster virus
- Human herpesvirus 6
- Smallpox
- Molluscum contagiosum
- Human papillomavirus
- Parvovirus B19
- Rubella
- Measles
- Coxsackie A virus

Gastroenteritis
- Adenovirus
- Rotavirus
- Norovirus
- Astrovirus
- Coronavirus

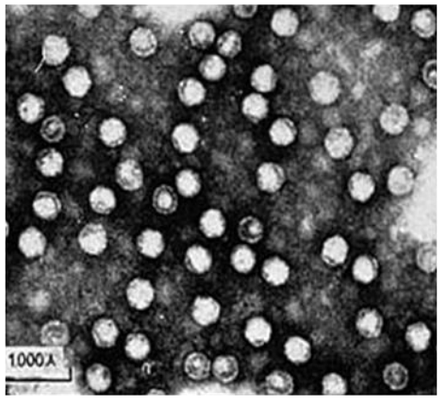
Sexually transmitted diseases
- Herpes simplex type 2
- Human papillomavirus
- HIV

Pancreatitis
- Coxsackie B virus

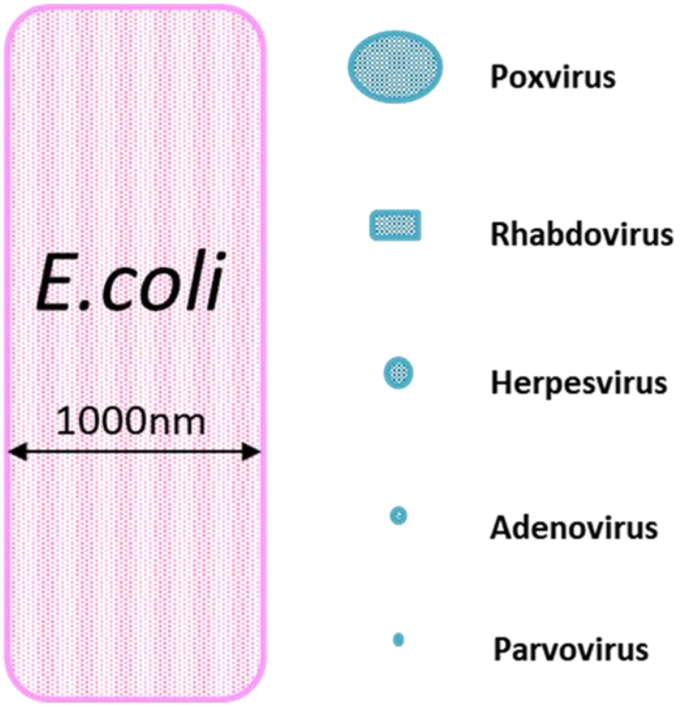
What is a Virus?

A virus is an infectious, obligate intracellular parasite, comprising genetic material (DNA or RNA) surrounded by a protein coat and/or membrane.

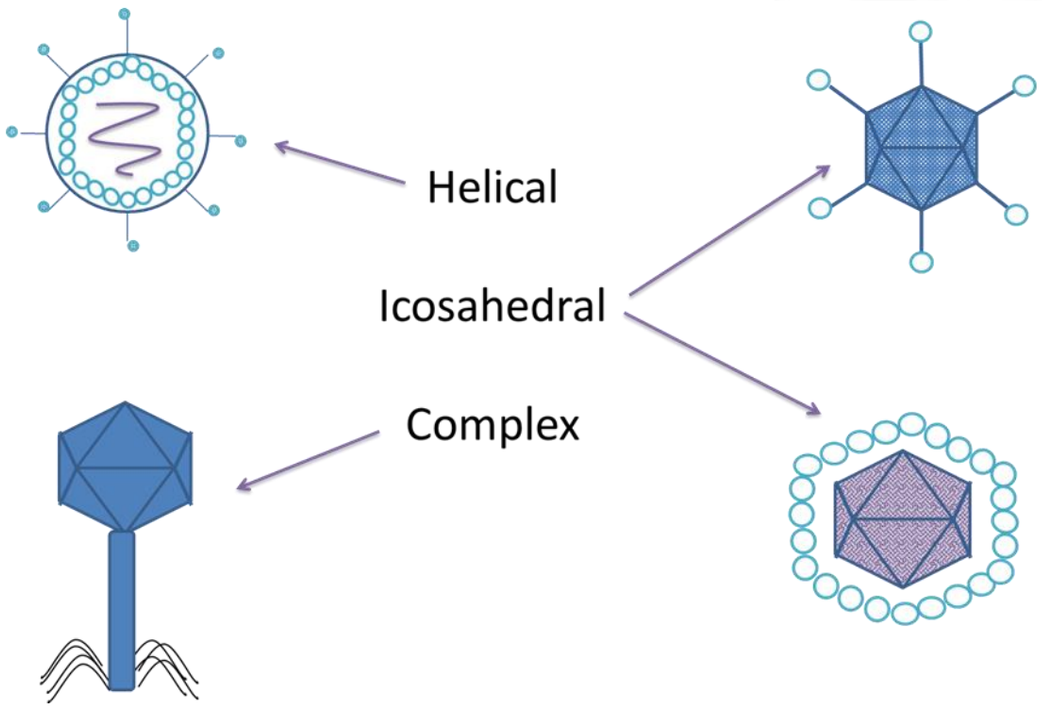
Polio – electron microscopy



Different Sizes



Different Shapes



Virus Properties

Viruses have no replication machinery and are not living, these are the main differences from bacteria:

	Virus	Bacteria
Cell wall?	✗	✓
Organelles?	✗	✓
DNA and RNA?	✗	✓
Dependant on host cell?	✓	✗
Living?	✗	✓

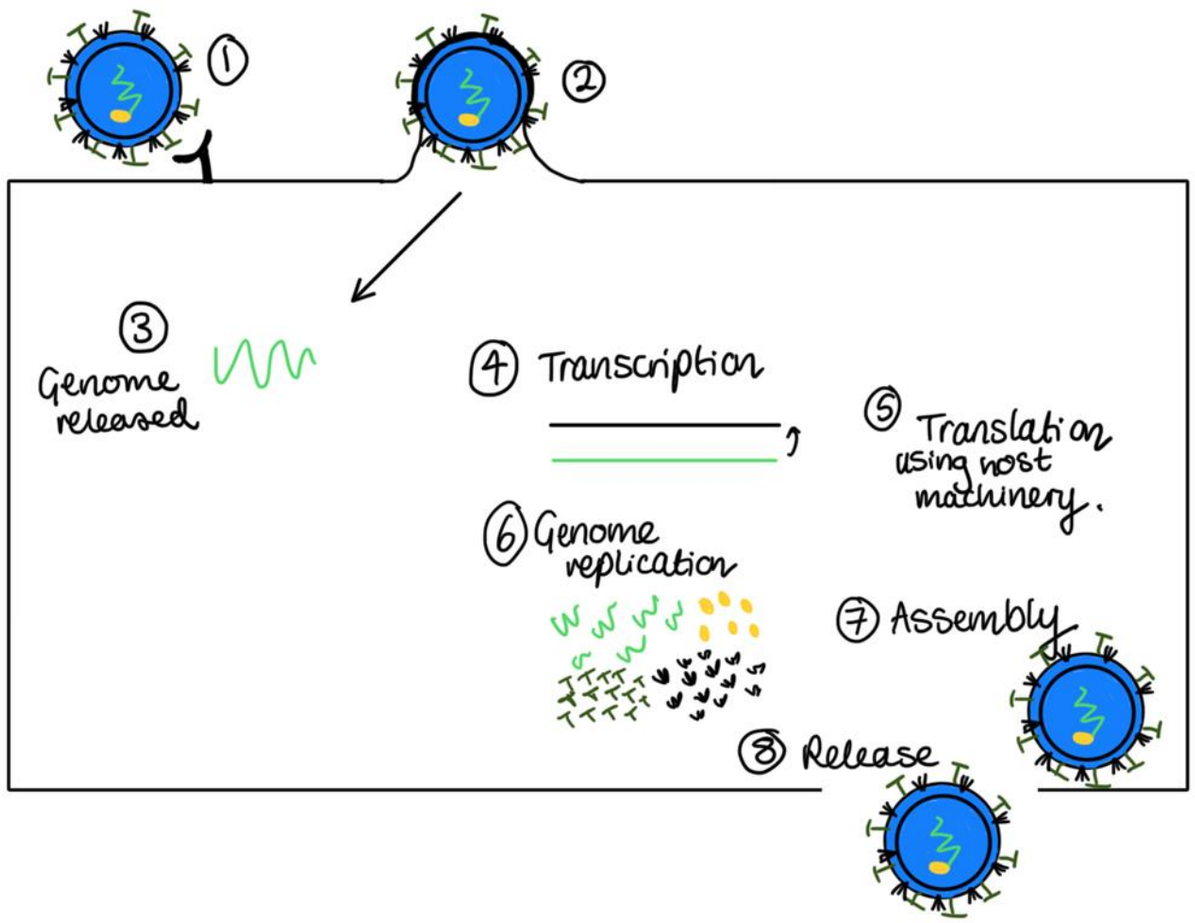
The Baltimore classification system is based on the nature of the nucleic acid and there are 7 groups:

	Genome	Examples
Group I	dsDNA	Herpes, Pox, Adenovirus
Group II	ssDNA	Parvovirus, HPV
Group III	dsRNA	Rotavirus
Group IV	ssRNA (+ sense)	Polio, Hep A, C, E
Group V	ssRNA (- sense)	Influenza, Measles, Rabies, Hep D
Group VI	ssRNA (+) with RT	HIV
Group VII	dsDNA with RT	Hepatitis B

Virus Life Cycle

Virus life cycles differ depending on which nucleic acid they use and how they attach and enter the cell. However, a generic virus life cycle looks like this:

1. Attachment
2. Entry
3. Genome released
4. Transcription
5. Translation
6. Genome replication
7. Assembly
8. Exit



How do viruses cause disease?

Direct destruction of host cells

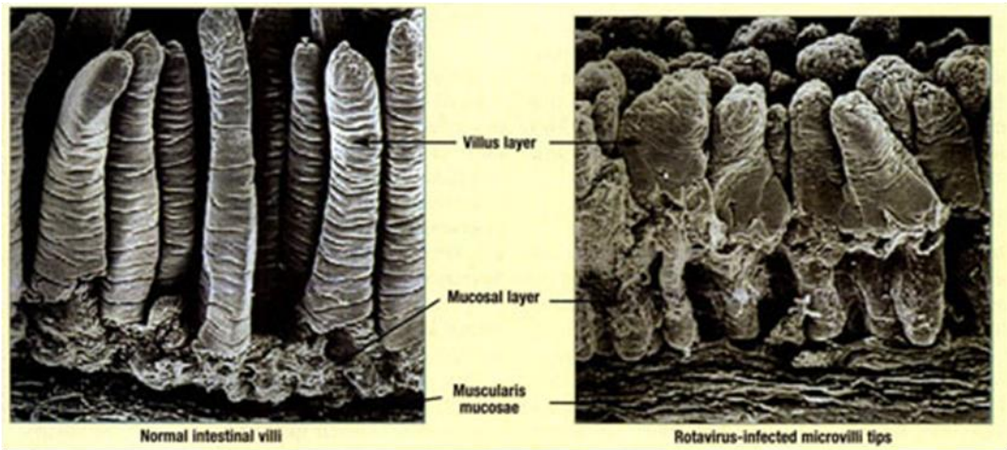
poliovirus causes host cell lysis and death after a viral replication period of 4 hours

Lysis of neurons = paralysis

HIV lyses CD4 cells

Modification of host cell

rotavirus atrophies villi and flattens epithelial cells, this means sugars can't be absorbed properly and a hyperosmotic state leads to diarrhoea

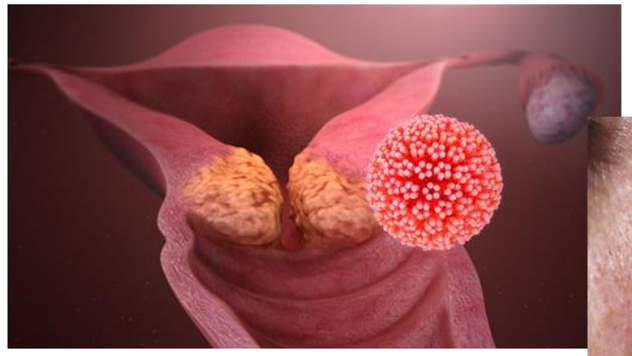


"Over-reactivity" of immune system

The immune response to HBV during chronic HBV infection results in low-level liver cell destruction leading to cirrhosis

Damage through cell proliferation

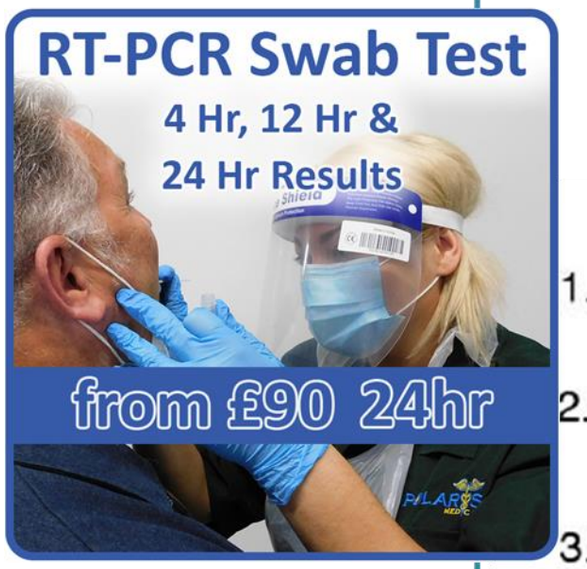
human papillomavirus integrates into cervical endothelial cells, expresses oncoproteins which can lead to over proliferation of cells and cervical cancer



Evasion of host defences

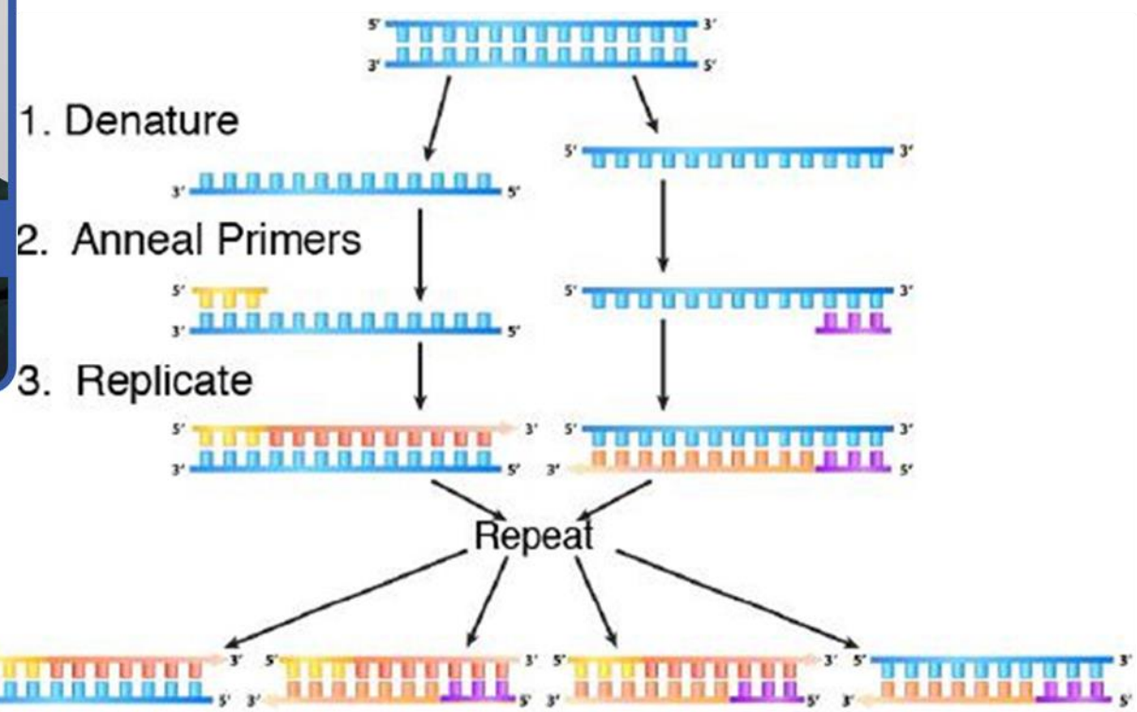
varicella zoster virus can lay dormant after chickenpox infection and reactivate years later as shingles, due to successful latency via evasion

Diagnosing Viral Infections



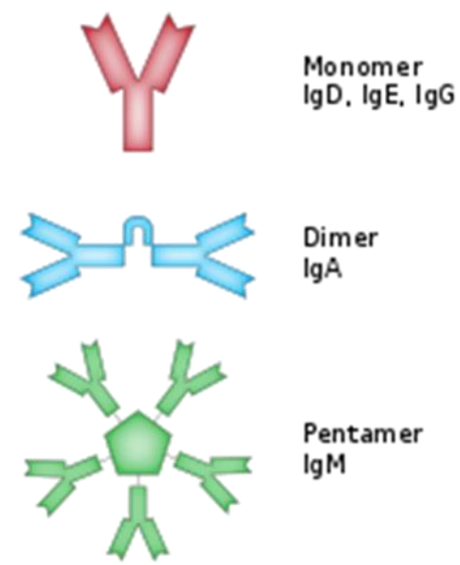
PCR

Is viral genetic material present?

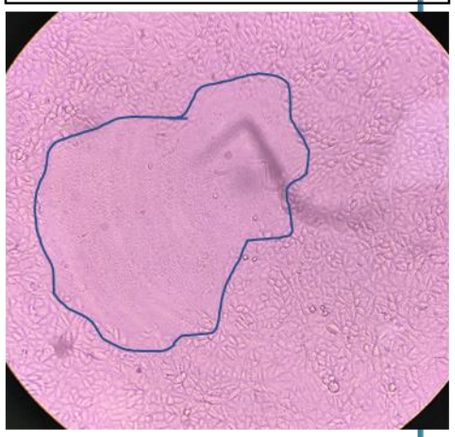


Serology

Is there immune memory to a virus? i.e. exposure

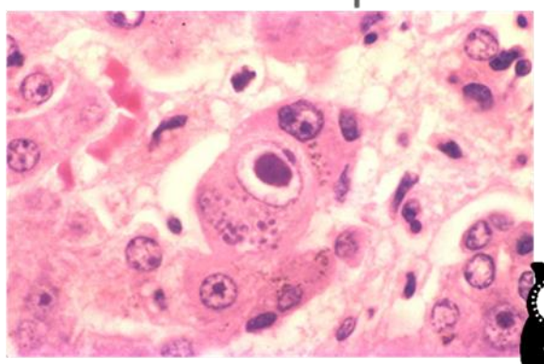


Measles – cytopathic effect, a syncytia (outlined)



Histopath.

Are there any features of viral infection present?



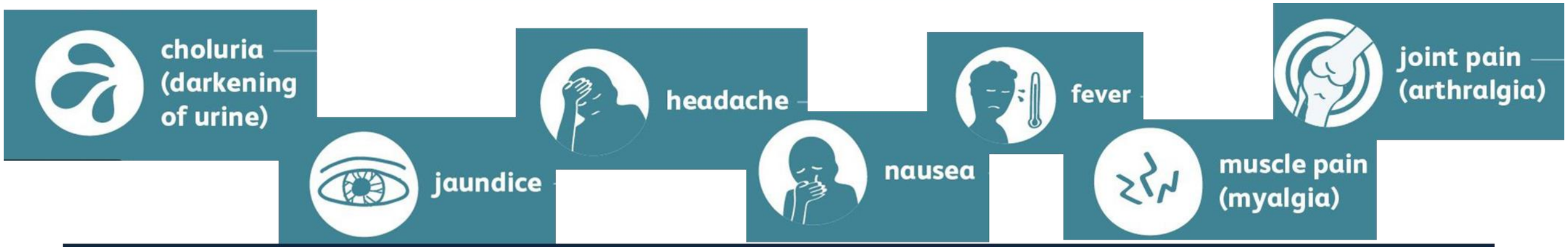
(cytomegalovirus)

Limited Clinical Use:

- Viral culture + light microscopy
- Electron Microscopy

→ Too much time
→ Too much effort
→ Too expensive

Hepatoviridae - exam q fodder!

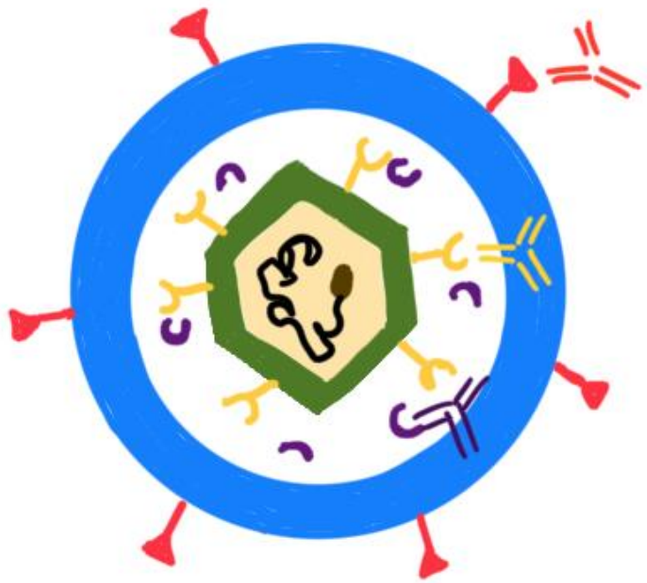


Type of Hepatitis

	A	B	C	D Dependent on B	E
Source of virus	faeces	blood/ blood-derived body fluids	blood/ blood-derived body fluids	blood/ blood-derived body fluids	faeces
Route of transmission	faecal-oral	percutaneous permucosal	percutaneous permucosal	percutaneous permucosal	faecal-oral
Chronic infection	no	yes	yes	yes	No/yes?
Prevention	pre/post-exposure immunization	pre/post-exposure immunization	blood donor screening; risk behavior modification	pre/post-exposure immunization; risk behavior modification	ensure safe drinking water

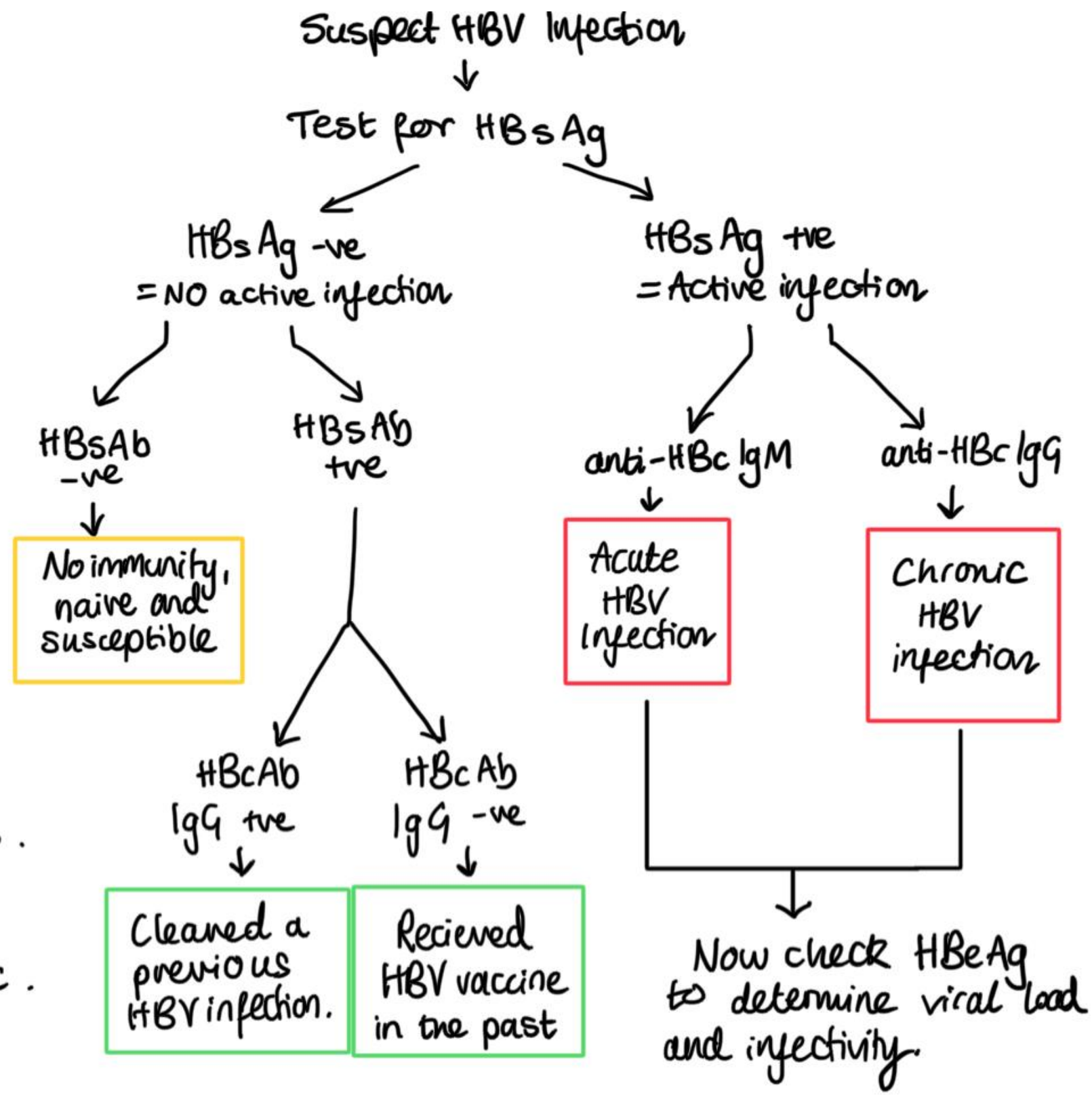
Hepatitis B Serology

HBV virion and antibodies



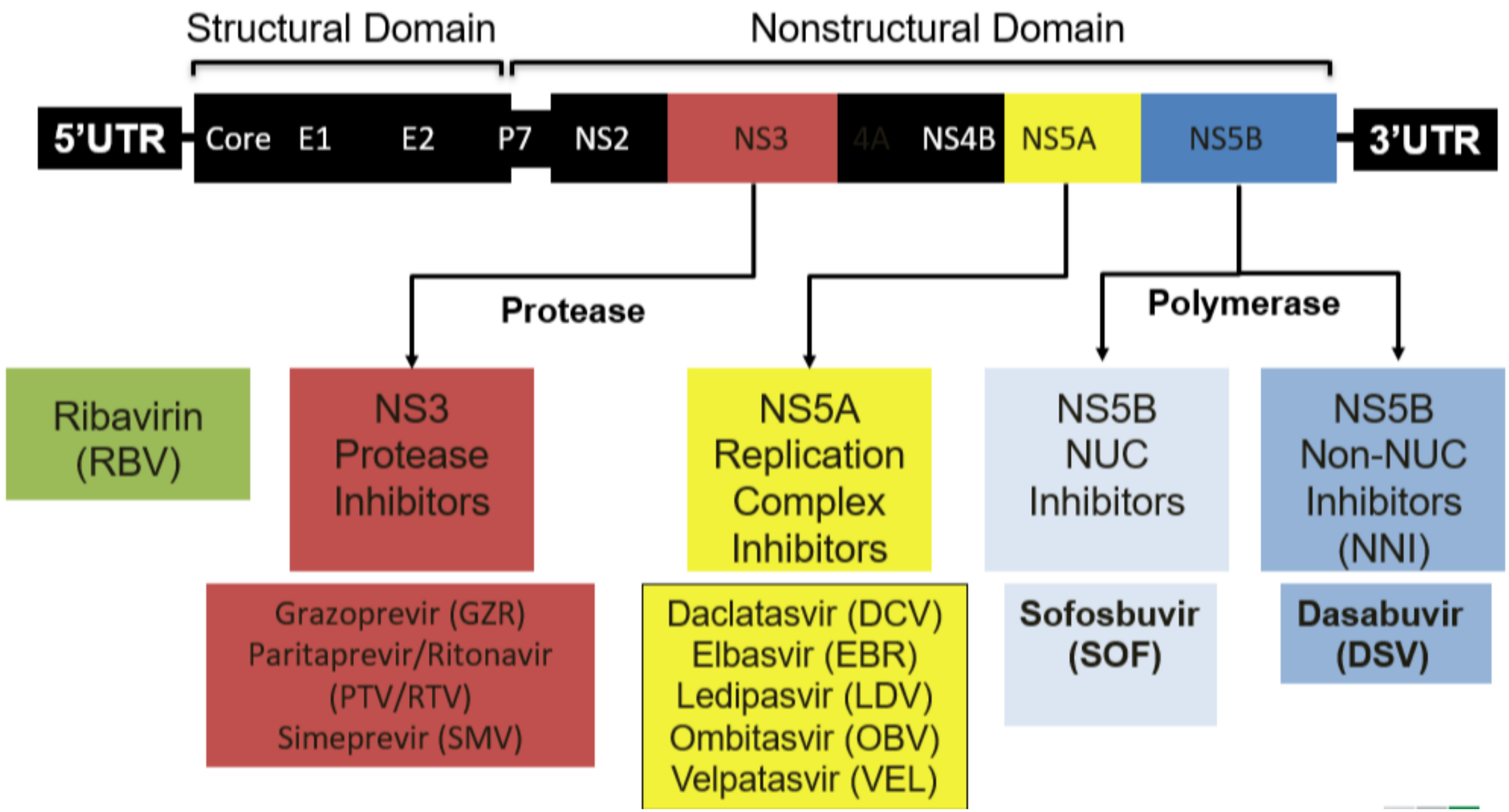
Key:

- = Surface Antigen, HBsAg.
- = E Antigen, HBeAg.
- = Core Antigen, HBcAg.
- = Surface Antibody, anti-HBs.
- = E Antibody, anti-HBe.
- = Core Antibody, anti-HBc.



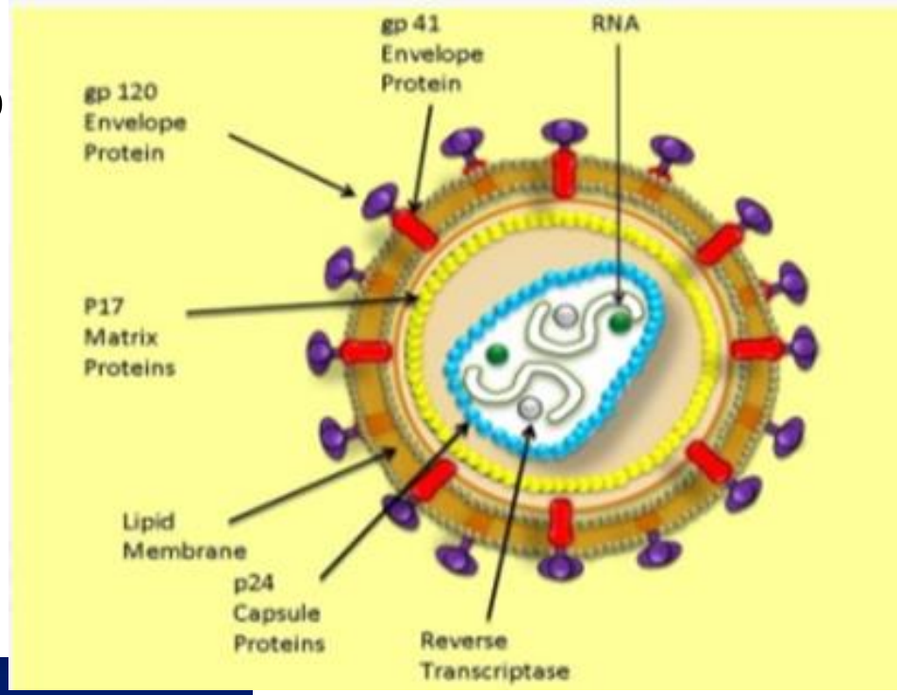
Hepatitis C is treated with Direct Acting Antivirals

Approved DAAs From Multiple Classes: Basis of 2016 Combination HCV Regimens



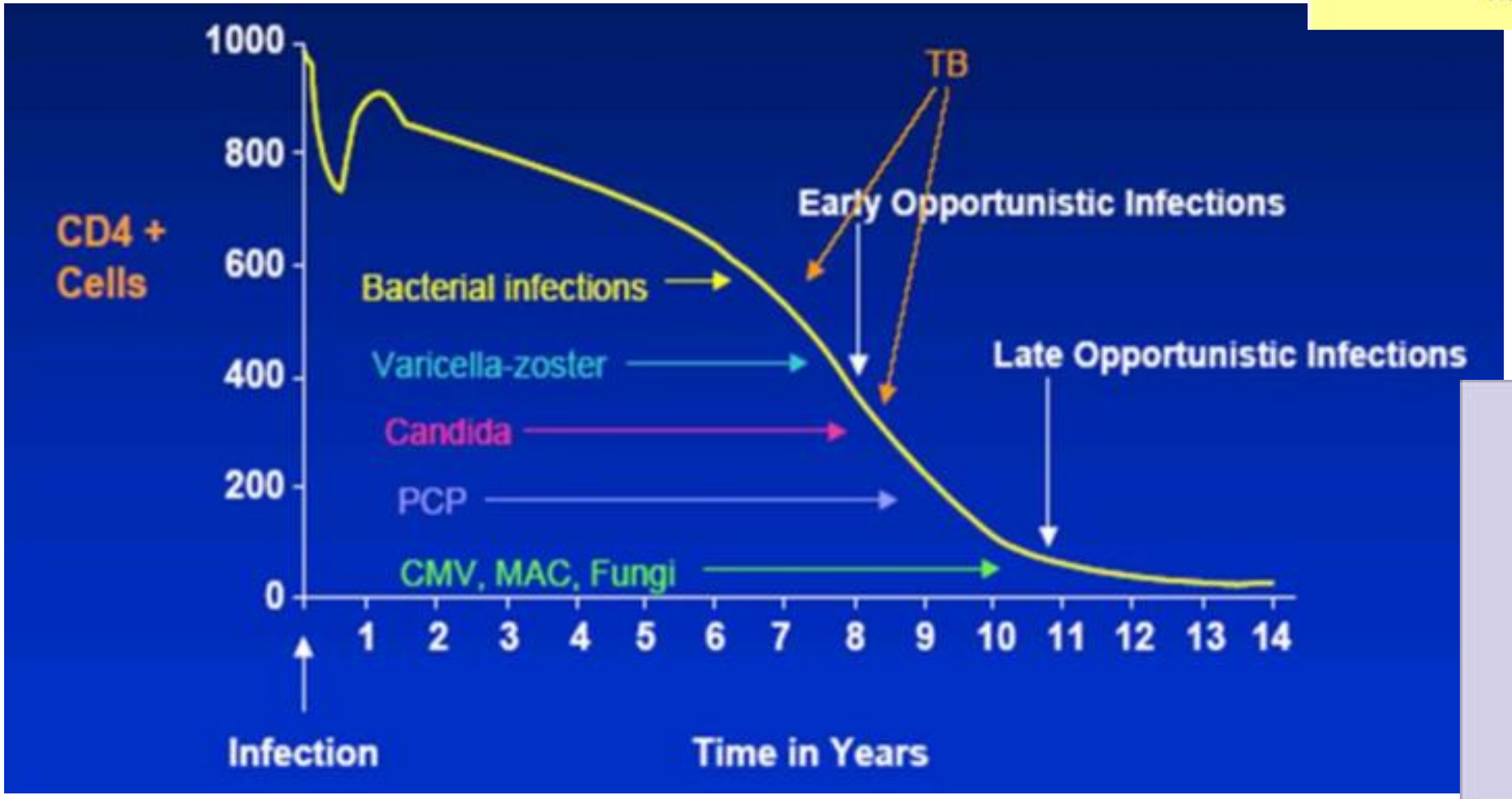
Clinical Picture: HIV

HIV is a member of the retrovirus family. Retrovirus basically means it encodes a reverse transcriptase for an extra life cycle step where RNA is transcribed into DNA, a protease and integrase.



Infects CD4+ T cells and macrophages. Transmission = bodily fluids.

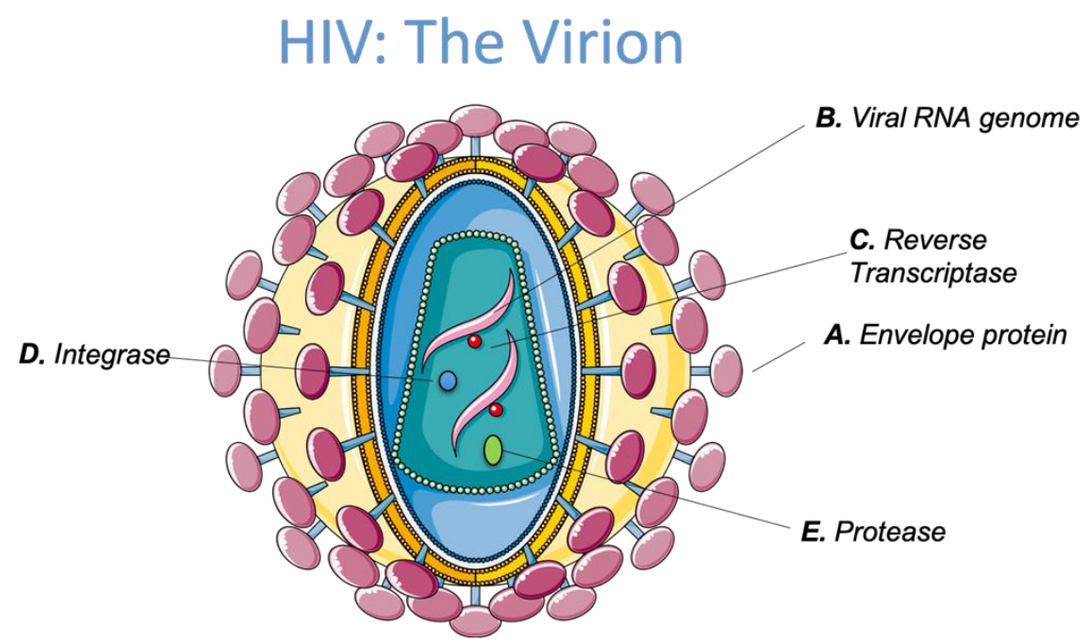
Sx: 50% have initial non-specific malaise. Then mainly asymptomatic until CD4+ drops so low that opportunistic infections occur. Maintain high index of suspicion.

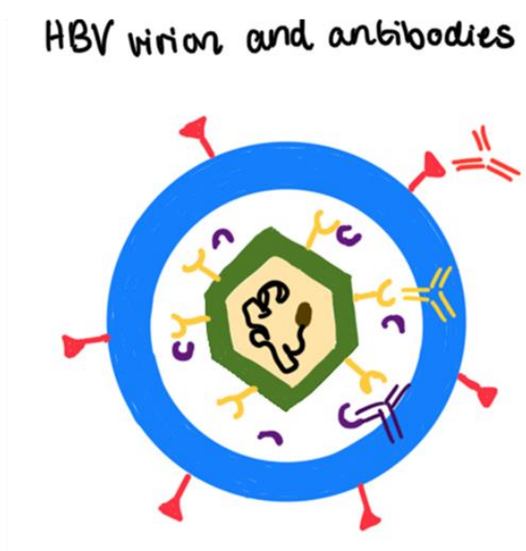


Don’t get confused by the drug acronyms…

Highly acting anti-retroviral therapies (HAART) are for HIV infection.

Direct acting antivirals (DAA) are for Hepatitis C Infection.





Treating severe viral infections – Herpes viral sepsis

"Medics treating Ms Sampson and Mrs Mulcahy assumed they were suffering from a bacterial infection and didn't prescribe the anti-viral medication that may had saved their lives."

IV Acyclovir

NEWS

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Health

New mothers who died of herpes could have been infected by one surgeon

By James Melley & Michael Buchanan
BBC News

22 November

thebmj covid-19 Research Education News & Views Cam

News

Sepsis guidance may change as result of deaths of two women from herpes infection after giving birth

BMJ 2021 ; 375 doi: https://doi.org/10.1136/bmj.n2881 (Published 23 November 2021)
Cite this as: *BMJ* 2021;375:n2881

Article | Related content | Metrics | Responses

Gareth Iacobucci

Author affiliations

The BMJ

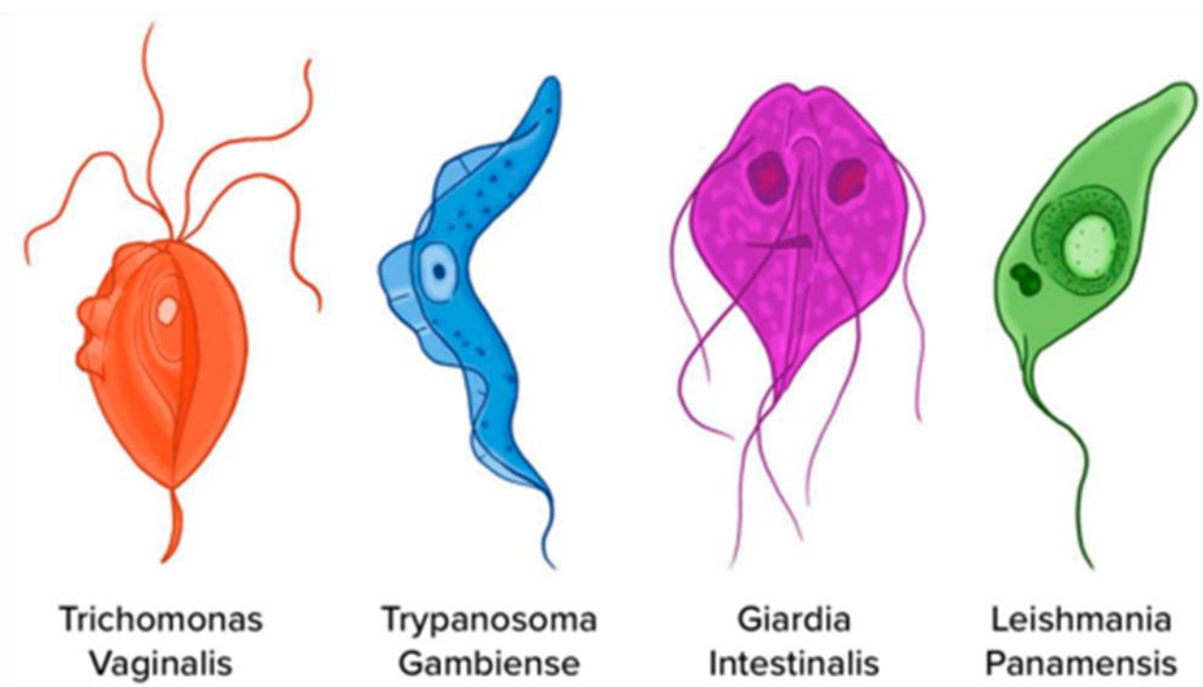
Clinical advice on how to treat new mothers with sepsis could change after two women died of a postpartum herpesvirus infection.

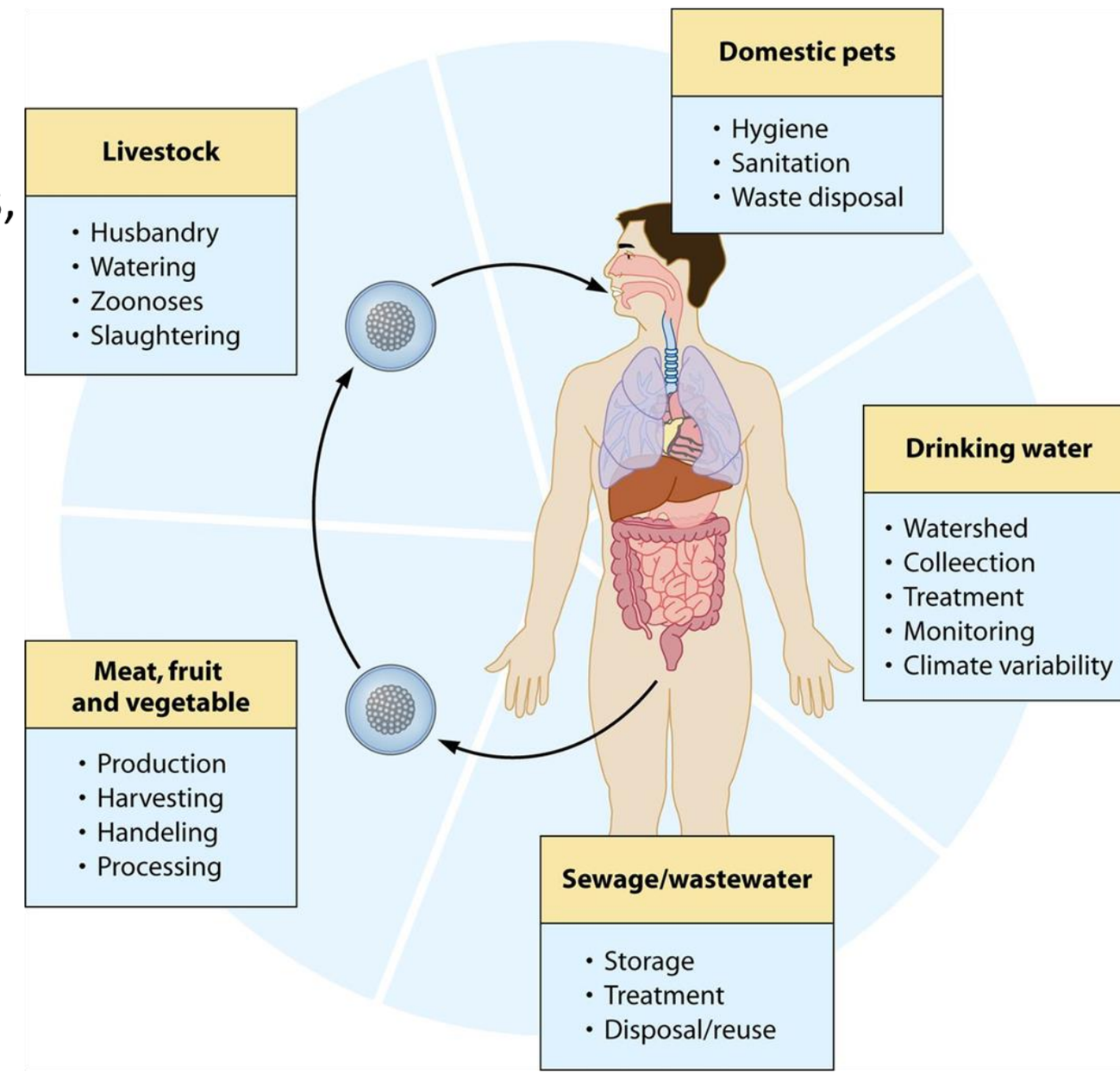
BMJ article about case - https://www.bmj.com/content/375/bmj.n2881.full

PROTOZOA

Protozoa

- Microscopic unicellular eukaryotes
- Can be free living OR parasitic
- Classification based on movement: ameoboids, ciliates, sporozoan, flagellates.
- Examples are malaria, *Giardia lamblia*, *Toxoplasmosis* spp., *Trichomonas vaginalis*.
- **2a need-to-know** = malaria



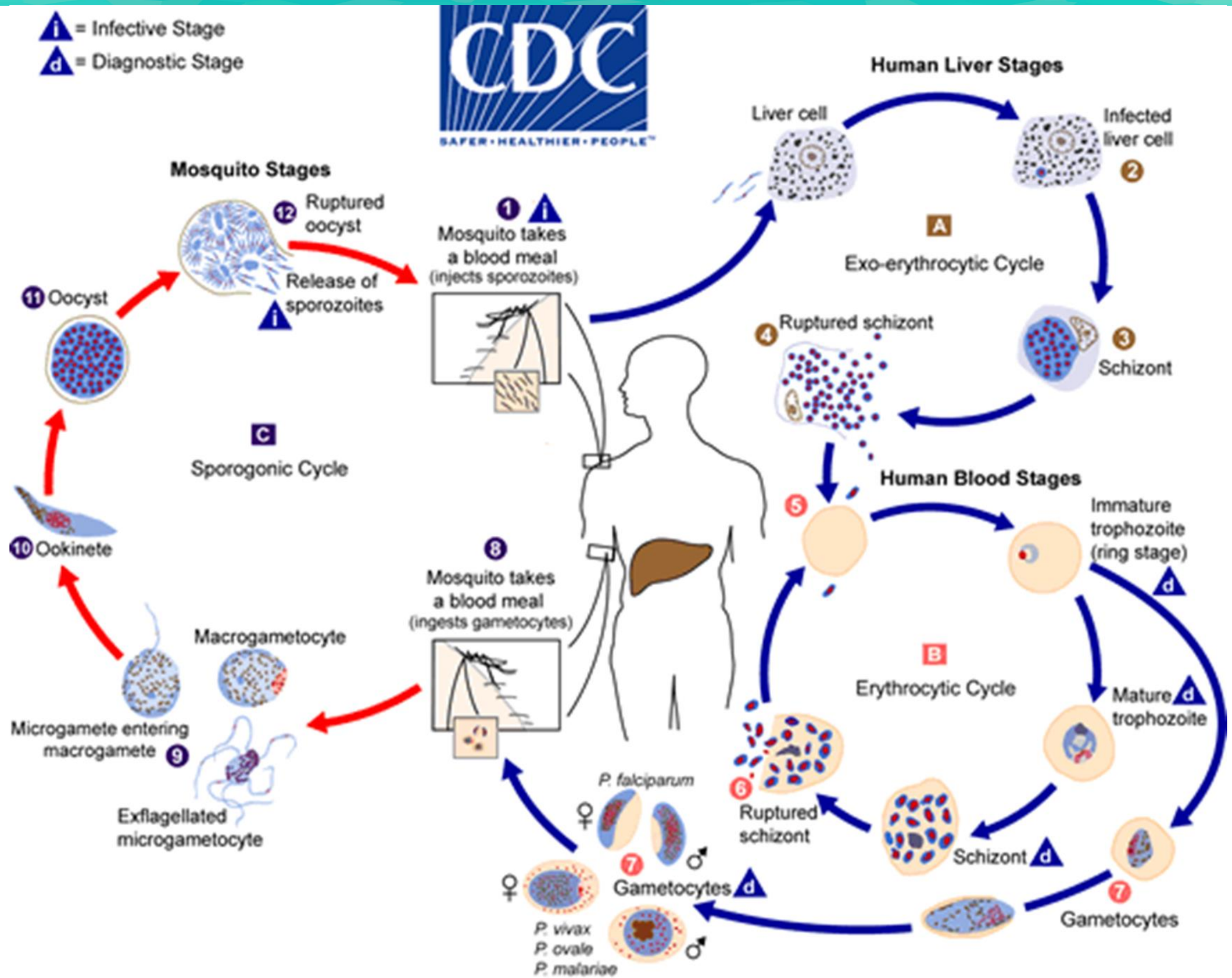


Malaria

Not essential to learn whole cycle, just know:

- vector is *Anopheles*
- host = humans and animals
- spread in blood
- infects liver and RBCs
- some species can go dormant.

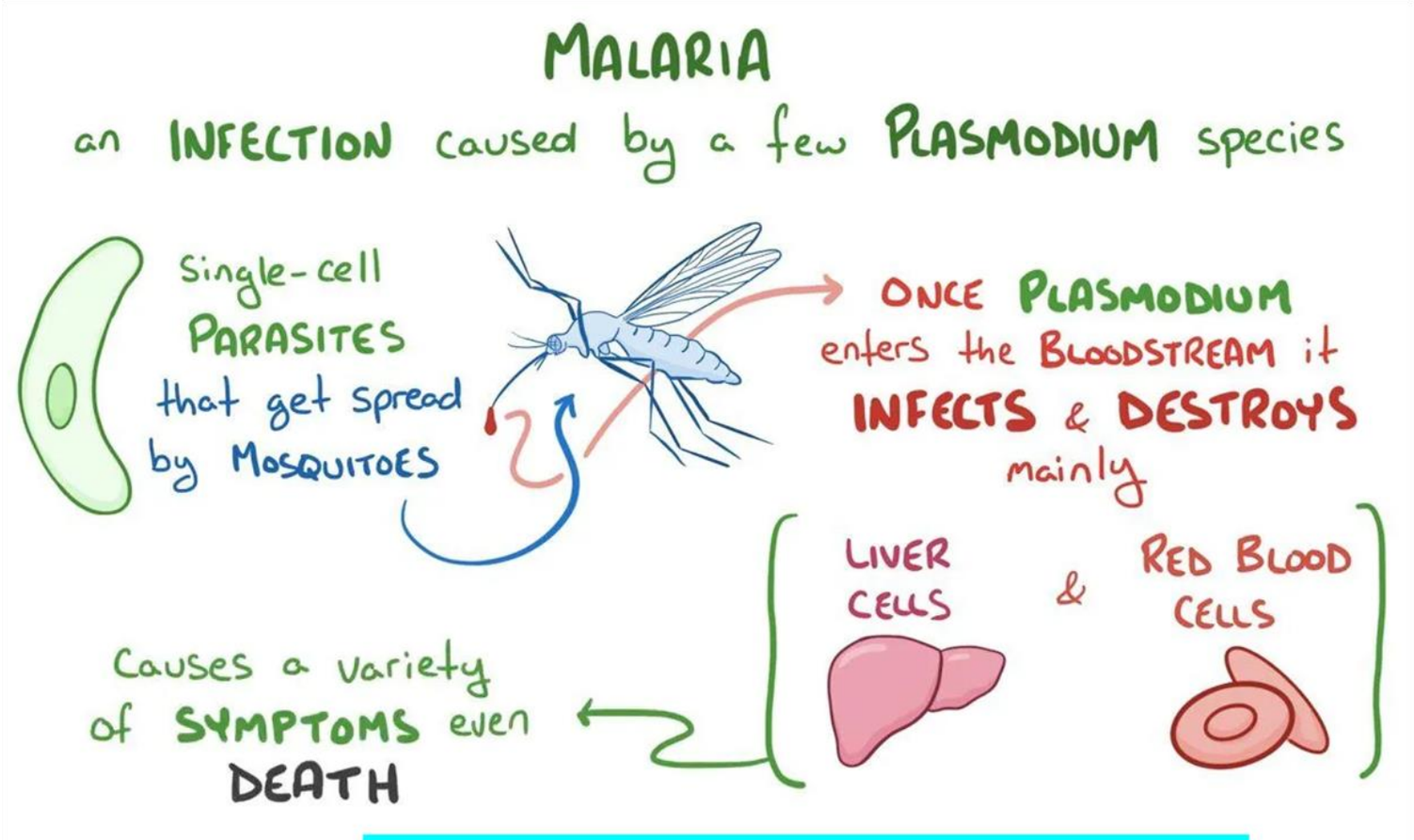
Life cycle written simple explanation is in the text box of the slide. Too complex for this presentation!



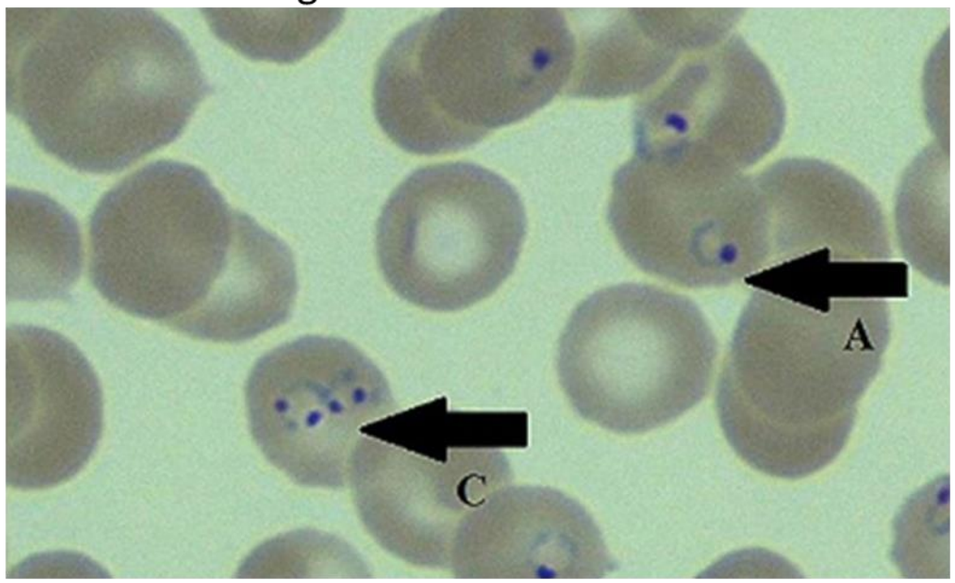
Clinical Picture: Malaria

Non-specific symptoms such as fever, chills, sweats, headache, and myalgia.
Hx of recent travel, +/- Hx of insect bite or non-compliance with prophylaxis.
Treat with oral chloroquine for uncomplicated and IV artesunate for complicated.

Bite from an infected *Anopheles* mosquito



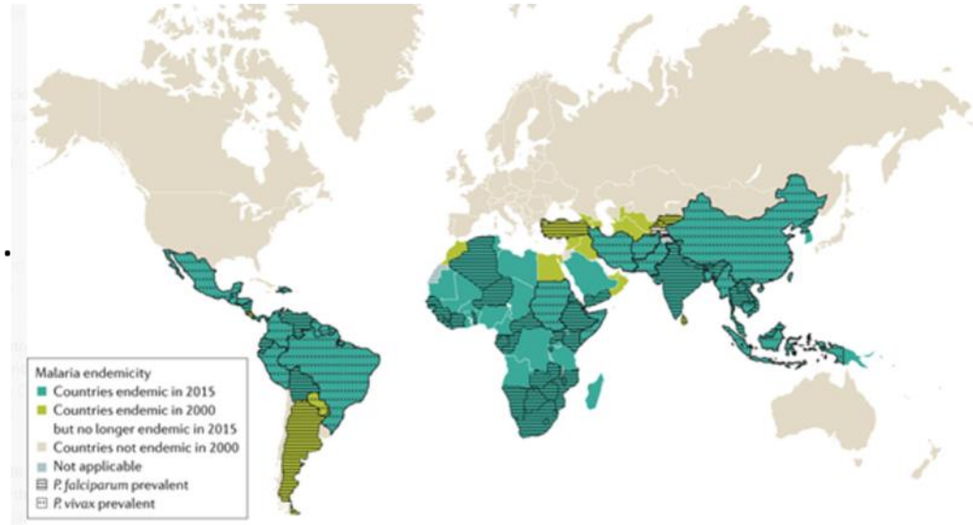
Giemsa-stained thin and thick blood film smear remains the diagnostic test of choice.



5 Malaria species (BLUE can go dormant in liver causing relapse)

1. *P falciparum* **most common** species, widespread in Sub-Saharan Africa, Southeast Asia, Oceania, South America.
2. *P malariae* has similar distribution to *P. falciparum* but lower prevalence.
3. *P vivax* found in Asia, Americas, Eastern Europe, and North Africa. 80% cases occur Ethiopia, India, and Pakistan.
4. *P ovale* is found in Africa and the West Pacific.
5. *P knowlesi* is found in certain forested areas of Southeast Asia.

Endemic areas of the world



Feedback Form – please fill in thank you!

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Mock Questions

Question 1

Which of these is an example of Group A streptococci?

a) S. pneumoniae
b) S. agalactiae
c) S. pyogenes
d) S. viridans
e) S. mitis

Question 1 –Answer and Explanation

c) Streptococcus pyogenes

Explanation:

The grouping referred to here is Lancefield latex grouping for Streptococcal bacteria.

Group A Streptococcus (GAS) consist of one species, *Streptococcus pyogenes*. *Strep pyo* is particularly virulent and causes severe infection.

Presents clinically with rapid spread and highly invasive infection. Infections are toxin-mediated, so patients often require organ support. Clindamycin is often added into antibiotic cover, as it has good antitoxin activity.

S. Pneumoniae ->
S. agalactiae
S. pyogenes
S. viridans
S. mitis

Question 2

A 28-year-old homeless man attends A and E with a 3-month history of night sweats, weight loss and a persistent, productive cough. For the last week he has also begun coughing up bright red blood. Which of these stains would you use on his sputum sample?

a) Loeffler's methylene blue
b) Spore stain
c) Capsule stain (India ink)
d) Giemsa stain
e) Ziehl-Neelson stain

Question 2 –Answer and Explanation

e) Ziehl-Neelson stain

Explanation:

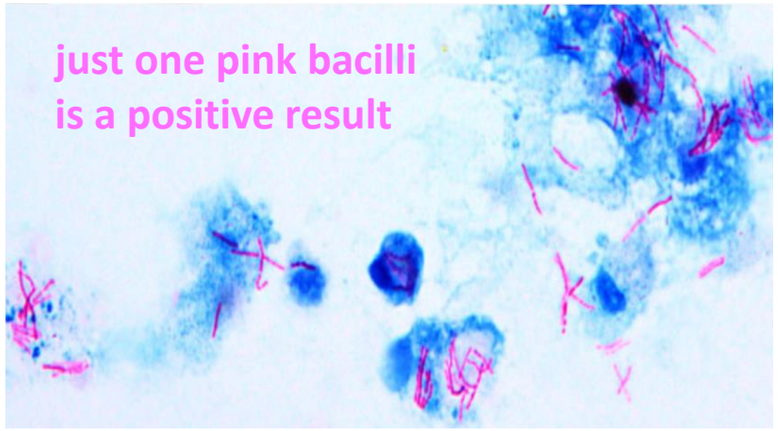
Night sweats, weight loss, a cough and haemoptysis is classic history of TB. Epidemiology – TB is more common in people from certain geographical areas where TB is endemic, and also people living in poor quality or overcrowded housing where TB can spread through prolonged, close contact. As this man is homeless he may be living in such accommodation so this was also a factor that may indicate ?TB.

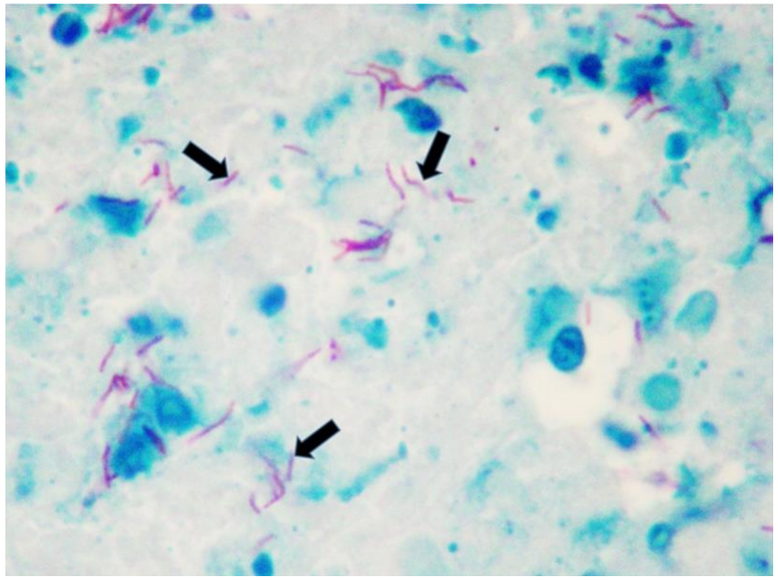
Remember Mycobacteria have a waxy, lipid cell wall that is impenetrable to the Gram stain.

Z-N stain lets us visualise *Mycobacteria* by using heat to drive a dye called carbol fuchsin inside the cell wall. This makes the Mycobacterium resistant to decolourisation with acid, so they are said to be “acid-fast”.

Interpretation of Z-N stain:

Positive Mycobacterium = pink bacilli (just one is enough).
The background of the stain e.g. sputum is blue after decolourisation with acid.





Question 3

The man is diagnosed with TB and commenced on standard treatment regimen. One week later he attends an urgent same-day GP appointment. He is very worried as he has been passing orangey-red urine. Which of his new drugs is likely causing this side effect?

a) Rifampicin
b) Isoniazid
c) Retinoic acid
d) Nitrofurantoin
e) Pyrazinamide

Question 3 –Answer and Explanation

Answer: a) Rifampicin

TB treatments and side effects:

Isoniazid – numb/tingly extremities
Ethambutamol – ocular side effects
Rifampicin – orange/red urine
Pyrazinamide – arthralgia

Ison- I can’t feel my hands
EYEthambutamol
Red and orange picin

Rifampicin urine sample.

Nitrofurantoin causes neon yellow urine sample.

Question 4

Staphylococcus aureus gives a positive result with the coagulase test, true or false?

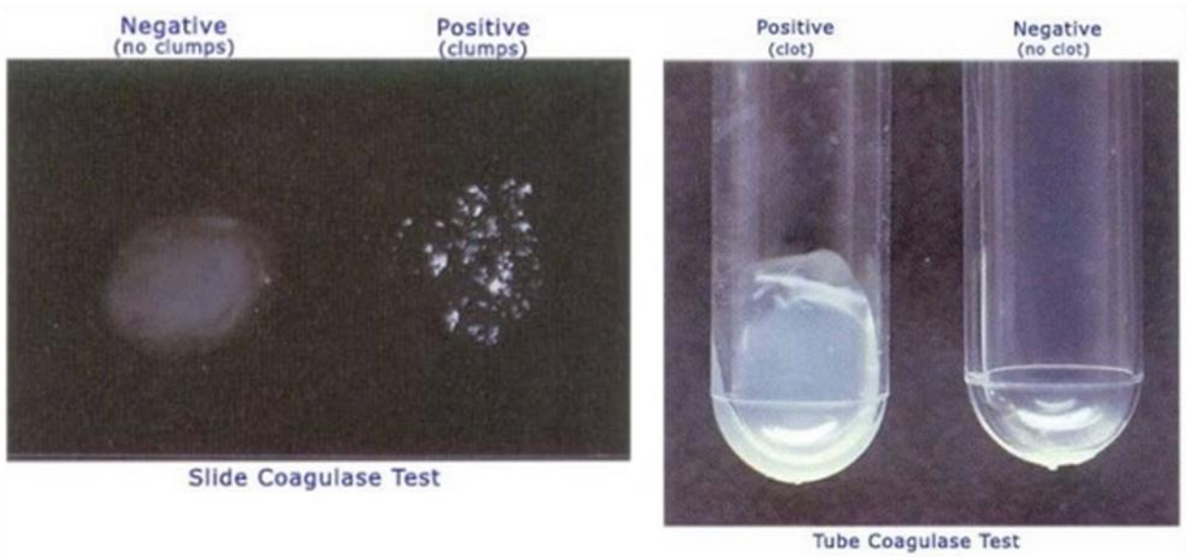
Question 4 –Answer and Explanation

The answer is TRUE.

The coagulase test differentiates *Staph aureus*, which produces the enzyme coagulase, from *S. epidermis* and *S. saprophyticus*, which do not produce coagulase. i.e Coagulase Negative Staphylococcus (CONS).

Staph aureus = Coagulase +ve
Coag neg staph (epi and sapro) = Coagulase –ve

Method: apply rabbit plasma to a small sample of pure colony. Observe for fibrin clot clumps.



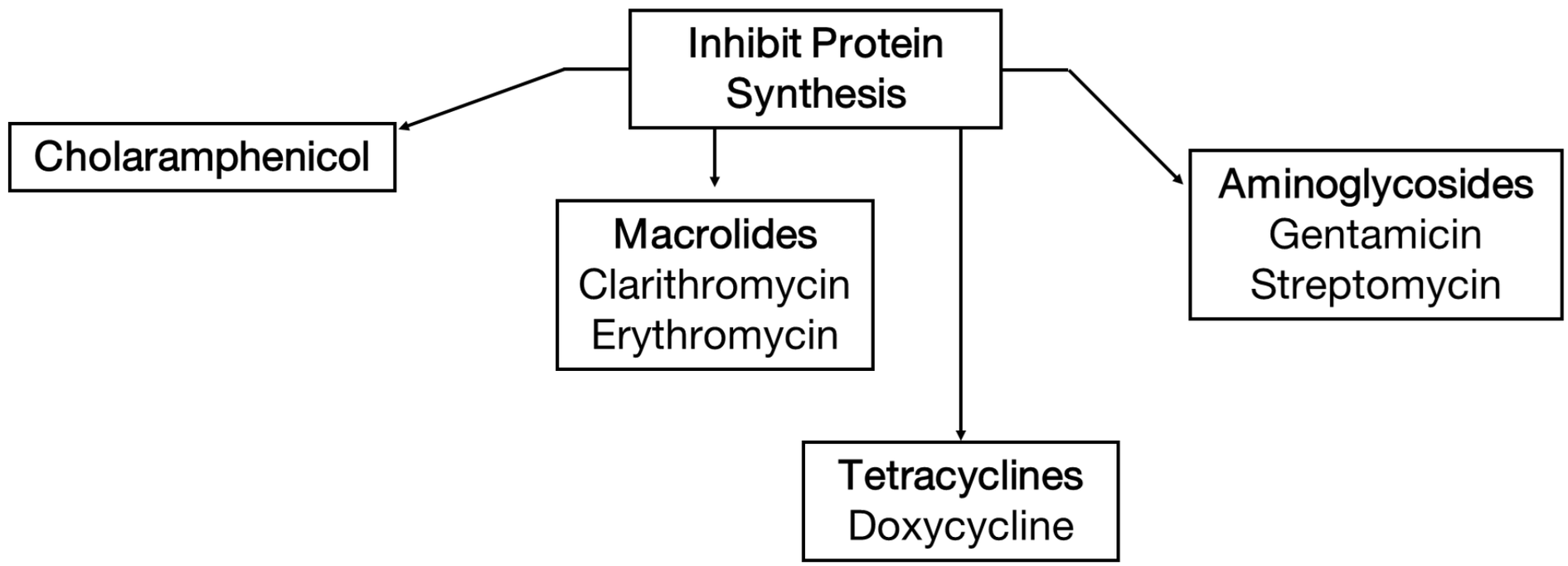
Question 5

Which class of antibiotic does NOT target bacterial cell wall synthesis?

a) Glycopeptides
b) Macrolides
c) Cephalosporins
d) Penicillins
e) Carbapenems

Question 5 –Answer and Explanation

Answer: B) Macrolides



Handy Hint
We generally use macrolides instead of penicillin for those with a penicillin allergy

Question 6

Which two species of malarial parasite can persist in humans long-term without proper treatment?

a) *P. knowlesi* and *P. mirabilis*
b) *P. vivax* and *P. falciparum*
c) *P. ovale and P. vivax*
d) *P. ovale* and *P. aeruginosa*
e) *P. malariae* and *P. knowlesi*

Question 6 –Answer and Explanation

Answer: c) *P. ovale* and *P. vivax*

These two species can persist in the liver as hypnozoites.

To prevent relapses, patients with *Plasmodium vivax* or *Plasmodium ovale* infection should be given a 14-day course of **primaquine**, which will treat the hypnozoite forms. It is not usually necessary to perform a follow-up blood film post-treatment.

Question 7

Which two species of malarial parasite can persist in humans long-term without proper treatment?

a) *P. knowlesi* and *P. mirabilis*
b) *P. vivax* and *P. falciparum*
c) *P. ovale and P. vivax*
d) *P. ovale* and *P. aeruginosa*
e) *P. malariae* and *P. knowlesi*

Question 7 –Answer and Explanation

Answer: c) *P. ovale* and *P. vivax*

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Question 8

A 40-year-old man presents for a routine visit with an elevated alanine aminotransferase level (55 IU/mL). He has a history of IV drug use, and a friend has recently been diagnosed with hepatitis B infection. He has a normal physical examination and has no stigmata of chronic liver disease. You want to investigate him for HBV, which serology test would indicate active HBV infection?

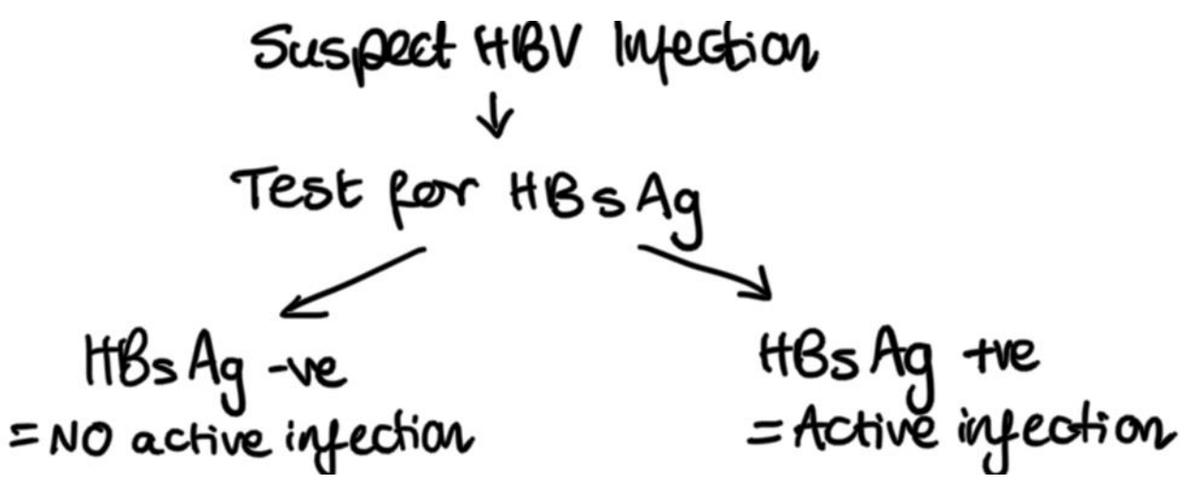
a) Core Antigen, HBcAg
b) Surface Antibody, HBsAb
c) E Antibody, HBeAg
d) Surface Antigen, HBsAg
e) Core Antibody, HBcAb

Question 8 –Answer and Explanation

Answer: d) hepatitis B surface antigen (HBsAg)

A positive hepatitis B surface antigen (HBsAg) result establishes the diagnosis and indicates active infection.

See diagram on previous slide for full explanation.



Question 9

A 75-year-old man attends A and E with a 3 day history of productive cough, dyspnoea and pleuritic chest pain. On examination, he has a low-grade fever.

What is the most common causative pathogen of community-acquired pneumonia?

a) *Legionella multophila*
b) *Streptococcus pneumoniae*
c) *Staphylococcus aureus*
d) *Streptococcus pyogenes*
e) *Haemophilus influenzae*

Question 9 –Answer and Explanation

Answer: b) *Streptococcus pneumoniae*

Streptococcus pneumoniae (the pneumococcus) is the most common causative pathogen of CAP across a range of severities and patient ages.

The most commonly reported atypical bacterial causes of CAP are Mycoplasma pneumoniae, Chlamydophila pneumoniae, and Legionella pneumophila.

Top tip: Remember *Legionella* is waterborne – classic history is of patient went on holiday and stayed in hotel, now having pneumonia symptoms. *Legionella* classically caught from contaminated shower water and air con systems.

Question 10

A 35-year-old woman attends A and E. She has a high BMI, and a 6-month history of confirmed gallstones with biliary colic pain. Over the last few days, she has had worsening abdominal pain, felt hot and sweaty and her partner said she looked yellow this morning.

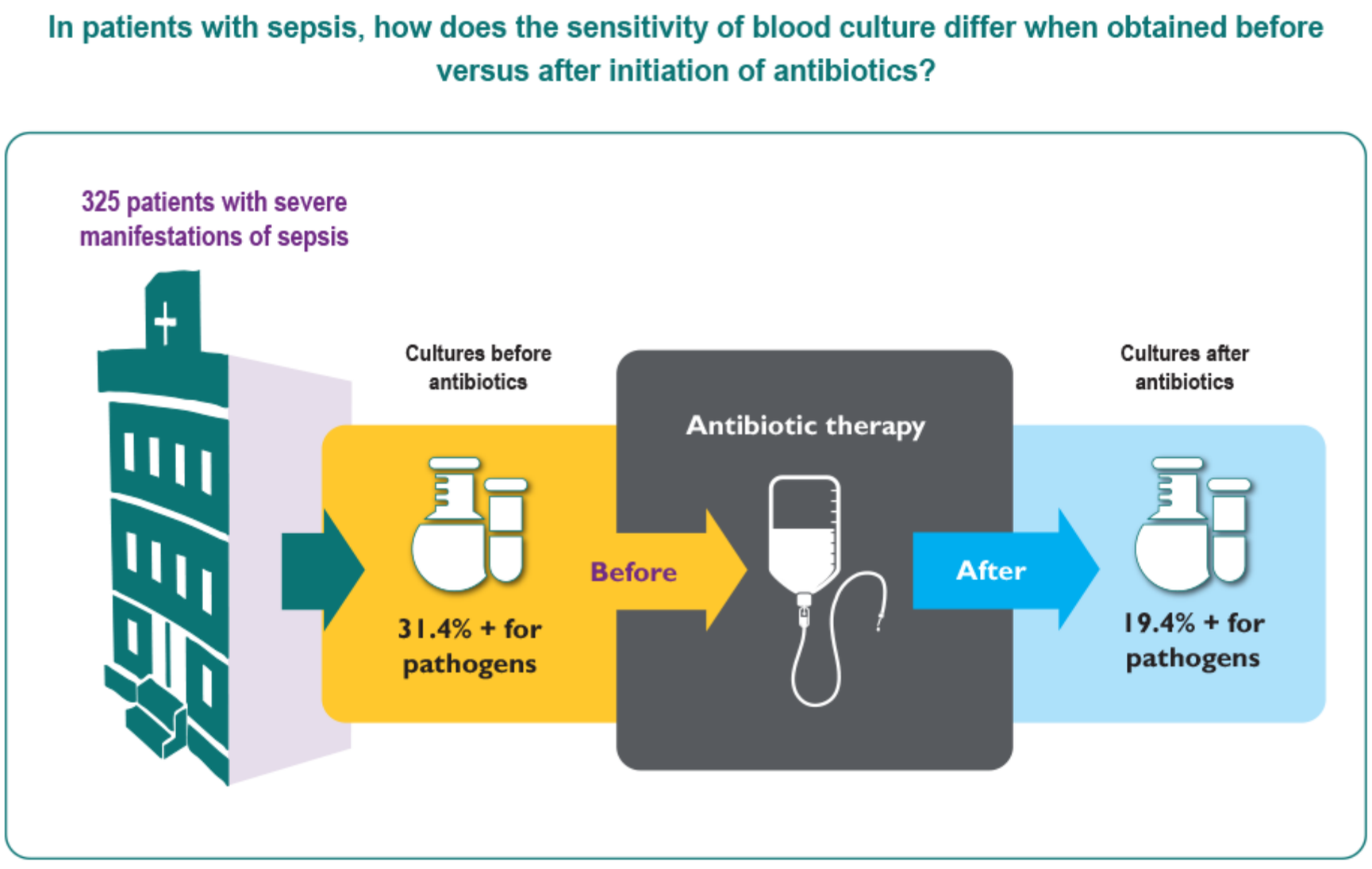
She is systemically very unwell and you suspect biliary sepsis. Thinking about blood cultures, should you take them straight away or get her some IV antibiotics first?

a) Straight away, you need to send them to the lab asap.

b) Get the antibiotics into her system before fiddling around trying to get bloods.

Question 10 –Answer and Explanation

Answer: a) Get bloods before antibiotics



Question 11

A 19-year-old university student attends A and E with a history of fever, non-blanching rash and photosensitivity. Their housemate tells you over the last few hours the pt was confused, and on examination the pt GCS is decreased. You perform an emergency lumbar puncture. Results were:

The CSF sample looks turbid and the opening pressure was high.
WBC content = 1100 cells per ul (normal value <8 cell/ul)
Glucose content = 20 mg per dl (normal value 50 – 80 mg/dl).

Is this a:

a) a viral meningitis
b) a bacterial meningitis?

Question 11 –Answer and Explanation

Answer: b) Bacterial meningitis

	Appearance	Opening Pressure	WBC cell/ul	Protein mg/dL	Glucose mg/dL
Normal	Clear	90-180	<8	15-45	50 - 80
Bacterial	Turbid	High	>1000	>200	<40
Viral	Clear	90-180	<300 (lymphocytes)	<200	Normal

Question 12

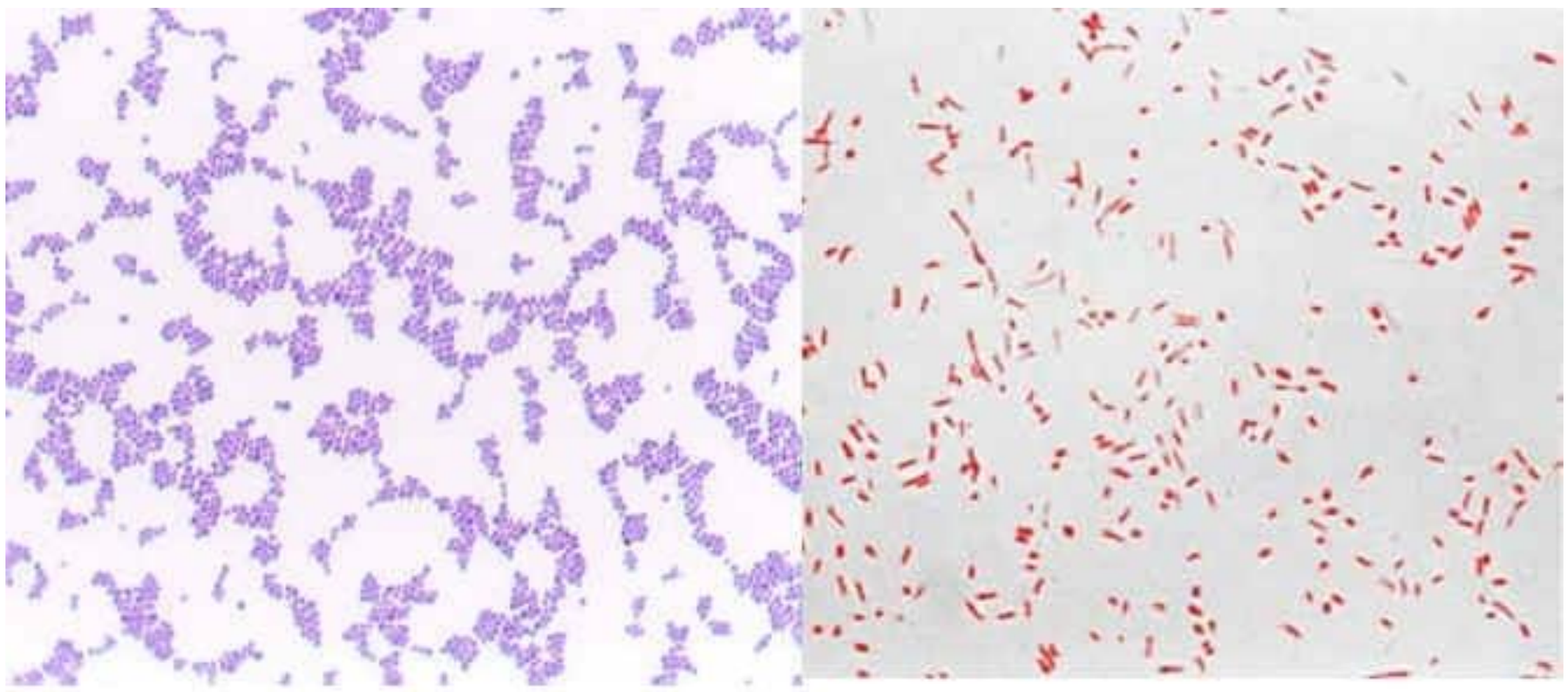
What colour does Staphylococcus aureus Gram stain?

a) Purple
b) Pink

Question 12 –Answer and Explanation

Answer = a) purple

Staph aureus is a Gram positive bacteria.



Gram +ve Bacteria

Gram -ve Bacteria

Question 13

Which of these is the most appropriate way of detecting viruses?

a) Light microscopy
b) Culture on an agar plate
c) PCR
d) API Strip

Question 13 –Answer and Explanation

Answer = c) PCR

- Rapid
- Accurate
- Fairly cheap nowadays

Question 14

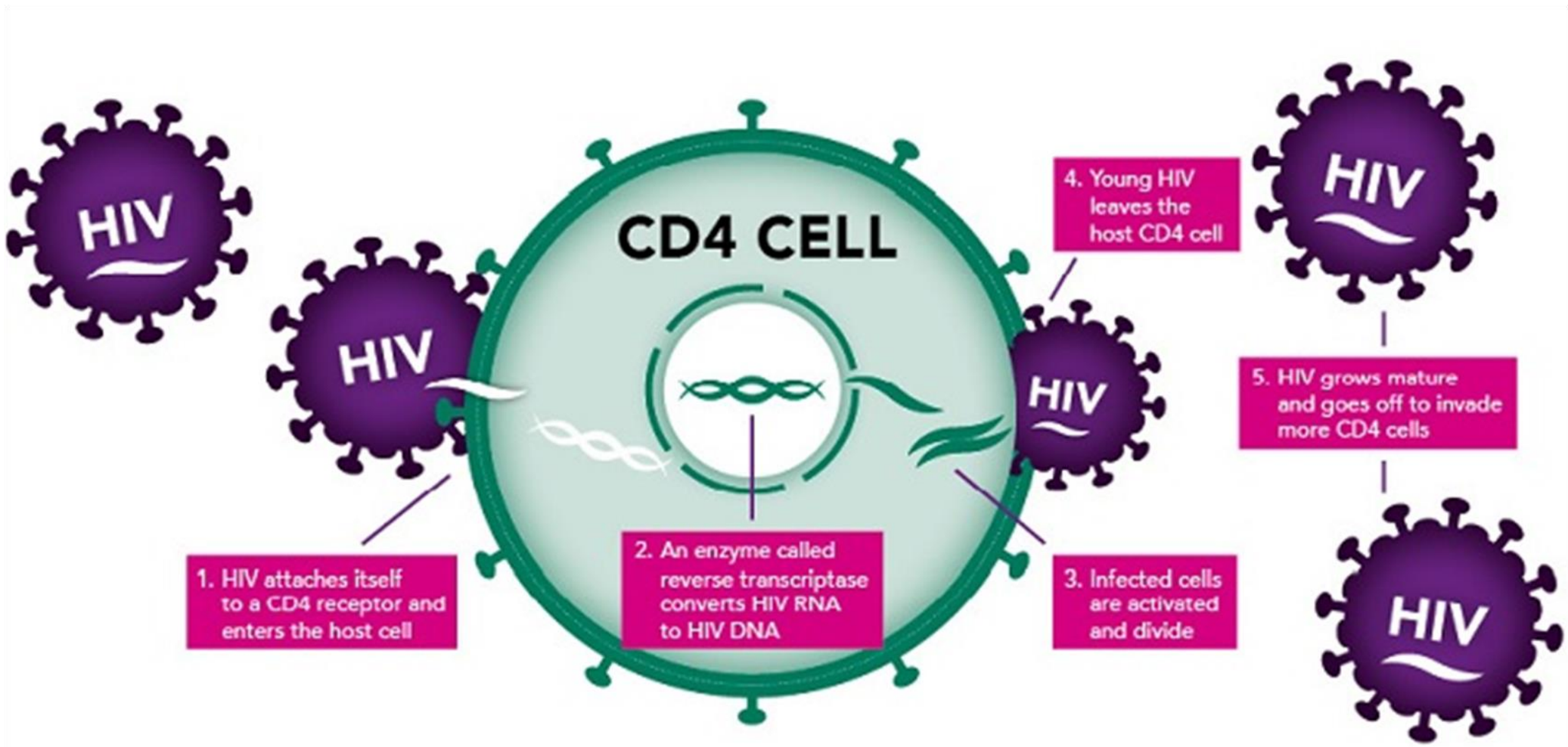
Which cells does HIV infect?

a) CD4+
b) CD10+
c) Neutrophils
d) CD4-
e) CD3+

Question 14 –Answer and Explanation

Answer = a) CD4+

Just one to learn!



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Tips and Resources

Tips for Studying Microbiology

Revise bugs in a clinically-focussed method, so learn by condition, rather than by microorganism.

- e.g. revise top causes of UTIs, pneumonia, osteomyelitis etc (common exam questions).
- Use THE CONCISE LIST to guide which common infections you should know inside out (find it on the drive).

Helpful to incorporate epidemiology and patient factors into microbiology

- e.g. fungal conditions often in immunocompromised patients
- e.g. the top causes of septic arthritis and pneumonia vary with the age of the patient

Know the Gram status and morphology of the common bacterial culprits.

- Staphs, Streps, and Enterococci are Gram + cocci, Neisseria and Moraxella are Gram – cocci.
- Pseud, Haemophilus and Enterobacteriaceae are Gram – rods and Clostridium difficile is a Gram + club-shaped rod.
- Remember Ziehl Neelson for TB!

Small amount of agar knowledge is very helpful

- Know your appearance on basic agar (Columbia or TSI), like Pseud is green and Staph aureus is gold
- e.g. blood agar for Streptococcus haemolysis status (green haloes = alpha/viridans group; transparent haloes = beta)
- e.g. MacConkey agar for lactose fermentation status (useful for Gram – rods)

Revise the <u>key</u> phenotypic tests – draw out diagrams if you are visual!

- Catalase, coagulase, and optochin are key for differentiating the Gram + cocci.
- Lancefield latex grouping key for Streptococci classification.
- Motility status, oxidase, indole and lactose fermentation status are key for Gram – rods.

Tips for Treating Infections

- Try and pick up patterns to remember which antibiotics to use - eg Staph infections will generally be responsive to penicillin - give Flucloxacillin! Use Microguide app or BMJ best practice to inform yourself.
- Group antibiotics by system – e.g. for UTIs always treat with Nitrofurantoin or Trimethoprim unless pregnant or allergy.
- In reality, prescribing is more complex but for 2a you really just need to know first line (possibly second if you are keen bean).
- Some bugs have definitive antibiotics, e.g. Vancomycin for MRSA
- Remember penicillin allergies - they are common and an easy way you could slip up, make sure you read the whole question!
- Some antibiotics you will need to learn side effects of - this will become very apparent and obvious – e.g. TB antibiotics see slide.

Resources for Studying Microbiology

Download Microguide from Appstore for your placement. Provides centre specific antibiotic guidance

Make your own phenotypic classification maps for these four topics:

- Gram + cocci
- Gram – cocci
- Gram + rod
- Gram – rod

Use the maps to learn your phenotypic tests like coagulase, catalase, oxidase etc.

Agar Bible – information I collected about agars and how each bacteria appears on the agar with pictures, I can email out to you.

Sid's Microbiology Guide PDF – search for Microbiology Guide on the google drive, this lists the microbes by clinical problem and will be helpful to you in run up to exam. If you can't find it email me (efbeswick1@sheffield.ac.uk).

Microbenotes – this website has useful note summaries of key topics, open the link and navigate by using the topics/categories dropdown on the right hand side of the screen https://microbenotes.com/

Microbiology Info – this website I found particularly useful for biochemical (phenotypic) test because it has pictures and clearly states examples for positive and negative results, as well as what to use as controls https://microbiologyinfo.com/

Microbiology Nuts and Bolts website – decent amount of info on here to get started with the basics of clinical microbiology. Can buy accompanying book if you find useful http://www.microbiologynutsandbolts.co.uk/

BMJ Best Practice – gain access by logging in through StarPlus (contact library if problems), has very good information summaries for infections and symptoms, also has aetiology section https://bestpractice.bmj.com/info/

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https://docs.google.com/forms/d/e/1FAIpQLSd1peco2UMuKyVaJvgd8kUaUdPvNiobFNMLjvRwS4aooRBvbw/viewform?usp=sf_link

ANY QUESTIONS?

Emily Beswick (Phase 2b) – please email me at
ebeswick1@sheffield.ac.uk

Happy to help with absolutely anything Micro or questions about phase 2a over email or google meets. No silly questions, please get in contact!

Hope you all have a lovely Christmas and remember to take time out to relax over the break.

WORMS

Nematodes (Worms)

Worms are common around the world, but rare in UK. Clinically they can cause:

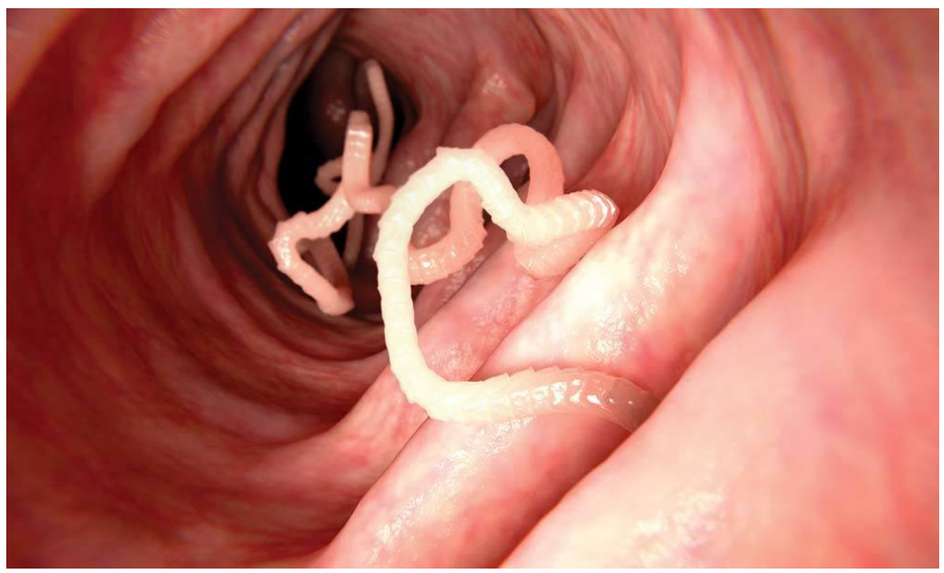
- Schistosomiasis – contracted by freshwater swimming or drinking in endemic areas like Africa, can cause bladder cancer if left untreated.
- Hookworm

3 groups: nematodes (roundworms), trematodes (flatworms) and ceratodes (tapeworms).

Life cycle includes periods of growth both inside and outside of hosts (which may be human or animal). The pre-patent period is the interval between infection and the appearance of eggs/larvae in the stool.

Transmission – faecal to oral.

The body mounts a poor immune response to nematode infection, mainly IgG and IE mediated.



Clinical Tables for Bacterial Infection

GRAM +VE	Differentiation:	Bacterium	Associated Infections	Commensal Location	Sensible Antibiotics	Notes
Clusters	Coagulase +ve	**S.aureus**	Impetigo, boils, cellulitis, endocarditis, toxic shock syndrome	Nasal passages + skin	Flucloxacillin, Co-Amoxiclav, Macrolides (susceptible to most)	Skin infections
		MRSA			Gentamicin, Vancomycin	
	Coagulase -ve	S.epidermidis	Surgical wound infections, septicaemia, endocarditis	Skin	Co-Amoxiclav,	Usually only harmful to immunocompromised patients
		S.saphrophiticus	Acute cystitis	Female genital tract + perineum	Nitrofurantoin, Trimethoprim	Causes 5-8% of cystitis infections, most commonly in young women
Chains	Alpha-haem	**S. pneumoniae**	Pneumonia, otitis media, sinusitis, meningitis	Nasopharynx (+ rest of upper resp tract)	Oral Amoxicillin, IV Benzylpenicillin	Think ENT!
		Viridans group streptococci	Oral strep, deep organ abscesses	Oral cavity mostly, also upper resp tract, female genital tract + GI tract	Amoxicillin	S.milleri = most virulent
	Beta-haem	**Group A strep (S.pyogenes)**	Cellulitis, tonsillitis, impetigo, scarlet fever, pharyngitis	Resp tract	Amoxicillin (any penicillins)	Throat + skin infections
		Group B strep (S.agalactiae)	Postpartum infection, neonatal sepsis, neonatal meningitis	Lower GI tract + female genital tract	Amoxicillin	Most common cause of bacterial infections in newborn babies
		Group G strep (S.dysgalactiae)	Severe mastitis, rheumatic fever, throat infections	GI tract + genital tract	Amoxicllin	
	Non-haem	Group D strep (S.bovis, Enterococcus)	S.bovis: endocarditis, UTIs, sepsis Enterococcus: endocarditis, UTIs, intra-abdominal + pelvic infections	GI tract	Amoxicillin	S.bovis associated with colorectal

GRAM -VE	Differentiation:	Bacterium	Associated Infections	Commensal Location	Sensible Antibiotics	Notes
Lactose Fermenting		**E.coli**	UTIs, traveller's diarrhoea, cholecystitis, cholangitis	GI tract	Co-amoxiclav, Trimethoprim, Nitrofurantoin	Causes majority of UTIs
		Klebsiella	Pneumonia, meningitis, surgical wound infections	GI tract	Do sensitivity testing	Difficult to treat (some strains highly resistant)
Non-Lactose Fermenting	+ve Oxidase	Pseudomonas	Pneumonia	GI tract	Tazocin, Gentamicin, Quinolones	Do not usually cause infections in healthy people - if so infection is mild
	-ve Oxidase	**Shigella**	Shigellosis (diarrhoea, fever)	N/A (from water)	Quinolones, Azithromycin	
		Salmonella	Salmonellosis (diarrhoea)	Intestines (from raw meats, poultry, eggs)	Amoxicillin, Quinolones, Macrolides	
		Proteus	UTIs, related to stone formation	Intestines	Penicillins, cephalosporins	
Cocci		**Neisseria**	Gonorrhoea, meningitis	Some species commensal, some pathogenic only	Cephalosporins	