

Practical Pathogenic Bacteria

LAB: 1

Methods of Bacterial Identification

1- Microbial Causes of Infection:

- Bacteria, viruses, fungi, and parasites.
- The pathogen may be exogenous (acquired from environmental or animal sources or from other people) or endogenous (from the normal flora).

2- Specimen Selection, Collection, and Processing:

- The quantity of specimens must be adequate. Specimens are selected on the basis of symptoms, should be representative of the disease process.
- Contamination of the specimen must be avoided by using a sterile equipment and aseptic precautions.
- The specimen must be taken to the laboratory and examined promptly. Special transport media may be helpful. Reliable specimens must be secured for diagnosis of the bacterial infection before antimicrobial drugs are administered.

3- Microscopic Examination:

- Gram staining is a differential staining technique that differentiates bacteria on their ability to retain color.
- Gram stain procedure. This staining method will differentiate gram-positive (purple) bacteria from gram-negative (red) bacteria.
- It is based on the composition of the bacterial cell wall of both Gram-negative and Gram-positive bacteria. The mechanism of Gram staining in the decolorizing step acts differently in both bacteria resulting in two different colors for identification.

Practical Pathogenic Bacteria

LAB: 1

- Gram-positive bacteria: *Staphylococcus* and *Streptococcus*.

Gram-negative bacteria: *Escherichia coli* and *Klebsiella*.

- Microbial morphology and arrangement can be observed. For example, Staphylococci are Gram-positive cocci which are arranged in clusters (grapes)

4- Microbiological Culture Examination (Media Selection):

- It is based on the specimen source (urine, blood, etc.).

- The appropriate bacteriological media are selected to grow the organism for further work-up.

- Selection of the appropriate temperature and incubation conditions (aerobic versus anaerobic) allow the optimal bacterial growth.

- Colony morphological characteristics on growth media. For example, size, color, odor and the ability to lyse (break apart) blood cells are important for bacterial identification.

- Nonselective media permit the growth of many microorganisms.

- Selective media contain inhibitory substances that permit the isolation of specific types of microorganisms.

5- Biochemical Identification:

- The ability of a bacterial species to use a sugar, an amino acid or an enzymatic substrate is very useful for bacterial identification.

- These tests can be used individually (coagulase for *Staphylococcus aureus*) to identify an organism, or in a set of tests to identify Gram-negative bacilli.

Practical Pathogenic Bacteria

LAB: 1

- Many commercially prepared kits are available to identify bacteria using biochemical and enzymatic tests.

6- Serologic Methods:

- These methods typically involve testing an unknown antibody against a known antigen bound to a latex particle or similar structure.
- After mixing the antigen and antibody together and rotating, a visible agglutination (clumping) will appear if positive for the organism tested.

7- Molecular Methods

- These methods are the latest and most specific methods available in the laboratory.
- These tests are based on the ability to detect, identify and characterize microorganisms based on their DNA or RNA.
- Polymerase chain reaction (PCR) is a common molecular method used to identify bacteria.

8- Antibiotics Susceptibility Examination

- Microorganisms, particularly bacteria, are tested *in vitro* to determine whether they are susceptible to antimicrobial agents.

Practical Pathogenic Bacteria

LAB: 1

Laboratory Specimens

- 1- Blood : Septicemia
- 2- Urine: Urinary tract infections
- 3- Stool: Gastrointestinal infections.
- 4- Sputum: Respiratory infection
- 5- Vaginal swabs: Vaginal infections .
- 6- Nose & ear swabs : Nose & ear infections
- 7- Cerebral spinal fluid : CNS infections
- 8- Food & vomit : Food poisoning
- 9- Pus : Acne , burns , wounds.
- 10- Seminal fluid : Urethral discharge