

VIRAL INFECTIONS

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Viral Infections : Viral infections are type of infection caused by a virus , a type of microbe.

A viral infection occurs when a virus enters the body through contaminated air with virus , eating contaminated food or by having sexual contact with a person who is infected with a virus.

Aetiology :-

Causative Organism	Type of Viral Infection
Rhino virus	Common cold
Influenza virus	Flu
Varicella Zoster virus	Shingles, Chicken pox
Rubella virus	Mumps, Polio
Rabies Virus	Rabies
Human papilloma virus	HPV infection, Cervical cancer
Human immune deficiency virus	HIV/AIDS

Mechanisms of Infection and Pathogenesis

- **Viral disease:** Proliferation of a harmful **virus** inside the body and cause disease.
- **Viral pathogenesis:** Interaction of viral and host factors that leads to disease production.

Steps of a virus life cycle that shape pathogenesis

- 1-Entry of the virus into the body.
- 2- Local replication in susceptible cells (tissue tropism,modulate the host innate immune response)
- 3-Dissemination and spread to secondary tissues and target organs
- 4-Secondary replication in susceptible cells
- 5-Shedding of the virus into the environment
- 6- Transmission to new host

Entry of the virus

-Entry via the Respiratory Tract

- attaching to specific receptors on epithelial cells of mucosa
- remain localized (adenoviruses, influenza).
- become systemic by disseminated via lymphatics or bloodstream (rinderpest virus, Newcastle virus).

-Entry via the Oropharynx and Intestinal Tract

- acquired by ingestion swallowed, reach the stomach and intestine directly or may first infect oropharynx.
- esophagus is rarely infected (it's tough epithelium and the rapid passage of swallowed material over its surface)
- rotaviruses, caliciviruses, and enteroviruses

-Entry via the Skin

- Breaches in skin (e.g.cuts, punctures, abrasions, or wounds)
- bite of arthropods(mosquitoes,ticks,Insects), animal(**rabies**).
- **either** remain in skin (**Papillomaviruses**) **or** Deeper trauma introduce viruses into the dermis with its rich supply of vessels, lymphatics, nerves ,underlying subcutaneous tissue and muscle.

- Entry via the Genitourinary Tract

- abrasions to the vaginal, rectal, and urethral epithelium during sexual activity can facilitate virus entry (e.g., papillomaviruses,Herpes simplex virus 2)
- HIV-1 and 2, human T-lymphotropic viruses 1 and 2 and hepatitis B and C viruses, do not produce local lesions but are sexually transmitted.

- Entry via the Eyes

Virus can reach the eye by aerosol, rubbing with contaminated fingers, ophthalmic procedures with improperly sterilized instruments, swimming pool water. (e.g., some adenoviruses, influenza viruses, South American arenaviruses, and enteroviruses)

Typical sites of virus entry into the body: The first steps of viral infection is determined by the site at which the virus implants into the body. This would subsequently dictate the mechanisms of viral pathogenesis.

Local replication and spread

- Following initial entry to the host,. Here, the virus must modulate the host innate immune response to prevent its elimination by the body while facilitating its replication.
- Replicated virus from the initially infected cell then disperse to infect neighbouring susceptible cells, this results in a localised infection, like ,common cold (rhinovirus), flu (parainfluenza), gastrointestinal infections (rotavirus) or skin infections (papillomavirus)
- **Virus tropism:** refers to the virus' preferential site of replication in discrete cell types within an organ. In most cases, tropism is determined by the ability of the viral surface proteins to fuse or bind to surface receptors of specific target cells to establish infection.
- **Host Factors** : Several viral infections have displayed a variety of effects, ranging from asymptomatic to symptomatic or even critical infection, solely based of differing host factors alone. In particular, genetic factors, age and immunocompetence play an important role is dictating whether the viral infection can be modulated by the host

Dissemination and secondary replication

via blood stream (viremia)

- Virus can cause systemic disease through a disseminated infection spread throughout the body via **blood** or lymphatic system, e.g., chickenpox (varicella zoster virus), smallpox (variola), HIV (human immunodeficiency virus).
- A minority of viruses can disseminate via the nervous system.
- **This early viremia** is called **primary viremia** (*may be clinically silent*).
- Virus replication in major target organs leads to the sustained production of much higher concentrations of virus producing **a secondary viremia** *which can in turn lead to the establishment of infection in yet other parts of the body.*

Shedding and secondary transmission

- The viruses spread to sites where shedding into the environment can occur.
- The respiratory, alimentary and urogenital tracts and the blood are the most frequent sites of shedding in the form of bodily fluids, aerosols, skin, excrement(**the same body opening is involved in entry and exit**).
- The virus would then go on to be transmitted to another person, and establish the infection cycle again.

Viral Disease mechanisms

- A viral infection does not always cause disease. A viral infection simply involves viral replication in the host, but disease is the damage caused by viral multiplication. An individual who has a viral infection but does not display disease symptoms is known as a carrier.

❑ Damage caused by the virus

- ❑ Viruses can destroy cells through a variety of mechanisms.
 - ❑ Viruses often induce direct cytopathic effects to disrupt cellular functions through releasing enzymes to degrade host metabolic precursors,
- or**
- ❑ Releasing proteins that inhibit the synthesis of important host factors, proteins, DNA and/or RNA

❑ **Damage caused by host immune system**

- ❑ Sometimes, instead of cell death or cellular dysfunction caused by the virus, the host immune response can mediate disease and excessive inflammation.
- ❑ The stimulation of the innate and adaptive immune system in response to viral infections destroys infected cells, which may lead to severe pathological consequences to the host.
This damage caused by the immune system is known as virus induced immunopathology.
- ❑ Specifically immunopathology is caused by the excessive release of antibodies, interferons and Pro inflammatory cytokines, secretion of interferons and other cytokines can trigger cell damage, fever and flu like symptoms.
- ❑ In severe cases certain viral infections as in Avian H5 N1 influenza in 2005, induction of the host immune response can elicit a flaring release of cytokines known as cytokine strome

Diagnostic tests for viral infections

In general, diagnostic tests can be grouped into 3 categories:

(1) Direct detection: In direct examination, the clinical specimen is examined directly for the presence of virus particles, virus antigen or viral nucleic acids

(2) Indirect examination (virus isolation): In indirect examination, the specimen into cell culture, eggs or animals in an attempt to grow the virus: this is called virus isolation

(3) Serology: Serology actually constitute by far the bulk of the work of any virology laboratory

Three Approaches

1. Direct detection of virus

- Electron Microscopy
- Light Microscopy "Inclusion Bodies"
- Antigen detection tests
- Molecular Methods: PCR & Nucleic Acid Probes

2. Virus Isolation (Indirect detection)

- Animal inoculation
- Inoculation of eggs
- In vitro Cell Culture

3. Serology (Detection of Antibodies)

I. Direct Examination of Specimen

- a) Electron Microscopy morphology/immune electron microscopy
- b) Light microscopy histological appearance - e.g. inclusion bodies
- c) Antigen detection immunofluorescence, ELISA etc.
- d) Molecular techniques for the direct detection of viral genomes

II. Indirect Examination

- i. Cell Culture:
 - a) Cytopathic effect,
 - b) haemadsorption,
 - c) confirmation by neutralization,
 - d) interference,
 - e) immunofluorescence.
- ii. Eggs pocks on CAM - haemagglutination, inclusion bodies
- iii. Animals disease or death confirmation by neutralization

III. Serology : Detection of rising titres of antibody between acute and convalescent stages of infection, or the detection of IgM in primary infection

A. Classical Techniques

- a) Complement fixation tests (CFT)
- b) Haemagglutination inhibition tests
- c) Immunofluorescence techniques (IF)
- d) Neutralization tests
- e) Single Radial Haemolysis

B. Newer Techniques

- a) Radioimmunoassay (RIA)
- b) Enzyme linked immunosorbent assay (EIA)
- c) Particle agglutination
- d) Western Blot (WB)
- e) Recombinant immunoblot assay (RIBA), line immunoassay (Liatek)

1. Direct Examination

- ❑ Direct examination methods are often also called rapid diagnostic methods because they can usually give a result either within the same or the next day.
- ❑ This is extremely useful in cases when the clinical management of the patient depends greatly on the rapid availability of laboratory results e.g. diagnosis of RSV infection in neonates, or severe CMV infections in immunocompromised patients.

i. Antigen Detection: Antigen detection include:

- Immunofluorescence testing of nasopharyngeal aspirates for respiratory viruses e.g.. RSV, flu A, flu B, and adenoviruses,
- Detection of rotavirus antigen in faeces, the pp65 CMV antigenaemia test,
- Detection of HSV and VZV in skin scrapings, and the detection of HBsAg in serum.

The main advantage of these assays is that they are rapid to perform with the result being available within a few hours.

ii .Electron Microscopy (EM)

- Virus particles are detected and identified on the basis of morphology. A magnification of around 50,000 is normally used.
- EM is now mainly used for the diagnosis of viral gastroenteritis by detecting viruses in faeces e.g. rotavirus, adenovirus, astrovirus, calicivirus and Norwalk-like viruses.
- Detection of viruses in vesicles and other skin lesions, such as herpesviruses and papillomaviruses.

iii. Light Microscopy

- Replicating virus often produce histological changes in infected cells. These changes may be characteristic or non-specific.
- Viral inclusion bodies are basically collections of replicating virus particles either in the nucleus or cytoplasm. Examples of inclusion bodies include the negri bodies and cytomegalic inclusion bodies found in rabies and CMV infections respectively.

iv. Viral Genome Detection

- Methods based on the detection of viral genome are also commonly known as molecular methods.
 - However in practice, although the use of these methods is indeed increasing, the role played by molecular methods in a routine diagnostic virus laboratory is still small compared to conventional methods.
- Classical molecular techniques for the quantification of DNA/RNA such as Southern-blot depend on the use of specific DNA/RNA probes for hybridization.
- However, it is often found that the sensitivity of these techniques is not better than conventional viral diagnostic methods.
- Newer molecular techniques such as the polymerase chain reaction (PCR), ligase chain reaction (LCR), nucleic acid based amplification (NASBA), and branched DNA (bDNA) depend on some form of amplification, either the target nucleic acid, or the signal itself. bDNA is essentially a conventional hybridization technique with increased sensitivity.

2. Virus Isolation

Cell cultures, eggs, and animals may be used for isolation. However, eggs and animals are difficult to handle and most viral diagnostic laboratories depend on cell culture only.

There are 3 types of cell cultures:

1. Types of cell cultures

- **Primary cells**-e.g. Monkey Kidney. These are essentially normal cells obtained from freshly killed adult animals. These cells can only be passaged once or twice.
- **Semi-continuous cells** – e.g. Human embryonic kidney and skin fibroblasts. These are cells taken from embryonic tissue, and may be passaged up to 50 times.
- **Continuous cells** e.g. Hela, Vero, Hep2, LLC-MK2, BGM. These are immortalized cells i.e. tumour cell lines and may be passaged indefinitely.

NOTE:- Primary cell culture are widely acknowledged as the best cell culture systems available since they support the widest range of viruses. However, they are very expensive and it is often difficult to obtain a reliable supply. Continuous cells are the most easy to handle but the range of viruses supported is often limited

2. Identification of growing virus

- ❖ The presence of growing virus is usually detected by: Cytopathic Effect (CPE) may be specific or non-specific e.g. HSV and CMV produces a specific CPE, whereas enteroviruses do not.
- ❖ Hemadsorption cells acquire the ability to stick to mammalian red blood cells.
- ❖ Hemadsorption is mainly used for the detection of influenza and parainfluenza viruses.
- ❖ Confirmation of the identity of the virus may be carried out using neutralization, hemadsorption-inhibition, immunofluorescence, or molecular tests.

NOTE:-Problems with cell culture. The main problem with cell culture is the long period (up to 4 weeks) required for a result to be available.

Also, the sensitivity is often poor and depends on many factors, such as the condition of the specimen, and the condition of the cell sheet.

Cell cultures are also very susceptible to bacterial contamination and toxic substances in the specimen. Lastly, many viruses will not grow in cell culture at all e.g. Hepatitis B and C, Diarrhoeal viruses, parvovirus etc.

3. Rapid Culture Techniques

- ❖ Rapid culture techniques are available whereby viral antigens are detected 2 to 4 days after inoculation.
- ❖ Examples of rapid culture techniques include shell vial cultures and the CMV DEAFF test. Detector of Early Antigen Fluorescent Foci test
- ❖ In the CMV DEAFF test, the cell sheet is grown on individual cover slips in a plastic bottle.(Detection of early antigen fluorescent foci)
- ❖ After inoculation, the bottle then is spun at a low speed for one hour (to speed up the adsorption of the virus) and then incubated for 2 to 4 days. The cover slip is then taken out and examined for the presence of CMV early antigens by immunofluorescence.

NOTE: The role of cell culture (both conventional and rapid techniques) in the diagnosis of viral infections is being increasingly challenged by rapid diagnostic methods i.e. antigen detection and molecular methods. Therefore, the role of cell culture is expected to decline in future and is likely to be restricted to large central laboratories.

3. Serology

Serology forms the mainstay of viral diagnosis.

- This is what happens in a primary humoral immune response to antigen.
- Following exposure, the first antibody to appear is IgM, which is followed by a much higher titre of IgG.
- In cases of reinfection, the level of specific IgM either remain the same or rises slightly. But IgG shoots up rapidly and far more than earlier than in a primary infection.

Many different types of serological tests are available.

- With some assays such as EIA and RIA, one can look specifically for IgM or IgG, whereas with other assays such as CFT and HAI, one can only detect total antibody, which comprises mainly IgG.
- The sensitivity and specificity of the assays depend greatly on the antigen used.

Assays that use recombinant protein or synthetic peptide antigens tend to be more specific than those using whole or disrupted virus particles