The laboratory evaluation of infectious diseases

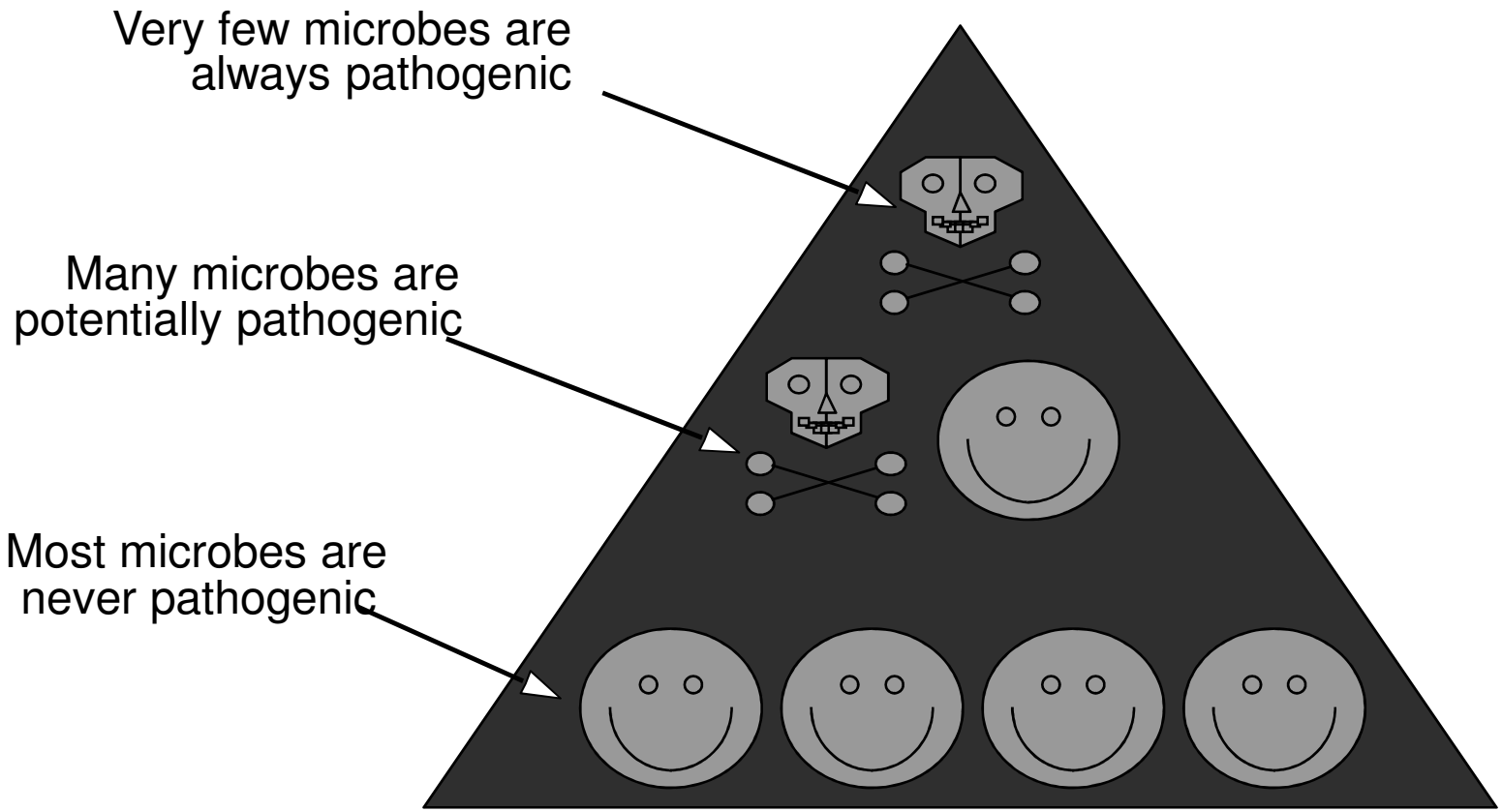
Dr Ruth Lekalakala
Tshwane Academic Division
Microbiology Dept.

Laboratory Diagnosis of Infections

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

Professor Mark Pallen

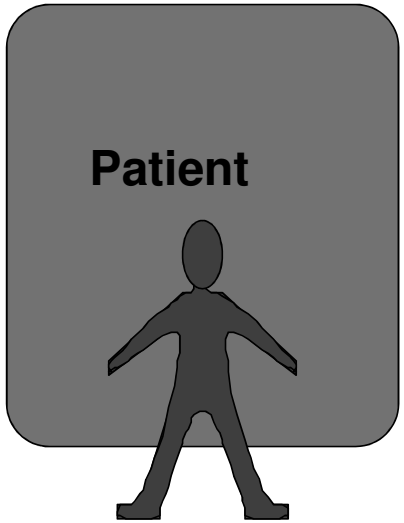
Microbes and humans

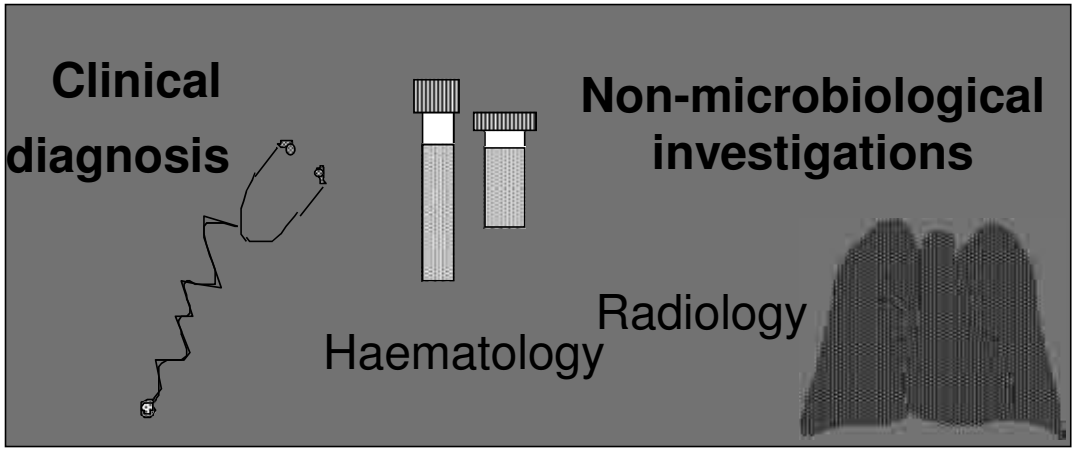


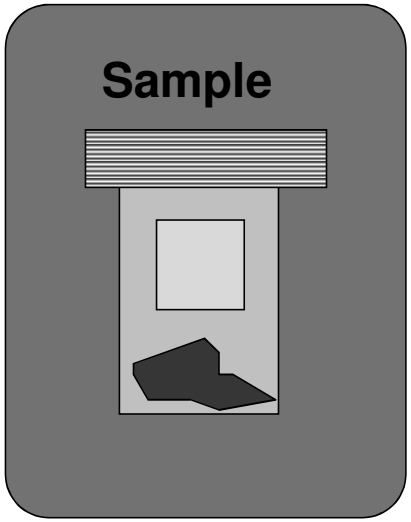
OBJECTIVES

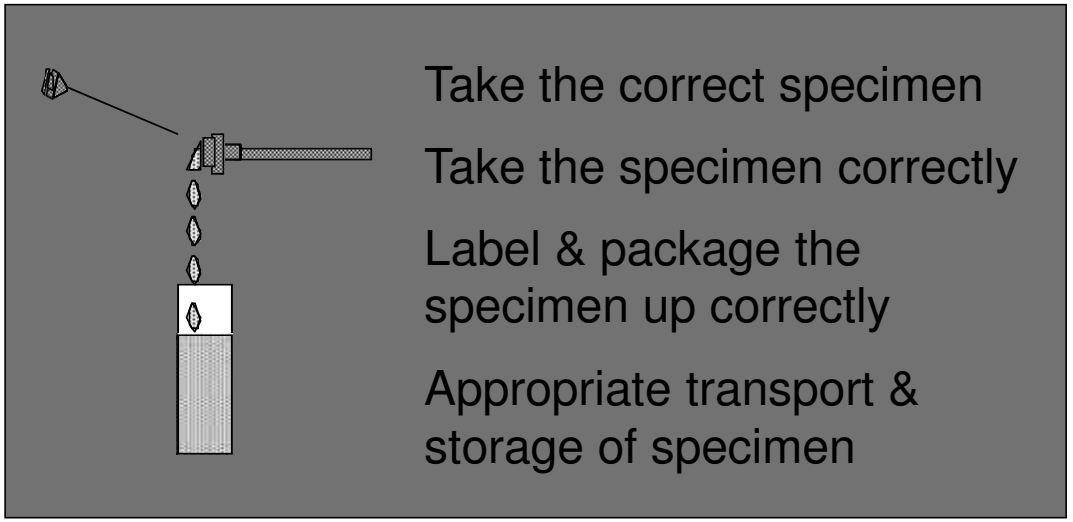
- Know available diagnostic technologies for Infectious diseases
- Understand specific specimen for specific diagnostic test
- Understand procedure for specimen collection
- Interpretation of the lab report

Diagnosis of Bacterial Infection









Which specimens/samples are processed in micro lab

- Site or origin
  - Central nervous system
  - Cardiovascular
  - Gastrointestinal
  - Genitourinary
  - Respiratory
  - Skin and soft tissue

Classification in the lab…….

- Direct
  - Collected from a sterile site
    - CSF, blood cultures, aspirates and biopsies
- Indirect
  - Passes through sites known to be colonized with normal flora
    - sputum and urine
- Stool (containing normal flora)

Common samples from paediatrics

- Cerebrospinal fluid - CSF
- Blood cultures
- Gastric aspirates – TB culture
- Sputum/nasopharyngeal aspirates
- Stool

Diagnostic technologies

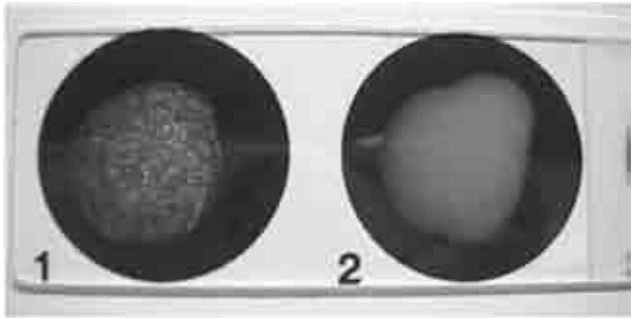
- Antigen Detection Assays
- Immunochromatographic tests
- Microscopy
- Cultures
- Sensitivities
- Molecular tests

CSF

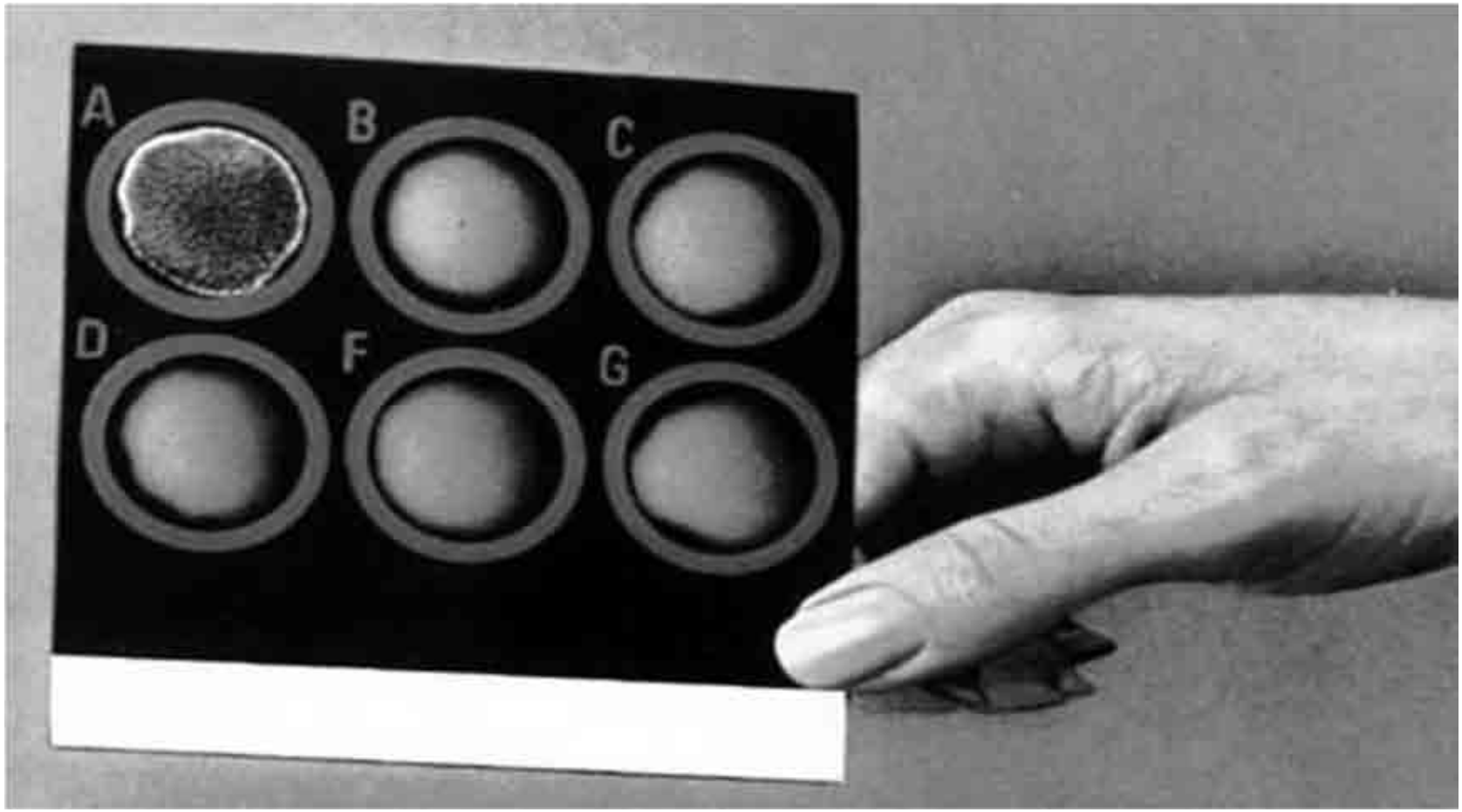
- Collected when CNS infection is suspected- meningitis
- Tests done in the lab include:
  - Rapid bacterial agglutination
  - Microscopy
    - Gram stain
    - Cell count (bacterial vs viral)
  - Culture & sensitivity

Rapid agglutination

- Point of Care Tests
  - Presence of antigen cause particles to agglutinate
  - wrong technique result in incorrect results
  - Prozone effect



Multiple tests on one card



Advantages of rapid diagnostic tests

- High sensitivity and specificity
- High negative and positive predictive values
- High accuracy compared to gold standard
- Simple to perform
- Rapid turn around time
- Cost effective

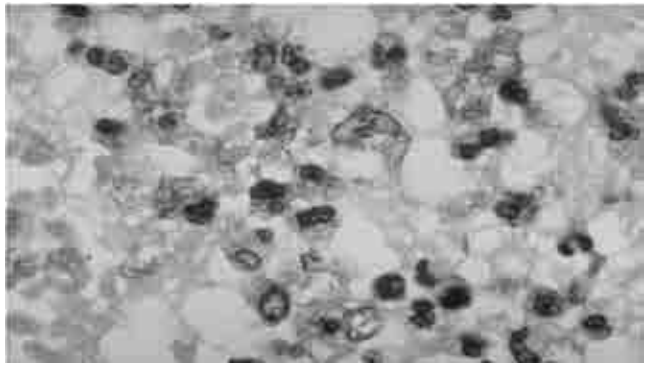
Microscopy

- Gram stain is the most commonly used
  - Wet prep or acid fast stain are less common
- Used for diagnosis
  - Presence of organisms/cells in the specimen
  - Gram stain is much less sensitive than culture
- Used to assess the quality of the specimen “Q scoring”

Microscopy
stained preparations

- Gram-stain

- Acid-fast stain
  - Ziehl-Neelsen



- Fluorescence
  - Direct, e.g. auramine
  - Immunofluorescence

Culture

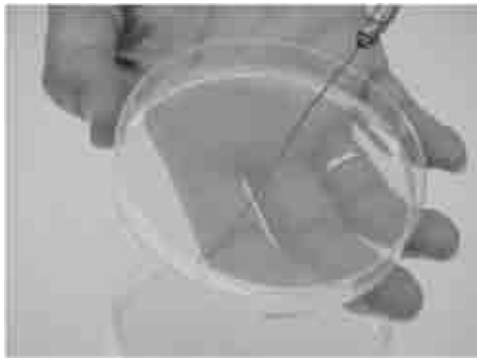
- Generally one of the most definitive methods
  - Sensitivity high (less for viruses, some rare bacteria)
  - Specificity high
- Provides isolates for further testing
- Often relatively slow
  - May take days (most commonly) to weeks (fungi, TB)
- Influenced by:
  - Types of media
  - Incubation conditions
  - Identification method

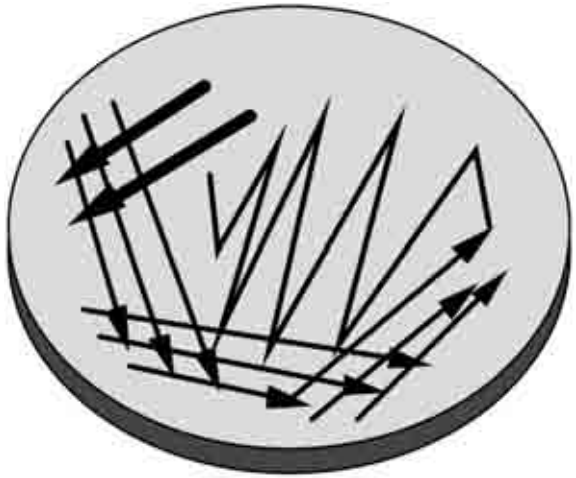
Culture Media

- May be liquid (“broth”) or solid (“plates”)
- Broth is used for
  - Detecting very low numbers of organisms
    - e.g. blood cultures

Culture of Bacteria

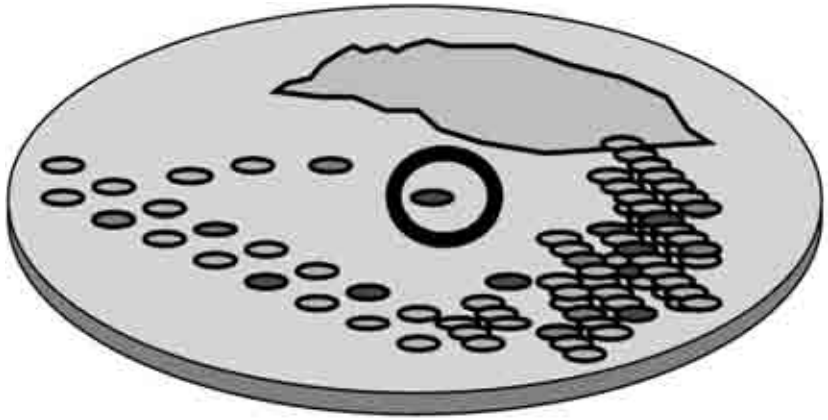
- Solid media
  - Agar plates
    - For Identification
    - For Enumeration
  - Slopes
  - For safe long-term culture, e.g. Lowenstein-Jensen media for TB
- Liquid media (broth)
  - For enrichment or maximum sensitivity

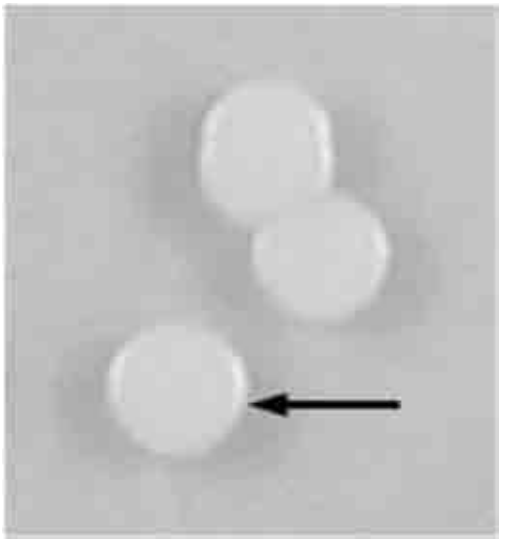




Advantages of Solid Media

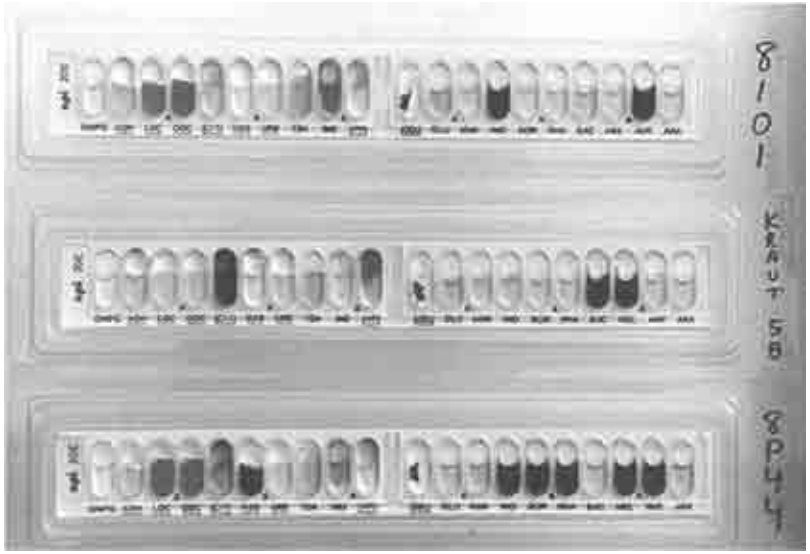
- isolation of single clonal colonies
  - get bacterium in pure culture
- identify by colonial morphology
- quantification by colony-forming units

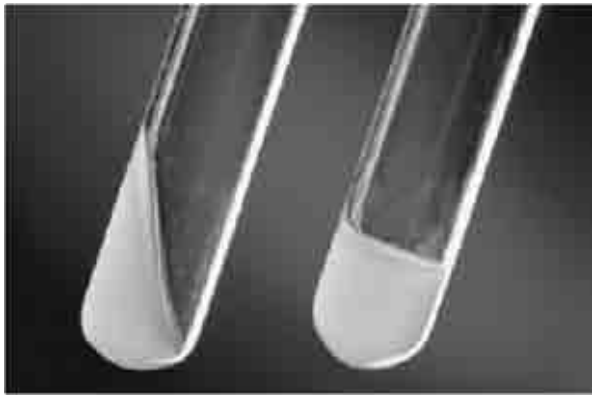




Identification of Bacteria

- Morphology
- Growth requirements
- Biochemistry
- Enzymes
- Antigens





Other Identification Methods

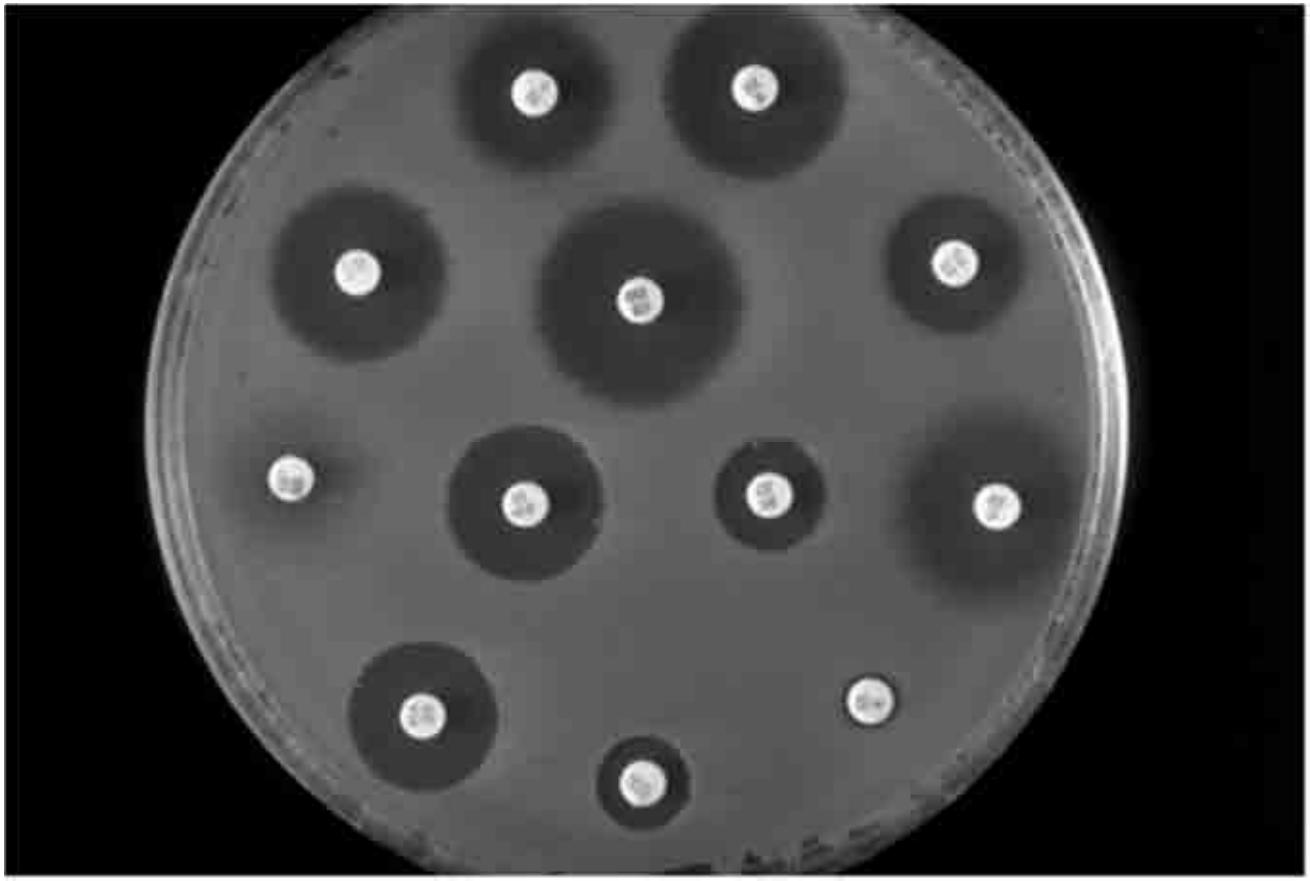
Include:

- Identification of specific antigens on the organisms surface
  - Agglutination tests
  - Immuno-fluorescent microscopy
- Molecular methods
  - Nucleic acid probes
  - Genome sequencing

Susceptibility testing methods

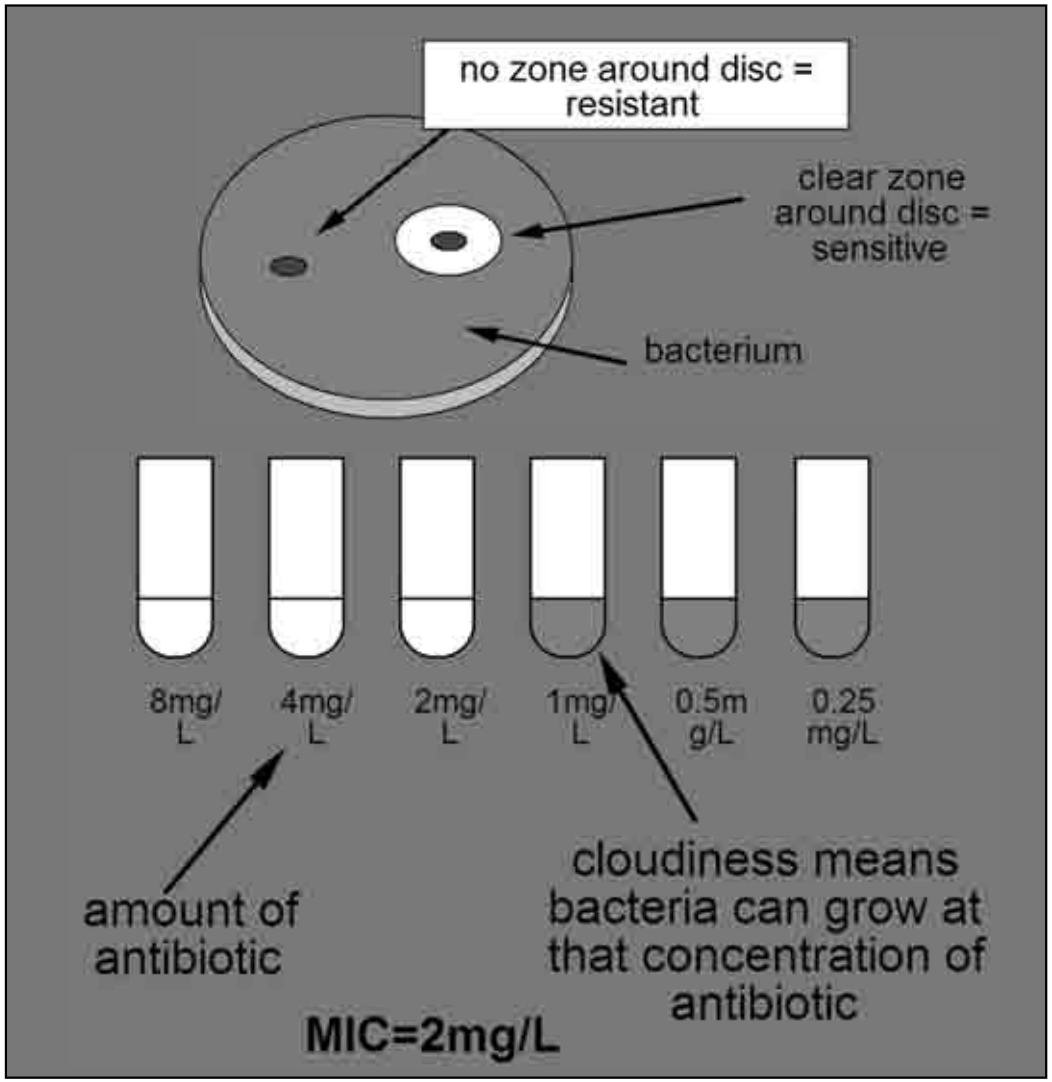
- Expose organism to concentration of antimicrobial
  - Antimicrobial may be in a disc
  - Antimicrobial may be in broth
  - Antimicrobial may be dissolved in the agar
- Lack of growth indicates inhibition
  - If the concentration is measured it is called the Minimal Inhibitory Concentration (MIC)
  - With discs a zone size correlates with a susceptible MIC, if the zone is bigger it is “Susceptible” if smaller it is “Resistant”.

Disk diffusion method

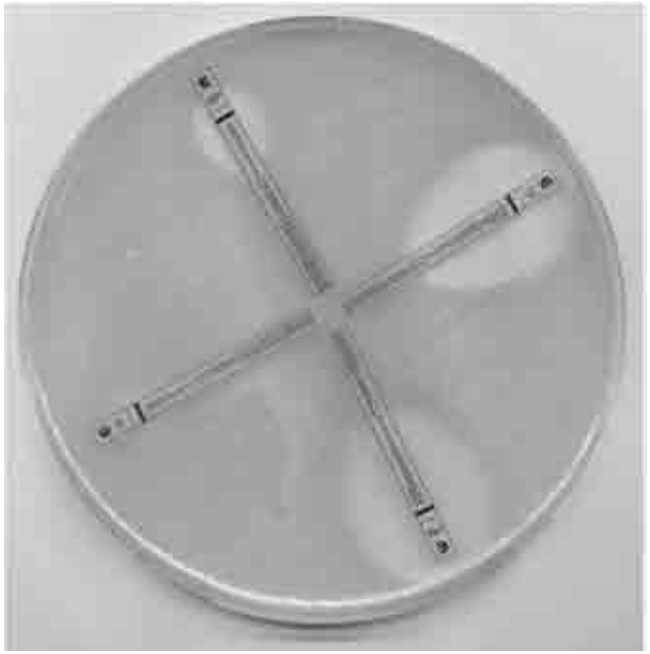


Sensitivity tests

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



E-test



Report from the lab

CSF Findings in Bacterial Meningitis

a. CSF pressure - usually elevated
b. CSF cells count and chemistry
   leukocytosis- >1000/cu mm
   % PMN - 90%
   Glucose- <40 mg/dl
   CSF blood to glucose ratio <0.40
   Protein 50-500 mg/dl
c. stained smears of CSF
   gram stain - (+) for bacteria
   AFB smear - (-)
   India ink - (-)
d. CSF culture
   *a negative culture does rule out meningitis

Blood culture

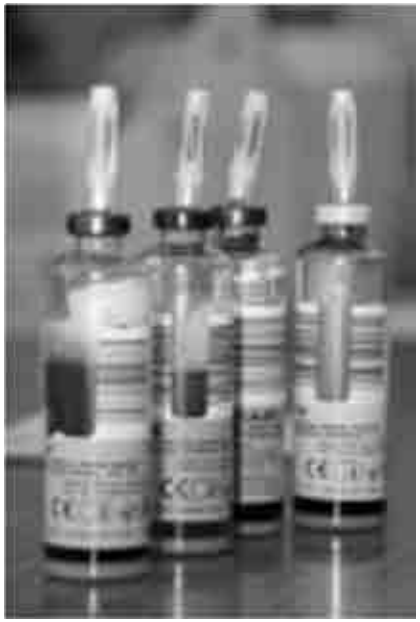
- Disinfect the skin properly
- Use the appropriate culture bottles
- Inoculate the recommended amount of blood
- Pediatric Aerobic,
- volume according to the manufacturer
- Min 1ml Max: 4ml
- Putting too much will not improve the yield.

Blood culture bottles

Blood culture machines

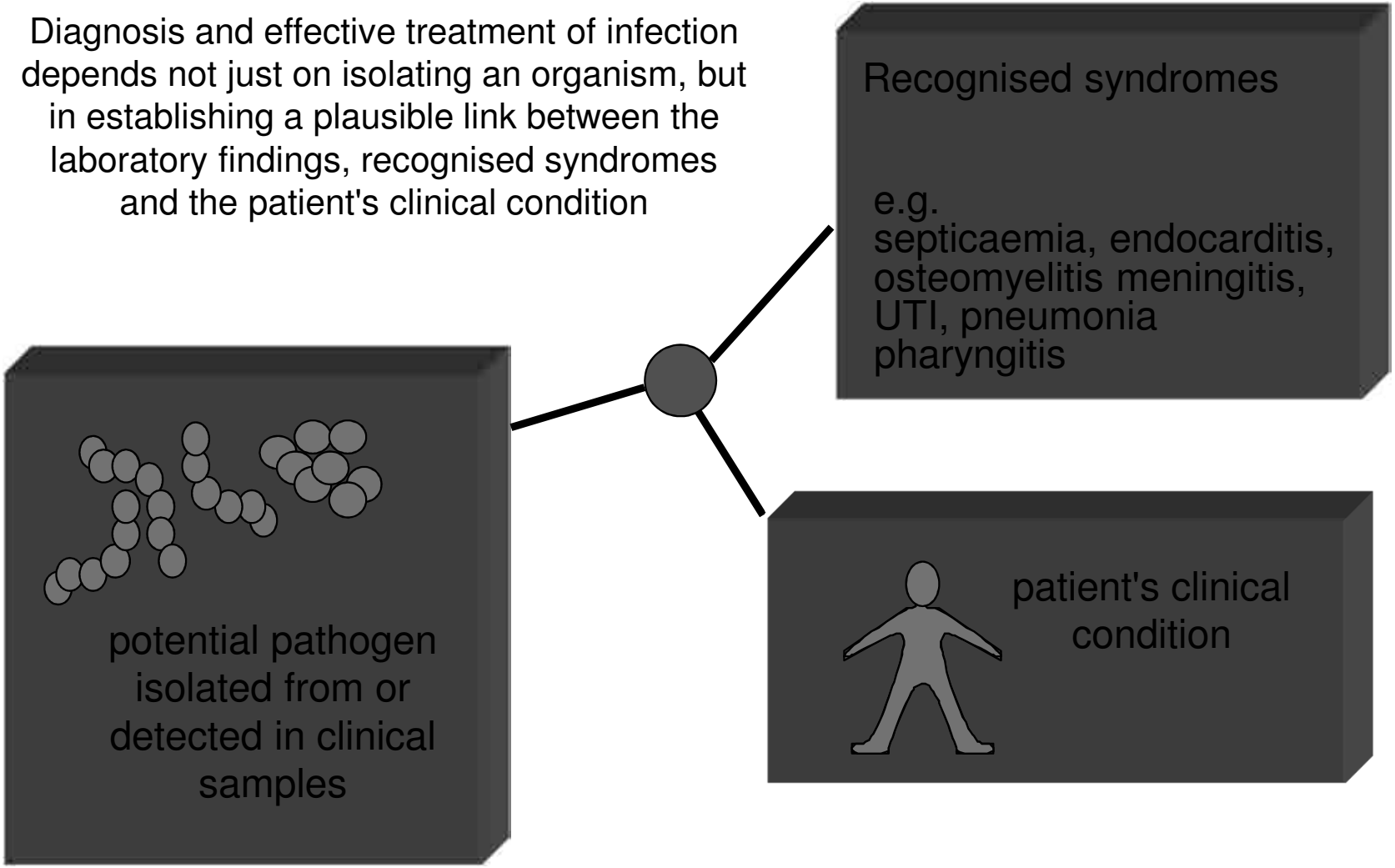
Positive signal

- Take the bottle out of the machine
- Do microscopy, culture and sensitivity as described with the CSF



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



LIMITATIONS OF CONVENTIONAL CLINICAL MICROBIOLOGY

- Culture
  - Labor intensive
  - Need for special media
  - Prolonged period of time to culture
  - Some organisms are uncultivable on artificial media
  - Potential health hazards
- Antigen Detection
  - Negative tests require confirmation
  - A ffected by poor specimen collection
  - Low microbe burden

Automated technologies for id sens

- Vitek 2
- Microscan
- Phoenix

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

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?

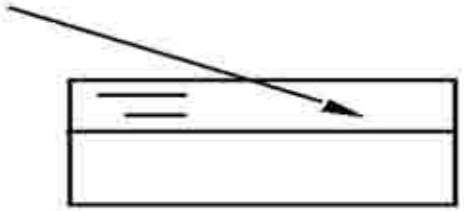
no

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

Thank you

- Comments and question are welcomed