



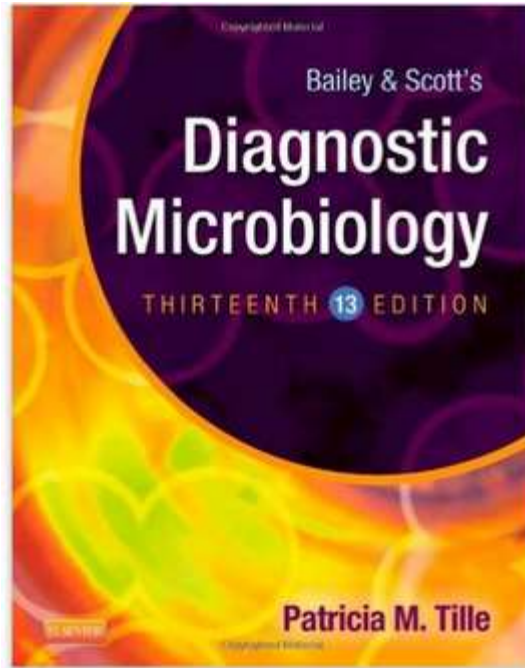
# Microbial Diagnosis

## 320 MIC

### Lecture 2:

# Specimen Management

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# General Concepts

- Microbiologists work in public health laboratories, hospital laboratories, reference or independent laboratories, and physician office laboratories (POLs). Depending on the level of service of each facility, the type of test differs, but in general microbiologist will perform one or more of the following functions:
  - Cultivation, identification, and AST of microorganisms
  - Direct detection of infecting microorganisms by microscopy
  - Direct detection of specific products of infecting organism using chemical, immunological, or molecular techniques
  - Detection of antibodies produced by the patients in response to an infecting organism (serodiagnosis).

# 1- Specimen Collecting and Handling

- Collection and transportation of specimen are critical considerations since any results the laboratory generates is limited by the **quality** of specimen and its **condition** on the arrival in the laboratory.
- Specimen should be obtained to minimize the possibility of introducing **contaminating microorganisms** that are not involved in the infection.
  - **Example:** Avoiding the normal flora in the mucous membrane infection when collecting specimen of infectious microorganism like ***Klebsiella pneumonia***.
  - This might be limited by using special techniques to bypass the areas containing the normal flora such as careful skin preparation before blood culture or spinal taps.

# 1- Specimen Collecting and Handling

## Appropriate Collection Techniques:

- It is the microbiologist's responsibility to provide **clinicians and nursing** staff with a collecting manual listing optimal specimen collecting techniques and transport information.
- Instructions should be written so specimen collected by **patient** (e.g. urine, sputum, or stool) are handled properly.

## 2- Specimen Transport

- The specimen should be transported to the laboratory within 2 hours of collection.
- All specimen containers should be leak-proof, and the specimen should be transported within sealable, leak-proof, plastic bags with separate section for paperwork; resealable bags or permanent seal are common.
- Bags should be marked with biohazard label.
- Many microorganisms are susceptible to environmental conditions such as oxygen (anaerobic bacteria), changes in temperature (*Neisseria meningitides*), or changes in pH (*Shigella*).
- Thus, the use of special preservatives or holding media for transportation is important to ensure organism viability.

# 3- Specimen Preservation

**Preservatives** are designed to maintain the appropriate colony counts for urine or the integrity of trophozoites and cysts for Ova & Parasites (O&P).

**Example:** boric acid for urines, or polyvinyl Alcohol (PVA) and buffered formalin for stool (For O&Ps).

**Transport or Holding Media** maintain the viability of microorganism present in the specimen without supporting the growth of any of the organisms. This maintains the organisms in a state of suspended animation.

**Example:** Stuart's media and Amie's media. Charcol might be added to absorb fatty acids\* present in the specimen.

# 3- Specimen Preservation (Count.)

**Anticoagulants** are used to prevent clotting\* of specimen such as blood, bone marrow, or synovial fluid.

**Example:** *Sodium Polyanethol Sulfonate (SPS)* for *Neisseria* or other anaerobic bacteria in bone marrow, *heparin* for viral cultures which is also inhibitor for G+ve bacteria and yeast.

- The colour of the collection tube might be inaccurate without specifying the anticoagulant. For instance, some yellow cap tube containing either *SPS* or *ACD* (trisodium citrate/Citric acid/ dextrose) which is not appropriate for use in microbiology.\*

# 4- Specimen Storage

Several storage methods are used if the specimen cannot be processed within 2 hours, depending on the transport media, etiologic agent:

- **Refrigerated temperature 4°C**, i.e. urine, stool, viral specimen, sputa, swabs and foreign device such as catheters.
- **Ambient room temperature 22°C**
- **Body temperature 37°C**, i.e. Cerebrospinal fluid (CSF) ,anaerobic bacteria)
- **And freezer temperature -20 °C** i.e. serum for serologic studies for 1 week **or -70 °C**, i.e. tissues or specimens for long term storage.



# 5- Specimen Labelling

- It should be labelled at the very least with the patient's name, identifying number, or birth date, and source.
- Labelling is important for the requisition to be matched up when received in the laboratory.

# 6- Specimen Requisition

- **The requisition (test)** is an order sent to the laboratory with the specimen.
- It is often a hard copy but could be sent electronically.
- The information on it include patient's history, diagnosis, and immunization record.
- It helps the microbiologist to work up the specimen and determine which organisms are significant in the culture.

- The patient's Name
- Hospital number
- Age or DOB
- Sex
- Collection date and time
- Ordering physician (UPIN number)
- Exact nature and source of the specimen
- Diagnosis
- Immunization history
- Current antimicrobial therapy

# 7- Rejection of Unacceptable Specimens

Specimens are unacceptable if any of the following conditions apply:

- The information on labels does not match that on the requisition.
- The specimen has been transported at the improper temperature
- It has not been transported in the proper medium
- Its quantity is insufficient for testing.
- It is leaking
- Its transport time exceeds 2 hours post-collection and the specimen not preserved.
- It arrived in a fixative (formalin) which, in essence, kills any organism present.
- It has been received for anaerobic culture from a site known to have anaerobes as part of normal flora (vagina, mouth).
- The specimen is dried up.
- Processing the specimen would procedure information of questionable medical value.

# 8- Specimen Processing:

- Priority should be given to the critical specimen such as **CSF, tissue, blood, and sterile fluids**.
- **Urine, throat, sputa, stool, or wound drainage** specimen can be saved for later.
- On arrival in the laboratory, the time and date received should be recorded.

# 9- Direct Microscopic Examination

## The direct examination serves several purposes:

1. The quality of specimen addressed\*.
  2. The microbiologist and clinical can be given an early indication.
  3. The work up of the specimen can be guided by comparing what grows in culture to what was seen on smear.
- Direct examinations are usually performed on specimen from most sources except **throat, stool, and nasopharyngeal**.

# 9- Direct Microscopic Examination

- The most common stain in **bacteriology** is **gram stain** to visualise rods, cocci, white blood cells, red blood cells, or squamous epithelial cells.
- The most common direct **fungus** stain are **KOH (potassium hydroxide)**, **PAS (periodic-acid Schiff)**, and **calcoflour white**.
- The most common **Acid-fast stains** are **AR (auramine rhodamine)**, **ZN (Ziehl-Neelsen)**, and **Kinyoun**.