BT601 – VIROLOGY

MID TERM IMPORTANT QUESTIONS

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1. What is virus Neutralization test?

A virus neutralization test (VNT) is a laboratory method used to detect and measure antibodies that can block a virus from infecting host cells. In this test, a patient's serum is mixed with a known amount of virus and then added to susceptible cell cultures. If neutralizing antibodies are present, they bind to the virus and prevent it from entering and damaging the cells. By observing whether infection occurs, the test helps determine the presence and level of protective, functional antibodies. VNTs are widely used to assess immunity after infection or vaccination and to support the diagnosis of various viral diseases.

2. Which in vitro genetic technique is used in virology?

An important in vitro genetic technique used in virology is Polymerase Chain Reaction (PCR). PCR is widely used to amplify and detect viral nucleic acids (DNA or RNA after reverse transcription), making it essential for diagnosing viral infections, studying viral genetics, monitoring viral load, and identifying mutations. Other in vitro techniques used in virology include RT-PCR, qPCR, DNA cloning, site-directed mutagenesis, and cell-free viral replication assays, but PCR/RT-PCR is the most commonly referenced one in exams.

3. Write names of 10 positive sense RNA plants virus.

Here are 10 positive-sense RNA plant viruses:

1. Tobacco Mosaic Virus (TMV)
2. Potato Virus Y (PVY)
3. Cucumber Mosaic Virus (CMV)
4. Tobacco Etch Virus (TEV)
5. Tomato Mosaic Virus (ToMV)
6. Pepper Mild Mottle Virus (PMMoV)
7. Tomato Bushy Stunt Virus (TBSV)
8. Brome Mosaic Virus (BMV)
9. Cowpea Mosaic Virus (CPMV)
10. Barley Yellow Dwarf Virus (BYDV)

4. Write principle of Transmission Electron Microscope.

The principle of a Transmission Electron Microscope (TEM) is based on passing a beam of high-energy electrons through an ultra-thin specimen to produce a detailed image. When electrons travel through the specimen, some pass straight, some are scattered, and some are absorbed depending on the density and structure of the material. Electromagnetic lenses then focus these transmitted electrons to form a highly magnified image on a screen or detector. Because electrons have a much shorter wavelength than visible light, a TEM can resolve extremely fine structural details at the nanometer scale.

5. What is host range?

Host range is the variety of living organisms—such as specific species, tissues, or cell types—that a virus, bacterium, or parasite can infect. It depends mainly on whether the pathogen can attach, enter, and replicate inside those hosts. Some pathogens have a narrow host range (infecting only one species), while others have a broad host range (infecting many different species).

6. What is the role of terminase enzyme in a genome packaging?

The terminase enzyme is essential in viral genome packaging. Its main role is to recognize the viral DNA, cleave it at specific sites, and actively insert it into the preformed viral capsid. Acting like a molecular motor, terminase uses energy from ATP to pump the DNA tightly into the capsid, ensuring the virus is properly assembled and ready for infection.

7. Write function of five different parts of light microscope?

1. ***Eyepiece (Ocular lens)*** – The lens you look through; usually magnifies 10×, further enlarging the image produced by the objective lens.
2. ***Objective lens*** – The primary lenses near the specimen; they provide different magnifications (e.g., 4×, 10×, 40×, 100×) and form the initial magnified image.
3. ***Stage*** – The flat platform where the slide is placed; it often has clips or a mechanical stage to hold and move the specimen precisely.
4. ***Light source/Illuminator*** – Provides illumination through the specimen, making details visible; can be a mirror reflecting light or an electric bulb.
5. ***Focus knobs (Coarse and Fine)*** – Move the stage or lenses up and down to bring the specimen into clear view; coarse for rough focus, fine for sharp detail.

8. What are some unique properties of virus?

Some unique properties of viruses that distinguish them from other microorganisms are:

1. ***Obligate intracellular parasites*** – Viruses can only replicate inside a living host cell; they cannot grow independently.
2. ***Acellular structure*** – Viruses are not made of cells; they consist of genetic material (DNA or RNA) enclosed in a protein coat, sometimes with a lipid envelope.
3. ***Genetic material can be DNA or RNA*** – Unlike cells, which always have DNA, viruses may have either DNA or RNA, single-stranded or double-stranded.
4. ***Small size*** – They are much smaller than bacteria and can only be seen with an electron microscope.
5. ***Specific host range*** – Most viruses infect only particular species or cell types.
6. ***Ability to evolve rapidly*** – High mutation rates allow viruses to adapt quickly to new hosts or environments.

9. Describe the different function of protein of paramyxoviridae.

The Paramyxoviridae family of viruses has several structural and non-structural proteins, each with specific functions:

1. ***Nucleoprotein (N)*** – Encapsidates the viral RNA genome, protecting it and forming the ribonucleoprotein (RNP) complex, which is essential for replication and transcription.
2. ***Phosphoprotein (P)*** – Acts as a cofactor for the viral RNA-dependent RNA polymerase (L protein) and assists in replication and transcription of the viral genome.
3. ***Large protein (L)*** – The RNA-dependent RNA polymerase responsible for viral RNA synthesis and mRNA capping.
4. ***Matrix protein (M)*** – Plays a key role in virus assembly and budding by linking the nucleocapsid to the viral envelope.
5. ***Fusion protein (F)*** – Mediates fusion of the viral envelope with the host cell membrane, allowing viral entry; also causes cell-to-cell fusion (syncytia formation).
6. ***Hemagglutinin–neuraminidase protein (HN/H/G)*** – Involved in attachment of the virus to host cell receptors and helps release newly formed virions by cleaving sialic acid residues.
7. ***Non-structural proteins (V, C, etc.)*** – Often involved in antagonizing host immune responses, such as inhibiting interferon signaling.

10. Describe the two types through which mutants induce? Example of category of mutagens.

Mutations in viruses or other organisms can be induced through two main types:

1. ***Spontaneous mutations*** – These occur naturally due to errors during DNA or RNA replication, such as base mispairing, slippage, or spontaneous chemical changes in nucleotides. They happen randomly without any external influence.
    - ***Example:*** A point mutation in a viral genome during replication.
2. ***Induced mutations*** – These are caused by external agents called mutagens, which increase the mutation rate. Mutagens can be chemical, physical, or biological.
    - ***Examples of mutagen categories:***
        - **Chemical mutagens:** Nitrous acid, alkylating agents (e.g., ethyl methanesulfonate)
        - **Physical mutagens:** Ultraviolet (UV) radiation, X-rays
        - **Biological mutagens:** Certain viruses or transposons that integrate into the genome

11. Differentiate interference in contrast microscopy principle and applications.

Interference (phase contrast) microscopy works on the principle of converting phase shifts in light—caused by differences in refractive index within a transparent specimen—into variations in brightness, making invisible structures visible. Unlike regular bright-field microscopy, it does not require staining, allowing observation of living cells. Its applications include studying the morphology, motility, and internal structures of microorganisms and eukaryotic cells, monitoring cell growth, and examining delicate specimens that could be damaged by staining. In short, the principle focuses on light interference to enhance contrast, while the applications emphasize observing living, unstained specimens in detail.

12. Explain Types of centrifuge used to isolate virus.

Centrifugation is a key technique used to isolate and purify viruses based on their size, shape, and density. There are several types of centrifuges used in virology:

1. ***Low-speed centrifuge*** – Spins at relatively low speeds (around 1,000–10,000 × g). It is used to remove cell debris and large particles from infected tissue or culture supernatants before further purification.
2. ***High-speed or ultracentrifuge*** – Operates at very high speeds (up to 100,000 × g). It is used to pellet **viruses** from clarified supernatants or culture fluids.
3. ***Density gradient centrifugation*** – Involves layering the viral suspension over a density gradient medium (like sucrose or cesium chloride) and centrifuging at high speed. Viruses **s**ettle at their buoyant density, allowing high-purity separation from other components.
4. ***Zonal or swinging-bucket centrifuge*** – A type of ultracentrifuge where samples are spun in swinging-bucket rotors, often used for gradient separation and for handling larger sample volumes gently.

13. Name the sub families of herpesviridae.

The Herpesviridae family is divided into **three subfamilies** based on biological properties and host range:

1. ***Alphaherpesvirinae*** – Characterized by a short reproductive cycle and the ability to establish latent infections in sensory nerve ganglia.
2. ***Betaherpesvirinae*** – Have a slow replication cycle and cause enlargement of infected cells (cytomegaly); latency is established in secretory glands, lymphoreticular cells, and kidneys.
3. ***Gammaherpesvirinae*** – Mainly infect lymphoid tissues and can transform cells; latency is established in lymphocytes.

14. Write short note on Topovirus.

Topovirus is a genus of plant viruses that belongs to the Tospoviridae family. These viruses are negative-sense single-stranded RNA viruses and are mainly transmitted by thrips (insect vectors). Topoviruses infect a wide range of plants, causing symptoms such as necrotic lesions, chlorotic spots, stunted growth, and leaf deformation, leading to significant agricultural losses. The virus has a spherical, enveloped structure, and its genome is segmented into three RNA molecules (L, M, and S segments) that encode proteins essential for replication, movement, and vector transmission. Examples include Tomato spotted wilt virus (TSWV), one of the most economically important plant pathogens.

15. Write short note on M13 phage.

M13 bacteriophage is a filamentous, single-stranded DNA virus that infects Escherichia coli carrying the F pilus. Unlike lytic phages, M13 causes a chronic infection, releasing new phage particles without lysing the host cell. Its genome is circular and encodes proteins for replication, assembly, and secretion of phage particles. M13 is widely used in molecular biology and biotechnology, including phage display, cloning, and DNA sequencing, because of its ability to produce single-stranded DNA efficiently.

16. Write composition of retrovirus.

The composition of a retrovirus includes several key components:

1. ***Envelope*** – A lipid bilayer derived from the host cell membrane, embedded with viral glycoproteins (e.g., gp120, gp41 in HIV) that help the virus attach and enter host cells.
2. ***Matrix protein (MA)*** – Lies beneath the envelope, providing structural support and assisting in viral assembly.
3. ***Capsid (Core, CA)*** – A protein shell that encloses the viral RNA genome and associated enzymes.
4. ***Genome*** – Two identical copies of single-stranded positive-sense RNA.
5. ***Viral enzymes*** – Include reverse transcriptase (converts RNA to DNA), integrase (inserts viral DNA into host genome), and protease (processes viral proteins during maturation).
6. ***Nucleocapsid protein (NC)*** – Binds and stabilizes the RNA genome inside the capsid.

17. What is Baltimore classification?

The Baltimore classification is a system used to categorize viruses based on the type of their genetic material and their method of replication. Developed by David Baltimore, 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:*** Single-stranded RNA viruses with reverse transcriptase (ssRNA-RT, e.g., retroviruses)
7. ***Group VII:*** Double-stranded DNA viruses with reverse transcriptase (dsDNA-RT, e.g., Hepadnaviruses)

18. Write properties of picornavirus.

Here are the key properties of Picornaviruses:

1. ***Genome*** – Single-stranded, positive-sense RNA (+ssRNA).
2. ***Capsid*** – Non-enveloped icosahedral structure, about 22–30 nm in diameter.
3. ***Size*** – Very small (“pico” means small).
4. ***Replication*** – Cytoplasmic; does not require a nuclear phase.
5. ***Stability*** – Resistant to heat, acid, and detergents; can survive in the environment.
6. ***Host range*** – Infect humans and animals, often causing respiratory, gastrointestinal, or systemic diseases.
7. ***Examples*** – Poliovirus, Rhinovirus, Coxsackievirus, and Hepatovirus.

19. What are possible effect of viral genome on host cell?

The viral genome can have several effects on the host cell, depending on the type of virus and its replication strategy:

1. ***Cell death (cytopathic effect)*** – Viruses can disrupt cellular machinery, leading to lysis or apoptosis.
2. ***Transformation*** – Some viral genomes integrate into the host DNA and can induce uncontrolled cell division, potentially causing cancer (oncogenic viruses).
3. ***Persistent infection*** – Viral genomes may remain in the host cell without causing immediate death, leading to latent or chronic infection.
4. ***Alteration of host metabolism*** – Viruses can redirect host cellular resources to produce viral components, affecting normal cellular functions.
5. ***Immune modulation*** – Viral genes may interfere with host immune responses, such as inhibiting interferon production or antigen presentation.
6. ***Genetic recombination or mutation*** – Some viral genomes can recombine with host DNA or undergo mutations, contributing to viral evolution.

20. Write 3 functions of viral capsid.

The viral capsid serves several important functions:

1. ***Protection of the genome*** – It encases and safeguards the viral nucleic acid from physical, chemical, and enzymatic damage.
2. ***Attachment and entry*** – Capsid proteins can interact with host cell receptors, helping the virus attach to and enter target cells.
3. ***Structural support and assembly*** – Provides the overall shape and stability of the virus, aiding in the correct assembly of new viral particles.

21. What is Induced mutation?

Induced mutation is a genetic change that occurs in an organism due to the influence of external agents called mutagens. Unlike spontaneous mutations, which happen naturally during DNA replication, induced mutations are caused intentionally or accidentally by chemicals, physical agents, or biological factors that alter the DNA sequence.

Examples of mutagens:

- **Chemical:** Alkylating agents (e.g., ethyl methanesulfonate), nitrous acid
- **Physical:** Ultraviolet (UV) radiation, X-rays
- **Biological:** Certain viruses or transposable elements

22. What is Genetic makeup of retro virus?

The genetic makeup of a retrovirus consists of:

1. ***Genome*** – Two identical copies of single-stranded positive-sense RNA (+ssRNA).
2. ***Genes*** – Typically organized into three main genes:
    - ***gag*** – Encodes structural proteins like capsid and matrix proteins.
    - ***pol*** – Encodes viral enzymes such as reverse transcriptase, integrase, and protease.
    - ***env*** – Encodes envelope glycoproteins required for attachment and entry into host cells.
3. ***Regulatory sequences*** – Long terminal repeats (LTRs) at both ends of the genome that regulate transcription, integration, and replication.

23. Differentiate between segments of Bunyavirus genomes in terms of the proteins they encode.

Bunyaviruses have a segmented, single-stranded negative-sense RNA genome divided into three parts: L (Large), M (Medium), and S (Small) segments. Here's a differentiation based on the proteins they encode:

1. ***L segment*** – Encodes the RNA-dependent RNA polymerase (L protein), which is essential for viral RNA replication and transcription.
2. ***M segment*** – Encodes the envelope glycoproteins (Gn and Gc) that mediate virus attachment and entry into host cells, and sometimes a nonstructural protein (NSm).
3. ***S segment*** – Encodes the nucleocapsid protein (N), which binds and protects the viral RNA, and in some viruses, a nonstructural protein (NSs) involved in modulating host responses.

In short, **L = polymerase**, **M = envelope proteins**, and **S = nucleocapsid and accessory proteins**, each playing distinct roles in the virus life cycle.

24. In order to enhance the contrast what kind of "dye" should be used for specimens of transmission electron microscope?

In Transmission Electron Microscopy (TEM), specimens are generally electron-transparent and have low natural contrast. To enhance contrast, heavy metal stains are used because they scatter electrons strongly**.**

Common dyes (stains) used in TEM:

- ***Uranyl acetate*** – Binds to nucleic acids and proteins, providing high contrast.
- ***Lead citrate*** – Enhances contrast of cellular structures.
- ***Osmium tetroxide (OsO_4)*** – Fixes and stains lipids, giving membrane visibility.

25. Write contribution of Edward Jenner in the field of Vaccines.

Edward Jenner made a landmark contribution to the field of vaccines by developing the first successful smallpox vaccine in 1796. He observed that milkmaids who had contracted cowpox were protected from smallpox, a deadly disease at the time. Jenner tested this by inoculating a boy with material from cowpox lesions and later exposed him to smallpox; the boy did not develop the disease. This experiment demonstrated the principle of immunization using a related, less virulent agent. Jenner's work laid the foundation for modern vaccinology and the eventual global eradication of smallpox.

26. What is microscopy?

Microscopy is the science and technique of using microscopes to observe objects and structures that are too small to be seen with the naked eye. It allows scientists to study the morphology, structure, and behavior of cells, microorganisms, tissues, and other tiny specimens. Microscopy can be classified based on the type of illumination or imaging used, such as light microscopy, fluorescence microscopy, and electron microscopy, each providing different levels of magnification and resolution.

27. Define Latency in detail.

Latency is a state in which a virus remains present in a host cell without producing active infection or causing immediate disease, effectively "hiding" from the host immune system. During this phase, the viral genome may persist as episomal DNA in the nucleus or integrate into the host genome, but no new infectious virions are produced**.**

Key features of viral latency include:

1. ***Persistence*** – The virus can remain in the host for long periods, sometimes for the lifetime of the host.
2. ***Reactivation potential*** – Latent viruses can reactivate under certain conditions (stress, immunosuppression, or other triggers) to produce active infection.
3. ***Minimal gene expression*** – Only a few viral genes, often regulatory or latency-associated, are expressed to maintain the latent state.
4. ***Examples*** – Herpes simplex virus (HSV) persists in sensory ganglia, Varicella-zoster virus (VZV) in dorsal root ganglia, and HIV can remain latent in memory T cells.

Latency is a survival strategy for viruses, allowing them to evade the immune system and persist in the host until conditions favor reactivation and transmission.

28. Explain Fluorescence antibody principle.

The principle of the fluorescence antibody (FA) technique is based on the specific binding of antibodies to their corresponding antigens and the visualization of this binding using a fluorescent dye. In this method, antibodies are labeled with a fluorescent molecule (such as fluorescein isothiocyanate, FITC). When these labeled antibodies bind to the target antigen in a sample and are exposed to ultraviolet or blue light under a fluorescence microscope, they emit visible light of a specific color. This allows the direct detection, localization, and identification of specific pathogens or cellular components with high specificity and sensitivity.

29. Define replication of togavirus.

Replication of Togavirus refers to the process by which these positive-sense single-stranded RNA (+ssRNA) viruses reproduce inside host cells. After the virus attaches to the host cell and enters via endocytosis, the viral RNA is released into the cytoplasm. Since it is positive-sense, the RNA can directly serve as mRNA for the translation of viral nonstructural proteins, including RNA-dependent RNA polymerase. This polymerase then synthesizes a negative-sense RNA intermediate, which serves as a template for producing new positive-sense genomic RNA. Structural proteins are translated from subgenomic RNA, and new virions are assembled at the plasma membrane, acquiring their envelope by budding. Finally, the mature virions are released to infect new cells.

30. Name vectors of plant virus.

Vectors of plant viruses are organisms that transmit viruses from an infected plant to a healthy plant. Common vectors include:

1. ***Insects*** – The most important vectors:
    - Aphids (e.g., transmit Potyviruses)
    - Whiteflies **(**e.g., transmit Begomoviruses)
    - Leafhoppers (e.g., transmit Rice tungro virus)
    - Thrips (e.g., transmit Tospoviruses)
    - Beetles (e.g., transmit Bean pod mottle virus)
2. ***Nematodes*** – Soil-borne, transmit viruses like Tobacco rattle virus.
3. ***Fungi*** – Certain plasmodiophorid fungi transmit viruses like Potato mop-top virus.
4. ***Mechanical transmission*** – Tools, hands, or contact between plants can also spread viruses.
5. ***Seed and pollen*** – Some viruses are transmitted through infected seeds or pollen.

31. Define transposons and its types.

Transposons, also called **"**jumping genes**,"** are DNA sequences that can move from one location to another within a genome. They can insert themselves into new positions in the DNA, potentially disrupting genes or regulating gene expression, and play a role in genome evolution and variability.

Types of Transposons

1. ***Class I (Retrotransposons)*** – Move via an RNA intermediate using a "copy-and-paste" mechanism: the DNA is transcribed into RNA, then reverse-transcribed back into DNA and inserted elsewhere.

Examples: LINEs (Long Interspersed Nuclear Elements), SINEs (Short Interspersed Nuclear Elements)

2. ***Class II (DNA transposons)*** – Move directly as DNA using a "cut-and-paste" mechanism, catalyzed by the enzyme transposase.

Examples: Tn elements in bacteria, Ac/Ds elements in maize

32. Write role of Beijerinick and Inkowski in Tobacco mosaic virus.

Beijerinck and Ivanovsky played key roles in the discovery and understanding of Tobacco Mosaic Virus (TMV):

1. ***Dmitri Ivanovsky (1892)*** – Using diseased tobacco plants, he showed that the infectious agent causing mosaic disease passed through porcelain filters that retained bacteria. He concluded that the disease was caused by a **"**filterable agent**"**, smaller than bacteria, but he did not identify it as a virus.
2. ***Martinus Beijerinck (1898)*** – He repeated Ivanovsky's experiments and went further, demonstrating that the infectious agent could replicate only in living plants, not in any growth medium. Beijerinck coined the term **"**virus**"** (meaning poisonous fluid) and established that TMV was a contagious, living pathogen smaller than bacteria.

33. Explain coronavirus replication.

Replication of Coronaviruses involves several key steps, as they are positive-sense single-stranded RNA (+ssRNA) viruses**:**

1. ***Attachment and Entry*** – The viral spike (S) protein binds to specific host cell receptors (e.g., ACE2 in SARS-CoV-2), and the virus enters the cell via endocytosis or membrane fusion.
2. ***Uncoating*** – The viral RNA genome is released into the cytoplasm.
3. ***Translation of Replicase Proteins*** – The +ssRNA acts as mRNA and is translated by host ribosomes into large polyproteins (pp1a and pp1ab), which are cleaved into non-structural proteins (nsps) that form the replication-transcription complex (RTC)**.**
4. ***RNA Replication and Transcription*** – The RTC synthesizes a negative-sense RNA intermediate, which serves as a template to produce new genomic RNA and subgenomic RNAs for structural and accessory proteins.
5. ***Translation of Structural Proteins*** – Structural proteins (S, M, E, N) are translated from subgenomic RNAs. Membrane proteins (S, M, E) enter the endoplasmic reticulum (ER) and move to the ER–Golgi intermediate compartment (ERGIC)**.**
6. ***Assembly*** – Nucleocapsid (N protein + RNA) associates with structural proteins at the ERGIC to form new virions.
7. ***Release*** – Mature virions are transported via vesicles and released from the cell by **exocytosis** to infect new cells.

34. Write the function of a normaski prism condenser and polarizer.

In Differential Interference Contrast (DIC) microscopy, which uses a Nomarski prism, the functions of the components are:

1. ***Nomarski Prism (Condenser Prism)*** – Splits a polarized light beam into two slightly sheared beams that pass through the specimen at slightly different paths. Differences in optical path length caused by variations in specimen thickness or refractive index create interference, enhancing contrast and producing a 3D-like image of transparent specimens.

2. ***Polarizer*** – Polarizes the light before it enters the prism and specimen, ensuring that the interference effect occurs properly. The polarizer controls the orientation of light waves, which is essential for creating the high-contrast, relief-like image in DIC microscopy.

35. Write formula for magnification of microscope.

Total Magnification=Magnification of Objective Lens×Magnification of Eyepiece (Ocular Lens)

Example**:** If the objective lens is 40× and the eyepiece is 10×, the total magnification is:

40x10=400x

This formula applies to compound light microscopes.

36. Write a note on hybridization of nucleic acid.

Nucleic acid hybridization is a molecular biology technique in which single-stranded DNA or RNA molecules bind (hybridize) to complementary sequences through base pairing. This process relies on the principle that adenine pairs with thymine (or uracil in RNA) and cytosine pairs with guanine, allowing the identification of specific sequences in a complex mixture.

Key Points:

- ***Purpose:*** Used to detect, quantify, or locate specific nucleic acid sequences in samples.
- ***Types:***
  - ***DNA–DNA hybridization*** – Detects complementary DNA sequences.
  - ***DNA–RNA hybridization*** – Used to study gene expression.
- ***Applications:*** Diagnostic assays for viral or bacterial infections, gene mapping, studying mutations, and recombinant DNA research.