BT601 – VIROLOGY

FINAL TERM IMPORTANT QUESTIONS

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Admin: **BS Biological Sciences** (One place to all your solutions)

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1. Write down the stages of Phage growth curve.

The phage growth curve consists of the following stages:

1. ***Adsorption*** – The bacteriophage attaches to specific receptor sites on the bacterial cell surface.
2. ***Penetration (Injection)*** – The phage injects its nucleic acid into the host cell, while the protein coat remains outside.
3. ***Latent period*** – No complete phage particles are detected inside or outside the host; viral nucleic acid replication and synthesis of phage proteins occur.
4. ***Eclipse period*** – Sub-stage of the latent period during which individual phage components are synthesized but not yet assembled into mature virions.
5. ***Maturation (Assembly)*** – Newly synthesized phage DNA and proteins are assembled into complete phage particles.
6. ***Rise period (Burst)*** – Host cell lyses and a large number of progeny phages are released, increasing the phage count sharply.

2. Write genomic properties of Togavirus.

Genomic properties of Togavirus:

- Genome consists of single-stranded, positive-sense RNA (+ssRNA).
- RNA is linear, non-segmented, and approximately 10–12 kb in length.
- Genome acts directly as mRNA upon entry into the host cell.
- RNA possesses a 5′ cap structure and a 3′ poly-A tail.
- Genome contains two major open reading frames (ORFs).
- The 5′ ORF encodes non-structural proteins involved in RNA replication.
- The 3′ ORF encodes structural proteins (capsid and envelope glycoproteins).
- Replication occurs in the cytoplasm of the host cell.

3. Which type of centrifugation is suitable for isolation of viruses?

Density gradient centrifugation is the most suitable type of centrifugation for the isolation of viruses.

4. How the viral growth can be detected on cell culture?

Viral growth in cell culture can be detected by the following methods:

- ***Cytopathic effects (CPE):*** Visible morphological changes in host cells such as cell rounding, vacuolation, syncytia formation, and cell lysis.
- ***Plaque formation:*** Clear zones (plaques) produced by virus-induced cell destruction in a cell monolayer.
- ***Inclusion bodies*:** Presence of characteristic intracellular inclusion bodies in infected cells.
- ***Hemadsorption:*** Adsorption of red blood cells onto virus-infected cells due to viral hemagglutinins.
- ***Immunofluorescence / immunocytochemistry:*** Detection of viral antigens using labeled antibodies.
- ***Interference phenomenon:*** Inhibition of replication of a second virus indicating presence of the first virus.

5. How prions are different from Bacteria and Viruses?

Prions differ from bacteria and viruses in the following ways:

- ***Nature:*** Prions are infectious proteins without nucleic acids, whereas bacteria and viruses contain DNA and/or RNA**.**
- ***Structure:*** Prions lack cellular structures and viral components such as cell wall, membrane, capsid, or envelope.
- ***Replication:*** Prions replicate by inducing abnormal folding of normal host proteins, not by nucleic acid synthesis; bacteria and viruses replicate using genetic material.
- ***Metabolism:*** Prions have no metabolic activity, while bacteria possess their own metabolism and viruses depend on host machinery.
- ***Size:*** Prions are smaller than viruses, whereas bacteria are much larger and viruses are intermediate in size.
- ***Sensitivity:*** Prions are highly resistant to heat, radiation, and chemical disinfectants compared to bacteria and viruses.
- ***Diseases caused:*** Prions cause slow, progressive neurodegenerative diseases, while bacteria and viruses cause a wide range of infectious diseases.

6. Write precautions of handling virus samples.

Precautions for handling virus samples include:

- Work in a biosafety cabinet appropriate to the risk group of the virus.
- Use personal protective equipment (PPE) such as gloves, lab coat, mask, and eye protection.
- Follow aseptic techniques to avoid contamination and accidental exposure.
- Avoid aerosol generation while pipetting, centrifuging, or vortexing samples.
- Clearly label and seal samples in leak-proof containers.
- Disinfect work surfaces and equipment with approved virucidal disinfectants before and after use.
- Dispose of viral waste by autoclaving or incineration according to biosafety guidelines.
- Wash hands thoroughly after handling samples and before leaving the laboratory.
- Restrict access to the laboratory and handle samples only by trained personnel.

7. Write down five parts of light microscope and their functions.

Parts of light microscope and their functions:

1. ***Eyepiece (Ocular lens)*** – Magnifies the image formed by the objective lens for viewing, usually 10×.
2. ***Objective lenses*** – Provide primary magnification of the specimen (e.g., 10×, 40×, 100×).
3. ***Stage*** – Supports and holds the glass slide containing the specimen.
4. ***Condenser*** – Focuses and concentrates light onto the specimen.
5. ***Diaphragm (Iris diaphragm)*** – Regulates the amount of light entering the condenser to improve contrast.

8. Write principle of Nucleic Acid hybridization.

Nucleic acid hybridization is based on the principle of complementary base pairing, in which single-stranded DNA or RNA molecules having complementary nucleotide sequences specifically recognize and bind to each other. When double-stranded nucleic acids are denatured by heat or chemicals, they separate into single strands. Upon cooling under appropriate conditions of temperature, pH, and salt concentration, complementary strands re-associate to form a stable DNA–DNA, DNA–RNA, or RNA–RNA hybrid. The extent of hybridization depends on the degree of sequence complementarity, allowing detection and identification of specific nucleic acid sequences.

9. Write genetic makeup of Retrovirus.

Genetic Makeup of Retrovirus:

- ***Genome type:*** Single-stranded positive-sense RNA (+ssRNA).
- ***Number of copies:*** Two identical RNA molecules per virion (diploid genome).
- ***Enzymes carried:*** Contains reverse transcriptase, integrase, and protease.
- ***Genes present:*** Typical genes include:
    - ***gag*** – encodes structural proteins (capsid, matrix, nucleocapsid)
    - ***pol*** – encodes enzymes (reverse transcriptase, integrase, protease)
    - ***env*** – encodes envelope glycoproteins (gp120, gp41)
- ***Unique feature:*** RNA genome is reverse-transcribed into DNA in host cell before integration into host genome.

10.Write principle of density gradient centrifugation.

Principle of Density Gradient Centrifugation:

Density gradient centrifugation is based on the principle that particles suspended in a fluid move through a density gradient during centrifugation until they reach a point where their density equals that of the surrounding medium. At this point, particles form distinct bands that can be separated. It allows separation of viruses, organelles, or macromolecules based on size, shape, and buoyant density.

11.Name the dyes which are used in transmission electron microscopy.

Dyes used in Transmission Electron Microscopy (TEM):

- ***Uranyl acetate*** – for staining nucleic acids and proteins
- ***Lead citrate*** – for enhancing contrast of cellular structures
- ***Osmium tetroxide (OsO_4)*** – for lipid and membrane fixation/staining
- ***Phosphotungstic acid (PTA)*** – for negative staining of viruses and macromolecules

12.What is latent occult viral infection? Write three examples.

Latent (Occult) Viral Infection:

A latent viral infection is one in which the virus persists in the host without causing apparent disease**,** remaining dormant in specific cells and may reactivate later under certain conditions. Examples**:**

1. ***Herpes simplex virus (HSV)*** – remains latent in nerve ganglia
2. ***Varicella-zoster virus (VZV)*** – remains latent in dorsal root ganglia
3. ***Epstein-Barr virus (EBV)*** – remains latent in B lymphocytes

13.Enlist some monochromic light sources used in microscope.

Monochromatic Light Sources Used in Microscope:

- Mercury vapor lamp
- Xenon arc lamp
- LED (Light Emitting Diode)
- Laser
- Sodium vapor lamp

14.Write three properties of Hepatitis Delta Virus.

Properties of Hepatitis Delta Virus (HDV):

1. ***Genome:*** Single-stranded circular RNA, negative-sense.
2. ***Defective virus:*** Requires Hepatitis B virus (HBV) for envelope proteins and replication.
3. ***Replication:*** Occurs via rolling-circle mechanism in the host cell nucleus.

15.Name ten viruses of positive sense RNA virus.

Ten Positive-Sense RNA (+ssRNA) Viruses:

1. Poliovirus (*Picornaviridae*)
2. Hepatitis A virus (*Picornaviridae*)
3. Coxsackievirus (*Picornaviridae*)
4. Rhinovirus (*Picornaviridae*)
5. Hepatitis C virus (*Flaviviridae*)
6. Dengue virus (*Flaviviridae*)
7. Zika virus (*Flaviviridae*)
8. Yellow fever virus (*Flaviviridae*)
9. Chikungunya virus (*Togaviridae*)
10. Rubella virus (*Togaviridae*)

16.Write benefits of Phage Therapy in comparison to Bactericidal Therapies.

Benefits of Phage Therapy Compared to Bactericidal Therapy:

1. ***Specificity:*** Targets only the pathogenic bacteria without harming normal microbiota.
2. ***Reduced resistance*****:** Bacteria develop resistance to phages less frequently than to antibiotics.
3. ***Self-amplifying:*** Phages replicate at the site of infection, increasing therapeutic effect.
4. ***Biofilm penetration:*** Can infect and lyse bacteria in biofilms where antibiotics often fail.
5. ***Fewer side effects:*** Minimal toxicity to human cells compared to broad-spectrum antibiotics.
6. ***Effective against MDR bacteria:*** Useful against multi-drug resistant (MDR) bacterial strains.

17.How plaque count experiment takes place in bacteriophage titration?

Plaque Count Experiment in Bacteriophage Titration:

1. ***Preparation of bacterial lawn:*** Mix host bacteria with soft agar and pour onto nutrient agar plate.
2. ***Serial dilution of phage:*** Prepare 10-fold serial dilutions of phage suspension.
3. ***Inoculation:*** Add diluted phage onto bacterial lawn and incubate at suitable temperature.
4. ***Plaque formation:*** Phages infect bacteria, replicate, and lyse host cells, forming clear zones called plaques.
5. ***Counting plaques:*** Count the number of plaques on plates with 30–300 plaques.

18.What are basic requirements for viral infection?

Basic Requirements for Viral Infection:

1. ***Susceptible host cell:*** Cell must have the appropriate receptors for viral attachment.
2. ***Permissive cell:*** Cell must allow viral replication and production of progeny viruses.
3. ***Viral entry:*** Virus must successfully attach and penetrate the host cell.
4. ***Availability of host machinery:*** Host must provide enzymes, nucleotides, and energy for viral genome replication and protein synthesis**.**
5. ***Overcoming host defenses:*** Virus must evade or suppress the immune response to establish infection.

19.Write a short note on M-13 phage.

M13 Phage:

M13 is a filamentous bacteriophage that infects *Escherichia coli*. It contains a single-stranded circular DNA genome of approximately 6.4 kb. Unlike lytic phages, M13 replicates by extruding from the host cell without causing lysis. Its flexible filamentous structure is composed of coat proteins surrounding the DNA. M13 phage is widely used in molecular biology for applications such as phage display, cloning, and DNA sequencing due to its stable and non-lytic nature.

20.What factors affect the titration of viruses?

Factors Affecting Virus Titration:

1. ***Host cell condition:*** Health, density, and susceptibility of host cells influence viral replication.
2. ***Incubation time and temperature:*** Optimal conditions are required for plaque formation or cytopathic effect.
3. ***Virus dilution accuracy:*** Proper serial dilutions are essential for reliable counting.
4. ***Medium composition:*** Nutrients, agar concentration, and pH can affect viral growth.
5. ***Adsorption efficiency:*** Time and conditions for virus attachment to host cells impact infection.
6. ***Technique consistency:*** Variations in plating, mixing, or handling can alter results.
7. ***Type of virus:*** Some viruses form clear plaques easily, others produce small or turbid plaques.

21.What is membrane technology application?

Membrane Technology Application:

Membrane technology is applied for separation, purification, and concentration of biological and chemical substances**.** It is widely used in:

- ***Water treatment:*** Removal of microbes, salts, and impurities (reverse osmosis, ultrafiltration).
- ***Biopharmaceuticals:*** Purification of vaccines, proteins, enzymes, and viruses.
- ***Food industry:*** Concentration of milk, juice, and other liquids.
- ***Waste management:*** Separation of pollutants and recovery of valuable products.
- ***Laboratory research:*** Virus concentration, cell fractionation, and protein isolation using microfiltration, ultrafiltration, or nanofiltration membranes.

22.What is protein hybridization?

Protein Hybridization:

Protein hybridization is a technique in which two different proteins or peptides are allowed to interact based on specific binding sites or complementary structures, forming a stable complex. It is used to detect, identify, or study protein–protein interactions, antibody–antigen binding, or fusion proteins in molecular biology and immunology.

23.Write three functions of Viral Capsid.

Functions of Viral Capsid:

1. ***Protection:*** Shields the viral nucleic acid from enzymatic degradation and environmental damage.
2. ***Attachment and entry:*** Facilitates binding to host cell receptors and assists in viral penetration.
3. ***Assembly:*** Provides structural framework for packaging the viral genome into a stable virion.

24.What are Obligate Intracellular Parasites?

Obligate Intracellular Parasites:

Obligate intracellular parasites are organisms that can replicate only inside living host cells because they lack the cellular machinery (enzymes, ribosomes, energy-generating systems) required for independent reproduction. Viruses are classic examples, as they rely entirely on the host's metabolic and synthetic machinery for replication.

25.What is Host-Virus genome?

Host–Virus Genome:

The host–virus genome refers to the combined genetic material of a virus and its host cell during infection. In some viruses, particularly retroviruses, the viral genome integrates into the host DNA, forming a provirus, which becomes a permanent part of the host genome and is replicated along with it. This integration allows the virus to persist, replicate, and sometimes alter host cell functions.

26.Write functions of protein and glycoprotein in Orthomyxovirus.

Functions of Protein and Glycoprotein in Orthomyxovirus:

1. ***Hemagglutinin (HA, glycoprotein):*** Mediates attachment of virus to host cell receptors and facilitates fusion with host membrane.
2. ***Neuraminidase (NA, glycoprotein):*** Helps in release of progeny virions from infected cells by cleaving sialic acid residues.
3. ***Matrix protein (M1, protein):*** Provides structural support and maintains virion shape.
4. ***M2 protein (ion channel protein):*** Involved in viral uncoating inside host cells.
5. ***Nucleoprotein (NP, protein):*** Encapsidates viral RNA and is essential for replication and transcription.

27.Write procedure for Rotter Culture tubes.

Procedure for Rotter Culture Tubes:

1. ***Preparation:*** Sterilize Rotter tubes and all required media.
2. ***Inoculation:*** Introduce the microbial or viral sample into the tube under aseptic conditions.
3. ***Incubation:*** Place the tubes at the appropriate temperature for the growth of the organism.
4. ***Observation:*** Monitor daily for turbidity, sediment, or cytopathic effects indicating growth.
5. ***Documentation:*** Record the results, including the rate and pattern of growth.
6. ***Disposal:*** After observation, properly decontaminate and dispose of the culture tubes according to biosafety guidelines.

28.Write characteristics and genetic makeup of Paramyxoviridae.

Paramyxoviridae

Characteristics:

- Enveloped, pleomorphic or spherical viruses.
- Negative-sense single-stranded RNA (-ssRNA) genome.
- Non-segmented, linear RNA (~15–19 kb).
- Replication occurs in the cytoplasm.
- Contains fusion (F) protein for cell entry and syncytia formation.
- Causes respiratory and systemic infections in humans and animals.

Genetic Makeup:

- Genome encodes 6–10 proteins, including:
    - ***Nucleocapsid protein (N)*** – encapsidates RNA
    - ***Phosphoprotein (P)*** – cofactor for RNA polymerase
    - ***Matrix protein (M)*** – structural integrity
    - ***Fusion protein (F)*** – mediates membrane fusion
    - ***Hemagglutinin-neuraminidase (HN)*** – attachment and release
    - ***Large protein (L)*** – RNA-dependent RNA polymerase

29.What is Lysogenic cycle?

Lysogenic Cycle:

The lysogenic cycle is a type of viral replication in which the viral genome integrates into the host cell's DNA and remains dormant (as a prophage) without causing immediate lysis. The viral DNA is replicated along with the host genome during cell division. Under certain conditions, the prophage may be induced to enter the lytic cycle, producing new virions and lysing the host cell.

30.What are targets of viral evasion proteins?

Targets of Viral Evasion Proteins:

Viral evasion proteins target host immune defenses to avoid detection and destruction. Major targets include:

1. ***Interferon signaling pathways*** – Inhibit interferon production or response.
2. ***Antigen presentation machinery*** – Block MHC class I or II expression to escape T-cell recognition.
3. ***Apoptosis pathways*** – Prevent programmed cell death of infected cells.
4. ***Complement system*** – Inhibit activation to avoid lysis by host serum.
5. ***Cytokine signaling*** – Modulate or mimic cytokines to suppress immune response.

31.Write subfamilies of Herpesviridae.

Subfamilies of Herpesviridae:

1. ***Alphaherpesvirinae*** – Rapid replication, latent in neurons (e.g., Herpes simplex virus, Varicella-zoster virus).
2. ***Betaherpesvirinae*** – Slow replication, latent in monocytes and lymphocytes (e.g., Cytomegalovirus, Human herpesvirus 6 & 7).
3. ***Gammaherpesvirinae*** – Latent in lymphoid tissue (e.g., Epstein-Barr virus, Kaposi's sarcoma-associated herpesvirus).

32.Write a note of replication of Retrovirus.

Replication of Retrovirus:

Retroviruses are single-stranded positive-sense RNA viruses that replicate through a DNA intermediate. After attachment to the host cell via specific receptors, the viral envelope fuses with the cell membrane, releasing the viral RNA and enzymes (reverse transcriptase, integrase, protease) into the cytoplasm. The reverse transcriptase enzyme converts viral RNA into double-stranded DNA, which is then transported into the nucleus. Using integrase, the viral DNA integrates into the host genome as a provirus. The provirus is transcribed by the host machinery to produce viral RNA and proteins. Finally, viral assembly and budding occur at the cell membrane, forming new infectious virions.

33.Write a note on replication of Coronavirus.

Replication of Coronavirus:

Coronaviruses are positive-sense single-stranded RNA (+ssRNA) viruses that replicate in the cytoplasm of host cells. After binding to the host cell receptor via the spike (S) protein, the virus enters the cell by membrane fusion or endocytosis. The viral RNA genome acts as mRNA and is immediately translated to produce replicase polyproteins, which are processed into non-structural proteins forming the replication–transcription complex. This complex synthesizes full-length genomic RNA and a set of subgenomic RNAs for structural protein production. Viral structural proteins (S, M, E) are inserted into the endoplasmic reticulum–Golgi intermediate compartment (ERGIC), where assembly occurs. Finally, new virions are budded into vesicles and released by exocytosis.

34.What is Red Queen Hypothesis?

Red Queen Hypothesis:

The Red Queen Hypothesis proposes that organisms must continuously evolve and adapt to survive in a constantly changing environment, especially due to co-evolution with other species such as predators, parasites, and pathogens. In virology and host–pathogen context, it explains the ongoing evolutionary arms race between viruses and their hosts, where each adapts in response to changes in the other to maintain relative fitness.

35.Write steps of Lytic cycle of bacteriophage.

Steps of Lytic Cycle of Bacteriophage:

1. ***Adsorption:*** Phage attaches to specific receptors on the bacterial cell surface.
2. ***Penetration:*** Viral DNA is injected into the host cytoplasm; the protein coat remains outside.
3. ***Biosynthesis:*** Viral genome is replicated, and phage proteins are synthesized using host machinery.
4. ***Maturation (Assembly):*** Newly synthesized viral DNA and proteins are assembled into complete virions.
5. ***Lysis and Release:*** Host cell bursts (lysis), releasing progeny phages to infect new cells.

36.Write characteristics of ideal viral drug.

Characteristics of an Ideal Antiviral Drug:

1. ***Selective toxicity:*** Kills or inhibits the virus without harming host cells.
2. ***Broad-spectrum activity:*** Effective against multiple strains or types of viruses.
3. ***Low resistance development:*** Virus should rarely develop resistance to the drug.
4. ***Good bioavailability:*** Easily absorbed, distributed, and retained in the body.
5. ***Minimal side effects:*** Safe for long-term or repeated use.
6. ***Target specific viral stage:*** Interferes with attachment, replication, assembly, or release of the virus.
7. ***Stable and easy to administer:*** Chemically stable, cost-effective, and preferably oral or injectable.

37.Enlist viral vectors used in plants.

Viral Vectors Used in Plants:

1. ***Tobacco mosaic virus (TMV)*** – widely used for gene expression and silencing**.**
2. ***Cauliflower mosaic virus (CaMV)*** – used for promoter studies and transgene delivery.
3. ***Potato virus X (PVX)*** – employed for transient expression of foreign proteins.
4. ***Barley stripe mosaic virus (BSMV)*** – used for virus-induced gene silencing (VIGS)**.**
5. ***Cowpea mosaic virus (CPMV)*** – used for vaccine antigen production and protein expression.

38.Write principle of SEM and TEM.

Principle of Electron Microscopy:

1. Transmission Electron Microscopy (TEM):

- TEM works on the principle that a beam of electrons is transmitted through an ultra-thin specimen**.**
- Electrons interact with the specimen, and regions of different density scatter electrons differently, forming a contrast image on a fluorescent screen or detector.
- Provides high-resolution internal structural details of cells, viruses, and organelles.

2. Scanning Electron Microscopy (SEM):

- SEM works on the principle that a beam of electrons scans the surface of a specimen, causing secondary electrons to be emitted**.**
- These emitted electrons are detected to form a three-dimensional image of the specimen surface.
- Provides detailed information on surface topography and morphology.

39.Write three segments of Bunyaviridae.

Segments of Bunyaviridae:

Bunyaviridae have a negative-sense single-stranded RNA (-ssRNA) genome that is segmented into three parts**:**

1. ***L segment (Large):*** Encodes RNA-dependent RNA polymerase (L protein)**.**
2. ***M segment (Medium):*** Encodes glycoproteins (Gn and Gc) for viral envelope.
3. ***S segment (Small):*** Encodes nucleocapsid (N) protein and sometimes non-structural proteins.

40.Write genetic makeup of Picronavirus.

Genetic Makeup of Picornavirus:

- ***Genome type:*** Single-stranded positive-sense RNA (+ssRNA)**.**
- ***Structure:*** Linear, non-segmented RNA (~7–8 kb).
- ***Polarity:*** Acts directly as mRNA for protein synthesis.
- ***Proteins encoded:***
    - ***Structural proteins (VP1, VP2, VP3, VP4):*** Form the capsid.
    - ***Non-structural proteins:*** Involved in replication, protease activity, and RNA synthesis**.**
- ***Unique feature:*** Genome contains a 5′-linked viral protein (VPg) instead of a cap, and a poly-A tail at 3′ end**.**

41.What is principle of Fluorescent Antibody Technique?

Principle of Fluorescent Antibody Technique (FAT):

The technique is based on the principle that antibodies specifically bind to their corresponding antigens. These antibodies are labeled with fluorescent dyes (fluorochromes). When the labeled antibodies bind to the target antigen and are exposed to ultraviolet (UV) light under a fluorescence microscope, the antigen–antibody complexes emit visible fluorescence, allowing detection and localization of the antigen.

42.Write functions of antibiotics and trypsin.

Functions of Antibiotics and Trypsin:

1. Antibiotics:

- Inhibit bacterial growth or kill bacteria to prevent contamination in cell and viral cultures.
- Used in virus propagation to maintain sterile host cell cultures.
- Reduce competition from unwanted microorganisms in laboratory experiments.

2. Trypsin:

- A proteolytic enzyme that cleaves proteins.
- Used to detach adherent cells from culture flasks for subculture.
- Activates certain viruses (e.g., Influenza virus) by cleaving precursor proteins into infectious forms.

43.What is Baltimore classification?

Baltimore Classification:

The Baltimore classification is a system that classifies viruses based on their type of genome and method of mRNA production**.** It divides viruses into seven groups:

1. ***Group I:*** Double-stranded DNA (dsDNA) viruses
2. ***Group II:*** Single-stranded DNA (ssDNA) viruses
3. ***Group III:*** Double-stranded RNA (dsRNA) viruses
4. ***Group IV:*** Positive-sense single-stranded RNA (+ssRNA) viruses
5. ***Group V:*** Negative-sense single-stranded RNA (−ssRNA) viruses
6. ***Group VI:*** Positive-sense single-stranded RNA viruses with reverse transcriptase (ssRNA-RT, retroviruses)
7. ***Group VII:*** Double-stranded DNA viruses with reverse transcriptase (dsDNA-RT, hepadnaviruses)

44.What is toxoid vaccine?

Toxoid Vaccine:

A toxoid vaccine is made from a bacterial toxin that has been inactivated (detoxified) so it is no longer harmful but still stimulates the immune system to produce protective antibodies.

- It does not cause disease**.**
- Examples include diphtheria toxoid and tetanus toxoid.
- Provides immunity against the toxin, not the whole bacterium.

45.How a normal cell is converted in cancerous cell?

A normal cell is converted into a cancerous cell through a series of genetic and epigenetic alterations that disrupt normal cell growth and division. Mutations can activate proto-oncogenes into oncogenes, leading to uncontrolled proliferation, while tumor suppressor genes such as *p53* or *Rb* may be inactivated, removing critical cell cycle checkpoints. Accumulation of DNA damage caused by carcinogens, radiation, or oncogenic viruses further promotes malignant transformation. Cancerous cells also evade apoptosis, allowing damaged cells to survive, and acquire the ability for unlimited division and angiogenesis, ensuring a continuous supply of nutrients. These combined changes result in the transformation of a normal cell into a malignant, cancerous cell.

46.What are three theories of evolution?

Three Theories of Evolution:

1. ***Darwin's Theory of Natural Selection:*** Organisms with favorable traits survive and reproduce, passing those traits to the next generation. "Survival of the fittest" explains adaptation and evolution over time.
2. ***Lamarckism (Theory of Inheritance of Acquired Characteristics):*** Traits acquired during an organism's lifetime due to use or disuse of organs can be transmitted to offspring.
3. ***Mutation Theory (Neo-Darwinism / Modern Synthesis):*** Evolution occurs through random genetic mutations and recombination, with natural selection acting on these variations to produce new species.

47.Define replication of Togavirus.

Replication of Togavirus:

Togaviruses are positive-sense single-stranded RNA (+ssRNA) viruses that replicate in the cytoplasm of host cells. After attachment to specific cell receptors via the viral envelope glycoproteins, the virus enters the cell by endocytosis. The viral RNA genome acts directly as mRNA and is translated to produce non-structural proteins involved in RNA replication. Using these proteins, the virus synthesizes a full-length negative-sense RNA, which serves as a template for producing new positive-sense genomes and subgenomic mRNAs for structural protein synthesis. Viral structural proteins are assembled with the genome into new virions at the cell membrane, which are then released by budding.

48.Write structure of Berkefeld filter.

Structure of Berkefeld Filter:

The Berkefeld filter is a cylindrical water filter made of diatomaceous earth (fossilized silica). Its structure consists of:

1. ***Porous ceramic cylinder:*** Main filtering element with micropores that trap bacteria and other particles.
2. ***Support frame or housing:*** Holds the ceramic cylinder in place, usually made of metal or plastic.
3. ***Inlet and outlet taps:*** Allow water to enter the top and filtered water to exit from the bottom.
4. ***Chamber for unfiltered water:*** Water passes through the ceramic pores by gravity, leaving impurities behind.
5. ***Pore sizes:*** Available in different grades (V, N, W) for filtration of bacteria and suspended particles.

49.What is Viral Neutralization test?

Viral Neutralization Test (VNT):

The viral neutralization test is a serological assay used to detect antibodies against a specific virus in a sample. It is based on the principle that antibodies in the serum can neutralize viral infectivity, preventing the virus from infecting susceptible cells. In the test, the virus is mixed with the serum and then added to a cell culture. If neutralizing antibodies are present, no cytopathic effect (CPE) or plaque formation is observed, indicating immunity or previous exposure to the virus.

50.Differentiate between PrP^C and PrP^Sc.

PrP^C and PrP^Sc:

PrP^C (cellular prion protein) is the normal, non-infectious form of the prion protein found in healthy cells and is rich in α-helices, soluble, and involved in normal cellular functions. In contrast, PrP^Sc (scrapie prion protein) is the abnormal, infectious isoform that is rich in β-sheets, insoluble, and resistant to proteases. PrP^Sc can convert normal PrP^C into the pathogenic form, leading to the accumulation of misfolded proteins and causing prion diseases such as scrapie and Creutzfeldt-Jakob disease.

51.Write structure of Prions.

Structure of Prions:

Prions are infectious proteins that lack nucleic acids. They are composed entirely of misfolded prion protein (PrP^Sc). The structure of prions includes:

- ***Amino acid chain:*** Single polypeptide of about 250 amino acids.
- ***Secondary structure:*** High content of β-sheets (in PrP^Sc) instead of normal α-helices (in PrP^C).
- ***No membrane or capsid:*** Unlike viruses, prions lack lipids, nucleic acids, and a protein coat.
- ***Aggregation property:*** Misfolded prions tend to form amyloid fibrils, which accumulate in tissues, especially in the brain.

52.What changes occur in animal cell due to vacuolization?

Changes in Animal Cells Due to Vacuolization:

Vacuolization in animal cells leads to formation of large vacuoles in the cytoplasm, causing several structural and functional changes:

- ***Cytoplasmic swelling:*** Vacuoles occupy space, pushing organelles aside.
- ***Disruption of organelle function:*** Mitochondria, ER, and Golgi may be compressed or displaced.
- ***Impaired metabolism:*** Accumulation of water or debris in vacuoles interferes with cellular processes.
- ***Cell rounding or bloating:*** Morphological changes are visible under a microscope.
- ***Possible cell death:*** Severe vacuolization can lead to apoptosis or necrosis.

53.What is mechanism and types of latency?

Mechanism and Types of Viral Latency:

Mechanism of Latency:

Viral latency occurs when a virus persists in host cells without producing infectious progeny, evading the immune system. During latency the viral genome integrates into the host DNA (provirus) or exists as episomal DNA/RNA. Viral gene expression is minimal, producing only latency-associated proteins to maintain persistence. External or internal triggers (stress, immunosuppression) can reactivate the virus, leading to productive infection.

Types of Latency:

1. ***Lysogenic latency:*** Viral DNA integrates into host genome (e.g., bacteriophage λ).
2. ***Episomal latency:*** Viral genome remains as extrachromosomal DNA in the nucleus (e.g., Herpes simplex virus).
3. ***Chronic or persistent latency:*** Virus continuously produces low levels of viral particles without killing the host (e.g., HIV).

54.Define role of Antibiotics and Trypsin in cell culture medium.

Role of Antibiotics and Trypsin in Cell Culture Medium:

Antibiotics: Antibiotics are added to cell culture medium to prevent bacterial contamination and maintain a sterile environment. They inhibit or kill unwanted microorganisms, ensuring that the host cells or viruses grow without interference from bacteria.

Trypsin: Trypsin is a proteolytic enzyme used to detach adherent cells from culture vessels during subculture or passaging. It can also activate certain viruses (e.g., influenza) by cleaving viral proteins into their infectious form, facilitating viral replication.

55.What are safety levels of Lab?

Biosafety Level 1 (BSL-1):

BSL-1 laboratories handle non-pathogenic organisms that pose minimal risk to humans, such as *E. coli* K-12. Basic laboratory precautions, including wearing lab coats, gloves, and eye protection, are sufficient. No special containment equipment is required, and standard good laboratory practices like handwashing and proper disposal of materials ensure safety.

Biosafety Level 2 (BSL-2):

BSL-2 laboratories work with moderate-risk pathogens that can cause human disease but are generally treatable, such as Hepatitis B virus or HIV. Safety measures include restricted access, use of biosafety cabinets (Class II**)** for procedures that generate aerosols, proper waste decontamination, and personal protective equipment (PPE). Staff are trained to handle infectious materials safely.

Biosafety Level 3 (BSL-3):

BSL-3 laboratories are designed for high-risk pathogens that can cause serious or potentially lethal diseases, like Mycobacterium tuberculosis or SARS-CoV-2. These labs have **co**ntrolled access, HEPA-filtered ventilation, and directional airflow to prevent escape of aerosols. Laboratory personnel must wear appropriate PPE, including respirators, and follow strict containment and decontamination procedures.

Biosafety Level 4 (BSL-4):

BSL-4 laboratories handle extremely dangerous pathogens with high mortality rates and no available vaccines or treatments, such as Ebola or Marburg virus. These labs require maximum containment, including full positive-pressure suits, airlock entry, and specialized waste management systems. All work is conducted in sealed, highly secure facilities, and staff undergo extensive training to prevent exposure and ensure complete biosafety.

56.What safety precautions should be made before working in Virology lab?

Safety Precautions Before Working in a Virology Laboratory:

- ***Wear proper personal protective equipment (PPE):*** *Lab coat, gloves, mask, and eye protection.*
- ***Know the risk group of the virus:*** Handle according to its Biosafety Level (BSL) requirements.
- ***Work in a biosafety cabinet (BSC):*** Especially when handling infectious or aerosol-generating materials**.**
- ***Sterilize work surfaces and equipment:*** Disinfect benches and instruments before and after use.
- Avoid eating, drinking, or applying cosmetics in the lab.
- Label and seal all samples clearly in leak-proof containers.
- ***Follow proper waste disposal protocols:*** Autoclave or disinfect all infectious material.
- Wash hands thoroughly before leaving the laboratory.
- ***Restrict access:*** Only trained personnel should enter the laboratory.
- ***Be familiar with emergency procedures:*** Know protocols for spills, exposures, or accidents.

57.What is differential interference microscopy and its applications?

Differential Interference Contrast (DIC) Microscopy:

Differential Interference Contrast (DIC) microscopy is an optical microscopy technique that enhances contrast in unstained, transparent specimens. It works by splitting a beam of polarized light into two slightly offset beams that pass through the specimen. Differences in refractive index and optical path cause interference between the beams, producing an image with high contrast and a pseudo-3D appearance**.**

Applications:

- Observation of living cells without staining.
- Studying cell organelles, membranes, and motility.
- Examining microorganisms such as bacteria, protozoa, and viruses in culture.
- Useful in cell biology, microbiology, and developmental biology research.

58.Define uncoating of virus.

Uncoating of Virus:

Uncoating is the process by which a virus **removes its capsid or envelope** after entering a host cell, releasing the **viral genome** into the cytoplasm or nucleus. This step is essential for the **viral nucleic acid to become accessible** for replication and transcription. Uncoating can occur through **fusion with the host membrane, endocytosis, or enzymatic degradation** of the capsid.

59.How to identify initial viral disease?

Identification of Initial Viral Disease:

The initial detection of a viral disease is based on a combination of clinical, laboratory, and epidemiological methods:

1. ***Clinical symptoms:*** Fever, malaise, rash, respiratory or gastrointestinal signs, and other characteristic manifestations.
2. ***Epidemiological history:*** Recent exposure to infected individuals, travel history, or contact with vectors.
3. ***Laboratory tests:***
    - Serological assays to detect virus-specific antibodies (IgM for recent infection).
    - Antigen detection using techniques like ELISA or immunofluorescence.
    - Molecular tests (PCR or RT-PCR) to detect viral nucleic acids.
4. ***Virus isolation:*** Inoculation of samples into cell cultures or embryonated eggs to observe viral growth.
5. ***Rapid diagnostic kits:*** Point-of-care tests for specific viral antigens or RNA.

60.Write mechanism of viroid transmission.

Mechanism of Viroid Transmission:

Viroids are small, circular, single-stranded RNA molecules that infect plants. They lack protein coats and rely entirely on host machinery for replication. Transmission occurs through:

1. ***Mechanical transmission:*** Via contaminated tools, hands, or handling of plants.
2. ***Vegetative propagation:*** Through grafting, cuttings, or tubers from infected plants.
3. ***Seed transmission:*** Some viroids are transmitted through infected seeds to the next generation.
4. ***Insect vectors:*** Certain sap-sucking insects can transmit viroids between plants.

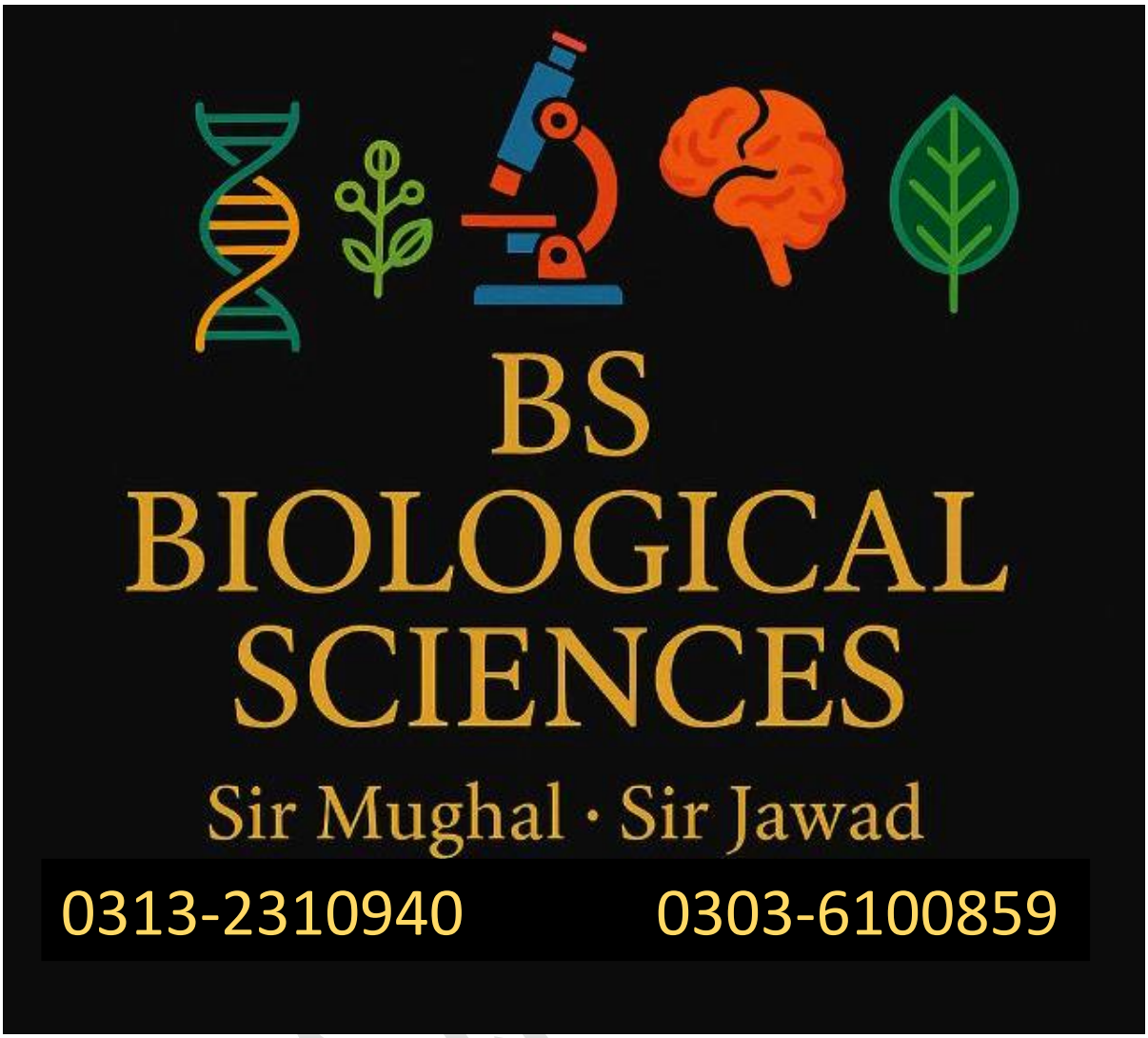
61.What is induced mutation? Write two types.

Induced Mutation:

An induced mutation is a genetic change in an organism's DNA caused by external agents (mutagens) such as chemicals, radiation, or physical factors, rather than occurring spontaneously.

Two Types of Induced Mutation:

1. ***Chemical-induced mutation:*** Caused by chemicals like alkylating agents, base analogs, or intercalating agents that alter DNA structure.
2. ***Physical-induced mutation:*** Caused by physical factors such as ultraviolet (UV) light, X-rays, or gamma rays that damage DNA.



Sir Mughal: https://wa.me/923132310940
Sir Jawad: https://wa.me/923036100859