Demonstrations from HDI laboratory sessions, bacteriology sections

Routine media used to grow bacteria

Routine broth: can't see what organisms really look like, must plate to a solid medium such as the ones that follow to identify

Blood Agar -general all-purpose

MacConkey Agar
- -selective: only gram negatives will grow
- -differential: detects biochemical patterns
  - -pink: organism ferments lactose and changes pH, leads to colour change
  - -clear: organism does not ferment lactose, no pH change

Chocolate Agar
-has special supplements to grow fastidious organisms
- *N.gonorrhoeae*

H.influenzae

Chocolate Medium for *Neisseria gonorrhoeae*

Note growth is present on one side only:

Green growth – normal flora

No growth – this side has antibiotics to suppress normal flora so the pathogen of interest can be detected

For gram positive organisms

Note growth on Blood Agar, but no growth on the MacConkey Agar

S. aureus

Other Staphs – Called "Coagulase negative staphylococci"

-Many species exist, common member of normal flora.

-*S.epidermidis* is most common species in this group

-*S. saprophyticus* is a common cause of UTI in young females

Many different media used for the routine set-up of stool specimens.

Stools are labour intense specimens.

E. coli

A gram negative rod

Enterobacteriaceae or “coliform”. Most strains normal flora in GI tract

Grows well in < 24 hrs

Lactose fermentor “LF” (Pink on MacConkey)

Common cause of UTI and many other infections

Klebsiella pneumoniae

A gram negative rod

Enterobacteriaceae or “coliform”. Most strains normal flora in GI tract

Grows well in < 24 hrs

Big “gloppy” colonies due to copious capsule

Lactose fermentor “LF” (Pink on MacConkey)

Causes many infections

Proteus mirabilis

A gram negative rod

Enterobacteriaceae or “coliform”. Most strains normal flora in GI tract

Grows well in < 24 hrs. NB: swarming on plate

Non-lactose fermentor “NLF” (Pale on MacConkey)

Causes many infections

Pseudomonas aeruginosa

A gram negative rod

Environmental, not common in GI tract

Growth slower, **24-48 hrs**

Note ground glass appearance, grape-like odor

Non-lactose fermentor “NLF” (Pale on MacConkey)

Common cause of opportunistic infections

Bi-plates:

Half plates of both Blood and MacConkey, used to culture urines

These are examples of urine cultures, set up with a calibrated loop (that is it gives a specific amount of urine to the plate) so that a colony count can be done to give a quantitative report

Antimicrobial Susceptibility Tests:

Disk Diffusion

Disks contain antibiotics which diffuse and inhibit bacterial growth

Zone sizes are measured to determine “Susceptible” or “Resistant”

Bigger zones do not mean more susceptible

Antimicrobial Susceptibility Tests:

E-test

Strip contains an antibiotic which diffuses at different concentrations and inhibits bacterial growth

Results give the MIC, minimum inhibitory concentration, the point where the zone intersects the strip

Automated Identification & Susceptibility Test Methods

Different colors in the wells show positive and negative reactions; the patterns can identify bacteria

Susceptibility to antibiotics is determined by whether there is growth in the wells

Fungal diagnosis:

Aspergillus

Example mold growth

If opened, the spores would spread readily

Aspergillus is everywhere. Serious infections occur in the compromised host

The characteristic spores & hyphae can be seen under the microscope (special stains may be needed)

Candida albicans

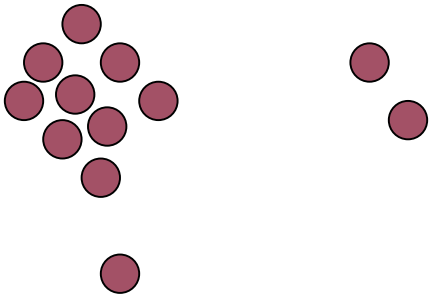
Example of yeast growth, similar to bacteria, but slower.
Do not grow on MacConkey

Many species of Candida are part of normal flora.

The characteristic budding yeasts can be seen under the microscope, usually with the gram stain

Staphylococcus

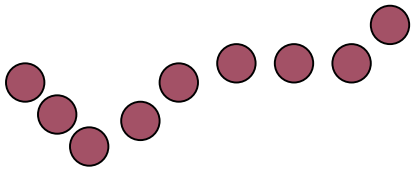
Gram positive cocci in clusters

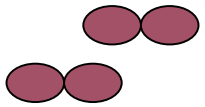


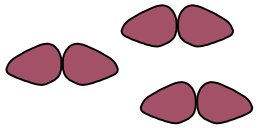
There are two main groups of Staphylococci:

S. aureus	***S. epidermidis***
Coagulase POSITIVE	Coagulase NEGATIVE
β haemolytic	Non-haemolytic
AGGRESSIVE INFECTION	Foreign Body Infection
FOOD POISONING	NO Food Poisoning
TOXIC SHOCK	NO Toxic Shock
Note that all staphylococci are gram positive cocci in clusters. These bacteria CAN NOT be differentiated by Gram Stain	

Streptococci
Gram positive cocci in

Chains 

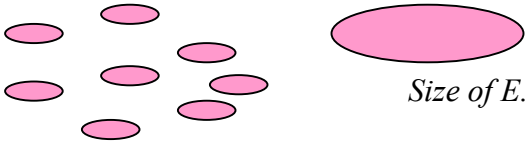
Doublets 

Lanceolate Doublets 

Streptococci are classified first by haemolysis:		
β haemolytic (complete)	α haemolytic (partial)	γ Haemolytic (No haemolysis)
Lancefield Groups	**Viridans (greening) Strep**	**Enterococci (formerly Group D Strep)**
A,B,C, (D), E, F,G…U	*S. pneumoniae*	*E. faecalis, E.fecium*
	S. mutans, S. sanquis	

Haemophilus

Gram negative coccobacilli



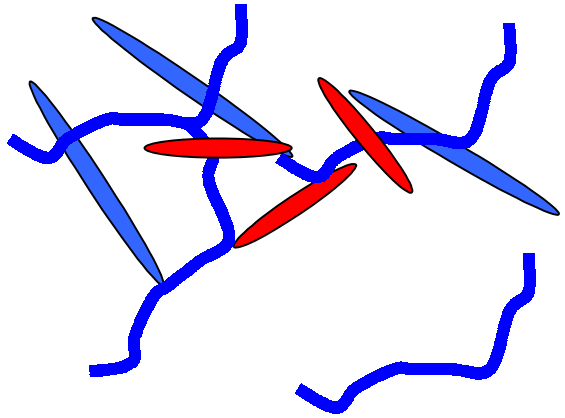
Size of E. coli in conparison

Note: sometimes it is difficult to see Haemophilus species because they get lost in the pink background

Although Haemophilus means "Blood Loving", this species will only grow if blood cells membranes are broken, releasing essential growth factors NAD, NADH.
Membrane lysis can occur by freeze-thaw (chocolate agar) or by enzymatic activity (Staphylococci). Note on the blood agar plate Haemophilus only grows around the staphylococci (Satellite Growth)

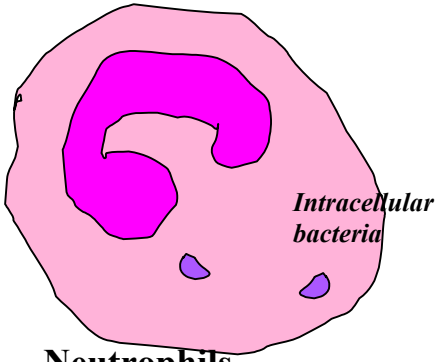
Mycobacteria
Bacteria with a thick waxy cell wall that **resists gram stain**.
M. tuberculosis is one example of a bacteria that does not stain with gram stain.

Kinyoun stain makes mycobacteria **red** against a blue background

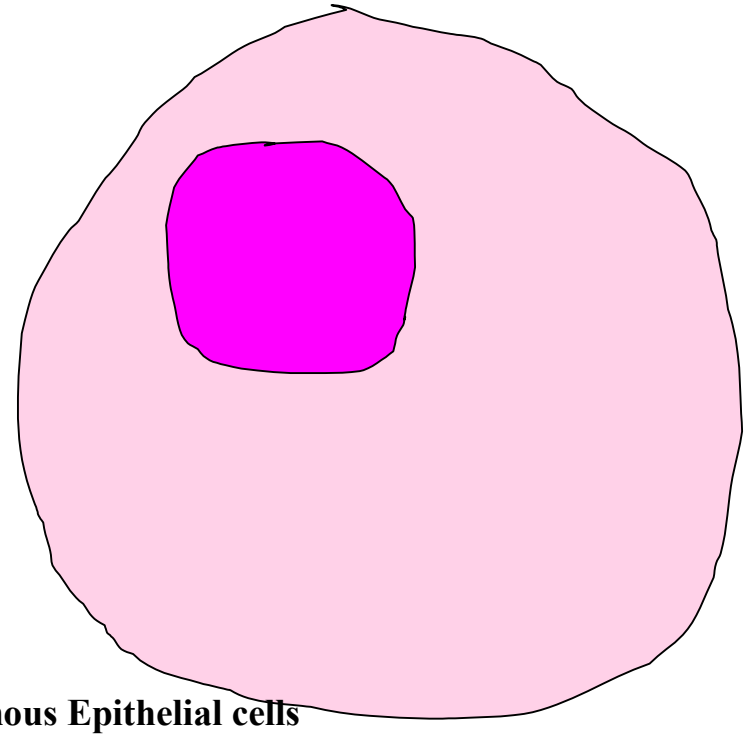


Gram stains demonstrate both bacterial cells and host elements including mucus, and cells.

*By observing and interpreting **all** the elements present, the gram stain provides a picture that can depict the quality of sample, the presence of normal and abnormal bacterial flora, and the response of the host. In short the **gram stain** provides a laboratory tool to visually differentiate between infection, colonization, and inflammation in the absence of infection, and also to provisionally identify bacteria and yeast that may be present. This information can be critical in decisions that effect initiation of anti-infective therapy.*



Neutrophils
Acute inflammatory response



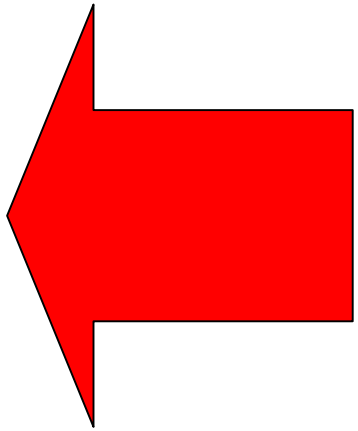
Squamous Epithelial cells

Evidence of mucosal cells in sample reflect on quality of sample either positively (viral nasopharyngeal) or negatively (sputum)

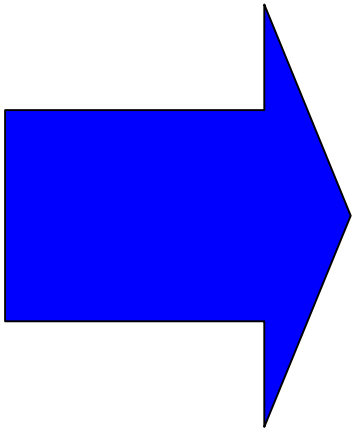
One of these sputum specimens is indicative of probable pneumonia, while the other is not interpretable because it contains too much saliva.

Can you tell which is which?

Remember that saliva contains many sqamous epithelial cells, while material from bronchi, bronchioles, and alveoli do not.



< 25 EPIs/LPF



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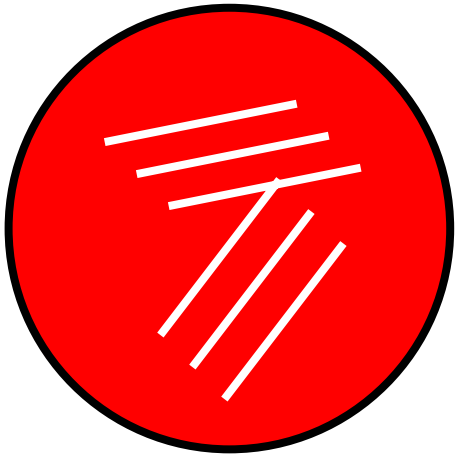
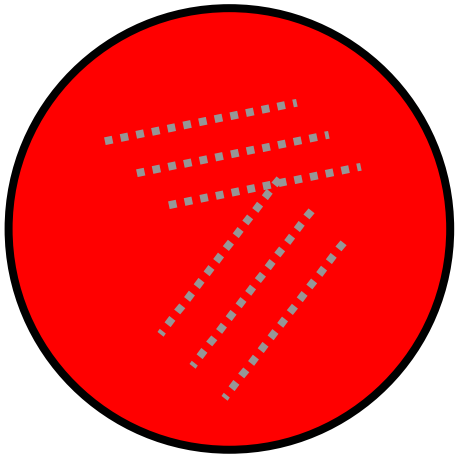
HAEMOLYSIS



Beta
Complete
Haemolysis



Alpha
Incomplete
Haemolysis

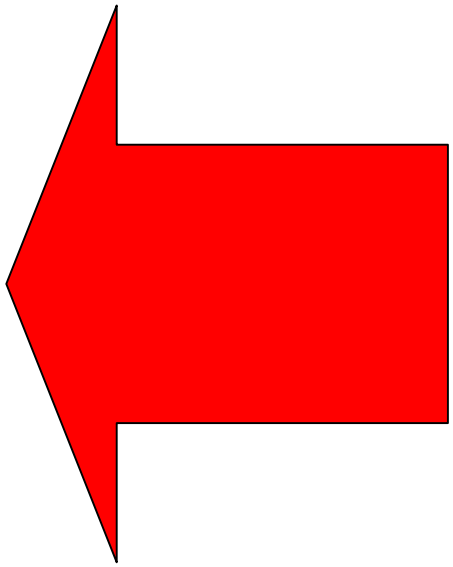


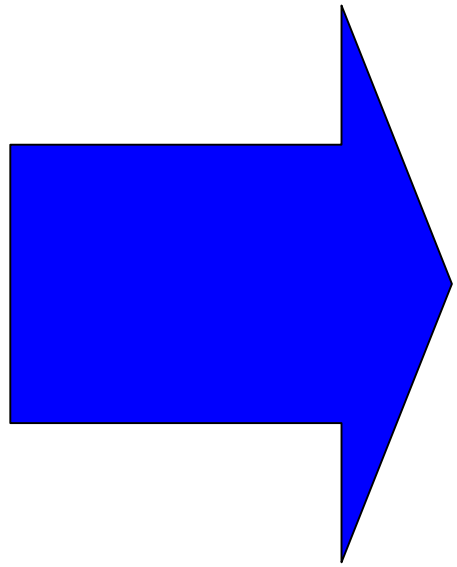
Gamma
No
Haemolysis

One of these specimens suggests the presence of infection. The other suggests the presence of many bacteria, but surface colonization only.

Can you tell which is which? Why?

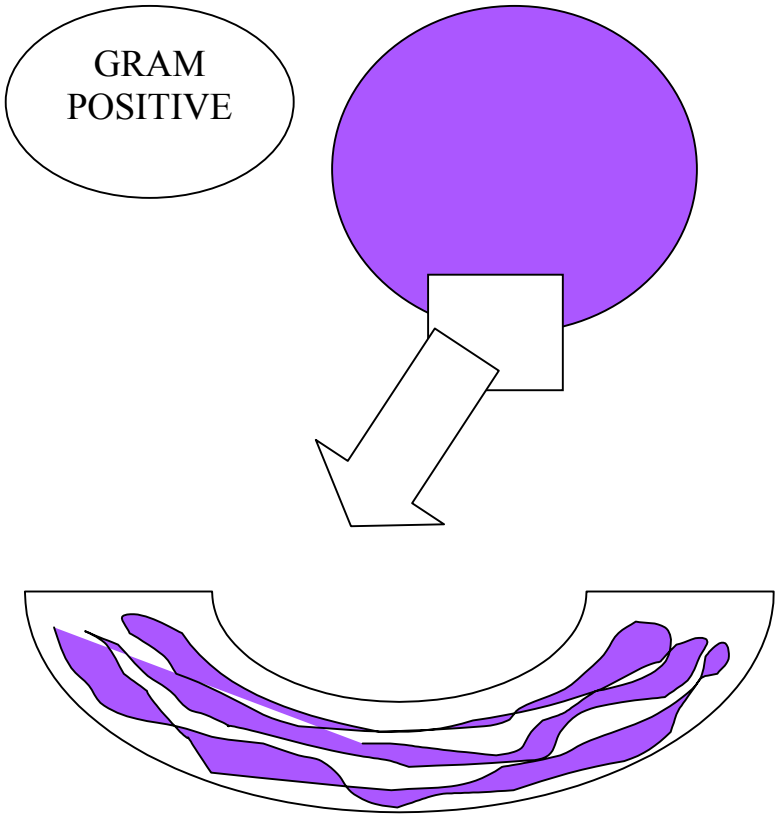
What is the likely identification of the predominant bacteria present?





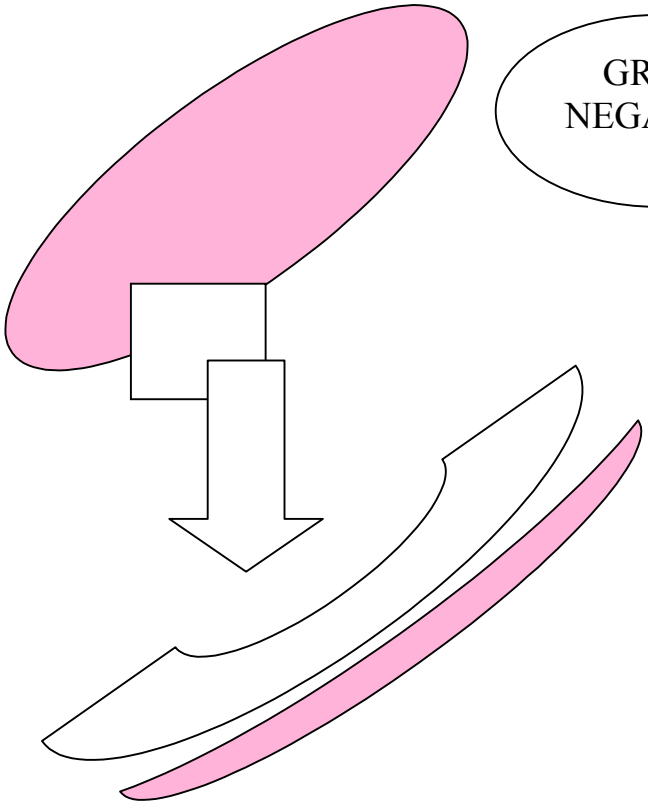
GRAM STAIN

GRAM POSITIVE



Peptidoglycan absorbs stain resulting in purple staining bacteria

GRAM NEGATIVE



Lipid outer membrane resists stain with crystal violet. Cells stain pink with safranin