### Collection of samples for Bacteriological examination

#### General rules should be applied to all specimens

1. Hands should be washed before and after the collection
2. The sample must be
   a. Taken before the start of antimicrobial therapy
   b. Representative of infectious process eg. Sputum (not saliva), swab from depth of wounds (not from surface)
   c. Adequate volume
   d. Collected aseptically in an appropriate, sterile container, which must be dated, appropriately labeled and the requisition form completed
   e. Transported rapidly to the laboratory and transport media may be helpful; eg. Pus and urine within 2 hours
   f. As fresh as possible fearing of death of delicate organism eg: Gonococci or Haemophilus or overgrowth of contaminants

3. Viral transport media containing (amino acid, salts, solution at appropriate pH with antibiotics) for transporting swabs for viral cultures if there is delay should be kept at -70°C
4. Eye infections, genital tract and CSF specimens are best taken at the bedside

#### Selected cases for sample collection

##### A) Respiratory Tract infections

<table>
<thead>
<tr>
<th>Area</th>
<th>Sample Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Throat</td>
<td>Throat swab</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>Nasopharyngeal washing or nasopharyngeal swab in meningitis and viral infection</td>
</tr>
<tr>
<td>Middle ear</td>
<td>Otitis media with discharging ear, the sample is taken by ordinary swab from the discharge deeply as you can</td>
</tr>
</tbody>
</table>

##### Lower respiratory

- Sputum is collected in sterile container; best sample is the early morning sputu. The patient should expectorate from deep down in the lung
- Trans – tracheal aspiration, lung biopsy, or bronchoalveolar lavage (this is done during examination or operation)

##### B) Acute intestinal infection

<table>
<thead>
<tr>
<th>Area</th>
<th>Sample Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces</td>
<td>Faeces should be passed into clean containers, and should be sent to the laboratory as soon as possible</td>
</tr>
<tr>
<td>Vomitus</td>
<td>May be taken in case of poisoning</td>
</tr>
</tbody>
</table>

##### C) Urinary tract infection (UTI)

<table>
<thead>
<tr>
<th>Area</th>
<th>Sample Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mid stream specimen</td>
<td>Cleaning of the external genitalia with soap and water</td>
</tr>
<tr>
<td>Adhesive bags</td>
<td>Used for collection of urine specimen especially in children</td>
</tr>
<tr>
<td>Supra – pubic aspiration</td>
<td>In infants or in case of retention or pregnancy</td>
</tr>
<tr>
<td>Catherization of urethra or ureter</td>
<td>For collection of urine if the patient is already catherized</td>
</tr>
</tbody>
</table>

##### D) Meningitis

Sample is taken is CSF, which is taken by lumbar puncture
SCF is collected in screw – capped bottles and sent to the laboratory at once

##### E) Wounds, abscesses, fluids, tissue

<table>
<thead>
<tr>
<th>Area</th>
<th>Sample Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ordinary swab</td>
<td>Which must be soaked well in pus or exudates deep form infected wounds</td>
</tr>
<tr>
<td>Aspiration by syringe</td>
<td>To obtain a specimen of the pus itself and transfer to a sterile tube</td>
</tr>
<tr>
<td>Pieces of tissues</td>
<td>Removed at operation or curettage from infected tissues, then homogenized in a tissue grinder with a little broth</td>
</tr>
</tbody>
</table>

##### F) Genital tract infections

<table>
<thead>
<tr>
<th>Gender</th>
<th>Sample Collection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Cervicitis: cervical swab</td>
</tr>
<tr>
<td></td>
<td>Vaginitis: vaginal swab, discharge</td>
</tr>
<tr>
<td>Male</td>
<td>Acute: urethral discharge</td>
</tr>
<tr>
<td></td>
<td>Chronic: morning drop, prostatic massage</td>
</tr>
</tbody>
</table>
### G) Septicemia

<table>
<thead>
<tr>
<th>Sample</th>
<th>The sample collected is blood</th>
</tr>
</thead>
</table>

**Principle**
Done for the diagnosis of bacterial endocarditis and also done in the following condition
- For diagnosis of bacterial endocarditis
- All diseases where bacteremia occurs as in typhoid fever during the first week, brucellosis and meningococcal meningitis etc
- Also in septicemia due to any organism

**Blood culture technique**

**Procedure**
1. A strict aseptic technique should be used
2. 5 to 10 ml of blood is withdrawn with a sterile syringe after through sterilization of the puncture are
3. The blood is added to 50ml broth in blood culture bottles, which are incubated at 37°C
4. Subcultures are made every day by withdrawing small amount (loopfull) of blood broth mixture and plating it out on blood agar
5. The colonies are identified and their antibiotic sensitivity is tested
6. If results of subculture are negative for 7 successive days discard the blood culture
7. The big volume of broth has the following advantages
   a. It provides good nutrition for multiplication of the organism, which is usually present in small numbers
   b. It dilutes out any antibiotic or any antibacterial substances in the serum

**Methods of isolation of bacteria**

**General**
Almost every procedure used in diagnostic microbiology is based on examining pure cultures eg; isolated growth of a single bacterium
This can be achieved by the plating out technique, which aims at spreading out bacteria on the surface of solid media. Each bacterium will then divide repeatedly to give rise to a separate colony

**Methods of anaerobiosis**
1. Use of deep media as deep agar tube and Smith – Noguchi media
2. Use of media containing reducing compounds as Robertson’s cooked meat media and thioglycolate
3. Absorption of O2 by Na-pyroallate using Buchner’s tube of McLeod’s plate
4. Replacement of oxygen with hydrogen using McIntosh and Fildes jar
5. Gas bag jar

**Anaerobic gas pack system**
- Hydrogen is generated inside the jar by placing a special gas pack envelope commercially prepared
- The presence of the catalyst in the jar allows the hydrogen released to combine with the oxygen in the jar to give strictly anaerobic condition
- An anaerobic indicator eg; methylene blue which is colorless and changes blue in presence of O2, is put in the jar to check the activity of the catalyst

**Anaerobic culture media**
- Consist of cooked minced meat to which broth is added
- The meat contains reducing substances eg; haematin and glutathione which maintain anaerobic conditions in the depth of this fluid medium
- Used for growing the Clostridium group and other anaerobes
- Sterilized in the autoclave

**Thioglycolate broth**
This medium contains sodium thioglycolate which is a reducing substance that renders the medium suitable for the growth of anaerobic bacteria
### Identification of isolated bacteria

<table>
<thead>
<tr>
<th>Microscopic examination</th>
<th>Examination of unstained preparations is used for demonstrating motility Examination of gram stained preparations determine; whether gram positive or gram negative, their morphology (c cocci, bacilli, etc) size and arrangement</th>
</tr>
</thead>
</table>
| Culture appearance      | 1. **Colony morphology:** its size and shape, whether it is opaque or translucent, mucoid or dry  
2. **Pigment production**  
   - If it is an endopigment producer, the pigment will be restricted to the colony as in S. aureus (golden yellow)  
   - If it is an exopigment producer, the color will diffuse in the surrounding medium as in Pseudomonas aeruginosa colonies  
   - Swariming growth on solid media as in Proteus  
3. **Type of hemolysis on blood agar**  
   - B hemolysis: S. aureus and Strep. Pyogenes  
   - A hemolysis: S. Viridans and Pneumococci  
4. **Lactose fermentation on MacConkey’s agar**  
   - Lactose fermenters (Coliform): give rose pink colonies  
   - Non lactose fermenters: colonies are pale (Salmonella & Shigella) |
| Biochemical reaction    | 1. **Sugar fermentation:** depends on the varying ability of bacteria to ferment sugars with acid production, which may or may not be accompanied by the evolution of gases  
2. **Indole production**  
3. **Voges Proskauer’s reaction**  
4. **Methyl red test**  
5. **Urease test:** some organisms eg: Proteus produce urease enzyme, which can be detected by alkalinity and increase PH of the surrounding medium  
6. **Oxidase test:** some bacteria eg: Neisseria, Vibrio, Campylobacter and Pseudomonas produce oxidase enzyme, which can reduce oxidase reagent to a deep purple color  
7. **Commercial kit system:**  
   - Packaged biochemical identification systems are readily available form commercial sources for example the API (Analytical Profile Index) system.  
   - It is composed of a plastic strips with cupules containing dehydrated substances that are used to determine the different biochemical reactions  
   - Biochemical profiles are determined by reading the color change and interpreting according to available charts  
   - These are then converted to numerical codes, which can be read form a key (index) giving the identification of the organism |
| Automated bacterial identification system | There are many system eg; the Vitek system which determines the presence of growth, identifies the organism and its antibiotic sensitivity by detecting turbidity and color changes in special cards inoculated with the organism |
| Serological identification | By slide agglutination with specific antibodies  
Detection of bacterial specific antigens or antibodies |
| Animal inoculated used to |  
- Identify certain pathogenic bacteria eg; tubercle bacilli and leptospira which produce characteristic lesions when injected in laboratory animals  
- Distinguish between pathogenic and non – pathogenic species of bacteria  
- Eg: Lab animal → guinea pig of TB, mouse in Pneumococci |
| Skin test | Can be used either for diagnosis of a case or diagnosis of susceptibility |
| Typing of isolates |  
- Antibiotic sensitivity patterns  
- Biotyping  
- Serotyping  
- Bacteriophage typing  
- Bactericine typing  
- DNA typing |
| Molecular identification and typing methods | For detection of microbial nucleic acid by  
1. Polymerase chain reaction (PCR): it is an advanced technique to generate many copies of a single DNA  
   a. Qualitative PCR: confirm the presence of an infection, differentiates between resolved and active infection  
   b. Quantitative PCR: document rapid (RVR) and early (EVR) virologic response. Guide duration of antiviral therapy  
2. DNA sequencing : it is the determination of the order of nucleotides through the whole length of DNA or RNA molecule  
3. DNA probe: a piece of single stranded DNA or RNA which is complementary to the sequence of interest (to be detected) and labeled by detectable material at its 5’ phosphatate end |
# Antibiotic sensitivity testing

| Kirby – Bauer disk – diffusion method | • Disk with exact amounts of different antimicrobial agents are placed on culture dishes inoculated with microorganisms to be tested  
• The organism’s growth (resistance to the drug) or lack of growth (sensitivity to the drug) is then monitored  
• The size of the zone of growth inhibition is influenced by the concentration and rate of diffusion of the antibiotic on the disk |

| Notice the following | 1. For urine samples use the following antibiotic disc: Nitrofurantoin, Nalidixic acid, Norfloxacin  
2. Quinolones and sulfa are contraindicated for pediatric use and in pregnancy  
3. Use pipracillin disc for pseudomonal infections  
4. Streptococci have natural resistance for gentamicin  
5. Use methicillin disk in case of Staphylococcus infection to detect MRSA  
6. Vancomycin is conserved for highly resistant strains of Staphylococcus  
7. If the patient is already on antibiotic therapy use the these antibiotic disks to test their efficacy |

<table>
<thead>
<tr>
<th>Broth dilution method</th>
<th>Minimum inhibitory concentration (MIC)</th>
<th>Minimal bactericidal concentration (MBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation</td>
<td>It is the lowest concentration of antibiotic required to kill a particular bacteria. Determine from broth dilution MIC tests by subculturing to agar media without antibiotic</td>
<td></td>
</tr>
</tbody>
</table>

## Figures

![Figure 1 Gas bag jar](image)