

METHODS OF ISOLATION OF BACTERIA

By:

Alaaeldeen Balal Ahmed

- **Isolation of bacteria:**

Isolation of bacteria forms a very significant step in the diagnosis and management of the illness. Isolation of bacteria involves various steps:

- 1- Specimen collection
- 2- Preservation and transportation of specimen
- 3- Microscopic examination of sample
- 4- Various methods used for isolation of bacteria

- **Specimen collection:**
- Many different specimens are sent for microbiological examination from patients with suspected bacterial infection.
- **Common specimens include:**
Urine, faeces, wound swabs, throat swabs, vaginal swabs, sputum, and blood.
- **Less common, but important specimens include:**
Cerebrospinal fluid, pleural fluid, joint aspirates, tissue, bone etc.

- Some types of specimen are normally sterile e.g. blood, CSF. These samples are usually obtained via a percutaneous route with needle and syringe, using appropriate skin disinfection and an aseptic technique. The culture of bacteria from such specimens is usually indicative of definite infection except if they are skin contaminants (bacteria inhabitants of normal skin).

- In contrast, many microbiological specimens are obtained from non-sterile sites e.g. vaginal or throat swabs, urine sample, stool sample. Such samples often contain bacteria of no clinical relevance in addition to possible pathogens, making the interpretation of culture results more difficult.
- It is preferred to obtain the samples for bacteriological culture before antibiotic therapy is started. This maximizes the sensitivity of the investigations and reduces false-negative results.

- Specimens must be accurately labelled and accompanied by a properly completed requisition form, indicating the nature of the specimen, the date of sample collection, relevant clinical information, the investigations required, and details of antibiotic therapy, if any. This allows the laboratory to perform the correct range of tests, and helps in the interpretation of results and reporting.

- **High-risk samples**

Certain bacterial infections are a particular hazard to laboratory staff, and specimens that might contain these pathogens should be labelled as 'high risk' to allow for additional safety measures if necessary. For example - blood cultures from suspected typhoid (*Salmonella typhi*) or brucellosis (*Brucella* species), and samples from suspected *Mycobacterium tuberculosis*.

- **Preservation and Transport of specimen**
 - Most specimens are sent to the laboratory in sterile universal containers. Swabs are placed in a suitable transport medium (eg. charcoal medium) otherwise it leads to false negative reporting.
 - Specimens should be transported as soon as possible to the laboratory. In case a delay is anticipated the specimen should be stored at 4° C.

- Immediate transport is necessary in order to:
 - (i) Preserve the viability of the 'delicate' bacteria, such as *Streptococcus pneumoniae* or *Haemophilus influenzae* (delays in processing can cause false-negative culture results);
 - (ii) Minimize the multiplication of bacteria (e.g. coliforms) within specimens before they reach the laboratory.

- **Microscopy**

A Gram stain helps with the visualization of bacteria, and gives an indication of the type of bacteria present, based on the shape of the bacteria and the staining properties (Gram positive: purple; Gram negative: pink/red). A Gram stain also helps to identify mixtures of bacteria, helps to determine the appropriate range of agar plates to be used for subsequent culture, and helps with the interpretation of culture results.

For liquid specimens e.g. CSF, the sample is first centrifuged to concentrate any bacterial cells in the deposit, and Gram stain and culture is performed from the deposit after the supernatant is decanted. This helps increase the sensitivity of both microscopy and culture.

Ziehl-Neelsen (ZN) stain is used to demonstrate the presence of Mycobacteria. Mycobacteria can also be visualized using the fluorescent dye auramine and a fluorescence microscope.

Direct immunofluorescence is employed to detect certain pathogens (e.g. *Legionella*, *Pneumocystis*) using specific antibodies conjugated to a fluorescent dye.

Another microscopic technique is **dark ground microscopy**. This is mainly used to detect the thin spirochaetal cells of *Treponema pallidum* (syphilis bacteria).

- **Methods of Isolation of Bacteria**

Methods of isolation of bacteria can be broadly classified into two:

1- Culture methods

On Solid media

On Liquid media

Automated systems

2- Non-culture methods

- **Culture methods**

The specimens received in the laboratory are plated on the culture media. The appropriate culture media is selected depending upon the bacteria suspected. The following precautions need to be taken into consideration when the culture methods are processed

- 1- Optimal atmospheric conditions
- 2- Optimal temperature
- 3- Growth requirement of the bacteria

- ***Atmospheric conditions:***

Colonies of bacteria are usually large enough to identify after 18–24 hours of incubation (usually at 37°C), but for some bacteria longer incubation times are required (from 2 days to several weeks). Culture plates are incubated (1) in air, (2) in air with added carbon dioxide (5%), (3) anaerobically (without oxygen) or (4) micro-aerophilically (a trace of oxygen) according to the requirements of the different types of bacteria that may be present in specimens.

In case of Mycobacteria especially the scotochromogen the culture bottles are placed in dark or the bottles are covered with black paper and kept for incubation at 37°C.

- ***Temperature:***

Most of the bacteria requires a temperature of 37°C for optimal growth. This temperature is provided placing the inoculated culture plates in the incubator set at 37°C temperature.

- **Growth requirement of the bacteria**

Different bacteria have different growth requirements. For e.g *Haemophilus influenzae* requires factor V and factor X for its growth, which are found in chocolate agar. Thus for sample suspected of *Haemophilus influenzae*, the samples are plated on chocolate agar. Similarly depending upon the growth requirements the appropriate culture media are used.

- **Culture on Solid Media:**

- The principal method for the detection of bacteria from clinical specimens is by culture on solid culture media. Bacteria grow on the surface of culture media to produce distinct colonies.

- Different bacteria produce different but characteristic colonies, allowing for early presumptive identification and easy identification of mixed cultures.

- **Culture in liquid media**

- Bacteria can also be grown in liquid media (broth). Like agar plates, broth cultures may be non selective or selective. Bacterial growth is easy to detect as the clear liquid turns turbid, usually within 24–48 hrs, but incubation may need to be extended to 14 days or more.
- **Advantage:** is that it is significantly more sensitive than direct culture on agar.

- **The disadvantage:** is that, by itself, it is not easy to determine the type of bacteria present or whether a mixed growth has occurred, and in most cases the broth must be subcultured onto solid agar plates. This causes an additional delay in culture results. Broth cultures are also prone to contamination.

- **Automated system**

Automated blood culture systems eg. BACTEC, BacteAlert utilize liquid culture. Bacterial growth may be detected by a variety of methods (e.g. detection of bacterial CO₂ production).

The advantage of automated system are:

1- Rapidity : they aid in faster growth of bacteria. Thus less time consuming.

2- The incidence of contamination during the processing of sample are minimised.

*One of the main limitations is the commercial viability.

- **Non culture methods**

Isolation of bacteria can also be carried out by non-culture methods. In particular the more advanced Amplification techniques like Polymerase chain reaction (PCR) and nucleic acid sequence based amplification (NASBA) etc are being used in clinical laboratories for isolation and identification of bacteria.

*The following are some of the factors that are considered in interpreting bacteriological culture results:

- 1- Type of specimen
- 2- Any delays in processing
- 3- Types of bacteria recovered
- 4- Knowledge of the normal human flora at different sites
- 5- Clinical information provided on the request form
- 6- Details of recent antibiotic therapy