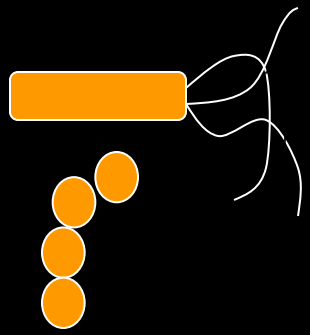
Laboratory Diagnosis of Infection

We ask the lab for a diagnosis, expecting a yes or no, but often end up with just a maybe…

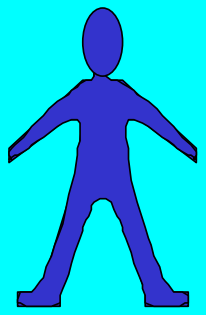


Outline

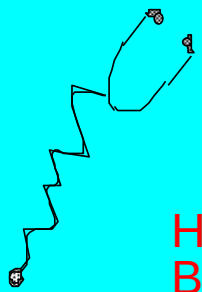
- Clinical assessment
- Collecting and transporting specimens
- Microscopy
- Culture
- Sensitivity
- Non-cultural diagnostic methods
- Virological diagnosis
- Interactions between humans and microbes
- Normal flora

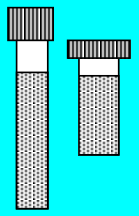
Diagnosis of Bacterial Infection

Patient



Clinical diagnosis

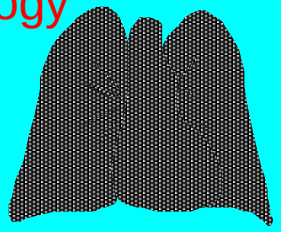




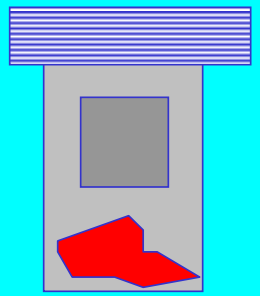
Haematology
Biochemistry

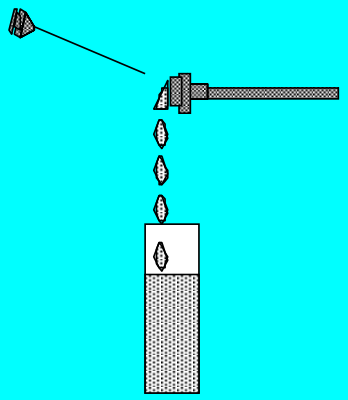
Non-microbiological investigations

Radiology



Sample





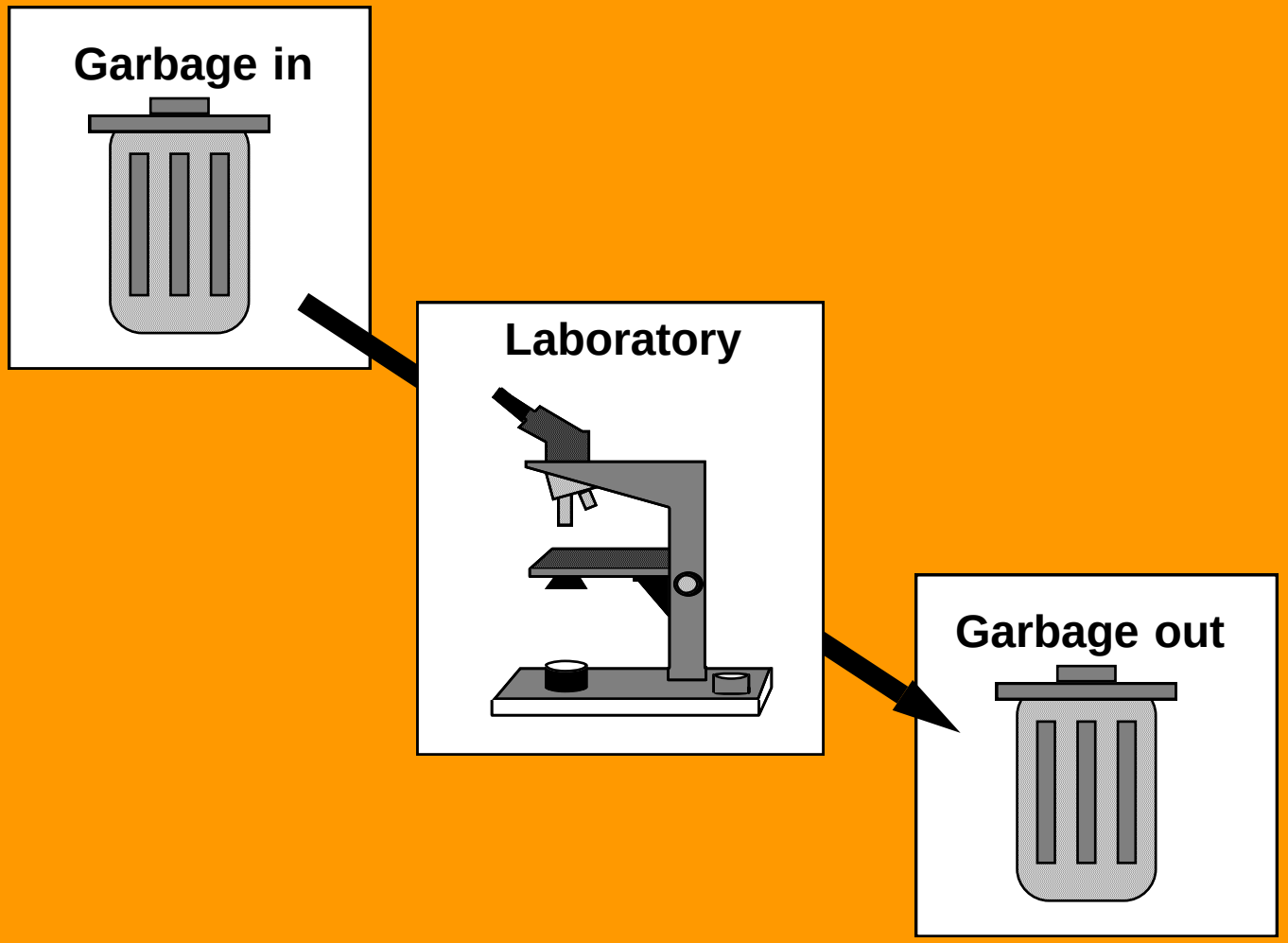
Take the correct specimen

Take the specimen correctly

Label & package the specimen up correctly

Appropriate transport & storage of specimen

A proper clinical assessment is essential for optimal use of laboratory services!



Is your investigation worthwhile?

Do you know what information you want?

no

yes

Is the information already available?

yes

no

Does it affect patient management?

no

yes

Can the lab provide this information?

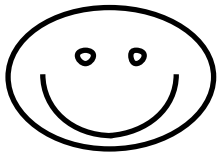
no

yes

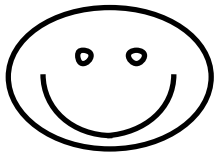
stop! think again

Contact the lab for info on
Best test
Type of sample
Timing of sample
Transport of sample
Interpretation of results

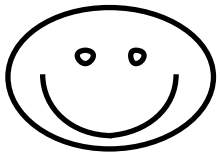
Give the lab all relevant clinical information
e. g. antibiotic treatment
recent travel
special risks etc



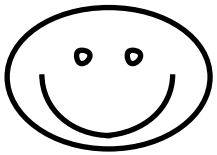
Happy clinician



Happy microbiologist



Happy patient



Happy manager

Collecting the correct specimen

- Endocervical swabs for GC
- Pernasal swabs for pertussis
- whole EMU for TB
- Sputum , not saliva
- Blood culture bottles, not clotted blood
- Correctly timed Gentamicin assays
- Pus, not swabs

Getting the specimen to the lab

- Problems in delay or inappropriate storage• delay in dignosis & treatment
  - pathogens die
  - contaminants overgrow
- Blood cultures directly into incubator
  - not refigerator!
- CSF straight to lab
- Don't put an entire surgical specimen into formalin!
  - Send a portion to microbiology in a sterile container

Collecting the specimen correctly

- Take an mid-stream urine
  - avoids contamination with perineal flora
- CSF
  - Avoid contamination
  - Avoid bloody tap
- Throat swab
  - Make the patient gag!
- Blood cultures
  - Avoid contamination with skin organisms

Specimens & Infection Control

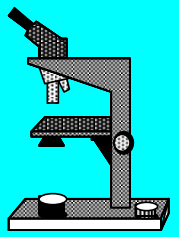
- Please be considerate to lab staff!!
  - Label hazardous specimens
- Don't send specimens to the lab without proper packing
  - Leaking or blood-stained specimens are not acceptable!!!

Factors limiting usefulness of bacteriological investigations

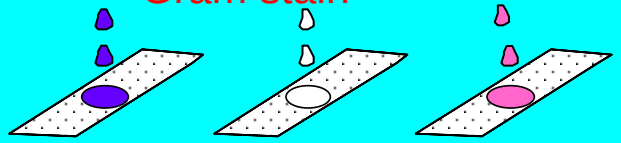
- wrong sample
  - e.g. saliva instead of sputum
- delay in transport / inappropriate storage
  - e.g. CSF
- overgrowth by contaminants
  - e.g. blood cultures
- insufficient sample / sampling error
  - e.g.in mycobacterial disease
- patient has received antibiotics

Diagnosis of Bacterial Infection

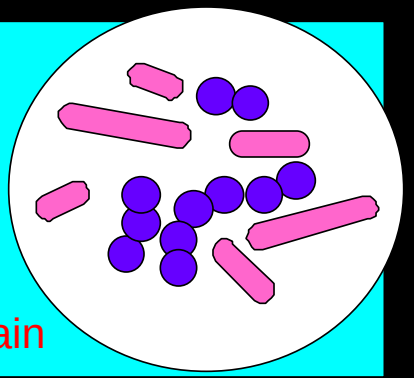
microscopy



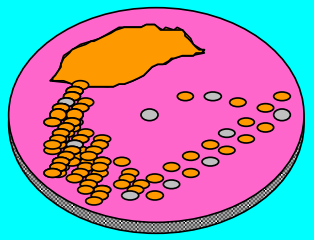
unstained or stained with e.g. Gram stain

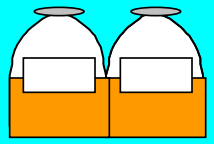


Stain Decolorise Counterstain



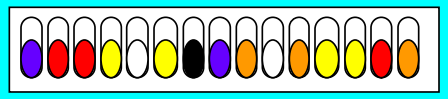
culture



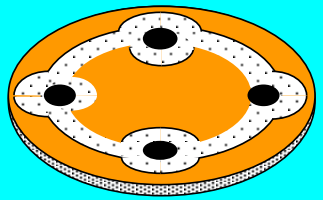


identification by biochemical or serological tests on pure growth from single colony

on plates or in broth

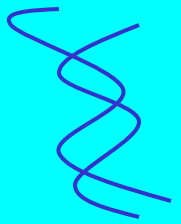


sensitivities



by disc diffusion methods, breakpoints or MICs

Serodiagnosis

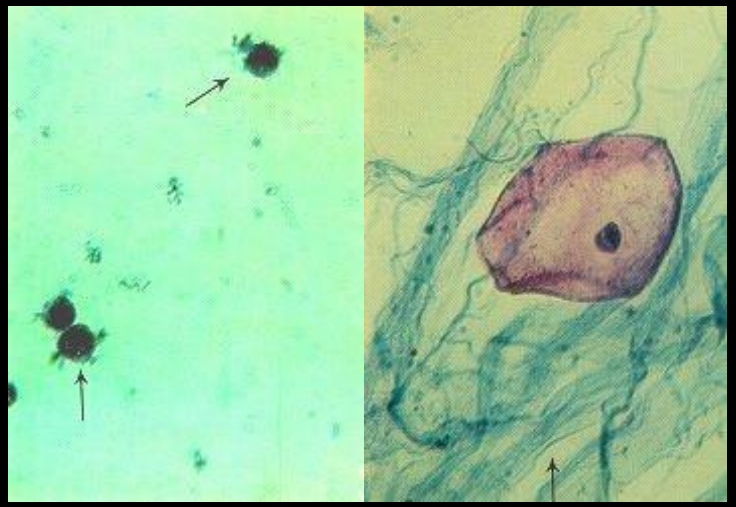


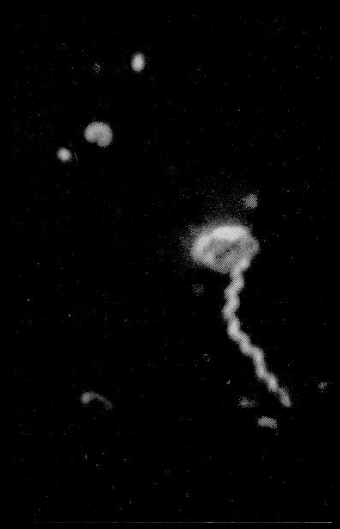
DNA technologies

Microscopy

Unstained preparations

- “Wet prep”
- Dark-ground illumination for syphilis

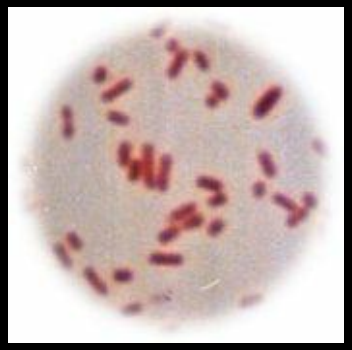


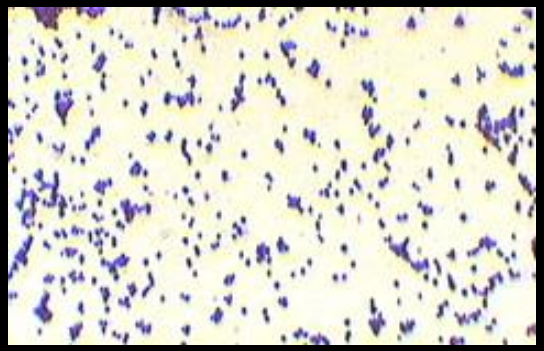


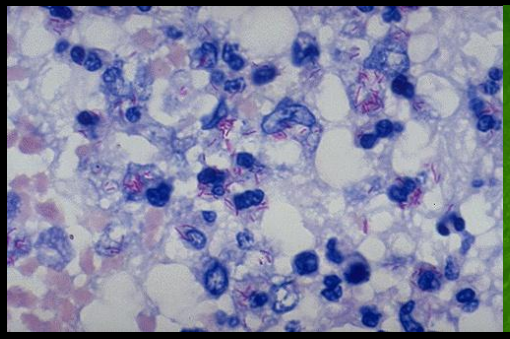
Microscopy

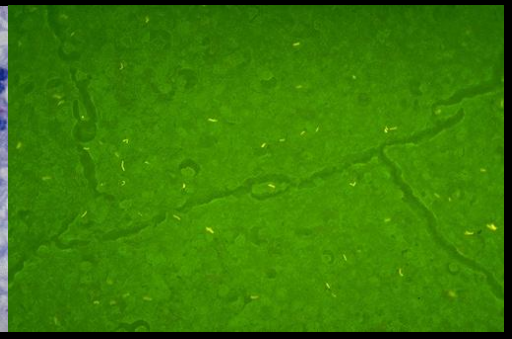
Stained preparations

- Gram-stain
- Acid-fast stain
  - Ziehl-Neelsen
- Fluorescence
  - Direct, e.g. auramine
  - Immunofluorescence



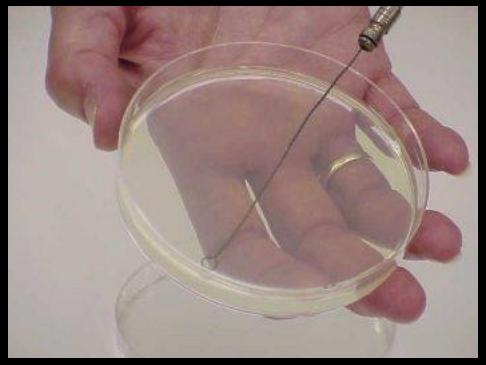


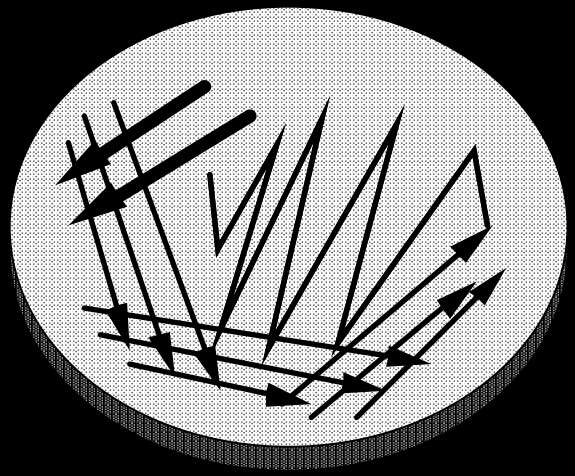




Culture of Bacteria

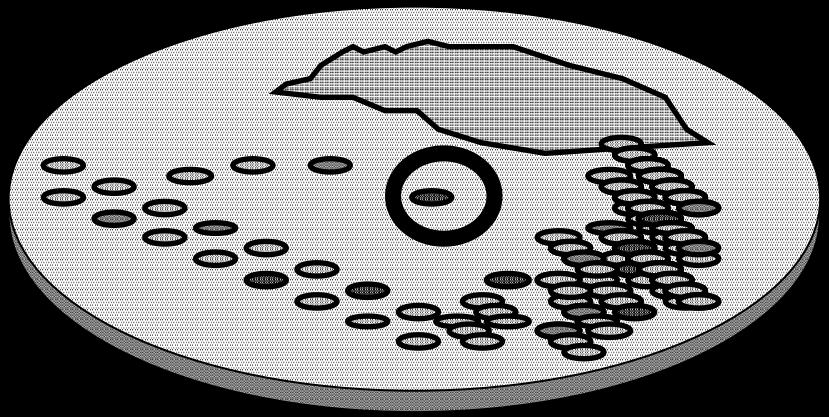
- Solid media
  - Agar plates
    - For Identification
    - For Enumeration
  - Slopes
  - For safe long-term culture, e.g. Lowenstein-Jensen media for TB
- Liquid media (broth)
  - For enrichment or maximum sensitivity

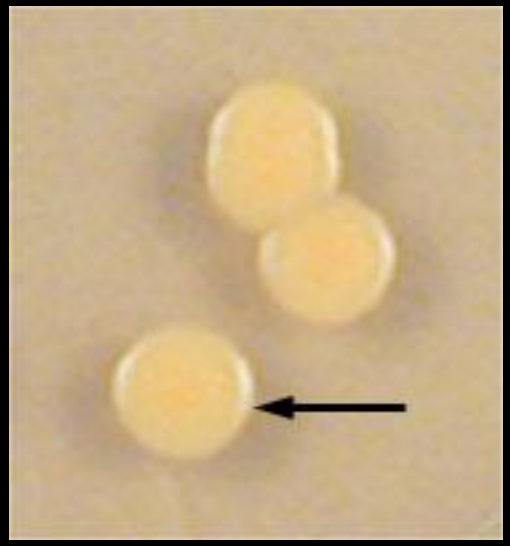




Advantages of Solid Media

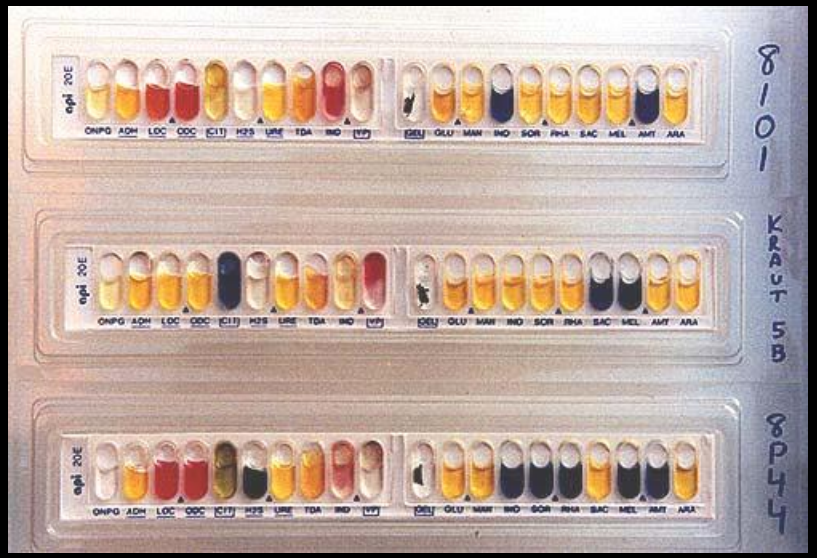
- isolation of single clonal colonies
  - get bacterium in pure culture
- identify by colonial morphology
- quantification by colony-forming units

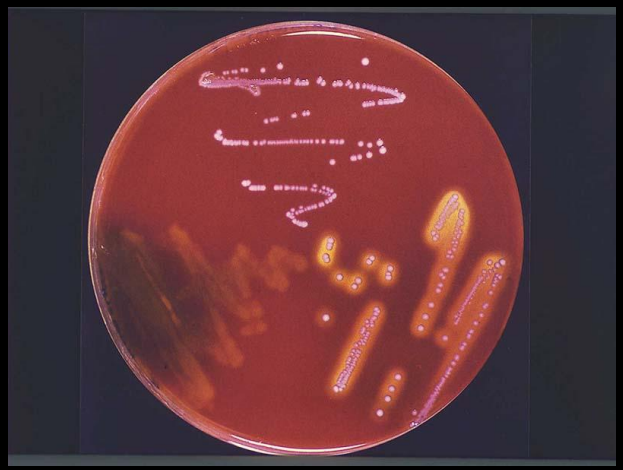




Identification of Bacteria

- Morphology
- Growth requirements
- Biochemistry
- Enzymes
- Antigens



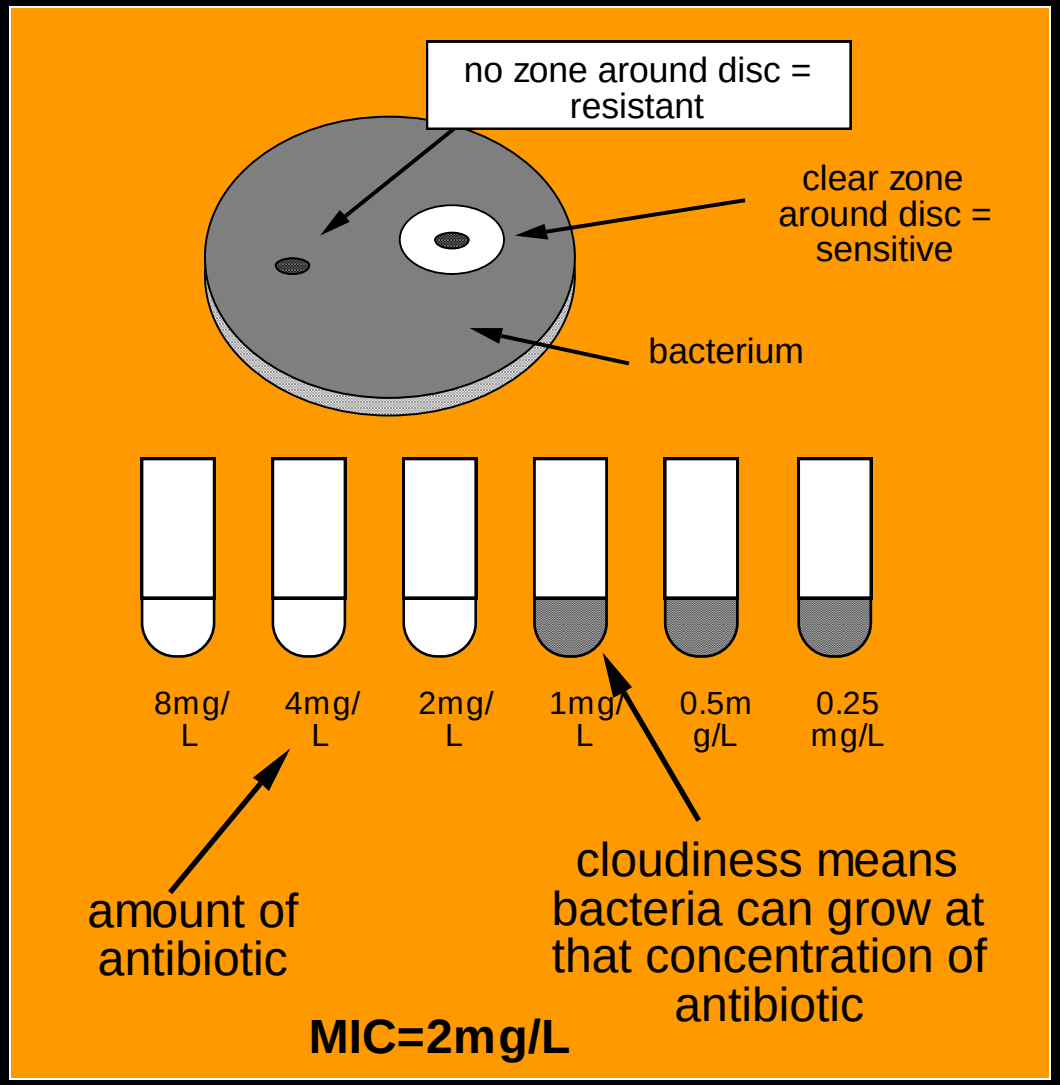


Non-cultural diagnostic methods

- Antigen detection
  - e.g. latex agglutination
- Antibody detection
  - e. g. agglutination tests, complement fixation tests, indirect immunofluorescence
- Molecular methods
  - Polymerase Chain Reaction

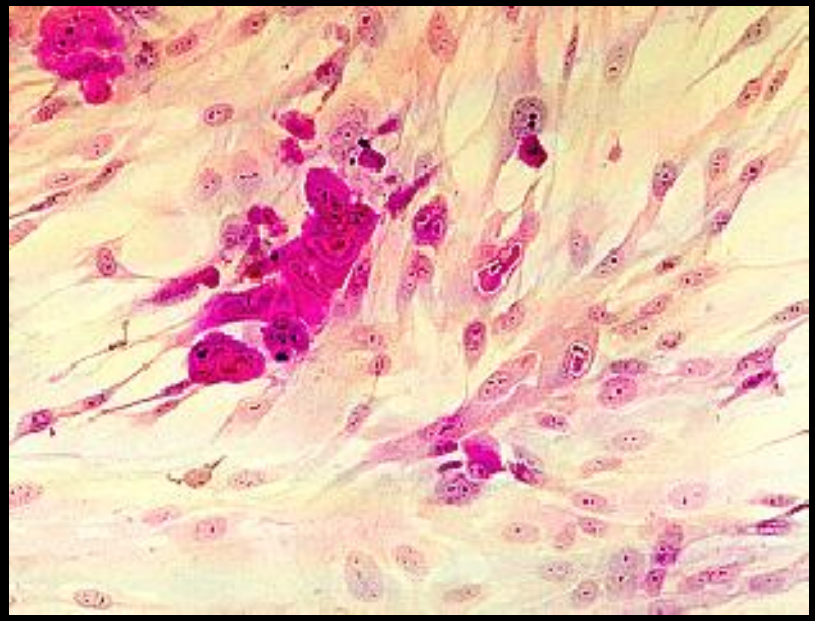
Sensitivity tests

- on solid media
  - disc diffusion technique
- in liquid media
  - minimum inhibitory concentration (MIC) test
- Breakpoint methods
- E-test



Diagnosis of Viral Infection

- Electron microscopy
- Antigen detection
- Antibody detection
- Virus culture
  - Detect cytopathic effect or antigen
- Molecular methods
  - Polymerase Chain Reaction
  - Sequencing (e.g. for sensitivities)

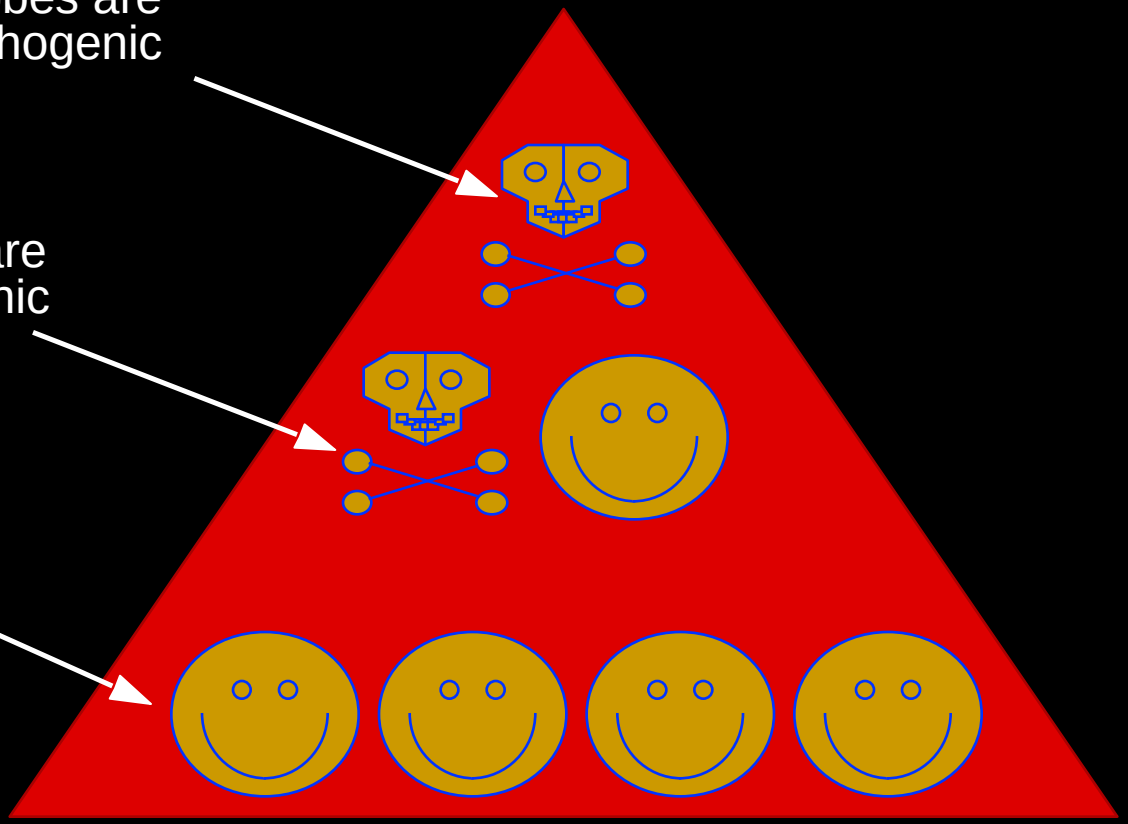


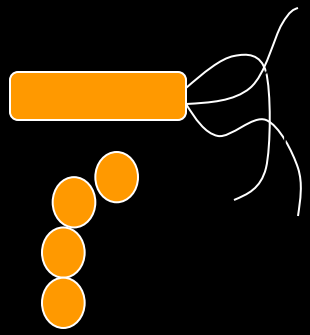
Microbes and humans

Very few microbes are always pathogenic

Many microbes are potentially pathogenic

Most microbes are never pathogenic





Microbes and humans

Disease can come about in several overlapping ways

1. Some bacteria are entirely adapted to the pathogenic way of life in humans. They are never part of the normal flora but may cause subclinical infection, e.g. *M . tuberculosis*
2. Some bacteria which are part of the normal flora acquire extra virulence factors making them pathogenic, e.g. *E. coli*
3. Some bacteria which are part of the normal flora can cause disease if they gain access to deep tissues by trauma, surgery, lines, e.g. *S. epidermidis*
4. In immunocompromised patients many free-living bacteria and components of the normal flora can cause disease, especially if introduced into deep tissues, e.g. *Acinetobacter*

How do we know that a given pathogen causes a specific disease?

- Koch's postulates
  - the pathogen must be present in every case of the disease
  - the pathogen must be isolated from the diseased host & grown in pure culture
  - the specific disease must be reproduced when a pure culture of the pathogen is inoculated into a healthy susceptible host
  - the pathogen must be recoverable from the experimentally infected host

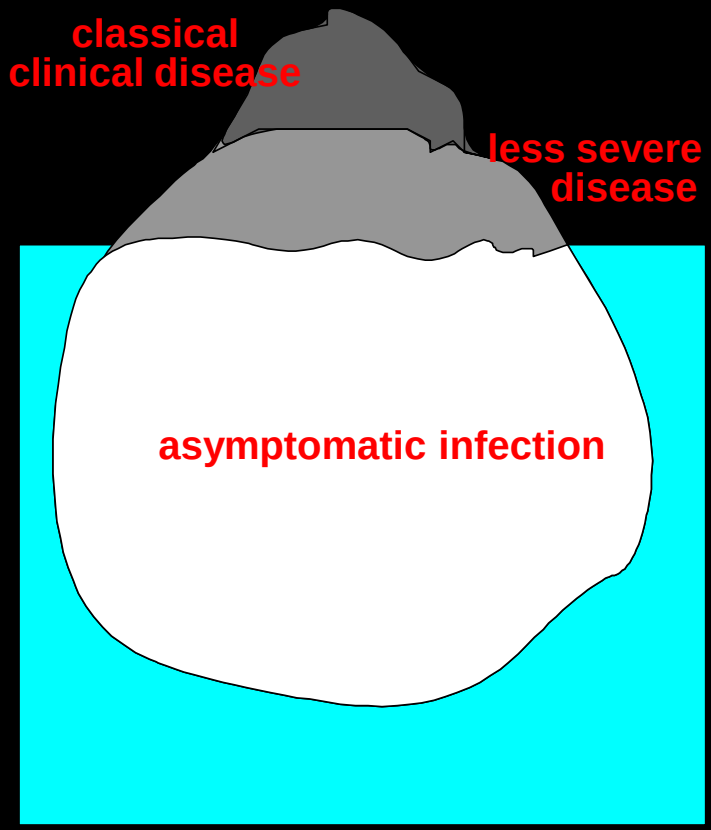
The iceberg concept of infectious disease

poliomyelitis in a child
0.1-1% of infections are clinically apparent

rubella
50% of infections are clinically apparent

Spectrum of virulence

rabies
100% of infections are clinically apparent

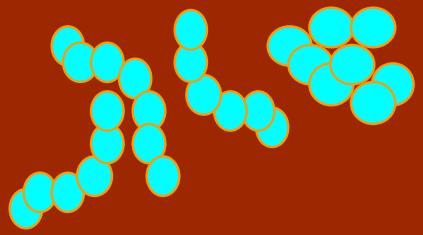


How do we know that a given pathogen causes a specific disease?

Diagnosis and effective treatment of infection depends not just on isolating an organism, but in establishing a plausible link between the laboratory findings, recognised syndromes and the patient's clinical condition

Recognised syndromes

e.g.
septicaemia, endocarditis, osteomyelitis meningitis, UTI, pneumonia pharyngitis

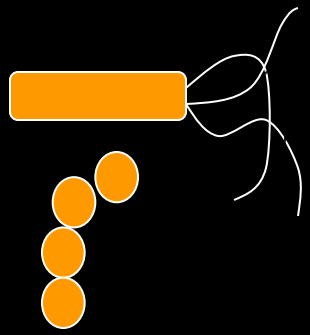


potential pathogen isolated from or detected in clinical samples

patient's clinical condition

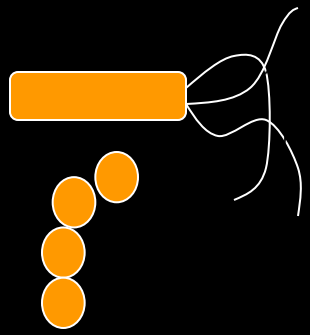
Microbes and humans

- Evidence for a potential pathogen being clinical significant (particularly for bacteria)
  - Isolated in abundance
  - Isolated in pure culture
  - Isolated on more than one occasion
  - Isolated from deep tissues
  - Evidence of local inflammation
  - Evidence of immune response to pathogen
  - Fits with clinical picture



Normal flora

- All body surfaces possess a rich normal bacterial flora, especially the mouth, nose, gingival crevice, large bowel, skin
  - This can be a nuisance in that
    - it can contaminate specimens
    - it can cause disease
  - This is beneficial in that
    - it can protect against infection by preventing pathogens colonising epithelial surfaces (*colonisation resistance*)
    - removal of the normal flora with antibiotics can cause superinfection, usually with resistant microbes
- Endogenous viruses reside in the human genome
  - worries about similar pig viruses in xenografts



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