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 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?
  yes
  no

Is the information already available?
  yes
  no

Does it affect patient management?
  yes
  no

Can the lab provide this information?
  yes
  no

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 diagnosis & treatment
  - pathogens die
  - contaminants overgrow
- Blood cultures directly into incubator
  - not refrigerator!
- 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

- cloudiness means bacteria can grow at that concentration of antibiotic
- MIC = 2 mg/L

no zone around disc = resistant
clear zone around disc = sensitive
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

**Spectrum of virulence**

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

*Rubella*
50% of infections are clinically apparent

*Rabies*
100% of infections are clinically apparent

Asymptomatic infection

Less severe disease

Classical clinical disease
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:
- septicaemia
- endocarditis
- osteomyelitis
- meningitis
- UTI
- pneumonia
- pharyngitis

Potential pathogen isolated from or detected in clinical samples.

Patient's clinical condition.
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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