Microbiology
Sample Collection

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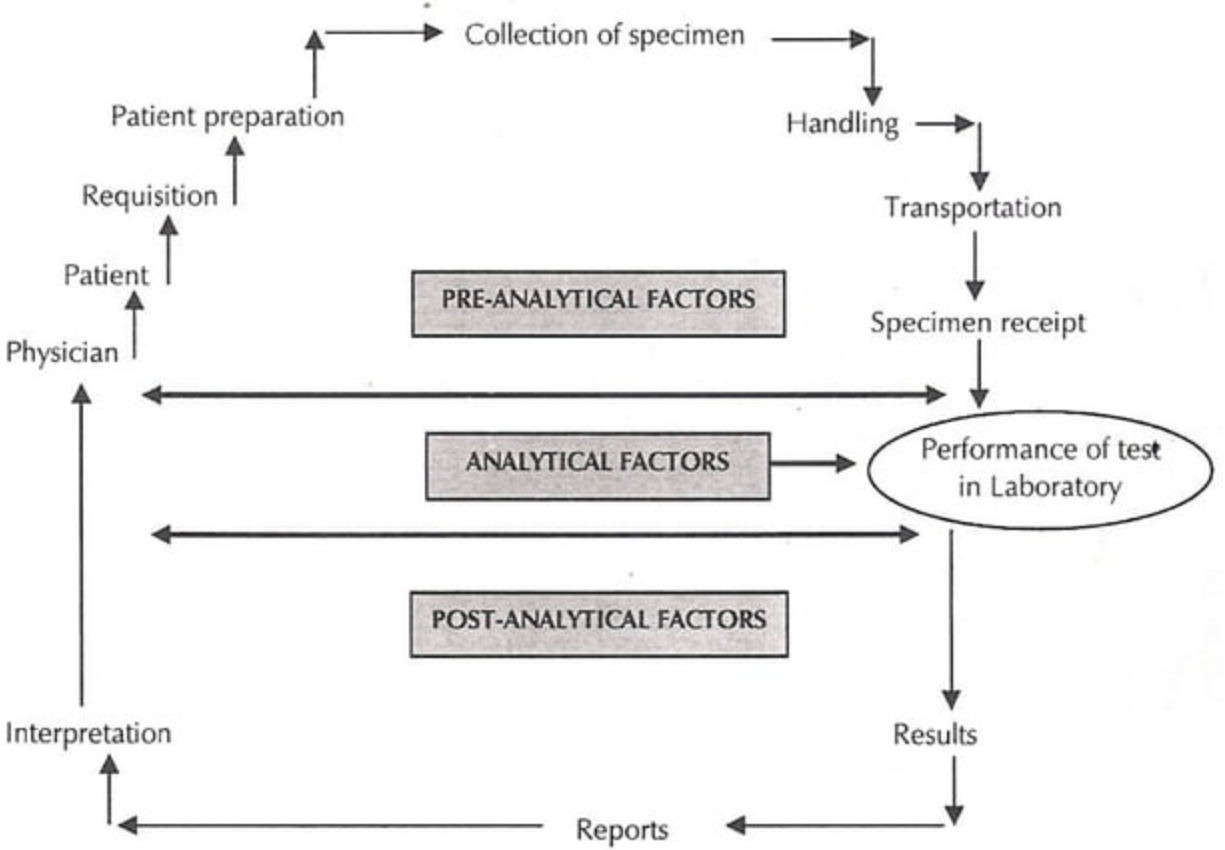
GIGO

- Diagnostic results depend on quality of specimens
- Poorly collected / transported specimens
  - Fail to isolate causative microorganism
  - Recover contaminants / normal flora
- Before the administration of Antimicrobials (or trough levels)


Collection of specimen
Patient preparation
Handling
Requisition
Transportation
Patient
PRE-ANALYTICAL FACTORS
Specimen receipt
Physician
ANALYTICAL FACTORS
Performance of test in Laboratory
POST-ANALYTICAL FACTORS
Interpretation
Results
Reports

Factors affecting Quality of Lab results

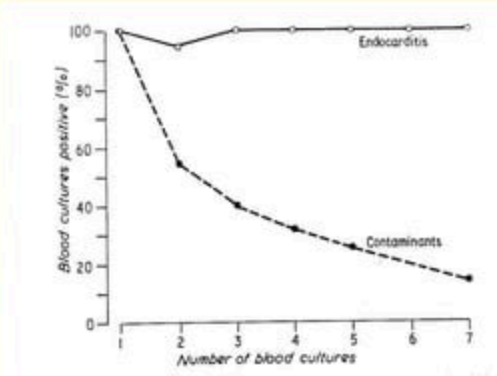
- **Pre-analytical**
  - **Appropriate samples**
  - Test method
  - Clinical details
  - **Sample collection**
  - Labelling (incorrect specimen identification)
  - Transportation / storage
- **Analytical** – equipment, reagents, calibration, SOPs, specificity, precision, accuracy, updated knowledge, technique, Quality Control, EQAS
- **Post-analytical** – transcription, communication

Help the Lab to help patients

- Work "blindfolded" without clinical details
- Provide clinical details
  - Symptoms / signs
  - Diagnosis
  - **Antimicrobial therapy** – current / prior
  - **Prosthetic devices**
  - **Immunosuppression**
  - Previous results
  - Other relevant investigations

Blood cultures

- Acute sepsis - 2-3 sets of blood cultures from separate sites
- Endocarditis - 3 cultures over 1-2 hours, ≥15 minutes apart (repeat if negative after 24 hours)
- PUO - 2 cultures, at least 1 hour apart (if negative - repeat 2 cultures 1 hour apart, after 24-36 hours)
- Single sample does not help
  - Intermittent bacteraemia
  - Opportunistic pathogens

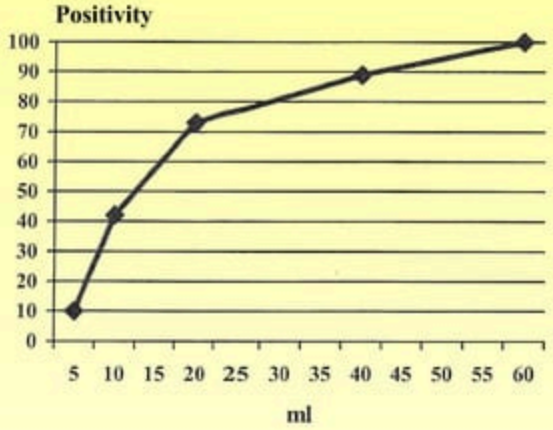


Blood culture

- Sample collection
  - Site
  - Strict aseptic technique
  - Palpation
  - Skin disinfection
    - 0.5% Chlorhexidine in 70% alcohol
    - OR Tincture Iodine or 10% Povidone Iodine
    - 3 times, allow to dry
  - First sample should be blood culture
  - Clean top of the bottle (alcohol)

Blood culture

- Bacteraemia / Enteric fever
  - Volume - critical
    - Adults: 5-20 ml
    - Children: 2-5 ml
    - Neonates: 0.5-1 ml
  - No of samples
  - 2 Sets – different sites, within 10 minutes



Blood culture

- CRBSI - DTP cultures
  - Equal Volumes
  - Timing
    - Simultaneous
    - Trough levels
  - Central line hub - clean with 70% alcohol
  - Line tip should be sent for culture after removal in CRBSI

Interpretation of CRBSI - DTP cultures

Central	Peripheral	Interpretation
+	-	Colonisation
+	+	<2 hours – Bacteraemia
+	+	>2hours – **CRBSI**
-	+	Bacteraemia
+	+	Discordant isolates - repeat

Blood culture

- Anaerobic culture
  - Brain abscess
  - Gastrointestinal infections
  - Closed abscesses
- Miliary TB – Rapid TB Culture

Blood culture

- Do NOT
  - Palpate the vein after skin disinfection
    - Palpate only before skin disinfection
  - Use iodine to clean bottle caps
    - Instead use alcohol / chlorhexidine

Device tips

- Central line / intravascular devices
  - Long (distal tip and proximal transcutaneous segment)
    - long term
      - Percutaneous: Broviac, Hickman, etc
      - Subcutaneous ports
    - short term percutaneous: Swan-Ganz, Intracath, etc
  - Short (entirety, remove hub)
    - peripheral, umbilical, arterial, etc
- Foley's or ETT tips NOT recommended for culture

Respiratory Samples

- Sputum
  - Patient instructions
  - Induced sputum
  - Quality of sample
    - Acceptable specimen: PMN: SEC ratio ≥ 2:1
    - Unacceptable specimen: PMN: SEC ratio < 2:1
- BAL
- ET aspirates – suction extractor
- Throat swab
  - Sample collection
  - One swab / investigation

LRT

- Sputum (expectorated)
  - rinse mouth and gargle before collection
- Sputum (induced)
  - use a wet toothbrush to clean buccal mucosa, tongue and gums
  - rinse the mouth thoroughly
  - 20-30 ml of 3-10% saline
- Tracheostomy secretions
- Bronchoscopy secretions - lavage, bronchial brush, biopsy
- Lung aspirations
- Lung biopsies

Respiratory samples

- Aerobes: 3-5 ml Sputum (not saliva)
- Fungi (3-5ml) - 3 early morning samples; lung biopsy / aspirate
- Mycobacteria (5-10ml) - 3 early morning samples; lung biopsy / aspirate
- Anaerobes (1ml) - aspirate from sinus, middle ear, trachea, lung or biopsy
- Pneumocystis (2ml) - induced sputum, BAL, or lung biopsy
- Parasites (3-5ml) Amoeba, helminth eggs (*Paragonimus westermani*), hooklets of Echinococcus, larvae of hookworm, ascaris and Strongyloides sp.

Body fluids

- Skin disinfection; aseptic technique
- Pleural, pericardial, peritoneal, CAPD*, synovial
- Bacteria (1-5ml); whole bag for CAPD
- Fungi (>10ml) - Blood for *Histoplasma capsulatum, Cryptococcus sp, Candida sp.*
- Anaerobes (1-5ml)
- Mycobacteria (>10ml)
- Greater volumes increase the chances of organism recovery

CSF

- Aseptic technique
- 3 sets – Microbiology, Biochemistry and Haematology
  First sample (or most turbid) to Microbiology (unless haemorrhagic)
- Recommended minimum volumes (greater volumes increase the chance of organism recovery)
  - Routine culture: 1 ml
  - Fungal culture: 2 ml
  - Mycobacterial culture: 2 ml
- *Microbiology does not mean microscopic samples!*

Pus

- Surgically obtained pus / fluid
- Pus better than swabs
- Tissue in small amount of saline
- Aspirates from closed abscesses
- If anaerobes suspected
  - Ask for and inoculate in anaerobic transport medium / BacT/Alert bottle
  - Immediate transport

Skin and s/c tissue

- Burns - punch biopsy (3-4mm) of deeper tissue - quantitative culture
- Superficial wounds
  - Syringe aspiration better than swabs
  - Fungal - scrape the periphery of the lesion
- Ulcers and nodules - curette base, exudate in syringe or swab
- Viral recovery (HSV, Varicella zoster) is highest from young vesicles, than from pustules, ulcers or crusted lesions.

Wounds, aspirates and tissue

Biopsies are better than aspirates

- Bite wounds - aspirate pus
- Bone - kept moist in sterile saline
- Deep wounds / abscesses
- Punch skin biopsies
- Soft tissue aspirate

Anaerobic culture

- Pus from closed abscesses
- Blood
- Bone marrow
- Bile
- CSF
- Peritoneal fluid
- Suprapubic aspirate (Urine)

Anaerobic culture

Unacceptable specimens

- Gastrointestinal
  - Faeces
  - Gastric / small bowel contents
  - Rectal swab
- Urogenital
  - Urine
  - Urethral swab
  - Vaginal Swabs
- Respiratory
  - Throat swabs
  - Coughed sputum

Anaerobic cultures

- Collection technique
- Exclusion of air
- Blood (anaerobic) culture before all other samples
- Rapid transport

Urine

- Direct collection; first morning sample (3 samples for AFB culture) ideal – highest counts; in practice best time to collect is when patient is able to provide an adequate sample
- 24 hour urine cultures not recommended
- Collect clean catch, mid-stream urine (MSU); do not stop flow of urine
- Transport within 2 hours; otherwise refrigerate at 2-8°C

Urine

- Supra-pubic aspirate (SPA)
- Bladder washout
- **Foley's catheter sample**
  - Maintain a sterile, closed catheter system at all times
  - Urine specimens should he obtained aseptically without opening the catheter-collection junction
  - Urine should be aspirated from the distal end of the catheter with sterile needle and syringe after disinfecting the area

Urine

- Bacteria (0.5-1ml)
- Fungi (>20ml)12
- Mycobacteria (100ml)
- Anaerobes (1ml)
- Virus (10-50ml)
- Parasites (24 hour collection) -*Schistosoma haematobium* eggs, *Trichomonas vaginalis* trophozoites and *Ochocerca volvulus* microfilariae

Urine culture

- Suprapubic / Foley's aspirates – clearly labelled, different interpretation
- Operative sample – details
- Foley's catheter tip not recommended
- Catheter sample collection; label
- AFB / Mycobacteria
  - Early morning sample; 100ml
  - 3 consecutive days

Doubtful significance

- Foley's catheter tip
- Burns - wounds swabs
- Colostomy discharge
- Vomitus
- Lochia
- Gastric aspirates of new born
- Bed sore swabs
- ET tube / suction catheter tips

Rejection criteria

- Leaking container
- Unlabelled / wrongly labeled / mismatched samples
- Specimen received in fixative
- Insufficient quantity
- Specimen unsuitable for request
- Prolonged transport
- Dried specimen
- Duplicate specimens on the same day for the same test (except blood, CSF, tissue, sterile body fluids exc. urine)

Labelling

- Label specimen container & form
  - Patient name
  - Identification number
  - Source / site
  - Date / time of collection
  - Collector (esp in case of blood cultures)

Transportation

- ASAP / within two hours
- For Anaerobic / amoebic abscess aspirate
  - 15-30 minutes
- If delay unavoidable
  - Refrigerate, 2-4°C / ice (2-4 hours)
  - Exceptions - CSF, genital, eye, internal ear specimens, amoebic abscess aspirate.

Thank you

- Good samples are essential for good results