microbiology

Definition of microbiology

Microbiology is the study of microscopic organisms, such as bacteria, fungi, and parasites. It also includes the study of viruses, which are not technically classified as living organisms but contain genetic material. Microbiology research encompasses all aspects of these microorganisms such as their behavior, evolution, ecology, biochemistry, and physiology, along with the pathology of diseases that they cause.

Branches of Microbiology

By Taxsonomy

- **Bacteriology**: the study of bacteria.
- **Immunology**: the study of the immune system. It looks at the relationships between pathogens such as bacteria and viruses and their hosts.
- **Mycology**: the study of fungi, such as yeasts and molds.
- **Nematology**: the study of nematodes (roundworms).
- **Parasitology**: the study of parasites. Not all parasites are microorganisms, but many are. Protozoa and bacteria can be parasitic; the study of bacterial parasites is usually categorized as part of bacteriology.
- **Phycology**: the study of algae.
- **Protozoology**: the study of protozoa, single-celled organisms like amoebae.
- **Virology**: the study of viruses.

While applied microbiology research includes:

- **Agricultural microbiology**: the study of microorganisms that interact with plants and soils.
- **Food microbiology**: the study of microorganisms that spoil food or cause foodborne illnesses. Can also study how microorganisms are used in food production, such as fermentation of beer.
- **Medical microbiology**: the study of microorganisms responsible for human disease.
- **Microbial biotechnology**: using microbes in industrial or consumer products.
- **Pharmaceutical microbiology**: the study of microorganisms used in pharmaceutical products, such as vaccines and antibiotics.

What is basic microbiology equipments?

Microbiology equipments include microscopes; slides; test tubes; petri dishes; growth mediums, both solid and liquid; inoculation loops; pipettes and tips; incubators; **autoclaves**, and laminar flow hoods

What is the scope of microbiology?

The scope of Microbiology is huge because of the involvement of microbiology in various fields such as Pharmacy, Medicine, clinical research, agriculture, dairy industry, water industry, nanotechnology & chemical technology. ... Microbiologists can make careers in research and non-research fields

How to Study Medical Microbiology?

Fundamentals of Microbiology

Bacteriology

Virology

Mycology

- **Biological Properties**
  - Morphology, identification,
  - Antigenic structure
- **Pathogenesis and Pathology**
  - Clinical findings
- **Diagnostic Laboratory Tests**
- **Immunity**
- **Treatment & Prevention**
  - Epidemiology & Control

Basic Classification of Microorganism

• Eukaryotes	Prokaryotes
Large in size	Small in Size
Mitochondria Present	DNA not separated from cytoplasm
Membrane bound Nucleus	Mitochondria absent
Eg Algae, Protozoa, Fungi, Slime Moulds	Eg Bacteria
Contains all enzymes for production of metabolic energy	Contains all enzymes like Eukaryotes

Sampling and sample preparation

Sample preparation, in analytical chemistry, the processes in which a representative piece of material is extracted from a larger amount and readied for analysis. **Sampling and sample preparation** have a unique meaning and special importance when applied to the field of analytical chemistry

What is the sample collection?

Introduction to Specimen Collection

Laboratory tests contribute vital information about a patient's health. Correct diagnostic and therapeutic decisions rely, in part, on the accuracy of test results. Adequate patient preparation, specimen collection, and specimen handling are essential prerequisites for accurate test results. The accuracy of test results is dependent on the integrity of specimens.

There are four steps involved in obtaining a good quality specimen for testing: (1) preparation of the patient, (2) collection of the specimen, (3) processing the specimen, and (4) storing and/or transporting the specimen.

Preparation

Prior to each collection, review the appropriate test description, including the specimen type indicated, the volume, the procedure, the collection materials, patient preparation, and storage and handling instructions.

Preparing the Patient. Provide the patient with appropriate collection instructions and information on fasting, diet, and medication restrictions when indicated for the specific test.

Preparing the Specimen. All primary specimen containers must be labeled with at least two identifiers at the time of collection. Submitted slides may be labeled with a single identifier, but two identifiers are preferred. Examples of acceptable identifiers include (but are not limited to): patient's name (patient's first and last name exactly as they appear on the test request form), date of birth, hospital number, test request form number, accession number, or unique random number.

Avoiding Common Problems

Careful attention to routine procedures can eliminate most of the potential problems related to specimen collection. Materials provided by the laboratory for specimen collection can maintain the quality of the specimen only when they are used in strict accordance with the instructions provided.

General Specimen Collection. Some of the common considerations affecting all types of specimens:

- Please examine specimen collection and transportation supplies to be sure they do not include **expired containers**.
- Label a specimen correctly and provide all pertinent information required on the test request form.
- Submit a quantity of specimen sufficient to perform the test and avoid a QNS (quantity not sufficient).
- Use the container/tube indicated in the test requirements for appropriate specimen preservation.
- Follow patient instructions prior to specimen collection.

- Carefully tighten specimen container lids to avoid leakage and/or potential contamination of specimens.
- Maintain the specimen at the temperature indicated in the test requirements.

Serum Preparation. The most common serum preparation considerations:

- Separate serum from red cells within two hours of venipuncture.
- Mix specimen with additive immediately after collection.
- Allow specimens collected in a clot tube (eg, red-top or gel-barrier tube) to clot before centrifugation.
- Avoid hemolysis: red blood cells broken down and components spilled into serum.
- Avoid lipemia: cloudy or milky serum sometimes due to the patient's diet

Urine Collection. The most common urine collection considerations:

- Obtain a clean-catch, midstream specimen.
- Store unpreserved specimens refrigerated or in a cool place until ready for transport.
- Provide patients with instructions for 24-hour urine collection.
- Add the preservative (as specified in the test requirements) to the urine collection container prior to collection of the specimen.
- Provide sufficient quantity of sample to meet the minimum fill line on preservative transport container.

- Provide the proper mixing of specimen with urine preservative as specified in the test requirements.
- Use the collection container as specified in the test requirements, and refrigerate the specimen when bacteriological examination of the specimen is required.
- Carefully tighten specimen container lids to avoid leakage of specimen.
- Divide specimen into separate containers for tests with such requirements.
- Provide a complete 24-hour collection/aliquot or other timed specimen.
- Provide a 24-hour urine volume when an aliquot from the 24-hour collection is submitted.
- Preservatives vary for each test; refer to test information for the required preservative.

Timing of sample withdrawal

Single Specimens. Here are some instances in which timed single specimens may be required.

- Fasting plasma glucose alone or in conjunction with a random glucose determination, as recommended by the American Diabetes Association, to diagnose diabetes. Fasting here is defined as no caloric intake for at least eight hours.
- Postprandial glucose may be performed two hours after a meal for a timed test that is helpful in diabetes detection.

- Blood glucose determinations may be ordered at a specific time to check the effect of insulin treatment.
- Blood cultures may be ordered for a specific time if a bloodstream bacterial infection is suspected.
- Therapeutic monitoring of patients on medication.

Multiple Specimens. Here are some instances in which timed multiple specimen tests may be ordered.

- The most common timed procedure is a glucose tolerance test.
- To test the effect of a certain medication, a physician may order the same test to be obtained on consecutive days, before, during, and after the patient has received a medication.
- Collection of an acute and convalescent serum to aid in the diagnosis of a viral infection when culturing is not feasible.
- Other examples include such tests as occult blood, ova and parasites, and blood cultures.

Collection of blood

Blood must be collected with care and adequate safety precautions to ensure test results are reliable,

contamination of the sample is avoided and infection from blood transmissible pathogens is prevented.

Protective gloves should be worn when collecting and handling blood samples. Lancets, needles, and syringes must be sterile, and dry, and blood collecting materials must be discarded safely to avoid injury from needles and lancets.

Capillary blood

Capillary blood is mainly used when the patient is an infant or young child and the volume of blood required is small, e.g. to measure haemoglobin, perform a WBC count, and to make thick and thin blood films.

In district laboratories, capillary blood is also used to monitor anaemia during pregnancy and post-operatively. Haemoglobin and PCV values are slightly higher in capillary blood than in venous blood. Thick blood films for malaria parasites are best made from capillary blood (anticoagulated blood is more easily washed from slides during staining).

Importance of collecting thick and thin blood films from children:

When a haemoglobin test is requested, always collect both thin and thick blood films at the same time. If a child is found to be moderately or severely anaemic, the blood films can often indicate with the minimum of delay the cause(s) of the anaemia, e.g. malaria or sickle cell disease.

Disadvantages in using capillary blood for blood tests include:

● Capillary blood can be used for only a few tests.

● Greater possibility of sampling errors particularly when the blood is not free-flowing, e.g. dilution of the sample with tissue juice can occur if the puncture area is squeezed excessively.

● Difficulty in obtaining sufficient blood particularly when it is required for more than one test. Rapid clotting of blood in a pipette is common, particularly in tropical temperatures.

● Tests cannot be repeated immediately or further tests performed when results are unexpected or seriously abnormal. A patient may have also received treatment following collection of the capillary sample, e.g. an antimalarial drug.

● It is not possible to estimate platelets in blood films made from capillary blood (platelets clump).

Sites suitable for collection:

- Capillary blood can be collected from: – The 'ring' finger of a child or adult "Do not stick the thumb or index finger as these are the most sensitive"
- The heel of an infant up to one year old "Care must be taken not to damage the heel by sticking it too near the edge or by holding it too forcibly".

Technique for collecting capillary blood

Make sure the puncture area is warm to allow the blood to flow freely. On cold days soak the hand or foot of an infant in warm water prior to collecting a sample. 1- Cleanse the puncture area with 70% ethanol. Allow the area to dry.

2- Using a sterile pricker or lancet, make a rapid puncture, sufficiently deep to allow the free flow of blood.

3- Wipe away the first drop of blood with a dry piece of cotton wool and use the next few drops for the test. Do not squeeze too hard because this will result in an unreliable test result.

4- When sufficient blood has been collected, press a piece of dry cotton wool over the puncture area until bleeding stops.

Note: Mouth suction pipetting must NOT be used.

Venous blood

Anticoagulated venous blood is used when more than 100 µl of whole blood is required or when serum from a clotted blood sample is needed, e.g. for compatibility tests or antibody tests.

Venous blood is preferable to capillary blood for the reasons previously described, particularly when the patient is an adult and several tests are required.

Anticoagulants For haematological tests

the anticoagulants used are EDTA (ethylenediamine tetra-acetic acid), also called sequestrene, and tri-sodium citrate. These chemicals prevent blood from clotting by removing calcium.

- **EDTA**

EDTA anticoagulated blood can be used for most tests, e.g. haemoglobin, PCV, WBC count, platelet count, reticulocyte count, and reporting blood cell morphology. It is not suitable for coagulation tests.

Dipotassium EDTA This is recommended in preference to disodium EDTA because it is more soluble.

Importance of correct concentration of EDTA: The correct amount of blood must be added to EDTA to prevent blood cell changes. Excess EDTA causes shrinkage and degenerative changes, e.g. amounts in excess of 2 mg/ml of blood can cause the disintegration of platelets, decrease in centrifuged PCV, and increase in MCHC. The blood must be well mixed with the EDTA.

- **Trisodium citrate**

is used to anticoagulate blood for:

– Measuring the ESR, with 1.6 ml of venous blood (or previously collected EDTA blood) being mixed with 0.4 ml of sodium citrate anticoagulant.

– Coagulation tests, with 9 ml of venous blood being mixed with 1 ml of sodium citrate anticoagulant.

- **Heparin anticoagulant**

Heparinized blood is mainly used for clinical chemistry tests , osmotic fragility test and immunophenotyping. It is not recommended for routine haematological tests because it causes cells to clump and heparin gives a blue background to blood films.

Technique for collecting venous blood

Laboratory staff must not collect venous blood unless they have been adequately trained in the procedure. Newly qualified staff must be supervised until they have gained sufficient experience. Do not collect blood for haematological tests from intravenous lines.

- Select a sterile, dry, preferably plastic syringe of the capacity required, e.g. 2.5 ml, 5 ml, or 10 ml. Attach to it a 19 or 20 SWG needle (preferably a disposable one). If the patient is a child or adult with small veins, use a 23 SWG needle. **Note:** When not using a disposable syringe or needle, check the syringe for good suction and the needle for any blockage, directing the syringe and needle safely away from the patient. Ensure all air is expelled from the syringe. Whenever possible use a disposable needle and syringe.

Evacuated tube collection systems: These disposable blood collecting containers are available from several manufacturers but they are more expensive to use for collecting venous blood than a syringe and needle. The container has a vacuum which is used to draw the blood into the container. One end of the needle is situated in the patient's vein and the other end through the cap of the container. Evacuated collection systems minimize contact with blood, help to ensure the correct amount of blood is added to anticoagulant, and simplify multiple sample collection.

- Apply a soft tubing tourniquet to the upper arm of the patient to enable the veins to be seen and felt. Do not apply the tourniquet too tightly or for longer than 2 minutes. Ask the patient to make a tight fist which will make the veins more prominent.
- Using the index finger, feel for a suitable vein, selecting a sufficiently large straight vein that does not roll and with a direction that can be felt*. *If a vein cannot be felt, apply a pressure cuff

above the elbow and raise the pressure to 80 mm (deflate the cuff once the needle is in the vein).

- Cleanse the puncture site with 70% ethanol and allow to dry. Do not re-touch the cleansed area.
- With the thumb of the left hand holding down the skin below the puncture site, make the venepuncture with the bevel of the needle directed upwards in the line of the vein. Steadily withdraw the plunger of the syringe at the speed it is taking the vein to fill*. Avoid moving the needle in the vein. If the plunger is withdrawn too quickly this can cause haemolysis of the blood and the collapse of a small vein.
- When sufficient blood has been collected, release the tourniquet and instruct the patient to open his or her fist. Remove the needle and immediately press on the puncture site with a piece of dry cotton wool. Remove the tourniquet completely. Instruct the patient to continue pressing on the puncture site until the bleeding has stopped.
- Remove the needle from the syringe and carefully fill the container(s) with the required volume of blood. Discard the needle safely. Do not attempt to re-sheath it because this can result in needle-stick injury. Important: Do not fill a container with the needle attached to the syringe. Forcing the blood through the needle can cause haemolysis.
- Mix immediately the blood in an EDTA or citrate anticoagulated container. When required, make a thick blood film from the blood remaining in the syringe. Immediately label carefully all the blood samples.
- Check that bleeding from the venepuncture site has stopped. Cover the area with a small dressing. Haematoma can be avoided by ensuring an appropriate vein is selected and the needle is well positioned in it and not accidentally pulled out of the vein when withdrawing the plunger of the syringe. When removing the needle, always release the tourniquet first and apply pressure immediately to the puncture site, maintaining it until the bleeding has stopped completely (always check). Avoiding haemolysis of blood samples

Fig 8.3 Collection of venous blood. After collecting blood, the tourniquet is released by pulling the tubing end on the left (marked with

an arrow). Avoiding haematoma when collecting venous blood
Bleeding from a vein into the surrounding tissue (haematoma)

Haemolysis can be avoided by:

● Checking that the syringe and needle are dry and that the barrel and plunger of the syringe fit well.

● Not using a needle with too fine a bore.

● Not withdrawing the blood too rapidly or moving the needle once it is in the vein. Frothing of the blood must be avoided.

● Removing the needle from the syringe before dispensing the blood into the specimen container and allowing the blood to run gently down the inside wall of the container.

● Adding the correct amount of blood to anticoagulant. Do not shake the blood but gently mix it with the anticoagulant.

● Using clean dry glass tubes or bottles for blood from which serum is required. Allow sufficient time for the blood to clot and clot retraction to take place. Red cells are very easily haemolyzed by the rough use of an applicator stick to dislodge a clot.

● Centrifuging blood samples for a minimum period of time. Centrifuging for 5 minutes at about 1000 g is adequate to obtain serum or plasma.

● Not storing whole blood samples in, or next to, the freezing compartment of a refrigerator. Why??

1. Stability of anticoagulated blood EDTA anticoagulated blood When EDTA anticoagulated blood cannot be tested within 1–2 hours it must be refrigerated at 4–8 C to prevent cellular changes affecting test results. Manual or automated blood cell counts, reticulocyte counts, and PCV change little in EDTA blood at 4–8 C when stored for up to 24 hours. Haemoglobin concentration is stable for 2–3 days at 4–8 C providing there is no haemolysis. Important: Blood which has been refrigerated must be allowed to warm to room temperature and be well mixed before being tested.

Blood films: In EDTA anticoagulated blood, morphological blood cell changes occur soon after blood is collected when it is stored at room temperature (18–25 C) and within 3 hours when stored at 4–8 C. It is therefore recommended that blood films be made and methanol-fixed as soon as possible after blood is collected and never made after overnight storage. Some of the blood cell changes which occur in EDTA blood include:

● Neutrophil degeneration with neutrophils becoming more irregular in shape, nuclear lobes separating, and vacuoles appearing in the cytoplasm. There is also loss of granules.

● Segmentation (budding) of the nucleus of lymphocytes and monocytes and vacuoles appearing in the cytoplasm.

● Erythrocytes becoming crenated and spherocytic.

● Platelets disintegrating.

2. Citrate anticoagulated blood Even when citrated blood is stored at 4–8 C, there is a decrease in the ESR due to changes in erythrocyte shape affecting rouleaux. The ESR should be measured within 4 hours of collecting the blood. Coagulation tests should be carried out as soon as possible after blood is collected into citrate anticoagulant.

Immunity and Immune Response

Made up of two cellular systems:

Humoral or circulating antibody system: B cells

Cell mediated immunity: T cells

Humeral immunity is mediated by antibodies and is the arm of adaptive immune response that functions to neutralize and eliminate extracellular microbes and microbial toxins. Humeral immunity is more important than cell mediated immunity in defending against microbes with capsules rich in polysaccharides and lipids.

Human immune system begins to develop in the embryo. Starts with hematopoietic (from Greek, "blood-making") stem cells in the bone marrow. Stem cells differentiate into major cells in the immune system granulocytes, monocytes, and lymphocytes(B & T Cells) Stems cells also differentiate into cells in the blood that are not involved in immune function, such as erythrocytes (red blood cells) and megakaryocytes (for blood clotting).

IMMATURE B CELLS

1. Immature B cells are tested for auto-reactivity before they leave the BM

2. If Immature B cells encounter self-antigen in the periphery, they are eliminated or inactivated

B CELL SUBSETS AND EFFECTOR CELLS

1. Immature B cells arriving in the spleen are short-lived and require cytokines and BCR signals for maturation and survival

2. Different lymphocyte subsets are found in particular locations

3. Terminal B cell differentiation: B cell => plasma cell & Memory cell

Phases and types of humeral immune response

Naïve B lymphocytes recognize antigens but do not secrete antibodies, and activation of these cells stimulate their differentiation into antibody-secreting plasma cells

Naïve B lymphocytes express two classes of membrane bound antibodies, IgM and IgD, that function as receptors of antigens.

The activation of B cells results in proliferation of antigen specific-specific cells, called clonal expansion, and their differentiation into effector cells called plasma cells that actively secrete antibodies. Each B lymphocyte makes a unique antibody molecule (immunoglobulin or Ig).

Antibody responses to different antigens are classified as T-independent or T-dependent , based on the requirement of T cell help.

Antibody responses to the first and subsequent exposures to an antigens, called primary and secondary responses, differ quantitatively and qualitatively.

stimulation of B lymphocytes by antigen

Humeral immune responses are initiated when antigen specific B lymphocytes in the spleen, lymph nodes, and mucosal lymphoid tissues recognize antigen.

B cells specific for an antigen use their membrane bound Ig receptors to recognize antigen in its native conformation. This will trigger signaling pathways that initiate B cell activation.

In addition to antigen stimulation B cells require other signals and many of these second signals are produced during innate immune responses to microbes.