MUKONO DIOCESE SCHOOL OF NURSING AND MIDWIFERY SCIENCES
MICROBIOLOGY NOTES BY SR OLIVER
MICROBIOLOGY

It the study the study of microorganisms that is organisms that are of microscopic dimensions

The organisms are too small to be seen by naked eyes i.e. less than 0.1mm and these can only be seen using a microscope

Microbiology is a subject that began with Anton van Leeuwenhoek's discovery of microorganisms in 1675, using a microscope of his own design

IMPORTANCES OF MICROBIOLOGY

- Promotion betterment of human health
- Helps to conquer infectious and fatal infections through immunization
- It influences the prevention and preparing safe drinking water
- Used in effective disposal of sewage and waste

To a nurse

- Nurses learn how disease causing organisms enter the body and their spread
- Enables a nurse to understand the principles of disinfection and effects of drugs on microorganisms
- The nurse learns the importance of proper collection of specimens for bacteriological examination in the laboratory and disease prevention
- A nurse will understand the meaning of reports received from the laboratory
- The nurse will understand how sera and vaccines are used in treatment and disease prevention, their preparations and effects on the body

TYPES OF MICRORGANISMS INCLUDE:

- ❖ Bacteria
- ❖ Fungi
- ❖ Algae
- ❖ Protozoa
- ❖ Algae
- ❖ Viruses

These microorganisms are studied under 5 major divisions i.e.

- ✓ Virology-study of viruses
- ✓ Bacteriology-study of bacteria
- ✓ Mycology-study of fungi
- ✓ Phycology-study of algae
- ✓ Protozoology-study of protozoa

CLASSIFICATION OF MICROORGANISMS

1. Prokaryotic microorganisms' e.g. bacteria

These are small unicellular organisms with no defined nucleus and cell membrane

2. Eukaryotic microorganisms' e.g. protozoa, algae, fungi, plants and animals
   These have a defined nucleus enclosing their genetic material and a cell membrane enclosing other cell organelles

DEFINITION OF TERMS

AETIOLOGY: Is the study of the causation or origination of disease, the factors which produce or predispose toward a certain disease or disorder.

PATHOGENECITY: The ability of a microorganism to cause disease

PATHOGENESIS: The mode of infection and process of disease causation

PATHOLOGY: The scientific study of the nature of disease and its causes, processes, development, and consequences

EPIDEMIOLOGY: Study of a particular disease why it occurs, how it spreads among the group of people and what can be done to prevent it and improve the health of the community

ENDEMIC: Constant presence of a disease or agent of a disease in a community or region

EPIDEMIC: An acute outbreak of disease
OR an epidemic is the rapid spread of a disease to a large number of hosts in a given population within a short period of time
Many endemic diseases can rapidly become epidemic if the environment or host influences change in a ways which favors transmission i.e. they start to exist in excess of normal expectance

PANDEMIC: Is a disease which spreads to several countries and affect a large number of people e.g. cholera, influenza.

CONTORL: Is the suppression of infection in a community by vaccination, health education, treatment, and sanitation

SYMBIOSIS: Is a close and often long-term interaction between different biological species e.g. the enteric bacteria that form part of the normal flora of the GIT assist in the synthesis of vitamin K and some of the vitamin B complex

PARASITOSIS: Infestation or infection with parasite

COMMENSALISM: A relation between individuals of two species in which one species obtains food or other benefits from the other without either harming or benefiting the latter e.g. numerous birds feed on the insects turned up by grazing mammals. (This

kind of relation can be contrasted with **mutualism**, in which both species benefit, **parasitism** is an association where one organisms benefits and another is harmed)

HOST: Is an organism that harbors a parasite (that is, a virus, a bacterium, a protozoan, or a fungus), or a mutual or commensal, symbiont, typically providing nourishment and shelter.

A HOST CELL: is a living cell in which a virus reproduces.

A PRIMARY HOST or definitive host: is a host in which the parasite reaches maturity and, if applicable, reproduces sexually.

A SECONDARY HOST or **intermediate host:** is a host that harbors the parasite only for a short transition period, during which (usually) some developmental stage is completed. For trypanosomes, strictly, **humans** are the secondary host, while the **tsetse fly** is the primary host, given that it has been shown that reproduction occurs in the insect

ANAEOBES: Organisms that grow in the absence of free oxygen

OBLIGATE OR STRICT ANAEROBES are those that grow only in the absence of oxygen

FACULTATIVE ANAEROBES these are able to grow either with or without free oxygen

MICRO-AEROPHILES theses are able to grow best in the presence of low amounts of oxygen

AEROBES: Organism able to live and reproduce only in the presence of free oxygen (e.g., certain bacteria and certain yeasts)

OPPORTUNISTS: Organisms if a suitable opportunity arises become pathogens and cause disease normally by transfer of commensals from a usual place to another part of the body where it establishes its self and cause disease or when the immune system is low e.g. E.coli, a normal flora in the GIT but if it enters the urinary system it causes UTI.

BACTERIA

Characteristics of Bacteria

- Bacteria are unicellular (single-celled) microorganisms.
- All bacteria are prokaryotes, this means: they lack membrane defined nucleus, only they have coiled single circular chromosome.
- Typical bacterial cell has a rigid cell wall made of peptidoglycan (except Mycoplasma).

This cell wall is responsible for shape of the bacterial cells and its staining properties.

There are 3 basic shapes of bacteria:

- Spherical or oval in shape (cocci)
- Rod-like (bacilli)
- Spiral or corkscrew shape (spirilla).

- The inner structures of the bacterial cell, the **cytoplasm**, the **nucleoid**, the **ribosomes**, **plasmids** and the **inclusion bodies** are contained in the cell envelope which is made of three layers from inside out, the **cytoplasmic membrane**, the **cell wall**, and the **capsule**.
- Two surface appendages may be present, **flagella** which enable bacteria to move and **pili** which are used in attachment to host cells.

Prokaryote vs. Eukaryote

> All cells are divided into two groups according to the presence of the nucleus.
> Cells that have well-defined nucleus are called **EUKARYOTES**
> Cells that lack nucleus are called **PROKARYOTES**
> All bacteria are prokaryotic organisms.
> Eukaryotic organisms include **fungi**, **protozoa**, **helminthes**, **plants**, **animals**, as well as **humans**.

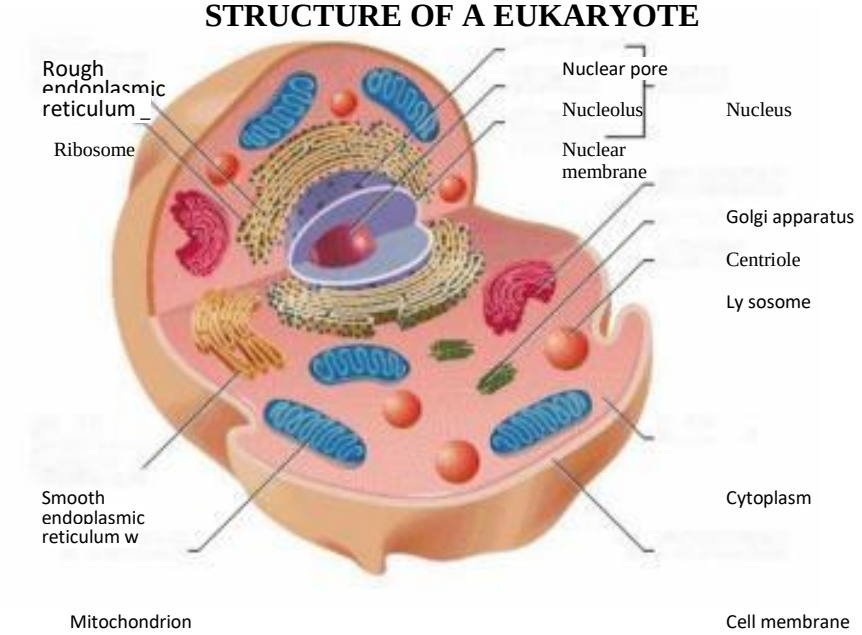
Prokaryotic cell

- Has **no** nucleus.
- Has **single, circular** chromosome.
- The nuclear body is called **nucleoid.**
- There is **no** nuclear membrane.
- There is **no** nucleolus.
- The cells divide by **binary fission.**

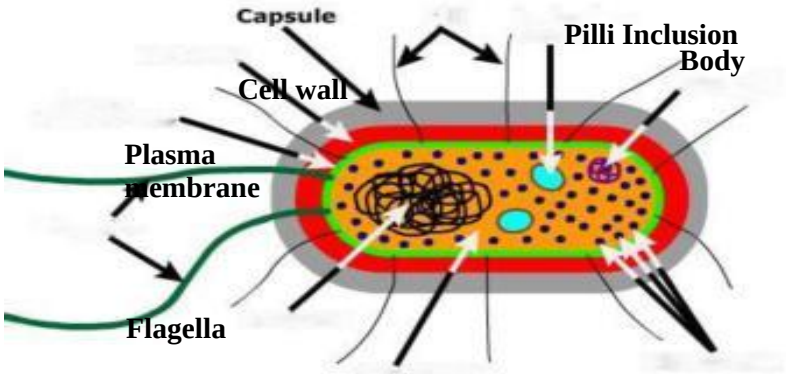
Eukaryotic cell

- Has a nucleus.
- Has one or more **paired, linear chromosome.**
- The nuclear body is called **nucleus.**
- The nucleus is bounded by a **nuclear membrane.**
- **Nucleolus** is present.
- The cell divides by **mitosis.**

STRUCTURE OF A EUKARYOTE



BACTERIA CELL STRUCTURE (prokaryote)



Nucleoid

Cytoplasm **Ribosomes**

Bacterial Cell Structure

1. **Cell envelope/ Capsule:**

Thick layer of viscous material--usually a polysaccharide--formed by many bacteria outside the cell wall

Some bacteria have only thin loose layer called **slime layer.**

Functions of the capsule:

- Allow bacteria to adhere to the surface of host cells.
- Protect bacteria from antibodies.
- protect bacteria from phagocytosis

2. **Cell wall:**

Thick rigid layer composed mainly of peptidoglycan.

The cell wall is responsible for:

- Shape of bacterial cell (spherical, rod like, or spiral)
- Reaction to gram stain (positive or negative)
- Bacterial cell protection from differences in osmotic pressure between the inside and outside environment

3. **Plasma membrane:**

Also called cytoplasmic membrane

It is a thin membrane that encloses the cytoplasm and is made of phospholipid bilayer and proteins.

Main function is:

- Selective permeability barrier which determines what enters and leaves the cell.
- energy production, peptidoglycan synthesis
- phospholipid synthesis
- waste removal
- Endospore formation.

4. **Flagella:**

Flagella are long hollow tubular filaments that enable bacteria to move. Bacterial cell may have one flagella at one pole (**Monotrichous**) for example Vibrio cholera, or many flagella arranged at one pole (**Lophotrichous**), at both poles (**Amphitrichous**), or distributed over the whole cell surface (**Peritrichous**).

5. **Pili:**

Pili; also called fimbriae, are thin hair-like appendages on the surface of many gram negative bacteria -- also found on very few gram positive bacteria -- they function as adhesion organs to attach the bacterial cell to the surface of the host cell.

6. **Cytoplasm:**

Cytoplasm is a complex fluid mixture — 80% water — containing amino acids, lipids, carbohydrates, ions, and enzymes. Nucleoid, ribosomes, inclusion granules, and plasmids (cellular organelles) are suspended and embedded in this fluid.

7. **Nucleoid:**

The nucleoid (chromosome) of the bacterial (prokaryotic) cell is the equivalent of the nucleus of the eukaryotic cell. There is no nuclear membrane but long coiled and supercoiled single circle of **D**eoxyribo-**N**ucleic **A**cid — **DNA**.

8. **Ribosomes:**

Ribosomes are the organelles where protein synthesis takes place. It is composed of **r**ibosomal **R**ibo-**N**ucleic **A**cid — **rRNA** — and proteins.

9. **Inclusion bodies:**

Inclusion bodies (also called **inclusion granules** or simply inclusions) are **storage particles** where bacteria store nutrients and some energy products. There are for example carbon, glycogen, sulfur and phosphate inclusions.

10. **Plasmids:**

Plasmids are Small pieces of circular DNA exist free in the cytoplasm outside the nucleoid.

Plasmids replicate independently of the chromosome. They carry the **genes** (codes) for toxin production and antibiotic resistance. A plasmid may have 5 to 100 genes compared to the chromosome that has 2000 to 4000 genes.

Endospore:

Endospore is an inactive form of the bacteria (dormant cell) which can survive and allow the organism to resist adverse environmental conditions, for example, drying, high temperatures, bactericidal agents, ultraviolet light and nutritional deprivation.

Endospores are produced only in bacillus and clostridia species (spore forming bacteria).

Spores can survive for long times until they are triggered to germinate (regrow).

Classification of bacteria

Medically important bacteria may be classified basing on any of the following:

1. Morphology (shape)
2. Growth requirement
3. Gram staining

Bacterial Morphology

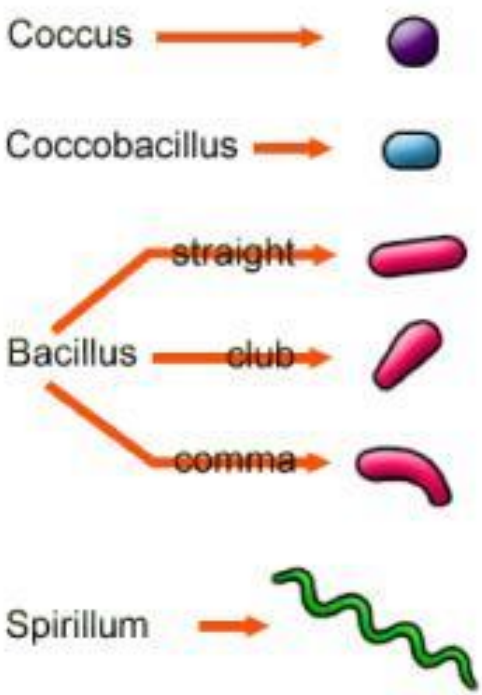
Bacterial cell shape:

Bacteria have **three** main characteristic shapes:

1. **Coccus** (plural = cocci) spherical or oval shaped.
2. **Bacillus** (plural = bacilli) rod-like, straight rod, club-shaped, or comma- shaped.
3. **Spirilum** (plural = spirilla) curved, spiral, or corkscrew shaped.

In between the cocci and the bacilli there is the **Coccobacilli**.

Some bacteria are pleomorphic (they may have different shapes) for example **Haemophilus influenza** that have shapes ranging from coccobacilli to long slender filaments.



(fig_1):
common shapes of bacteria

Bacterial cells arrangement:

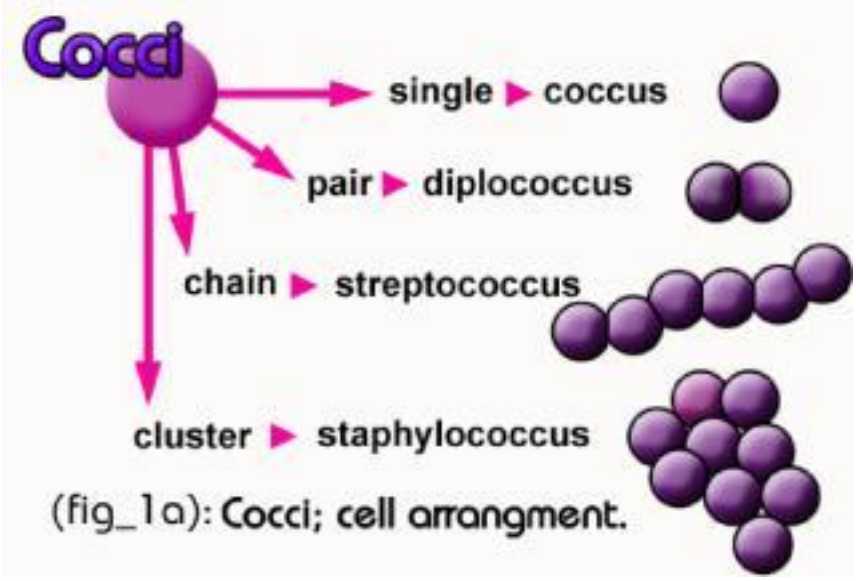
Many bacterial species show characteristic arrangement depending on the plane of division of the cell and the tendency of the cells to adhere to each other.

COCCI

Single = coccus

Double (pairs) = diplococci

Chains = streptococci Clusters = staphylococci

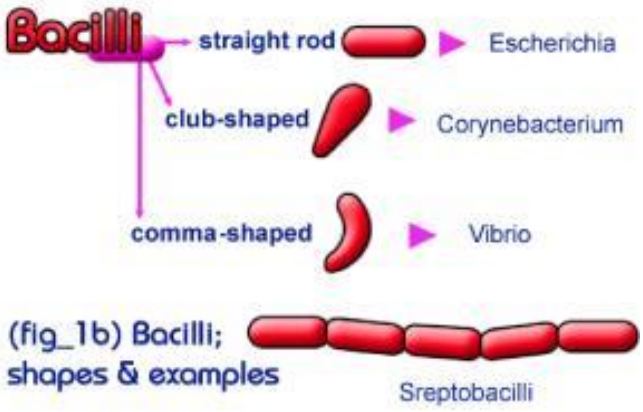


(fig_1a): Cocci; cell arrangment.

BACILLI single = bacillus

Double (pairs) = diplobacilli

Chains = streptobacilli



Bacterial staining methods

GRAM STAIN:

Gram staining is the most useful procedure in diagnostic microbiology, commonly used to identify unknown bacteria. It classifies bacteria into two groups, that is: Gram-positive (stains purple or violet) and Gram-negative (stains pink or red).

Procedure:

1. Prepare a smear and heat-fix it.
2. Apply **crystal violet** solution (leave it for one minute).
3. Wash the slide with water.
4. Apply **iodine solution** (leave it for one minute).
   At this time all bacteria appear violet (same color), that is, simple staining.
5. Wash the slide with water.
6. Decolorize with **acetone** (for 5 seconds only).
7. Now gram-positive bacteria are still visible (violet colored) but gram-negative bacteria are no longer visible.
8. Wash immediately in water.
9. Apply **safranin** (the counter stain) (for 30 seconds).
10. Wash the slide with water.
11. Blot and dry in air.

Now on examination under microscope types of bacteria are visible, the Grampositive bacteria appear purple or violet and the Gram-negative bacteria appear pink or red.

Gram-Negative Bacteria Examples:

There are many groups of Gram-Negative bacteria such as Cyanobacteria, Spirochaetes, and Proteobacteria etc. Out of which, proteobacteria is one of the major

Group of known Gram-Negative bacteria (it includes bacteria like E-coli, Salmonella, Pseudomonas, Moraxella, Helicobacter, Stenotrophomonas, Legionella, Acetic Acid Bacteria etc.).
Nesseria gonorrhoeae or N meningitidis

ZIEHL-NEELSON METHOD:

1. Prepare a smear and heat-fix it.
2. **Cover** the smear with a piece of blotting paper (absorbent paper).
3. Flood with **carbol fuchsin.**
4. **Steam for 5 minutes** by heating slide on a rack over a boiling water bath. Keep adding stain to avoid drying out the slide.
5. Allow the slide to cool.
6. Wash with water.
7. **Decolorize with acid-alcohol** adding it drop by drop until the dye no longer runs off from the slide.
8. Wash with water.
9. Apply **counterstain (methylene blue)** for one minute.
10. Wash with water.
11. Blot and dry in air.

On examination with light microscope acid-fast bacteria will appear **red**; non-acid-fast will appear **blue.**

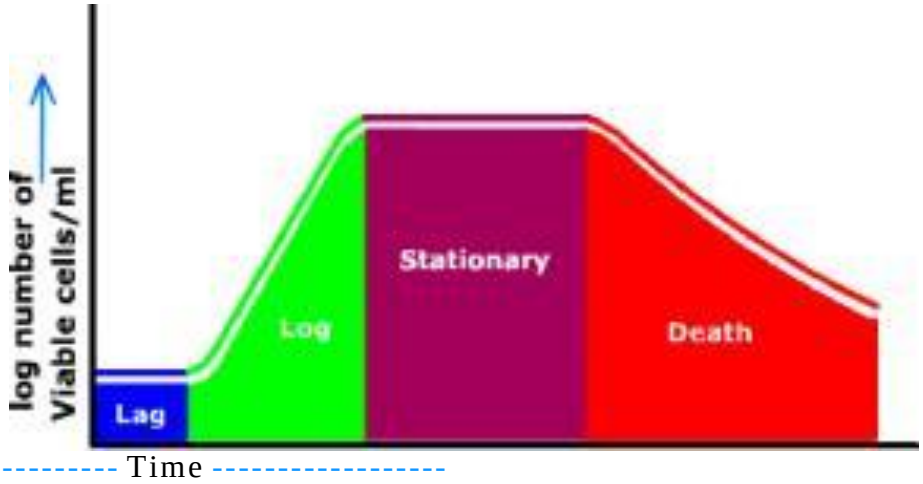
BACTERIAL GROWTH

Bacterial reproduction:

- Bacterial reproduction is by **binary fission.** This means that each cell grows and splits into two cells.
- **Generation time** is the period in which bacteria divide and each cell becomes two cells. In other words it is the period of time needed to double the number of cells (doubling time).
- This period is different from species to another and it can be as short as twenty minutes (20 min.) or as long as several days.
- For example The **doubling time** for
    - E. coli = 20 min
    - Mycobacterium tuberculosis = 24 hours o Treponema pallidum = 33 hours

Bacterial growth curve:

Growth of bacteria goes through **four (4) phases,** that is: lag phase, log phase, stationary phase, and decline (death) phase.



Bacterial growth curve

1 - LAG phase

The lag phase is a period of adjustment after the bacteria inters the body of a host or culture media.

- The bacteria are adjusting to the environment.
- Cells are active and there is an increase in cell size. They synthesize the components needed for cell division.
- Little or no cell division is occurring.

2 - LOG phase

The number of cells increases exponentially at a constant rate (the doubling time). BECAUSE

- Bacteria has adjusted to the environment
- Reproduction is taking place

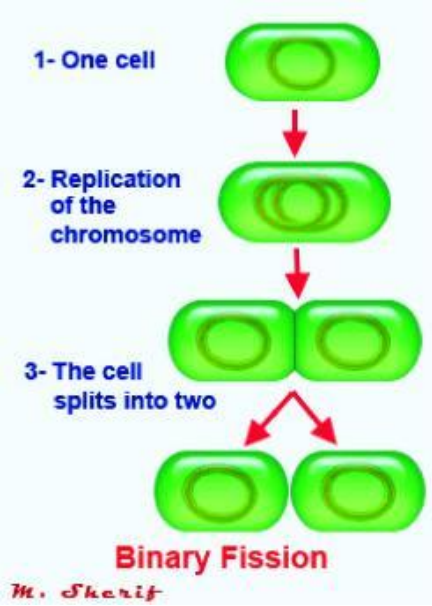
3 - STATIONARY phase

During this phase, the number of viable cells remains constant. The growth rate equals the death rate. Decreased growth rate and death is due to:

i. Depletion of nutrients.
ii. Accumulation of waste products.
iii. Change in pH.

4 - DEATH phase

In the decline (death) phase the death rate is more than growth rate that is, more bacteria die. The number of viable cells decreases.



Bacterial Growth Requirements

Nutrient requirements:
These are the chemicals and elements that are utilized for bacterial growth.
Major elements:
Such as Nitrogen, Carbon, Hydrogen, Phosphorus, Sulphur, Potassium, Magnesium, Calcium, and Iron
Nitrogen is used in the construction of amino acids, nucleic acids and other protein components of the cell.
Carbon is used in the construction of all organic components of the cell.
Bacteria are either:
Autotrophs i.e use inorganic carbon as their carbon source
Heterotrophs i.e use organ carbon for survival

Trace elements:
Trace elements are metal ions required by certain bacteria in very small amounts such as Manganese, Zinc, and Cupper.

Growth Factors:
These are organic compounds required in small amounts.
- Purines and pyrimidine's: required for synthesis of nucleic acids (DNA and RNA).Amino acids: required for the synthesis of proteins.

- Vitamins: used as coenzymes in several enzymatic reactions

Some bacteria (for example, E. coli) do not require any growth factors, other bacteria (for example, Lactobacillus) require purines, pyrimidine's, vitamins and several amino acids in order to grow. These compounds must be added in advance to culture media in order to grow these bacteria.

Oxygen requirements:

Oxygen is required for aerobic respiration and energy production.

Microorganisms are classified according to their oxygen requirements into:-

1. Obligate aerobes grow **only in presence** of oxygen.
2. Micro-aerophile grows in **low level** of oxygen. (No growth in absence of oxygen, high concentration of oxygen is toxic also).
3. Obligate anaerobe grow only in absence of oxygen (oxygen is toxic).
4. Facultative anaerobes grow in presence or absence of oxygen.
5. Aero tolerant anaerobes grow in absence of oxygen but are not affected if oxygen is present.

Temperature:

Microorganisms have different optimum temperature requirements in which they grow best. Microorganisms are classified according to their optimal temperature requirements into:-

1. **Psychrophiles:** grow best in cold temperatures between 0 — 20 °C.
2. **Mesophiles:** grow best in temperatures between 20 — 40 °C.
3. **Thermophiles:** grow best in temperatures between 40 — 90 °C.
4. **Extreme thermophiles:** grow best in temperatures above 90 °C.

Most bacteria are mesophiles especially pathogens that require temperature around 37 °C

PH:

PH = potential hydrogen = hydrogen ion concentration (the relative acidity or alkalinity of a solution)

Microorganisms have different optimum pH requirements.

1. **Acidophiles** grow in acid pH (less than 5.5).
2. **Neutrophils** grow in neutral pH (between 5.5 and 8.0).
3. **Alkalinophiles** grow in alkaline pH (more than 8.5).

Most pathogenic bacteria grow at pH between 6.5 and 7.5

PATHOGENIC MICROORGANSMS

Any microorganisms causing disease is called a **pathogen**

The ability of pathogens to cause disease is called **virulence**

The degree of pathogenicity is called **invasion**

The invasion of the body by a pathogen is called an **infection**

Characteristics of some clinically important bacteria

Gram positive bacteria

1. Bacillus anthracis

Xtics-

- ✓ they are gram positive
- ✓ Have large blunt ended bacilli (square ends) in pairs as along chain

- ✓ Are non-motile
- ✓ Encapsulated (ant phagocytic)
- ✓ Anaerobic
- ✓ Spore forming

Associated diseases

Anthrax

2. **CYANOBACTERIUM DIPTHERIAE**

Xtics-

Gram positive

Stain evenly

Have small club shaped rods which occur in arrangements resembling Chinese letters

Non motile

Facultative anaerobes

Associated diseases

Diphtheria

3. **STREPTOCOCCUS AUREAUS**

Xtics-

Gram positive

Stain darkly

Round cocci which occur in bundles like grapes non motile

Facultative anaerobes

Associated diseases

Skin infections

Cellulitis

Osteomyelitis

Pneumonia

Nosocomial infections

4. **CLOSTRIDIA**

Xtics-

Gram positive

Have large blunt ended rods

Spore forming

Anaerobes

Produce exotoxins

Associated diseases

Clostridium tetanii (tetanus)

Clostridium perfrigens (gas gangrene)

Clostridium batulinium (food poisoning)

5. **BORDETELLA PERTUSIS**

Xtics-

Gram positive

Small cocco bacilli

Obligate aerobes

Associated disesases

Pertussis (whooping cough)

GRAM NEGATIVE BACTERIA

6. **ESCHERICHIA COLI**

Xtics-

Gram negative

Motile flagellated rods

Facult**ative anaerobes**

Associated diseases

URTI's

Gastro-enteritius

E-coli associated with diarrhoea

7. **PSEUDOMONAS AUREGINOSA**

Xtics-

Gram negative

Motile rods with polar flagella

Obligate aerobes

Associated diseases

URTI's

Pneumonia

Burn infection

8. **SALMONELLA**

Xtics-

Gram negative

Have short flagellated rods

Facultative anaerobes

Associated diseases

Typhoid fever (enteric fever)

Enterocolitis

9. **VOBRIO CHOLERA**

Xtics-

Gram negative

Have Short curved rods with single polar flagellum

Facultative anaerobes

Associated diseases

Cholera

10. **MYCOBACTERIUM TUIBERCLOSIS**

Xtics-

Acid fast bacilli

Have long slander rods

Not stained with gram stain due to a lipid rich cell wall

They are obligate aerobes

Associated diseases

Tuberculosis

11. TREPONEMA PALLIDUM

Xtics-

Gram negative

Stain poorly

Spiral shaped

Highly motile

Associated diseases

syphillis

VIRUSES

General Characteristics of Viruses

Virus means **"poison"** (Latin)

Viruses are very small infectious agents, (20 - 300 nanometers)

A virus is a piece of nucleic acid (Genome) surrounded by a protein coat (Capsid).

The nucleic acid and capsid together are called **Nucleocapsid**

In some viruses the nucleocapsid is surrounded by a lipid envelope.

An intact complete infectious viral particle is called a **virion**.

They are **acellular** (not made up of cells); that is, they contain no cytoplasm or cellular organelles.

They do not grow or divide

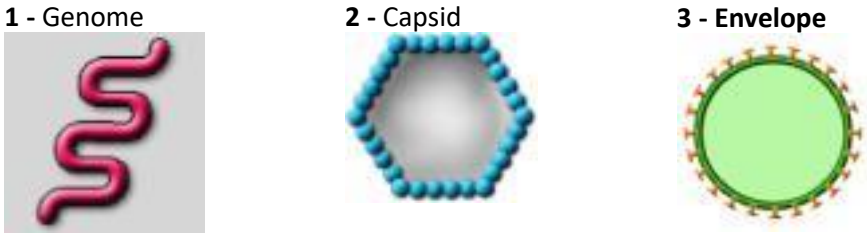
They are obligate intracellular parasites, (they are unable to multiply outside the living host cells)

They can infect animals, plants, and even other microorganisms.

Viruses which infect only bacteria are called **Bacteriophages**

Viruses that infect only fungi are termed **Mycophages**

A virus simply consists of:



Viral Genome (Nucleic Acid)

The viral genome has the genes (codes) for the synthesis of viral components and viral enzymes for replication.

The type of nucleic acid may be: **RNA** or **DNA** (but never both)

Capsid

The capsid is a protein shell surrounding the genome.

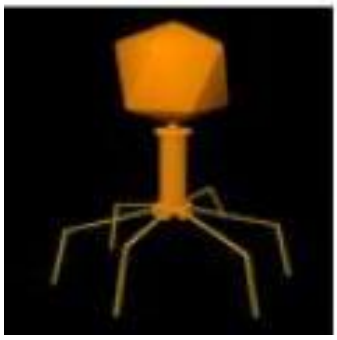
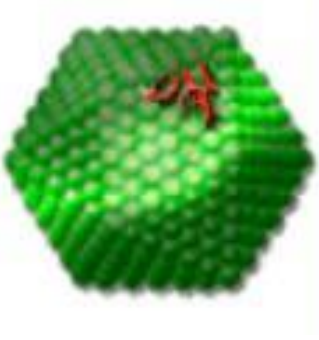
The capsid serves to:

a. Protect the viral genome.
b. Introduce the viral genome into host cells.

The shape of the capsid may be:

a. Icosahedral symmetry (spherical)
b. Helical symmetry (rod shaped or coiled)
c. **Complex symmetry** irregular shape (neither helical nor polyhedral)

Complex **Icosahedral** **Helical**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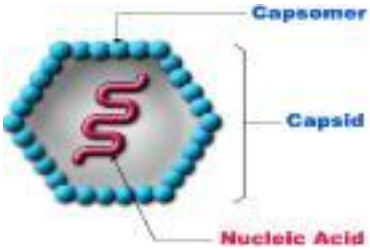
Envelope

- Some viruses have an additional covering, called the envelope.
- The envelope is a lipid bilayer containing proteins
- It is derived from host cell membranes, however its proteins are replaced by virus-specific proteins

A virus that is not enveloped is called **nonenveloped virus, nucleocapsid or naked virus**

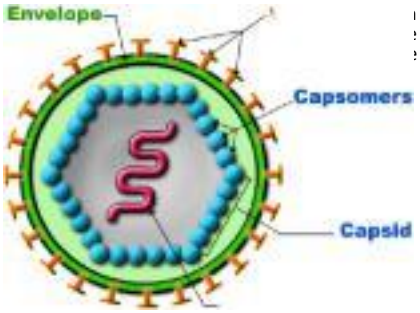
11. A virus which has envelope is called **enveloped virus**

Replication of Viruses



Qeqera! structure of a

Nor\eqveloped Virus



Geqeral Structure of aq

Trivet oped Virus

There are 5 major steps in the replication cycle of all viruses:

1. Adsorption (Attachment)
2. Penetration
3. Nucleic acid and protein synthesis
4. Assembly of virions
5. Release (Egress)

1 - Adsorption (Attachment)

Adsorption or attachment is the binding of the virus to the surface of the host cell
Specific proteins on the surface of the virion bind to special receptors on the host cell

2 - Penetration

Entry of the virus genome into the host cell

3 - Nucleic acid and protein synthesis

This involves
Synthesis of viral proteins,
Replication of the viral nucleic acid

4 - Assembly of virions

Assembly means combining viral nucleic acid with viral capsid
For enveloped viruses, it involves also the acquisition of an envelope
Some viruses assemble in the cytoplasm, others assemble in the nucleus

5 - Release (Egress)

This may occur by:
Disintegration or **lysis of the infected cell** which result in the release of intact infectious virions

1. **Budding from the cell surface,** as occurs with enveloped viruses

Characteristics of some clinically important viruses

Adenovirus

Characteristics

- Size: 80-110 nm in diameter
- Capsid: Icosahedral
- Nonenveloped
- Genome: DNA.
- Replicates in the nucleus Associated Diseases

1. Acute febrile pharyngitis
2. Viral pneumonia
3. Follicular conjunctivitis
4. Keratoconjunctivitis
5. Infantile gastroenteritis

Poliovirus

Characteristics

- Size: small, 30nm in diameter
- Capsid: icosahedral
- Nonenveloped
- Genome: RNA.

Associated Diseases

1. Poliomyelitis

Hepatitis A virus Characteristics

- Size: small, 27nm in diameter
- Capsid: icosahedral
- Nonenveloped
- Genome: RNA.

Associated Diseases

1. Hepatitis A (Infectious hepatitis)

Hepatitis B virus

Characteristics

- Size: 40 - 48 nm in diameter
- Capsid: icosahedral
- Enveloped
- Genome: DNA.

Associated Diseases

1. Hepatitis B
2. Primary hepatocellular carcinoma (HCC; hepatoma)

Hepatitis C virus

Characteristics

- Size: Estimated to be 40-50 nm in diameter. Capsid: icosahedral
- Enveloped
- Genome: RNA.

Associated Diseases

1. Hepatitis C

Human Immunodeficiency Virus (HIV)

Characteristics

- Size: 80 - 120nm in diameter
- Capsid: cone-shaped icosahedral
- Enveloped
- Genome: RNA
- The virion contains reverse transcriptase enzyme.

Associated Diseases

1. **Acquired Immunodeficiency Syndrome (AIDS)**

Rotavirus

Characteristics

- Size: 70 nm in diameter
- Capsid: icosahedral
- Nonenveloped
- Genome: RNA
- Replicates in the cytoplasm **Associated Diseases**

1. **Gastroenteritis** (nausea, vomiting, and watery diarrhoea in infants and very young children)

Herpes simplex virus, type 1

Characteristics

- Size: the virion is about 200 nm in diameter
- Capsid: icosahedral
- Enveloped
- Genome: DNA
- Replicates in the nucleus **Associated Diseases**

1. Primary HSV-1 infections
   Gingivostomatitis
   Keratitis
2. Latent HSV-1 infections
   Herpes labialis (cold sores) around lips

Influenza virus Characteristics

- Size: about 200 nm in diameter
- Capsid: helical
- Enveloped

- Genome: RNA
- Replication: occur in the nucleus

Associated Diseases

1. Influenza (flu)

Measles virus

Characteristics

- Size: about 200nm in diameter
- Capsid: Helical
- Enveloped
- Genome: RNA Associated Diseases

12. Measles

Rabies virus

Characteristics

- Size: 180 x 75 nm
- Capsid: Helical
- Enveloped (virion is bullet-shaped)
- Genome: RNA

Associated Diseases

1. Rabies (symptoms include salivation, vomiting, irritability, painful muscle spasms, hydrophobia, hallucination, paralysis, coma, and finally death)

FUNGI

General characteristics of pathogenic fungi

- ✓ Fungi are eukaryotes
- ✓ Fungal cells have a rigid cell wall composed mainly of chitin (polysaccharide).
- ✓ They are nonmotile \
- ✓ They are heterotrophs (require organic carbon source for growth).

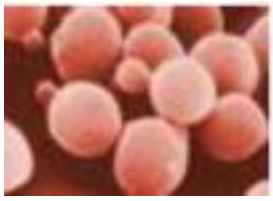
Morphology of pathogenic fungi

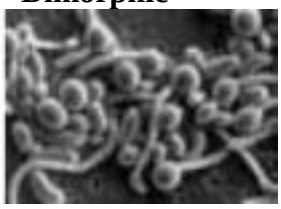
Pathogenic fungi of medical importance occur in two main shapes:

1. Yeast
   - Unicellular (single-celled)
   - Round to oval in shape
   - Size: 5 - 25 pm in diameter
2. Mould (Mold)
   - Multicellular
   - Long filaments (tube-like) known as hyphae (singular = hypha)
   - Size of hyphae cell: 2 - 5 pm in width and 5 - 50 pm in length
3. Dimorphic fungi
   - Are those fungi which grow in either forms (yeast-like and

mold like) under different environmental conditions, for example, temperature.

Yeast **Mold** **Dimorphic**





Nutrition

Fungi obtain their nutrients by absorption

> They secrete enzymes that breakdown the organic material then fungal cells absorb the nutrients through their cell walls.

Fungi may live as

1. Saprophytes on dead, decaying organic matter, or
2. Parasites on living organisms.

Growth reproduction

Fungi may reproduce asexually, sexually or both ways.

Sexual spores: result from the fusion of nuclei from two cells. Fusion of two haploid nuclei forming diploid and then division by meiosis (reduction division) producing sexual spores (Ascospores)

Asexual spores: arise from one cell only;

Budding (Yeast growth)

- Large cell forms a small bud (outgrowth)
- Bud gradually increases in size
- The nucleus of the parent cell divides (by mitosis) and one daughter nucleus migrates into the bud
- The bud completely separates from the parent cell

Fission

Simple division of a cell into two daughter cells

Asexual spores (conidia) are formed by mitosis in or on specialized hypha (moulds grow by elongation at the tips of their hyphae)

MYCOSES

Fungal diseases (Mycoses) are classified by the location of the infection

1. Superficial (cutaneous) mycoses:

   Organisms responsible for cutaneous mycoses are called dermatophytes Infections are limited to outer layers of skin, hair, and nails Some of the cutaneous mycoses include Pityriasis versicolor (Malassezia furfur), Tinea cruris, T. corporis, & Tinea unguium (Microsporum sp, Trichophyton sp. & Epidermophyton sp).

2. Subcutaneous mycoses:

   Infections involving the dermis, subcutaneous tissues, muscle, fascia, and bone

Usually result from puncture wounds and often form disfiguring Subcutaneous abscesses e.g. **Chromomycosi** (Cladosporium carionii), & **Madura foot** (Madurella grisea)

3. **Systemic mycoses:**
   Fungal infections of the internal organs
   Infections originate primarily in the lung and may spread to many organ systems
   Organisms responsible for systemic mycoses are **true pathogens** (can infect normal healthy individuals)
   Conditions include **Histoplasmosis** (Histoplasma capsulatum), C**occidioidomycosis** (Coccidioides immitis), & **Blastomycosis** (Blastomyces dermatitidis).
4. **Opportunistic mycoses:**
   Usually don't occur in healthy people Occur in Immuno-compromised persons
   May result from un-careful and random use of broad spectrum antibiotics
   Conditions include **Candidiasis** (Candida albicans), **Aspergillosis** (Aspergillus fumigatus), **Cryptococcosis** (Cryptococcus neoformans)**, Pneumonia** (Pneumocystis carinii)

PROTOZOA

General Characteristics of Protozoa

The protozoa are **unicellular** organisms
They are eukaryotic organisms
They are heterotrophs
The vegetative, reproducing, feeding form of a protozoan is called a "**trophozoite**"
Some protozoa produce a protective, hardened capsule called a "**cyst**"
Pathogenic protozoa are divided into four groups based upon their means of motility:

1. Amoebas
2. Flagellates
3. Ciliates
4. Protozoa

Medically important protozoa

Endameba histolytica

- The trophozoite is about 10 to 60 pm in diameter, actively motile
- The cyst form has one to four nuclei
- Infect the colon causing **Amoebic Dysentery (amoebiasis)**
- The parasite can spread to the liver and cause liver abscess **Giardia lamblia**
- The trophozoite is about 12 to 15 pm long
- It has two nuclei (looks like "owl face")
- It has four (4) pairs of flagella

- The cyst has four (4) nuclei
- The parasite infects the duodenum and causes diarrhoea **Trichomonas vaginalis**
- The trophozoite is pear-shaped, 15-18pm in length and 14-15pm in width
- The trophozoite has single nucleus
- There is no protective cyst
- Infects vagina and cervix in females
- Infects urethra and prostate in males **Trypanosoma**

1. Trypanosoma brucei
    o transmitted by the bite of the tsetse fly o causes meningoencephalitis “sleeping sickness”
2. Trypanosoma cruzi
    o transmitted by insect feces which contaminate the eye or skin wound o causes cardiomyopathy “Chagas' disease”

Leishmania

- Transmitted by the bite of a sand fly of the genus Phlebotomus or Lutzomia
- The flagellated form develop only in the intestine of the sand fly, and only the non-flagellated form is present in humans
- The intracellular non-flagellated form is 3 to 6 pm long by 1.5 to 3 pm in diameter
    1. Leishmania tropica causes cutaneous leishmaniasis (single or multiple skin ulcers) "Oriental sore"
    2. Leishmania donovani infects the reticuloendothelial system causing visceral leishmaniasis (hepatosplenomegaly, lymphadenopathy and anemia) "Kala-azar"

Balantidium coli

- The trophozoite is ovoid, 50 to 70 pm or longer (largest human protozoan parasite)
- The cyst form is 50 to 60 pm in diameter
- Balantidium infection is acquired by ingesting cysts in contaminated food or water
- Balantidiasis is accompanied by diarrhea or dysentery, abdominal pain, nausea, and vomiting

Plasmodium

- Four species cause disease “Malaria” in humans: P. falciparum, P. vivax, P. ovale and P. malariae
- They are obligate intracellular parasites
- Transmitted by the bite of an infected female Anopheles mosquito **Toxoplasma gondii**
- A sporozoan causes toxoplasmosis
- S Transmitted by:
    1. Ingesting food contaminated by cysts present in the feces of infected cats
    2. ingesting raw or undercooked meat of infected animal
    3. congenitally (during development in uterus) from infected mother
- Congenital infection may result in abortion, stillbirth, hydrocephalus or blindness

HELMINTHS

General Characteristics of Helminths

The helminths are **parasitic worms**

They are multicellular eukaryotic organisms

They generally possess digestive, circulatory, nervous, excretory and reproductive systems

There are three groups of helminths:

1. **Trematodes** (flukes)
2. **Cestodes** (tapeworms)
3. **Nematodes** (roundworms)

Medically important helminthes

1. The Cestodes

Characteristics

- Flat ribbon-like chain of segments
- Head of the tapeworm is called **Scolex** and it functions as Attachment organ
- Each segment bears a complete male and female system (i.e., **Hermaphrodite**)
- No mouth or digestive system; they get nutrients by absorption

Important Cestodes

1. Taenia saginata (beef tapeworm)
2. Taenia solium (pork tapeworm)
3. Diphylobothrium latum (broad fish tapeworm)
4. Hymenolepis nana (dwarf tapeworm)
5. Hymenolepis diminuta
6. Echinococcus granulosus The Flukes

2. Schistosoma species

1. Schistosoma mansoni (Intestinal bilharziasis)
2. Schistosoma haematobium (Urinary bilharziasis)
3. Schistosoma japonicum

Characteristics

- Adult worms are 0.6 to 2.5 cm long
- **Dioecious;** i.e., have separate male and female worms (separate sexes)
- The large male has marginal folds forming a canal in which the smaller female worm resides
- One (1) intermediate host: **Snail Clinical aspects**
- Infection occur by **cercaria** which penetrate the skin of people working or swimming in contaminated rivers
- Adult worms inhabit the veins of large intestine (S. mansoni and S. japonicum) or veins of urinary bladder (S. haematobium)

3. The Nematodes (Roundworms)

Characteristics

- Elongated, cylindrical shape, non-segmented with tapering ends
- **Dioecious**; i.e., have separate male and female worms (separate sexes) with the

male being smaller than the female

- They possess a complete digestive tract with **oral and anal openings**

Important Nematodes

1. Ascaris lumbricoides
2. Hookworms
3. Trichuris trichiura (whipworm)
4. Enterobius vermicularis (pinworm)
5. Strongyloides stercoralis
6. Wuchereria bancrofti (Lymphatic filariasis)

NORMAL FLORA

Normal floras are organisms that inhabit the body of a health person without causing disease under normal circumstances

They are usually bacteria or yeast, viruses, protozoa and worms are not considered to be the normal flora

Normal flora are non-pathogenic especially in their usual anatomic sites but may cause disease when they move to another site especially in the immunosuppressed patients and such diseases are referred to as **opportunistic infections**

Types of normal flora

Resident flora: they are commonly found in a particular area of the body at a given age

Transient flora: are microorganisms that are present at a given time and disappear or die off within hours, days, weeks or months

DISTRIBUTION OF NORMAL FLORA IN THE BODY

Normal floras usually occupy body parts that are in contact with the environment i.e The skin, mouth, nose, intestinal tract, vagina, eyes and others

Skin Flora

The varied environment of the skin results in locally dense or sparse populations, with Gram-positive organisms (e.g., staphylococci aureus, staphylococci epidermis, micrococci, and diphtheroids) usually Predominating.

Oral and Upper Respiratory Tract Flora

A varied microbial flora is found in the oral cavity, and streptococcal anaerobes inhabit the gingival crevice. The pharynx can be a point of entry and initial colonization for Neisseria, Bordetella, Corynebacterium, and Streptococcus spp.

Gastrointestinal Tract Flora

Organisms in the stomach are usually transient, and their populations are kept low (10^3 to 10^6/g of contents) by acidity. Helicobacter pylori is a potential stomach pathogen that apparently plays a role in the formation of certain ulcer types. In normal hosts the duodenal flora is sparse (0 to 10 /g of contents). The ileum contains a moderately mixed flora (10^6 to 10^8/g of contents). The flora of the large bowel is dense (10^9 to 10^{11}/g of

contents) and is composed predominantly of anaerobes. These organisms participate in bile acid conversion and in vitamin K and ammonia production in the large bowel. They can also cause intestinal abscesses and peritonitis.

Urogenital Flora

The vaginal flora changes with the age of the individual, the vaginal pH, and hormone levels. Transient organisms (e.g., Candida spp.) frequently cause vaginitis. The distal urethra contains a sparse mixed flora; these organisms are present in urine specimens (10^4/ml) unless a clean-catch, midstream specimen is obtained.

Conjunctival Flora

The conjunctiva harbors few or no organisms. Haemophilus and Staphylococcus are among the genera most often detected.

Host Infection

Many elements of the normal flora may act as opportunistic pathogens, especially in hosts rendered susceptible by rheumatic heart disease, immunosuppression, radiation therapy, chemotherapy, perforated mucous membranes, etc. The flora of the gingival crevice causes dental caries in about 80 percent of the population.

BENEFITS OF NORMAL FLORA TO THE HOST

- Prevents colonization by pathogens through competing for nutrients and receptor sites with pathogens
- Those in the gut secrete vitamin k and B complex which supplements food sources to the host
- Stimulates antibody mediated immune response that may cross react with future pathogens hence preventing diseases
- Lactobacilli in the vagina produces acids which maintains low PH hence inhibiting the growth of microorganisms such as candida albicans
- those found in the gut produce antimicrobial substances which kill or inhibit growth of pathogens

WHAT CAUSES NORMAL FLORAS TO BECOME PATHOGENIC

- Suppression of the normal flora by antibiotics allowing overgrowth of resistant species
- Immuno suppression
- Hormonal changes especially during pregnancy and menstruation

Introduction of microorganisms' to new sites eg E.coli from anus to the vagina leading to UTI

IMMUNITY/IMMUNOLOGY

Immunity is a state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion.

Immunology is the scientific study of the immune system and immune response

TERMS USED IN IMMUNOLOGY

Antigen: it's a substance that can provoke the body to produce antibodies

Antibody: this is an immunoglobulin produced by the body in response to stimulation from an antigen

Immunogens: are chemical compounds that cause specific immune response

Chemotaxis: it's a process whereby phagocytic cells are attracted to the area of invading pathogen in response to chemokines

Chemokines: it's a low molecular weight protein that stimulates a leukocyte movement

Haptens: are low molecular weights

Immunity involves both specific and non
-specific components.

The non-specific components act either as barriers to a wide range of pathogens irrespective of antigenic specificity.

The other components of the immune system adapt themselves to each new disease encountered and are able to generate pathogen-specific immunity.

Innate immunity (non-specific defense mechanisms)

Is the natural resistance with which a person is born.

It is present from birth and protects an individual from pathogens regardless of experiences.

It provides resistance through several physical, chemical, and cellular approaches.

There are 5 main innate defense mechanisms;

1. Surface barriers

- Intact skin forms a barrier against many pathogenic bacteria & its secretions (sweat & sebum) have antibacterial & antifungal properties.
- In certain situations where the number of bacteria is high the surfaces are moistened with a mucous secretion to entrap the organism until they can be removed; the nose, mouth & vagina are examples.
- Vaginal secretions serve as a chemical barrier following menarche, when they become slightly acidic, while semen contains defensives and zinc to kill pathogens.
- The hair in the nose filter the air & the cilia in the respiratory tract sweep the mucous & inhaled foreign bodies towards the throat for coughing it up or swallowing.
- Within the genitourinary and gastrointestinal tracts, commensal flora serve as biological barriers by competing with pathogenic bacteria for food and space and, in some cases, by changing the conditions in their environment, such as PH or available iron.

- ❖ This reduces the probability that pathogens will be able to reach sufficient numbers to cause illness.
- ❖ Risk of microbe invading the bladder is minimized by one way flow of urine from the bladder.

2. Phagocytosis

Is an important feature of cellular innate immunity performed by cells called 'phagocytes' that engulf pathogens or particles.

- . Phagocytes generally patrol the body searching for pathogens, but can be called to specific locations by cytokines.
- Once a pathogen has been engulfed by a phagocyte, it becomes trapped in an intracellular vesicle called a phagosome, which subsequently fuses with another vesicle called a lysosome to form a phagolysosome.
- The pathogen is killed by the activity of digestive enzymes
- Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens.
- During the acute phase of inflammation, particularly as a result of bacterial infection, neutrophils migrate toward the site of inflammation in a process called chemotaxis, and are usually the first cells to arrive at the scene of infection.
- Macrophages are versatile cells that reside within tissues and produce a wide array of chemicals including enzymes, complement proteins, and regulatory factors such as interleukin 1.
- Macrophages also act as scavengers, ridding the body of worn-out cells and other debris, and as **antigen-presenting cells** that activate the adaptive immune system.
- Dendritic cells (DC) are phagocytes in tissues that are in contact with the external environment; therefore, they are located mainly in the skin, nose, lungs, stomach, and intestines.
- Dendritic cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T cells, one of the key cell types of the adaptive immune system.

3. Natural antimicrobial substances

- ✓ **HCL** in the gastric juice kills the majority of ingested microbes.
- ✓ **Lysosome**, small protein with antibacterial properties, is present in granulocytes, tears, & other body secretions but not in sweat, urine or CSF.
- ✓ **Antibodies,** present in nasal secretions & saliva, inactivate microbes.
- ✓ **Saliva**, secreted into the mouth helps in washing away food debris that may otherwise encourage bacterial growth.

- ✓ **Interferons,** produced by T-lymphocytes & virus infected cells, help prevent viral replication within infected & healthy cells.
- ✓ **Complement**, system of about 20 proteins found in blood & tissues. It is activated by the presence of immune complexes & by foreign sugars on bacterial cell walls. The complement binds to bacterial cell walls thus destroying the microbe & also stimulating phagocytosis.

4. Inflammatory response

- ➢ Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response.
- ➢ They are most often associated with allergy and anaphylaxis.
- ➢ Basophils and eosinophil's are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites and play a role in allergic reactions, such as asthma.

5. Immulogical surveillance

- ➢ Natural killer (NK cells) cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses.
- ➢ Although they are lymphocytes, they are much less selective about their targets than the other T-cells & B-cells.

Through these approaches, innate immunity can prevent the colonization, entry, and spread of microbes.

Adaptive immunity (specific defense mechanisms)

Arises only after an infection or immunization and hence is "acquired" during life.
It allows for a stronger immune response as well as immunological memory, where each pathogen is "remembered" by a signature antigen.
The adaptive immune response is antigen-specific and requires the recognition of specific "non-self" antigens during a process called antigen presentation.
Antigen specificity allows for the generation of responses that are tailored to specific pathogens or pathogen-infected cells.
The ability to mount these tailored responses is maintained in the body by "memory cells". Should the pathogen affect the body more than once these specific memory cells are used to quickly eliminate it

Lymphocytes

The cells of the adaptive immune system are special types of leukocytes, called lymphocytes.
B cells and **T cells** are the major types of lymphocytes and are derived from **hematopoietic stem cells** in the **bone marrow**.

T cells;

- ✓ Are involved in **cell-mediated immune response**.
- ✓ are processed by the thymus gland & when mature they move out of the gland

- ✓ Are programmed to recognize only one type of antigen & during its subsequent travels through the body, it will react to no other antigen, however dangerous it might be.
- ✓ they recognize a "non-self" target, such as a pathogen, only after antigens (small fragments of the pathogen) have been processed and presented to it on the surface of an antigen presenting cell like macrophages, & dendrites
- ✓ To do this, after engulfing & digesting the antigen, they transport the most antigenic fragment to their own membrane & display it on the surface.
- ✓ When they come into contact with the T lymphocyte that has been processed to target that particular antigen, they will stimulate/activate it to divide & proliferate. This process is called **clonal expansion**.

There are four main types of specialized T-lymphocytes and each of these cells has important functions in an immune response;

1. **Cytotoxic/Killer T-cells;** directly inactivates any abnormal body cells (cancer cells & infected cells) carrying antigens by releasing powerful toxins.
2. **Helper T-cells;** essential for correct functioning of whole immune system- produces cytokines which support & promote cytotoxic cells & macrophages.
Cooperates with B-cells to produce antibodies

3. **Suppressor T-cells**; turns off activated lymphocytes. This limits the powerful & potentially damaging effects of the immune response.
4. **Memory T-cells.**

Clonal Expansion

B cells;

- ✓ Are involved in the Humoral Immune Response.
- ✓ Produced & processed in the bone marrow.
- ✓ Function- production of antibodies (immunoglobins) which are proteins designed to bind & destroy antigen.
- ✓ They target specific antigen
- ✓ They are fixed in lymphoid tissues like spleen & lymph nodes.
- ✓ Recognizes whole pathogens without any need for antigen processing. Once its antigen has been detected & bound with the help of T-helper cells, the B-cell enlarges & begins to divide (clonal expansion).

Functionally 2 distinct types of cell are formed;

1. Memory B-cells
2. Plasma cells;
   - ❖ Secrete antibodies (Ig) into the blood & are carried throughout the tissues.
   - ❖ Produce one type of Ig which targets the specific antigen that originally bound to the B-cell.
   - ❖ Ig bind to antigens making them targets for other defense cells
   - ❖ Ig also bind to bacterial toxins & neutralize them
   - ❖ Ig activate complement

There are 5 main types of antibody as shown in the table below;

IgA	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens. Also found in saliva, tears, and breast milk.
IgD	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens. It has been shown to activate basophils and mast cells to produce antimicrobial factors.
IgE	Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Also protects against parasitic worms.
1gG	Provides the majority of antibody-based immunity against invading pathogens. The only antibody capable of crossing the placenta to give passive immunity to fetus
IgM	They are the first to respond to an invading pathogen. They offer important protection during the early days of infection & are potent activator of the complement. These antibodies tend to stay in the bloodstream where they aid in killing bacteria.

ARTIFICIALLY ACQUIRED IMMUNITY

This develops only through deliberate actions such as vaccination.

Passive immunity is acquired through transfer of antibodies or activated T-cells from an immune host, and is short lived -- usually lasting only a few months

Active immunity is induced in the host itself by antigen, and lasts much longer, sometimes life-long.

Immunity can be acquired **naturally** or **artificially** & both forms may be **active** or **passive**.
Naturally acquired immunity occurs through contact with a disease causing agent, when the contact was not deliberate.
Artificially acquired immunity develops only through deliberate actions such as vaccination.
Passive immunity is acquired through transfer of antibodies or activated T-cells from an immune host, and is short lived -- usually lasting only a few months
Passive immunity is also provided through the transfer of IgA antibodies found in breast milk that are transferred to the gut of the infant, protecting against bacterial infections, until the newborn can synthesize its own antibodies

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Summary of the divisions of immunity

Artificially acquired passive immunity
Artificially acquired passive immunity is a short-term immunization induced by the transfer of antibodies, which can be administered in several forms; as human or animal blood plasma, as pooled human immunoglobulin for intravenous (IVIG) or intramuscular (IG) use, and in the other forms.
Passive transfer is used prophylactically in the case of immunodeficiency diseases, such as hypogamma globulinemia.
It is also used in the treatment of several types of acute infection, autoimmune diseases, and to treat poisoning.
Immunity derived from passive immunization lasts for only a short period of time, and there is also a potential risk for **hypersensitivity** reactions especially from globulin of non-human origin.
Passive transfer of cell-mediated immunity
Passive or "adoptive transfer" of cell-mediated immunity, is conferred by the transfer of "sensitized" or activated T-cells from one individual into another.
It is rarely used in humans because it requires histo-compatible (matched) donors, which are often difficult to find.
In unmatched donors this type of transfer carries severe risks of graft versus host disease.
It has, however, been used to treat certain diseases including some types of cancer and immunodeficiency.
This type of transfer differs from a bone marrow transplant, in which (undifferentiated) hematopoietic stem cells are transferred
.
Active immunity
When **B cells** and **T cells** are activated by a pathogen, memory B-cells and T- cells develop.

Throughout the lifetime of an animal these memory cells will “remember” each specific pathogen encountered, and are able to mount a strong response if the pathogen is detected again.
This type of immunity is both *active* and *adaptive* because the body's immune system prepares itself for future challenges.
Active immunity often involves both the cell-mediated and humoral aspects of immunity as well as input from the innate immune system.

Naturally acquired active immunity
Naturally acquired active immunity occurs when a person is exposed to a live pathogen, and develops a primary immune response, which leads to immunological memory.
This type of immunity is “natural” because it is not induced by deliberate exposure.
Many disorders of immune system function can affect the formation of active immunity such as immunodeficiency (both acquired and congenital forms) and immunosuppression.

Immunological memory
When B cells and T cells are activated and begin to replicate, some of their offspring will become long-lived memory cells. Throughout the lifetime of an animal, these memory cells will remember each specific pathogen encountered and can mount a strong response if the pathogen is detected again. This is "adaptive" because it occurs during the lifetime of an individual as an adaptation to infection with that pathogen and prepares the immune system for future challenges. Immunological memory can be in the form of either passive short-term memory or active long-term memory.

Acquired immunity
When antigens are encountered for the first time, a low level of Ig is produced though sufficient enough to clear the antigens. This is primary response.
When the antigens are encountered for the second time by the memory B-cells, a rapid response characterized by a marked increase in Ig production is achieved. This is secondary response.
This principle is used in active immunization against diseases.
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Passive immunity is also provided through the transfer of IgA antibodies found in breast milk that are transferred to the gut of the infant, protecting against bacterial infections, until the newborn can synthesize its own antibodies.

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ANTIBODIES OR IMMUNOGLOBULINS (Ig)

they are glycoproteins formed by plasma cells in response to antigen and counteract with antigens with great specificity. They are found in serum and other fluids such as gastric secretions and milk.

Serum containing antigen- specific antibody is called antiserum. Structure of antibody is given by GM Edelman and porter and won Nobel Prize in 1970 in physiology and medicine for this contribution.

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Classes or types of antibodies

The immunoglobulins are divided into five different classes:

1. Immunoglobulin G (IgG)
2. Immunoglobulin A (IgA)
3. Immunoglobulin M (IgM)
4. Immunoglobulin D (IgD)
5. Immunoglobulin E (IgE)

Immunoglobulin G

Immunoglobulin G (IgG) is termed as maternal antibody and is most abundant (80%) one

Immunoglobulin M

Immunoglobulin M (IgM) is the largest antibody and third most abundant (10%) in human serum. .

Immunoglobulin A

Immunoglobulin A (IgA) is the second most abundant (15%) of total antibodies in humans and occurs in body fluids such as saliva, tears, breast milk and mucosal

secretions from gastro intestinal, respiratory and genitourinary tracts. **Immunoglobulin D**
. They play an important role in secondary immune response..

Immunoglobulin E

Immunoglobulin E (IgE) occurs in extremely small amounts (0.002%). They mediate allergic reactions known as readings

Passive transfer or cell-mediated immunity

Passive or "adoptive transfer" of cell-mediated immunity, is conferred by the transfer of "sensitized" or activated T-cells from one individual into another.

It is rarely used in humans because it requires histocompatible (matched) donors, which are often difficult to find.

In unmatched donors this type of transfer carries severe risks of graft versus host disease. It has, however, been used to treat certain diseases including some types of cancer and immunodeficiency.

This type of transfer differs from a bone marrow transplant, in which (undifferentiated) hematopoietic stem cells are transferred.

Active immunity

When **B cells** and **T cells** are activated by a pathogen, memory B-cells and T- cells develop.

Throughout the lifetime of an animal these memory cells will “remember” each specific pathogen encountered, and are able to mount a strong response if the pathogen is detected again.

This type of immunity is both *active* and *adaptive* because the body's immune system prepares itself for future challenges.

Active immunity often involves both the cell-mediated and humoral aspects of immunity as well as input from the innate immune system.

Naturally acquired active immunity

Naturally acquired active immunity occurs when a person is exposed to a live pathogen, and develops a primary immune response, which leads to immunological memory.

This type of immunity is “natural” because it is not induced by deliberate exposure.

Many disorders of immune system function can affect the formation of active immunity such as immunodeficiency (both acquired and congenital forms) and immunosuppression.

Assignment

Draw the current immunization schedule

CYCLE OF INFECTION (TRANSMISSION CYCLE)

- Prevention and control of infection is of vital importance to the patient as well as to health care personnel.
- In order to provide proper care for patients’ with communicable diseases or infectious organisms, you should understand the components of infection and the

methods to control the cycle of infection.

- The cycle of infection (see diagram below) is like a chain consisting of six links.
- To produce disease, each link of the infectious process must be present in a logical sequence. Removing one link in the chain will control the cycle of infection

SOURCES OF INFECTION to man

Insects

Man

Animals

Vectors acting as reservoir hosts

Soil

Water

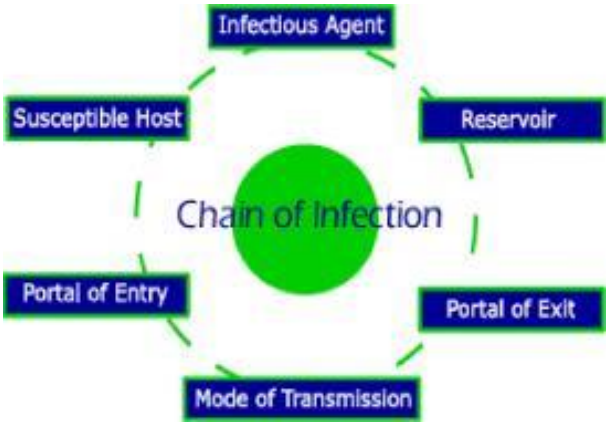
Food

NOTE

Methods of infection transmission

These are divided into 2 i.e. direct and indirect transmissions

- ✓ Direct includes: inhalation, ingestion, inoculation, insects, congenital (teratogenic), latrogenic and laboratory infection
- ✓ Indirect transmission: vehicle borne e.g. through water, food, ice, blood, serum, plasma
  Vector borne, air borne/ dust borne, unclean hands and finger



Infectious Microorganisms (Agent)

- These are the pathogens that cause communicable diseases.
- Most microbes do not cause disease automatically, rather their ability to do so depend on their virulence, which in turn depends on their structure, invasiveness and ability to manufacture toxins.
- Virulence is a measure of how effective the organism is at causing disease. 'High virulence means relatively low numbers are needed to cause disease in a health individual.
- Invasiveness means ability to enter and multiply in the host. Virulence & invasiveness often go together.
- Colonization and invasion of the body depend on the structure of the organism and on the susceptibility of the host. Some bacteria possess a capsule which can resist killing by WBC and others possess pili which help them to adhere on the host cell.
- Some bacteria produce toxins which damage the tissues. These organisms are divided into ***exotoxins*** and ***endotoxins*** **Exotoxins** are usually protein enzymes secreted by bacteria into their local environment.
- They can cause serious disease and death (diphtheria, tetanus, & botulism) and a range of food poisoning illnesses (V. cholerae, & E. coli).
- **Endotoxins** are liposaccharides contained in the cell wall of Gram- bacteria. They are not actively secreted by the cell but are released when it is destroyed & is broken open. Effects includes, fever, hypotension, & DIC. These effects constitute the ***septic*** or ***endotoxin*** shock.

Reservoir (source)

- This is where a microbe usually lives & obtains nutrients, moisture & the environmental conditions necessary for its growth.
- There are 3 sources of infection;
  - **o** Humans,
  - **o** Environment inanimate including food& water) **o**
  - Other animals and birds
- To cause infection microbes need a means of transferring to a susceptible host. When this occurs, the reservoir becomes a source of infection
- The ***human body*** is inhabited by many microorganisms, (mostly bacteria, some fungi and few other microorganisms) which under normal conditions in healthy individual are harmless. These microorganisms are termed **normal flora** or **commensals**.
- Sites of the body inhabited by normal flora
  - ✓ **Skin** (Most common organisms: Staphylococcus epidermis)
  - ✓ **Eye** (Most common organisms: Staphylococcus epidermis and Staphylococcus aureus)
  - ✓ **Mouth and nose** (most common organisms: streptococcus, diphtheroids

and Staphylococcus aureus)

- ✓ **Intestinal tract (**Many different species of bacteria; 99% anaerobes)
- ✓ **Urogenital tract** (Most common organisms: Lactobacilli)

• In addition to their normal flora, a person may be incubating an infection, acutely ill, recovering from an infection or be a chronic carrier of an organism but still be a potential source of an infection.

• Although almost any item (invasive equipment, soil, water, & food) harbors organisms, the majority do not to lead to overt infection unless there is a break in the aseptic techniques in the hospital.

• A variety of diseases can be spread from animals to man & these are called zoonosis'**.** Many animal products (infected poultry, eggs, meat & dairy products) can transmit infection

Portal of Exit

• This refers to the route by which the infectious microorganisms escape the reservoir. For example, pathogens that cause respiratory diseases usually escape through the respiratory tract (coughing, sneezing, and so forth)

• Modes of escape are Respiratory Tract, Gastrointestinal Tract, & Skin**.**

Mode of Transmission

There are 5 main routes of transmission;

1. **Contact transfer** - the most important in nosocomial infection:
   - ■ direct contact; via hands, transplacenta, sexual intercourse
   - ■ indirect contact; via fomites (equipment & inanimate objects)
2. **Airborne transfer**; microbes can only travel through air when they carried in airborne particles like dust, water & respiratory droplets. Droplets of moisture are expelled from the respiratory tract during talking, sneezing, or coughing. Most of them drop rapidly on the floor but those which are minute (droplet nuclei) remain suspended in the air to be inhaled by another individual or settle in open wound, e.g. during surgery.
3. **Common vehicle** - by contaminated food, water, solutions, drugs or blood products.
4. **Vector borne** - via arthropods e.g. ticks, mosquitoes, flea, human louse, flies, water snail, pigs, cattle, sheep, goats.
5. **Blood borne** - via inoculation injury. HBV & HIV are the main organisms of concern & inoculation injury is the main route for health care setting. Other viruses & syphilis may also be involved.

Portal/ Mode of Entry

• Refers to the method by which the pathogens enter the person (host) to cause disease microbes must have means of gaining access to the tissues of the host. Common points of access are;

1. **Respiratory Tract, by inhalation of** microbes e.g. TB, mumps virus etc.
2. **Gastrointestinal Tract by *ingestion.*** Pathogenic microorganisms enter the body of new host when food or water contaminated by faeces is ingested.
3. **Skin& mucous membrane *by inoculation to*** deeper tissues by surgery, ***insect bites, injection*** with contaminated products, ***trauma, & sexual contact.***
4. **Transplacenta.** A few organisms (CMV, rubella) can be transmitted from mother to feotus in utero.

Susceptible Host

- The host is the person who gets the disease.
- Once the host has the disease, he becomes a reservoir for future transmission of the disease.
- The body has a range of defenses designed to protect it against invasion by pathogens.
- Susceptibility to infections depends on the effectiveness of the defenses e.g. bacteria will not usually enter intact skin.
- Pathogens can be present on the body without invading tissue or causing infections. This is described as colonization.
- Infections caused by the transfer of microorganisms from one site on the body to another are called endogenous
- Infections acquired through the transfer of microorganisms form one person to another are called **exogenous infection** of cross infection.

The most susceptible persons to disease include;

1. Children who are very young.
2. People who are very old.
3. People on inadequate diets.
4. People who are chronically ill.
5. People receiving medical therapy.
6. People who are already ill.
7. People with open wounds.

An example of the Spread of Infection

An elderly patient, hospitalized with a gastrointestinal disorder, was on bed rest and required assistance for activities of daily living.

The patient had frequent uncontrolled diarrhoea stools and the nurse provided excellent care to maintain cleanliness and comfort. Following one episode of cleaning the patient and changing the bed linen, the nurse immediately went to a second patient to provide care. The nurse's hands were not washed before assisting the second patient.

Let's examine the chain of infection as it applies to this situation.

- ✓ Infectious agent -Escherichia coli

- ✓ Reservoir- Large intestine
- ✓ Portal of Exit- E. coli exited the body in faeces.
- ✓ Mode of transmission- the nurse removed the contaminated linen from the bed. The E. coli organism contaminated the hands of the nurse who then provided morning care to another patient.
- ✓ Portal of Entry-the second patient receiving care had a Foley catheter. The nurse manipulated the tubing attached to the catheter. The E. coli organism on the nurse's hands contaminated the catheter tubing and ascended to the patient's meatus and then into
  The urinary bladder
- ✓ Susceptible Host- the second patient with a Foley catheter. This patient was elderly and had a chronic illness necessitating complete bed rest. The Foley catheter contaminated by the E. coli organism provided a direct route into the urinary bladder.

Environmental factors influencing the spread of communicable diseases

A number of environmental factors influence the spread of communicable diseases that are prone to cause epidemics. The most important of these are:

- water supply

A lack of safe water, inadequate excreta disposal facilities, poor hygiene, poor living conditions and unsafe food can all cause diarrheal diseases. These diseases are a major cause of suffering and death in an emergency situation

- sanitation facilities

Poor waste disposal can expose the population to a lot of diseases

- food

Contaminated food can easily cause diseases especially diarrheal diseases

- Climate can affect disease transmission in a variety of ways. The distribution and population size of disease vectors can be heavily affected by local climate. Flooding after heavy rains can result in sewage overflow and widespread water contamination. In addition, there is some evidence to suggest that pathogens can be spread from one region to another along air streams or by wind.

Infection Control

Infection prevention and control measures aim to ensure the protection of those who

might be vulnerable to acquiring an infection both in the general community and while receiving care due to health problems, in a range of settings.
The basic principle of infection prevention and control is hygiene.
A breach in infection control practices facilitates transmission of infection from patients to health care workers, other patients and attendants.
It is therefore important for all health care workers, patients, their family members, friends and close contacts to adhere to the infection control guidelines strictly.
It is also imperative for health care administrators to ensure implementation of the infection control programe in health care facilities.
Infection control practices can be grouped in two categories

(1) Standard precautions;

(2) Additional (transmission-based) precautions.

Transmission of infections in health care facilities can be prevented and controlled through the application of basic infection control precautions which can be grouped into standard precautions, which must be applied to all patients at all times, regardless of diagnosis or infectious status, and additional (transmission-based) precautions which are specific to modes of transmission (airborne, droplet and contact). The terms “standard precautions” and “additional (transmission-based) precautions” have replaced previous terms such as universal blood and body fluid precautions, universal precautions and barrier nursing.

Standard precautions

Treating all patients in the health care facility with the same basic level of “standard” precautions involves work practices that are essential to provide a high level of protection to patients, health care workers and visitors. These include the following:

- hand washing and antisepsis (hand hygiene);
- use of personal protective equipment when handling blood, body substances, excretions and secretions;
- appropriate handling of patient care equipment and soiled linen;
- prevention of needle stick/sharp injuries;
- environmental cleaning and spills-management; and
- Appropriate handling of waste.

Hand washing and Antisepsis (hand hygiene)

Appropriate hand hygiene can minimize micro-organisms acquired on the hands during daily duties and when there is contact with blood, body fluids, secretions, excretions and known and unknown contaminated equipment or surfaces (for further details see Annex 1).

Wash or decontaminate hands:

- after handling any blood, body fluids, secretions, excretions and contaminated items;
- between contact with different patients;
- between tasks and procedures on the same patient to prevent cross contamination between different body sites;
- immediately after removing gloves; and
- Using a plain soap, antimicrobial agent, such as an alcoholic hand rub or waterless antiseptic agent.

The hospital setting is a good setting for communication about personal hygiene, such as informing visitors and the general public about hygiene rules such as washing hands.

Use of personal protective equipment

Using personal protective equipment provides a physical barrier between micro-organisms and the wearer. It offers protection by helping to prevent micro-organisms from:

- contaminating hands, eyes, clothing, hair and shoes;
- being transmitted to other patients and staff Personal protective equipment includes:
- gloves;
- protective eye wear (goggles);
- mask;
- apron;
- gown;
- boots/shoe covers; and
- Cap/hair cover.

Personal protective equipment should be used by:

- Health care workers who provide direct care to patients and who work in situations where they may have contact with blood, body fluids, excretions or secretions;
- support staff including medical aides, cleaners, and laundry staff in situations where they may have contact with blood, body fluids, secretions and excretions;
- laboratory staff, who handle patient specimens; and
- Family members who provide care to patients and are in a situation where they may have contact with blood, body fluids, secretions and excretions.

Principles for use of personal protective equipment

Personal protective equipment reduces but does not completely eliminate the risk of acquiring an infection.

It is important that it is used effectively, correctly, and at all times where contact with

blood and body fluids of patients may occur.
Continuous availability of personal protective equipment and adequate training for its proper use are essential. Staff must also be aware that use of personal protective equipment does not replace the need to follow basic infection control measures such as hand hygiene.

The following principles guide the use of personal protective equipment:

- Personal protective equipment should be chosen according to the risk of exposure.

The health care worker should assess whether they are at risk of exposure to blood, body fluids, excretions or secretions and choose their items of personal protective equipment according to this risk.

- Avoid any contact between contaminated (used) personal protective equipment and surfaces, clothing or people outside the patient care area.
- Discard the used personal protective equipment in appropriate disposal bags, and dispose of as per the policy of the hospital.
- Do not share personal protective equipment.
- Change personal protective equipment completely and thoroughly wash hands each time you leave a patient to attend to another patient or another duty.

Prevention of needle stick/sharps injuries

Take care to prevent injuries when using needles, scalpels and other sharp instruments or equipment.
Place used disposable syringes and needles, scalpel blades and other sharp items in a puncture-resistant container with a lid that closes and is located close to the area in which the item is used.
Take extra care when cleaning sharp reusable instruments or equipment.
Never recap or bend needles.
NB: Sharps must be appropriately disinfected and/or destroyed as per the national standards or guidelines.

Environmental Management Practices

A clean environment plays an important role in the prevention of hospital associated infections (HAI). Many factors, including the design of patient care areas, operating rooms, air quality, water supply and the laundry, can significantly influence the transmission of HAI.

Premises/buildings

Facility design and planning should ensure:

- adequate safe water supply;
- appropriate cleaning practices;

- adequate floor space for beds;
- adequate inter-bed space;
- adequate hand washing facilities;
- Adequate ventilation for isolation rooms and high-risk areas like operation theatres, transplant units, intensive care areas, etc.
- adequate isolation facilities for airborne, droplet, contact isolation and protective environment;
- regulation of traffic flow to minimize exposure of high-risk patients and facilitate patient transport;
- measures to prevent exposure of patients to fungal spores during renovations;
- precautions to control rodents, pests and other vectors; and
- Appropriate waste management facilities and practices.

Air (Ventilation)

Ventilation systems should be designed and maintained to minimize microbial contamination. The air conditioning filters should be cleaned periodically and fans that can spread airborne pathogens should be avoided in high-risk areas.

Water

The health care facility should provide safe water. If it has water storage tanks, they should be cleaned regularly and the quality of water should be sampled periodically to check for bacterial contamination.

Safe drinking water

Where safe water is not available, boil water for 5 minutes to render it safe
Alternatively, use water purification units.
Store water in a hygienic environment
Do not allow hands to enter the storage container.

Cleaning of the hospital environment

Routine cleaning is important to ensure a clean and dust-free hospital environment. There are usually many micro-organisms present in “visible dirt”, and routine cleaning helps to eliminate this dirt. Administrative and office areas with no patient contact require normal domestic cleaning. Most patient care areas should be cleaned by wet mopping. Dry sweeping is not recommended. The use of a neutral detergent solution improves the quality of cleaning. Hot water (80°C) is a useful and effective environmental cleaner.
Any areas visibly contaminated with blood or body fluids should be cleaned immediately with detergent and water.

Isolation rooms and other areas that have patients with known transmissible infectious diseases should be cleaned with a detergent/ disinfectant solution at least daily.
All horizontal surfaces and all toilet areas should be cleaned daily.

Management of health-care waste

Hospital waste is a potential reservoir of pathogenic micro-organisms and requires appropriate, safe and reliable handling. The main risk associated with infection is sharps contaminated with blood. There should be a person or persons responsible for the organization and management of waste collection, handling, storage and disposal. Waste management should be conducted in coordination with the infection control team.
Steps in the management of hospital waste include:

- generation,
- segregation/separation,
- collection,
- transportation,
- storage,
- treatment,
- Final disposal.

Waste management practices must meet national and local requirements; the following principles are recommended as a general guide:

Principles of waste management

Develop a waste management plan that is based on an assessment of the current situation and which minimizes the amount of waste generated.
Segregate clinical (infectious) waste from non-clinical waste in dedicated containers.
Transport waste in a dedicated trolley.
Store waste in specified areas with restricted access.
Collect and store sharps in sharps containers. Sharps containers should be made of plastic or metal and have a lid that can be closed. They should be marked with the appropriate label or logo, e.g. a biohazard symbol for clinical (infectious) waste Mark the storage areas with a biohazard symbol.
Ensure that the carts or trolleys used for the transport of segregated waste collection are not used for any other purpose - they should be cleaned regularly.
Identify a storage area for waste prior to treatment or being taken to final disposal area.

Treatment of hazardous and clinical/infectious waste

Each health care facility should identify a method for the treatment of clinical/infectious waste. This may consist of transportation of infectious waste to a centralized waste treatment facility or on-site treatment of waste.

Methods of disposal

Sharps:

- autoclave, shred and land-fill or microwave, shred and land-fill or treat by plasma pyrolysis of puncture- proof containers storing discarded sharps ;
- Deep burial in a secure area. Burial should be 2 to 3 meters deep and at least 1.5 meters above the groundwater table.

Waste requiring incineration:

- anatomical parts and animal carcasses;
- cytotoxic drugs (residues or outdated);
- Toxic laboratory chemicals other than mercury.

Waste that may be incinerated:

- Patient-contaminated non-plastics and non-chlorinated plastics.

Waste that should not be incinerated:

- chlorinated plastics;
- volatile toxic wastes such as mercury;
- Plastics, non-plastics contaminated with blood, body fluids, secretions and excretions and infectious laboratory wastes. (Such wastes should be treated by steam sterilization in autoclavable bags or microwave treatment. Shredding may follow both these methods. If neither method is available, chemical treatment with 1% hypochlorite or a similar disinfectant is recommended. However, excessive use of chemical disinfectants should be avoided as it may be a health and environmental hazard).

Radioactive waste (should be dealt with according to national laws).

Additional (transmission-based) precautions

Additional (transmission-based) precautions are taken while ensuring standard precautions are maintained. Additional precautions include:

- Airborne / Droplet precautions;
- Contact precautions.

Airborne /Droplet precautions

The following precautions need to be taken:

- Implement standard precautions.
- Place patient in a single room that has a monitored negative airflow pressure, and is often referred to as a “negative pressure room”. The air should be discharged to the outdoors or specially filtered before it is circulated to other areas of the health care facility.
- Keep doors closed.
- Anyone who enters the room must wear a special, high filtration, particulate respirator (e.g. N 95) mask.
- Limit the movement and transport of the patient from the room for essential purposes

only. If transport is necessary, minimize dispersal of droplet nuclei by masking the patient with a surgical mask.

Contact precautions

Diseases which are transmitted by this route include colonization or infection with multiple antibiotic resistant organisms, enteric infections and skin infections.
The following precautions need to be taken:

- Implement standard precautions.
- Place patient in a single room (or in a room with another patient infected by the same pathogen). Consider the epidemiology of the disease and the patient population when determining patient placement.
- Wear clean, non-sterile gloves when entering the room.
- Wear a clean, non-sterile gown when entering the room if substantial contact with the patient, environmental surfaces or items in the patient's room is anticipated.
- Limit the movement and transport of the patient from the room; patients should be moved for essential purposes only. If transportation is required, use precautions to minimize the risk of transmission.

Patient placement and transportation of patients

Patient placement

Appropriate or selective placement of patients is important in preventing the transmission of infections in the hospital setting. General principles in relation to the placement of patients include the following:

Spacing between beds

In open plan wards there should be adequate spacing between each bed to reduce the risk of cross contamination/infection occurring from direct or indirect contact or droplet transmission. Optimum spacing between beds is 1-2 meters.

Single rooms

Single rooms reduce the risk of transmission of infection from the source patient to others by reducing direct or indirect contact transmission. Where possible, single rooms should have the following facilities:

- hand washing facilities;
- Toilet and bathroom facilities.

Anterooms

Single rooms used for isolation purposes may include an anteroom to support the use of personal protective equipment.

Cohorting

For infection control purposes, if single rooms are not available, or if there is a shortage of single rooms, patients infected or colonized by the same organism can be cohorted (sharing of room/s).

When Cohorting is used during outbreaks these room/s should be in a well-defined area (a designated room or designated ward), which can be clearly segregated from other patient care areas in the health care facility used for non-infected/colonized patients

Transportation of patients

Limiting the movement and transport of patients from the isolation room/ area for essential purposes only will reduce the opportunities for transmission of micro-organisms in other areas of the hospital.

If transportation is required, suitable precautions should be taken to reduce the risk of transmission of microorganisms to other patients, health care workers or the hospital environment (surfaces or equipment). For example: when transporting a patient with pulmonary tuberculosis (open/active) placing a surgical mask on the patient while in transit is an appropriate precaution.

Laundry

General instructions

Linen

The basic principles of linen management are as follows:

- Place used linen in appropriate bags at the point of generation.
- Contain linen soiled with body substances or other fluids within suitable impermeable bags and close the bags securely for transportation to avoid any spills or drips of blood, body fluids, secretions or excretions.
- Do not rinse or sort linen in patient care areas (sort in appropriate areas).
- Handle all linen with minimum agitation to avoid aerosolisation of pathogenic micro-organisms.
- Separate clean from soiled linen and transport/store separately.
- Wash used linen (sheets, cotton blankets) in hot water (70°C to 80°C) and detergent, rinse and dry preferably in a dryer or in the sun. (Heavy duty washers/dryers are recommended for the hospital laundry).
- Autoclave linen before being supplied to the operating rooms/theatres.
- Wash woolen blankets in warm water and dry in the sun, in dryers at cool temperatures or dry-clean.

Bedding

- Mattresses and pillows with plastic covers should be wiped over with a neutral detergent.

• Mattresses without plastic covers should be steam cleaned if they have been contaminated with body fluids.

If this is not possible, contaminations should be removed by manual washing, ensuring adequate personnel and environmental protection.

• Wash pillows either by using the standard laundering procedure described above, or dry clean if contaminated with body fluids.

Reprocessing of instruments and equipment

The risk of transferring infection from instruments and equipment is dependent on the following factors:

(1) The presence of micro-organisms, the number and virulence of these organisms;

(2) The type of procedure that is going to be performed (invasive or non-invasive), and

(3) The body site where the instrument/and or equipment will be used (penetrating the mucosal or skin tissue or used on intact skin).

Any instrument or equipment entering into a sterile part of the body must be sterilized.

Where the instrument or equipment will be in contact with mucous membranes or non-intact skin, it must have undergone disinfection.

Where there will be contact with intact skin, disinfection or cleaning should be used.

Reprocessing of instruments and equipment in an effective way includes:

(1) Cleaning instruments and equipment immediately after use to remove all organic matter, chemicals and

(2) Disinfection (by heat and water or chemical disinfectants) or

(3) Sterilization.

Cleaning, disinfection and sterilization

Cleaning

Prior to any reprocessing to achieve disinfection or sterility all instruments and equipment MUST be cleaned. If not cleaned properly, organic matter may prevent the disinfectant or sterilant from having contact with the instrument/equipment and may also bind and inactivate the chemical activity of the disinfectant. If an instrument/equipment is unable to be cleaned then it is unable to be sterilized or disinfected.

After an instrument has been used, prior to it drying, it should be washed to remove any gross soiling. At this stage, detergent and water is appropriate to use.

There are four main methods used for cleaning of instruments and equipment:

1. Manual cleaning
2. Enzymatic cleaners

3. Ultrasonic cleaners and automated washers
4. Disinfection

Disinfection

Disinfection removes micro-organisms without complete sterilization.
Disinfection is used to destroy organisms present on delicate or heat-sensitive instruments which cannot be sterilized or when single use items are not available.
Disinfection is not a sterilizing process and must not be used as a convenient substitute for sterilization. Thermal disinfection is not appropriate for instruments that will be used in critical sites as these instruments must be sterile.
Certain products and processes will provide different levels of disinfection.
These levels are classified as:
(a) High-level disinfection: Destroys all micro-organisms except some bacterial spores (especially if there is heavy contamination).
(b) Intermediate disinfection: Inactivates *Mycobacterium tuberculosis* vegetative bacteria, most viruses and most fungi, but does not always kill bacterial spores.
(c) Low-level disinfection: Can kill most bacteria, some viruses and some fungi, but cannot be relied on to kill more resistant bacteria such as *M. tuberculosis* or bacterial spores.
The two methods of achieving disinfection are thermal and chemical disinfection.

1. Thermal disinfection (pasteurization)

If an instrument is able to withstand the process of heat and moisture and is not required to be sterile, then thermal disinfection is appropriate.
By using heat and water at temperatures that destroy pathogenic, vegetative agents, this is a very efficient method of disinfection.
The level of disinfection depends on the water temperature and the duration the instrument is exposed to that temperature.

Minimum surface temperature and time required for thermal disinfection

Surface Temperature (^{0}C)	Minimum disinfection time required (minutes)
90	1
80	10
75	30
70	100

2. Chemical disinfection

The performance of chemical disinfectants is dependent on a number of factors including: temperature, contact time, concentration, and pH, presence of organic or inorganic matter and the numbers and resistance of the initial bio-burden on a surface .Instrument grade disinfectants are classified as high, intermediate or low level. When used according to the manufacturers' guidelines, disinfectants will fall into one of these levels.

Chemical Disinfectant - level of disinfection achieved

Level of Disinfection Activity against Microbes	Activity against microbes
High level chemical Disinfectant	Inactivates all microbial pathogens except where there are large numbers of bacterial spores
Intermediate level disinfectant	Inactivates all microbial pathogens except bacterial spores
Low level disinfectant	Rapidly inactivate most vegetative bacteria as well as medium sized lipid-containing viruses, but may not destroy bacterial spores, mycobacteria, fungi or small nonlipid

Selection of disinfectant

There is no single ideal disinfectant. Different grades of disinfectants are used for different purposes. Only instrument grade disinfectants are suitable to use on

medical instruments and equipment. Monitoring of the disinfectant is important if it is a multi-use solution.

It is important that it is stored correctly and according to the manufacturer's instructions.

Be sure not to contaminate the solution when pouring out for use

Sterilization

Sterilization is the destruction of all micro-organisms and can be achieved by either physical or chemical methods.2 Sterilization is necessary for medical devices penetrating sterile body sites. Cleaning to remove visible soiling in reusable equipment should always precede sterilization. All materials must be wrapped before sterilization. Only wrapped/packed sterilized materials should be described as sterile. Before any instrument or equipment goes under the process of steam sterilization, the following should be checked:

(1) Ensure that the instrument can withstand the process (e.g. steam under pressure),

(2) Ensure that the instrument has been adequately cleaned,

(3) Ensure that the instrument does not require any special treatment,

(4) Ensure that records of the sterilization process and for the traceability of instruments are kept.

Instruments and equipment will only be sterile if one of the following sterilizing processes is used:

(1) Steam under pressure (moist heat),

(2) Dry heat,

(3) Ethylene oxide,

(4) Automated environmentally sealed low-temperature peracetic acid, hydrogen peroxide plasma and other chemical sterilant systems or sterilants, or

(5) Irradiation.

The above sterilizing methods are designed to give a sterility assurance level of at least one in a million or 10-6 (see glossary) as long as the process is validated and is according to the manufacturers' guidelines.

Ultraviolet light units, incubators, microwave ovens and domestic ovens must not be used for sterilizing.

1. Steam under pressure (moist heat) sterilization

This is the most efficient and reliable method to achieve sterility of instruments and equipment. This method sterilizes and dries the sterile package as part of the cycle. This is recommended in office-based practice.

There are several types of steam under pressure sterilizers (also called autoclaves):

Downward (gravity) displacement sterilizers (jacketed and non-jacketed)

- These are designed for the sterilization of waste, solutions and instruments.

Self-contained (bench-top) sterilizers - these are recommended for office based practice

as they are able to do small quantities or fairly simple items.
Bench-top sterilizers do not take wrapped items and therefore items must be used immediately after they are removed from the sterilizer. There will be differences in the models and types of features that are offered may vary.
These variations may include: drying stage, ability to take packaged and unwrapped items, systems to monitor temperature, pressure and holding time.
Prevacuum (porous load) sterilizers - these are not suited for liquid sterilization but are optimized for sterilization of clean instruments, gowns, drapes, toweling and other dry materials required for surgery.

2. Dry heat sterilization (hot air ovens)

Dry heat sterilization is caused by hot air that destroys pathogens by the process of oxidation. Dry heat sterilizers have had limited value because it is difficult to maintain the same temperature throughout the load, while the high temperatures and long time (170 ^{o} C for 1 hour) required to achieve sterility makes this method undesirable for many situations. The manufacturers' instructions must be followed; the door to the unit must not be opened while in sterilizing cycle.

3. Ethylene Oxide (EO)

Ethylene oxide gas is appropriate to use for sterilization of instruments/ equipment made from heat labile materials or those devices that contain electronic components. The time required to process the instrument is dependent on the temperature, humidity and concentration level of the gas.
The gas must penetrate the packaging and reach all surfaces of the instrument/equipment requiring sterilization. The time for such a process is between 12 hours to over 24 hours. Because EO is toxic, this gas is restricted in health care facilities and must be used according to strict guidelines to ensure staff safety. The manufacturer's instructions must be followed for the packaging, sterilization process, validation and aeration process.

4. Automated chemical (low temperature) systems3

Hydrogen peroxide plasma in a fully automated cycle can achieve low temperature, low moisture sterilization within a 45-80 minute cycle depending on the model of sterilizer used. The packaging used must be nonwoven/ non-cellulose polypropylene wraps.
Peracetic acid is a low-temperature sterilization method. Peracetic acid 0.2% is placed in an environmentally sealed chamber and fully automated processing system. The process achieves moist, low temperature sterilization within 25-30 minutes.

5. Irradiation

Gamma radiation is available from some commercial gamma irradiation facilities. However, it is not readily available for use in health care facilities.
Only those instruments and equipment that have undergone the entire sterilizing process can be regarded as sterile. Items must be wrapped or packaged appropriately to be considered sterile.2 Materials for packaging includes:

- Paper - this prevents contamination if it remains intact. It maintains sterility for a long period can act as a sterile field and can also be used to wrap dirty devices after the procedure.
- Non-woven disposable textiles.
- Containers - these can be used only if they contain material intended for a single treatment procedure for a single patient.
- The end-user must check the physical integrity of the package before use.

Boiling of medical devices for reuse is not recommended since it does not guarantee sterility.
However, in certain resource-poor situations where steam sterilization is not possible, these items should be thoroughly cleaned and subjected to a cycle in a pressure cooker for 30 minutes.

SIMPLE LABORATORY TESTS

SPECIMEN COLLECTION

Collection of material for bacteriological examination is the responsibility of the nurse. The specimen should be collected in such a manner that it does not become contaminated with other organisms.

- ➔ Preferably specimen should be obtained before antibiotic or other antimicrobial agents are administered. If culture has been taken after initiation of antimicrobial therapy, laboratory should be informed so that specific counteractive measures such as adding penicillinase or merely diluting the sample may be carried out.
- ➔ Material should be collected from a region where the suspected organism is most likely to be found and with as little external contamination as possible.
- ➔ Another important point to remember is the stage of the disease. Enteric pathogens are present in much larger number during other acute diarrheal stage of intestinal infections and they are most likely to be isolated at that time.
- ➔ Specimen should be quantitatively sufficient to permit complete examination and should be kept in sterile containers. Arrangements should be made for prompt delivery of specimens to the laboratory.
- ➔ The laboratory should be provided with sufficient clinical information to guide the microbiologist in the selection of suitable media and appropriate techniques.

Important points to remember are:

1. Strict aseptic precautions
2. Use always sterile containers labeled with patient's hospital number.
3. Avoid soaking outside of containers

4. Proper transport. If delay is expected, refrigerate the specimens

After microscopy, the microbiologist proceeds immediately to culture the specimen for pathologic bacteria in appropriate media. The cultures are grown at 370C in an incubator. Adhensives are refixed carefully. If anaerobic infection is suspected in the patient, additional media are to be used. After proper collection, the culture bottles are incubated at 370C and are never to be refrigerated.

SAMPLES USED

1. **URINE**

Midstream or clean-catch specimen is to be obtained. The specimen must be collected in a sterile, wide mouthed screw capped bottle, after thorough cleaning the genitalia with soap and water. Improperly collected urine specimens will lead to incorrect lab report. The specimen must reach the laboratory within 15 minutes of collection. If not, it should be refrigerated immediately.

If infection with tuberculosis is suspected, the entire early morning sample of urine should be sent in large special sterile bottle.

2. **FEACES**

A small quantity of formed stool is place in a sterile specimen container. About one third of the container should be filled with the stool. The container should never be completely filled with stool. Special care should be taken to see that the outside of the container is not contaminated. If mucus or flakes of tissue is present in the feces, these should be included in the collected specimen. In certain cases like suspected bacillary dysentery or Escherichia coli diarrhea, rectal swab is preferred. Sterile swabs, moistened in sterile saline are introduced well beyond the internal sphincter, twirled well, gently withdrawn and placed in a sterile test tube and sent to the laboratory immediately.

3. **PUS OTHER THAN PURULENT BODY FLUID**

About 1mL of pus is placed in a sterile test tube. If this is not possible as much as pus as possible is collected on two sterile swabs and replaced in a sterile test tube. The end of the swab sticks are never to be broken off. The tips of the swab sticks should project beyond the mouth of the tube to facilitate handling. The mouth of the test tube with projected tips of the swab sticks must be secured with the sterile cotton or gauze fastened with adhesive tapes soon after collection.

Ear, Nose, Eye and Throat Swabs

Two small stick swabs in a sterile test tube are used for collecting pus

The throat swabs are taken as follows;

The patient tongue is depressed and two swabs are passed well over the tonsils, surrounding areas and over the area where there is inflammation. The swabs with specimen are to be placed in the sterile test tube. Care should be taken not to touch inside the cheek or tongue.

Sputum

As far as possible, an early morning coughed up specimen is preferred. Instruct the patient to wash the mouth with plain water, a few minutes before taking the specimen. A few milliliter of the coughed-up specimen is placed in a sterile wide mouthed, screw-capped bottle and dispatched as early as possible.

4. BLOOD

Blood for serological Tests

For investigation 10mL of blood should be taken in a dry syringe and placed in a sterile test tube or bottle. The bottle should not contain any anticoagulant. The blood should be allowed to clot. The blood should be placed in the container directly from the syringe after removing the needle to avoid hemolysis due to frothing (forcing blood through the needle can cause hemolysis).

Blood for cultures

Cultures are made to determine the presence or absence of bacteria and therefore should be taken by venipuncture under careful aseptic conditions. The blood is added to the culture media in glass tubes and cultivated. It is observed after a period of time usually 2 to 3 days. Some of the organisms that can be determined by means of blood cultures are typhoid bacilli, pneumococci, streptococci and staphylococci.

Blood for Widal test

Widal test is a specific test for antibodies produced by typhoid and paratyphoid bacilli within the blood and tissues. Blood is collected by a sterile syringe and needle. The serum is allowed to separate from the clot. When various dilutions of the serum are made with normal saline and a standard antigen prepared with killed organism is mixed with the serum, agglutination will take place in a positive test.

Blood for Wassermann reaction

Wassermann reaction is a test to detect the presence of an antibody in patients of syphilis. For this, 5 mL of blood is taken from the veins; test is done on blood serum and also venereal disease research laboratory (VDRL) tests performed.