

# **Diagnostic Microbiology I**

# Approach to laboratory diagnosis

- Pre-laboratory work
  - a. Choosing specimen
  - b. Obtaining
  - c. Transporting
  - d. Providing essential information

**A. Bacteriologic**

**B. Immunologic**

# Specimen management

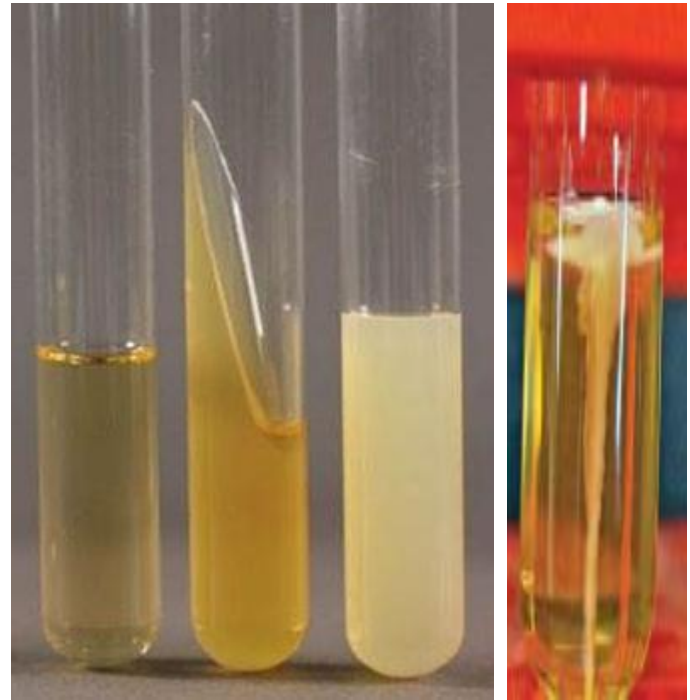
- Appropriate collection techniques
  - Instructions to nursing staff and clinicians
- Specimen transport
  - Preservation and storage
- Specimen labeling
- Specimen requisition
- Rejection of unacceptable specimens
- Specimen processing
- Gross examination

# General approach to the diagnosis of bacterial infection

- Direct microscopic examination (Staining)
  - The quality of the specimen
  - Early indication, guides empiric therapy
  - Comparing original smear with culture
- Selection of culture media
- Specimen preparation
- Inoculation on solid media
- Incubation methods
- Identify the organism
- AST

# Selection of culture media

- Culture media can be classified
  - Substratum
  - Nutritional component
  - Functional use



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# Bacterial cultivation

- **Evaluation of colony morphology**
  - Type of media
  - Relative quantity of each colony type
  - Colony characteristic
  - Gram stain and subculture

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# Identification of the organism

- Using phenotypic criteria
  - Microscopic morphology and staining characteristics
  - Macroscopic morphology
  - Environmental requirements of growth
  - Resistance or susceptibility to antimicrobial agents
  - Nutritional requirements and metabolic capabilities
- Using genotyping criteria
- Immunological criteria

# Phenotype-based identification schemes

- A. Selection and inoculation of ID test battery
- B. Incubation for substrate utilization
- C. Detection of metabolic activity
- D. Analysis of metabolic profiles

## A. Selection and inoculation of ID test battery

- Type of bacteria to be identified
- Clinical significance of the bacterial isolate
- Availability of reliable testing methods

## **B. Incubation for substrate utilization**

- **Conventional Identification**
- **Rapid Identification**
- **Matrix-Assisted Laser Desorption Ionization Time of Flight**

**Mass Spectrometry (MALDI-TOF)**

## C. Detection of metabolic activity

- **Colorimetry**
- **Fluorescence**
- **Turbidity**

## **D. Analysis of metabolic profiles**

- **Identification databases**
- **Use of the database to identify unknown isolates**

# Diagnosis of Negative culture

- Antibody detection
  - IgM
  - IgG
- Antigen detection
  - Fluorescent antibody
  - Latex agglutination
- Nucleic acids detection

# Immunochemical methods

- Direct whole pathogen agglutination assays
- Complement fixation assays
- ELISA
- Western blot immunoassays



# Nucleic acid-based analytic methods

- Hybridization
- Amplification
- Applications
  - Direct detection
  - Identification of microorganisms grown in a culture
  - Characterization of microorganisms