Collection of Samples for Bacteriological Examination

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General rules should be applied to all specimens

- Hands washed before and after the collection.
- The samples:
  - Taken before the start of antimicrobial therapy.
  - Representative
  - Adequate volume.
  - Collected aseptically; dated, and labelled
  - Transported rapidly to the laboratory.
  - As fresh as possible.
- Viral transport media.
- Bedside samples
A) Respiratory Tract Infection
1) Throat swab: URTI
Throat swab

Tonsilar fossa
2) **Nasopharynx**: nasopharyngeal washing or nasopharyngeal swab in meningitis and viral infection.
3) Middle ear:
4) **Lower respiratory:**

Early morning sputum.

If the patient failed to expectorate
Trans-tracheal aspiration, lung biopsy, or bronchoalveolar lavage

A bronchoscope is used to view the airways and check for any abnormalities.
in children???????????
B) Acute Intestinal Infection
Faeces

rectal swab

Rectal tube, bile, vomitus
C) Urinary tract infection (UTI)
1) Mid stream specimen

Delay ???
2) Supra-pubic aspiration

3) Adhesive bags
4) Catheterization of urethra
D) Meningitis
CSF

lumbar puncture
screw-capped bottles
E) Wounds, Abscesses, Fluids, Tissues
Ordinary Swab

Regranex stimulates angiogenesis (granulation tissue) for wound healing. Only a thin layer needs to be applied topically on a daily basis.
Aspirating pus by using a syringe

Pieces of tissues: Removed at operation or curettage from infected tissues, then homogenized in a tissue grinder with a little broth.
F) Genital Tract Infections
a) In females:

- **Cervicitis**: Cervical swab.

- **Vaginitis**: Vaginal swab, discharge.

b) In male:

- **Acute**: Urethral discharge.

- **Chronic**: Morning drop, prostatic massage.
G) Septicemia
Blood Culture Technique

Principle

- For diagnosis of bacterial endocarditis
- All diseases where bacteraemia occurs as in typhoid fever during the first week, brucellosis, and Meningococcal meningitis etc...
- Also in septicaemia due to any organism.

Procedure
METHODS OF ISOLATION OF BACTERIA
Plating out technique
Methods of anaerobiosis

1) Deep agar
2) Media containing reducing compounds
3) Absorption of O2 by Na-pyrogallate (Buchner’s tube or McLeod’s plate)
4) Replacement of Oxygen with hydrogen

**Gas-bag jar**
IDENTIFICATION OF ISOLATED BACTERIA
I. Microscopic Examination:

Unstained
Gram stained
2) Cultural appearance:

a) Colony morphology
Mucoid colonies
b) Pigment Production

Endopigment
Exopigment
C) Swarming Growth
**D) Type of Haemolysis on Blood Agar**

- **β haemolysis**
- **α haemolysis**
e) Lactose Fermentation on MacConkey's agar
3) Biochemical reactions:

a) Sugar Fermentation:
B) Indole production:

Demonstrates the ability of certain bacteria to decompose the amino acid tryptophane present in peptone to indole.
c) Voges Proskauer's Reaction (V.P.):

- Some bacteria ferment glucose with production of acetyl methyl carbinol.
c) Methyl Red Test (M.R.):

Detect the ability of some bacteria to produce large amounts of acid from fermentation of glucose, thus lowering the pH of the medium below 4.
E) Urease Test

Some organisms e.g. *Proteus* produce urease enzyme, which can be detected by alkalinity and increase pH of the surrounding medium.
F) Oxidase Test

Some bacteria e.g. *Neisseria*, *Vibrio*, *Campylobacter*, *Pseudomonas*, produce oxidase enzyme, which can reduce oxidase reagent to a deep **purple** colour.
G) Commercial Kit Systems:
H) Automated bacterial identification systems (ViteK): which determines the presence of growth, identifies the organism and its antibiotic sensitivity by detecting turbidity and colour changes in special cards inoculated with the organism

I) Serological identification (slide agglutination).

J) Animal inoculation
K) Skin tests
IX. Molecular identification and typing methods
L) Typing of Isolates

- Antibiotic sensitivity patterns.
- Biotyping.
- Serotyping.
- Bacteriophage typing.
- Bactericine typing.
- DNA typing.
M) Molecular Identification and Typing Methods:

- Detection of microbial nucleic acid:
  1. Polymerase Chain Reaction (PCR).
  2. DNA sequencing.
  3. DNA probe.
Polymerase Chain Reaction (PCR):

- It is an advanced technique to generate many copies of a single DNA molecule.
**Types:**

**A) Qualitative PCR:**
- Confirm the presence of an infection.
- Differentiates between resolved and active infection.

**B) Quantitative PCR:**
- **Follow up therapy:** Document rapid (RVR) and early (EVR) virologic response.
- Guide duration of antiviral therapy.
- Confirm resolution of infection and sustained virologic response (SVR).
2) DNA sequencing:

- It is the determination of the order of nucleotides (GCAT ....etc) through the whole length of DNA or RNA molecule.
3) DNA probe

- A piece of single stranded DNA or RNA (or PNA) (peptide nucleic acid) which is complementary to the sequence of interest (to be detected) and labeled by detectable material at its 5’ phosphate end.
ANTIBIOTIC SENSITIVITY TESTING
1) Kirby-Bauer Disk-diffusion method
Isolate in Mueller-Hinton broth.

Allow culture to soak in for 10 minutes.

Add antibiotic disks.

Swab entire surface of Mueller-Hinton agar with test bacterium.

Cartridges (Difco) can be used to dispense individual disks.

Push handle of dispenser down to dispense 12 disks.

Touch to surface of agar.

Incubate

Measure diameter of zones of inhibition to the nearest mm after 16-18 hours incubation.
Notice the following:

For urine samples use the following antibiotic disks: Nitrofurantoin, Nalidixic acid & Norfloxacine

Quinolones and sulfa are contraindicated for pediatric use and in pregnancy

Tetracyclines are contraindicated below the age of 18 years

Use pipracillin disk for pseudomonal infections

Streptococci have natural resistance for gentamycin
Use **methicillin** disks in case of *Staphylococcus* infection to detect **MRSA**.

**Vancomycin** is conserved for highly resistant strains of *Staphylococcus*.

If the patient is already on antibiotic therapy use these **antibiotic disks** to test their efficacy.
II- Broth dilution method:

a) Minimum inhibitory concentration (MIC):
   • Is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation.

b) MBC:
   • It is the lowest concentration of antibiotic required to kill a particular bacterium.
   • Determined from broth dilution MIC tests by subculturing to agar media without antibiotics.
1) dilution (halving of previous concentration)

1000  500  250  125  62.5  31.2  15.6  7.81
µmol.l⁻¹

2) inoculation (10³ germs per well)

3) incubation (35°C, 24 – 120 h)

no growth  growth

4) reading (MIC = 125 µmol.l⁻¹)
Thank You