Diagnostic Microbiology

Introduction to Diagnostic Microbiology Diagnostic microbiology is concerned with identifying the causative agents of infectious diseases. The laboratory methods used to diagnose infections include:

1. **Phenotypic Methods** – Microscopy, culture, and biochemical tests.
2. **Serological Analysis** – Detecting antigen-antibody interactions.
3. **Nucleic Acid Tests** – Identifying microbial DNA/RNA.
4. **Biological Assays** – Testing for pathogen-related biological activities.

Stages of Clinical Diagnosis

1. **Specimen Collection & Processing**
    - A specimen is any substance taken from a patient's body for testing (e.g., blood, urine, sputum, pus, cerebrospinal fluid, etc.).
    - Proper collection ensures accurate identification of the pathogen.
2. **General Rules for Specimen Collection**
    - Use aseptic techniques.
    - Collect samples before administering antibiotics.
    - Prevent contamination from normal flora.
    - Use appropriate transport media.
    - Label specimens correctly and accompany them with a request form.
3. **Types of Specimens & Collection Techniques**
    - **Sputum:** Collected in the morning before any mouthwash.
    - **Throat/Mouth Swabs:** Taken with a sterile swab, avoiding contamination with saliva.
    - **Nasal Swabs:** Collected using sterile cotton wool.
    - **Ear/Eye Discharges:** Collected with swabs and delivered to the lab promptly.
    - **Urine:** Midstream urine (MSU) is preferred; catheter collection for bedridden patients.
    - **Cerebrospinal Fluid (CSF):** Collected aseptically via lumbar puncture.
    - **Blood Culture:** Performed when bacteremia is suspected; collected before antibiotic administration.

Transport of Specimens

- Specimens should be transported to the lab within 2 hours.
- Refrigeration is required for delayed transport (except for blood, CSF, anaerobes, and fastidious organisms, which require room temperature storage).

Specimen Rejection Criteria

- Improper transport temperature or container.
- Prolonged transport time.

- Unlabeled or mislabeled specimens.
- Broken or leaking containers.
- Dried-out specimens or inadequate volume.

The laboratory diagnosis of infectious diseases uses bacteriologic and immunologic approaches. The bacteriologic approach identifies organisms through staining and culturing, while the immunologic approach detects antibodies in the patient's serum. Several steps are crucial in the bacteriologic approach before lab work: choosing the right specimen based on understanding the infection's pathogenesis, avoiding contamination, promptly transporting or storing the specimen, and providing essential information to lab personnel. Bacteriologic lab work involves observing the organism after staining, obtaining a pure culture on a bacteriologic medium, and identifying the organism using various methods like biochemical reactions and DNA probes.

The diagnosis of bacterial infections involves various approaches, including Gram staining and obtaining a pure culture. When organisms aren't recovered by culturing, serologic testing is employed to detect specific antibodies. A significant indicator is a fourfold rise in antibody titer between acute and convalescent-phase serum samples. Obtaining a pure culture requires culturing the organism on bacteriologic agar, with blood agar being initially used to support bacterial growth and observe hemolysis. However, blood agar doesn't support the growth of viruses or obligate intracellular bacteria like Chlamydia and Rickettsia due to the lack of a functioning nucleus in red blood cells.

TABLE 9–2 How to Diagnose a Bacterial Infection When the Culture Is Negative

1. Detect antibody in the patient's serum. Detection of immunoglobulin (Ig) M antibody indicates a current infection. A fourfold or greater rise in antibody titer between the acute serum sample and the convalescent serum sample also indicates a current infection. (A major drawback with the use of acute and convalescent serum samples is that the convalescent sample is usually taken 10–14 days after the acute sample. By this time, the patient has often recovered and the diagnosis becomes a retrospective one.) A single IgG antibody titer is difficult to interpret because it is unclear whether it represents a current or a previous infection. In certain diseases, a single titer of sufficient magnitude can be used as presumptive evidence of a current infection.
2. Detect antigen in the patient's specimen. Use known antibody to detect presence of antigens of the organisms (e.g., fluorescent antibody to detect antigens in tissue, latex agglutination to detect capsular polysaccharide antigens in spinal fluid).
3. Detect nucleic acids in the patient's specimen. Use polymerase chain reaction (PCR) and DNA probes to detect the DNA or RNA of the organism.

TABLE 9–3 Commonly Used Bacteriologic Agars and Their Function

Name of Agar[1]	Bacteria Isolated on the Agar	Function or Properties of the Agar
Blood	Various bacteria	Detect hemolysis
Bordet-Gengou	*Bordetella pertussis*	Increased concentration of blood allows growth
Charcoal-yeast extract	*Legionella pneumophila*	Increased concentration of iron and cysteine allows growth
Chocolate	*Neisseria meningitidis* and *Neisseria gonorrhoeae* from sterile sites	Heating the blood inactivates inhibitors of growth
Chocolate agar plus X and V factors	*Haemophilus influenzae*	X and V factors are required for growth
Egg yolk	*Clostridium perfringens*	Lecithinase produced by the organism degrades egg yolk to produce insoluble precipitate
Eosin-Methylene Blue	Various enteric gram-negative rods	Selects against gram-positive bacteria and differentiates between lactose fermenters and nonfermenters
Löwenstein-Jensen	*Mycobacterium tuberculosis*	Selects against gram-positive bacteria in respiratory tract flora and contains lipids required for growth
MacConkey	Various enteric gram-negative rods	Selects against gram-positive bacteria and differentiates between lactose fermenters and nonfermenters
Tellurite	*Corynebacterium diphtheriae*	Causes tellurite to become tellurium, which has black color
Thayer-Martin	*N. gonorrhoeae* from nonsterile sites	Chocolate agar with antibiotics to inhibit growth of normal flora
Triple sugar iron (TSI)	Various enteric gram-negative rods	Distinguishes lactose fermenters from nonfermenters and H_2S producers from nonproducers

[1]Names are listed in alphabetical order.

Serological Tests for Pathogen Identification

1. **Precipitation Reactions:** Antibody-antigen interaction forms an insoluble complex.
2. **Agglutination Reactions:** Used for blood grouping, bacterial identification, and serological testing.
3. **Fluorescent Antibodies & Immunofluorescence Testing:** Used for direct pathogen identification.
4. **Immunochromatography:** Used in rapid tests such as pregnancy tests and infectious disease diagnosis.
5. **Complement Fixation:** Diagnoses fungal, viral, and parasitic infections.
6. **Western Blot (Immunofixation):** Confirms antibodies against specific antigens (e.g., HIV).
7. **ELISA (Enzyme-Linked Immunosorbent Assay):** Detects pathogens and antibodies using color changes.

Immunologic methods for microbiologic diagnosi, highlights **two primary approaches**:

1. Using **known antibodies** to identify microorganisms.
2. Using **known antigens** to detect patient antibodies.

Identification of an Organism with Known Antiserum

Several serologic techniques are outlined:

- **Capsular Swelling (Quellung) Reaction**:
  Used for detecting encapsulated bacteria like *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis* (groups A & C). The capsule swells in the presence of homologous antiserum, visible microscopically.
- **Slide Agglutination Test**:
  Helps identify *Salmonella* and *Shigella* by clumping unknown organisms with specific antisera. Commonly used for detecting **O antigens**, while **H and Vi antigens** are used in epidemiology.
- **Latex Agglutination Test**:
  Uses latex beads coated with antibodies to detect specific bacteria or antigens. It is useful for identifying the capsular antigens of *H. influenzae*, *N. meningitidis*, certain *Streptococcus* species, and *Cryptococcus neoformans*.

Counterimmunoelectrophoresis (CIE) Test

This test involves applying an **electrical field** to move an **unknown antigen** and a **specific antibody** toward each other. If they are homologous, a **precipitate forms**, confirming the antigen's presence. Capsular Polysaccharides Detection: Used to detect H. influenzae, N. meningitidis, S. pneumoniae, and group B streptococci in spinal fluid.

Enzyme-Linked Immunosorbent Assay (ELISA): A highly assayed enzyme is linked to detect the presence of homologous antigen. Useful for detecting a variety of bacterial, viral, and fungal infections.

Fluorescent Antibody Tests: Uses fluorescent dyes to identify a variety of bacteria.

Slide or Tube Agglutination Tests: Serial dilutions of a patient's serum are mixed with standard bacterial suspensions. Used in the diagnosis of typhoid fever, brucellosis, tularemia, plague, leptospirosis, and rickettsial diseases.

Serologic Tests for Syphilis: Detect T. pallidum. Includes nontreponemal tests (cardiolipin-lecithin cholesterol) like VDRL and RPR, and treponemal tests (FTA-ABS and MHA-TP).

Cold Agglutinin Test: Detects Mycoplasma pneumoniae infections.

Nucleic Acid Tests

Nucleic Acid-Based Tests: Includes nucleic acid amplification tests (NAAT) and nucleic acid sequence analysis. Useful for Chlamydia and Mycoplasma species, N. gonorrhoeae, and T. pallidum. Also used to identify bacteria based on ribosomal RNA sequence (e.g., Tropheryma whippelii).

- **Southern Blot:** Detects DNA sequences.
- **PCR (Polymerase Chain Reaction):** Amplifies microbial DNA.
- **RT-PCR (Reverse Transcription PCR):** Converts RNA to DNA for viral detection.

Biological Assays

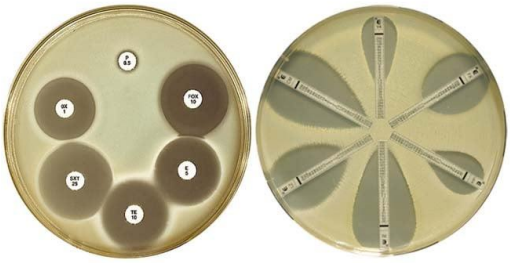
- Bioassays measure the effect of a substance on living cells or tissues.
- Used for hormone analysis, drug monitoring, and pathogen detection.

Antimicrobial Susceptibility Testing

- **Disk Diffusion Method:** Measures the inhibition zone of antibiotics.
- **Dilution Method:** Determines the minimum inhibitory concentration (MIC) of antibiotics

Antibiotic susceptibility testing (AST) is a laboratory method used to determine the effectiveness of antibiotics against bacterial pathogens. It helps guide appropriate antibiotic therapy, preventing the misuse of antibiotics and combating antimicrobial resistance. The most common AST methods include the **disk diffusion (Kirby-Bauer) test**, **broth dilution**, and **E-test**. In the disk diffusion method, antibiotic-impregnated paper disks are placed on an agar plate inoculated with the test bacteria. After incubation, zones of inhibition are measured to determine susceptibility. The **broth dilution method** determines the minimum inhibitory concentration (MIC) by exposing bacteria to different

antibiotic concentrations in liquid media. The **E-test** combines both methods, using a strip with a gradient of antibiotic concentrations to find the MIC.



Results are interpreted based on Clinical and Laboratory Standards Institute (CLSI) or European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Bacteria are classified as **susceptible (S), intermediate (I), or resistant (R)** to a given antibiotic. AST plays a crucial role in clinical microbiology, guiding patient treatment, tracking resistance trends, and supporting infection control. Regular testing is essential to combat antibiotic resistance and ensure effective treatment of bacterial infections.

Standard Precautions in Diagnostic Microbiology

- Treat all specimens as potentially infectious.
- Use personal protective equipment (PPE) (gloves, masks, gowns, etc.).
- Work in designated laboratory areas with appropriate safety measures.

Conclusion Diagnostic microbiology plays a crucial role in disease diagnosis and treatment. Proper specimen collection, transport, and processing ensure accurate identification of pathogens and effective patient management.