Course code: MCB 403
Course title: Virology and tissue culture
Number of Units: 3 units
Course duration: 3 hour per week

COURSE DETAILS:

Course details
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COURSE CONTENT:

Origin and nature of viruses; structure, properties and classification of viruses; the chemical and physical properties of bacterial, animal and plant viruses; principles of isolation; cultivation; purification and maintenance of bacteriophages and other viruses in vivo; transmission of viral diseases in plants and animals; interference phenomenom and interferon; systematic virology especially those endemic in Africa— polio, measles, rabies, oncogenic viruses, lassa virus, AIDS virus, viral genetics, viruses and genetic engineering; application of cell culture technique in virology.

COURSE REQUIREMENTS:

This course is offered both by Microbiology and Biology Students at 400L. Attendance is necessary.

READING LIST:

Madigan, M.T., Martinko, J.M. & Parker, J. (1997). Brock Biology of Microorganisms. Prentice Hall. USA.
Pelczar, M.J., Chan, E.C.S. & Krieg, N.R. (1986). Microbiology. McGraw Hill Book Co. New York.

1. Banor, J.D., Akermann, P.G., Toro, G.: Clinical Laboratory Methods. 8th Edition. The C.V. Mosby Company. Saint Louis, U.S.A. 1974
2. Brooks, G., Balel, J., Morge, S.: In: Jaetz, Melnick and Adelberg (Editor). Medical Microbiology. 21st Ed. Aphenton and Lange. Stanford, California. 1998
3. Davidson, I. and Henry, J.B.: Clinical Diagnosis. 15th Ed. W.B. Saunder Company. 1974

LECTURE NOTES

- Definition of viruses

- Chemical and physical properties of viruses
- Principles of isolation, cultivation and purification of bacteriophages
- Plant viruses endemic in Africa
- Viruses and genetic engineering
- Viruses are obligate submicroscopic microorganisms
- Physical properties of viruses include-
- The viral symmetry
- Presence/absence of an envelope
- Possession of spikes
- Viral symmetry- a virus may either be an icosahedral or helical
- Icosahedral –are spherical viruses e.g. *Cowpea mosaic virus* (CPMV) genus *Comovirus*
- Helical- rod shaped viruses e.g. *Tobacco mosaic virus* (TMV)
- Spikes – made up of glycoproteins e.g. *Influenza virus*

LECTURE NOTE

What is a Virus?

A virus is a small filterable and obligate intracellular parasite requiring a living host for its multiplication

Reasons for studying Viruses

- Viruses are important agents of human, animal and plant diseases eg rice yellow mottle virus of rice , foot and mouth disease of domestic animals and measle virus.

Potential application of viruses

- Phage typing of bacteria
- Sources of enzymes
- As pesticides

- Antibacterial agents
- Anticancer agents
- Gene vectors for protein production
- Gene vectors for treatment of genetic diseases

Origin of viruses

- Evidence for the existence of virus was first provided in the late 19th century by Martinus Beijerinck and Dimitri Ivanovski. They made extracts from disease plants infected with tobacco mosaic virus (tmv) and passed the extracts through fine filter. The filtrates contained an agent that was able to infect new plants but no bacteria could be cultured from the filtrates.

Characteristics of viruses

- They are all potentially infectious
- Presence of single nucleic acid
- Incapability to grow
- Reproduction from the genetic material only
- Absence of enzymes for energy metabolism
- Absence of ribosomes
- Absence of information for the production of enzymes in the energy cycle
- Absence of information for the synthesis of ribosomal proteins
- Absence of information for the synthesis of ribosomal RNA and soluble tRNA

occurrence

- Viruses occur in a wide hosts
- Plants--- angiosperms, gymnosperms, ferns, algae, bacteria and fungi
- Animals--- protozoans, insects, fish, amphibians, birds, mammals and human

Morphology

Shapes– viruses have different shapes such as:

- Spheroid or cubiod eg adenoviruses
- Elongated eg potato viruses
- Flexuous or coiled eg beet yellow virus
- Bullet shaped eg rabies virus
- Filamentous eg bacteriophage M13
- Pleomorphic eg alfalfa mosaic virus
- size
- Viruses have variable sizes but sizes vary from 20nm to 300nm in diameter

Viral structure

- A complete viral structure is made up nucleic acid core surrouded by a protein coat or capsid

nucleic acid * capsid = nucleocapsid

nucleocapsid can be naked or enveloped

Function of protein coat is for protection and attachment

Function of nucleic acid is for the synthesis of viral material

Classification of viruses

- International Committee on Taxonomy of Virus (ICTV) was formed in 1966. ICTV laid down rules for the classification and nomenclature of viruses

EVOLUTION OF VIRUSES

Three hypothesis have been proposed to explain the origin of viruses.

1. Viruses are descendants of ancient precethilar urgenisins that became paasites of the first cellular organisms. As organisms and animals evolved, viruses evolved with them. Many viruses do not cause any damage but may remain alent during the life of the host.

2. Viruses have evolved from pathogenic bacteria through a retrograde evolutionary process. Although rickettsiae and chlemydiae are examples of intracellular organisms that have undergone parasitic degeneration. There is at present no evidence to support the theory that true viruses have evolved from bacteria.
3. Viruses are components of normal cells that sometimes become autonomous. Within the cells the virus might exert an antrocatalytic influence so that replicas of itself are formed from the materials within the cell. Viruses might be said to resemble genes that have escaped regulatory control and continue to multiply as long as there is building material. For example, normal-appearing, apparently healthy cell cultures derived from infants who acquired reubella infection in utero produce rubella virus that is cytopathic for other cell limes.

Viruses vs Bacteria

Viruses differ fundamentally from bacteria in the following characteristics:

1. Small size and filterability
   Viruses are measured in millimicrons (one thousandth of a micron) and (for small viruses) in Angstrom wait (A°) (one tenth of a millioncron). Virus sial is usually determined by direct observation under the electron microscope.
2. Growth only in living cells
   Host cells are usually provided in one of three forms:
   a) The experimental animal
   b) Chick embryos
   c) Tissue cultures
3. Resistant to the action of antibiotics and other agents that destroy bacteria.
   a) Heat – generally inactivated by temperature of 56-60°C for 30 minutes
   b) pH – are usually destroyed at and pH below 5 and alkaline pH above 9
   c) Glycerol – most viruses survive 50% glycerol whereas bacteria are destroyed
   d) Bactericidal Agents – e.g. Phenol is not efficient as a viral disinfectant oxidizing agents are most effective with viruses
   e) Antibiotics and Chemotherapeutic Agents – Sulfonamides, penicillin, streptomycin and the tetracyclines have little effect on the viruses
4. Viral reproductive processes differ from the simple binary fission of bacteria.

5. Viruses contain only one kind of nucleic acid and it is covered by a protein coat.

What is Virology? This is the branch of microbiology which is concerned with viruses and viral diseases.

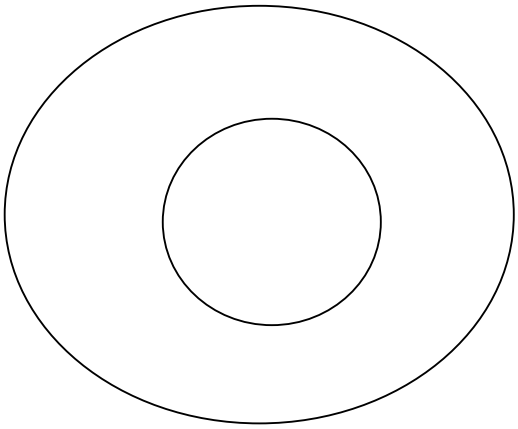
NATURE OF VIRUSES

Virus is one of a group of minutes infections agents with certain exceptions (e.g. Pox viruses) not resolved in the light microscope, and characterized by a lack of independent metabolism and by the ability to replicate only within living host cells. Like living organisms, they are able to reproduce with genetic continuity and the possibility of mutation. They are morphologically heterogeneous, occurring as rod-shaped, spherical or polyhedral and radpole-shaped form. The individual particle or virion, consists nucleic acid (the nucleoid), DNA or RNA (but not both) and a protein shell or Capsid, which contains and protects the nucleic acid. Viruses are customarily separated into three subgroups on the basis of host specificity namely – bacteria viruses, animal viruses and plant viruses. They are classified as to their origin (e.g. reoviruses), mode of transmission (arborviruses, tick born viruses) or the manifestation they produce (polioviruses, polymaviruses, poxviruses). They are sometimes warmed for the geographical location in which they were first isolated (e.g. coxsackevirus).

Viruses are the smallest infectious agents containing a molecule of nucleic acid (RNA or DNA) as their genome. The nucleic acid is encased in a protein shell and the entire infectious unit is termed a virion. Viruses infectious unit is termed a virion. Viruses replicate only in living cells. The viral nucleic acid contains information necessary for programming the infected host cell to synthesize a number of virus-specific Macoomolecules required for the production of virus progeny. During the replicative cycle, numerous copies of viral nucleic acid and coat proteins are produced. The coat protein assemble together to form the capsid, which encases and stabilized the viral nucleic acid against the extra cellular environment and facilitates the attachment and penetration of virions upon contact with new susceptible cells.

The nucleic acid, once isolated from the virion, can be hydrolyzed by either ribo- or deoxyribonuclease whereas the nucleic acid within the intact virus is not affected by such treatment. In contrast, viral antiserum will neutralize the virion because it reacts with the antigens of the protein coat. However, the same antiserum has no effect on the free infectious nucleic acid isolated from the virion.

The host range for a given virus may be extremely limited, but viruses are known to infect unicellular organisms such as mycoplasma, bacteria and algae and all higher plants and animals.



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Components of the complete virus particle or virion.

Capsid: The symmetric protein shell which encloses the nucleic acid genome. Often, empty Capsids are by-products of the viral replicative cycle. Nucleocapsid is the capsid together with enclosed nucleic acid.

A virion (virus particle) lacks certain components absolutely essential for its own replication and must depend on the host cell in which it is replicating to provide these missing factors. One component missing from all viruses is ATP – generating system. For independent life, a cell must carry out oxidations to provide energy to regenerate those high energy phosphate bonds used for biosynthetic reactions. No virion possess this regeneration system and hence, it must rely on the ATP-generating system present in the infected host cell. A second component that viruses lack and that the host cell must provide is the structural component for protein synthesis, that is ribosomes. The synthesis of any protein require that a ribonucleic acid (message RNA) be attached to a ribosome so that the individual amino acid can be joined to form the protein. The virion does not carry its own ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), but all viruses must use host cell ribosomes for protein synthesis. Another characteristic that is peculiar only to viruses is that, whereas all other forms of life contain both RNA and DNA. Viruses contain only one type of nucleic acid (RNA or DNA) but not both.

Many viruses are capable of producing cancer (tumor) in certain animals. There is a strong evidence that a human maliquancy called BURKITT'S LYMPHOMA is the result of a virus infection. DNA viruses accomplish this by incorporating their DNA into the host cell chromosomes. RNA viruses must first transcribe their RNA into DNA in order to render a cell malignant.

NOTE: 1) Transcription:- The mechanism by which specific information encoded in a nucleic acid chain is transferred to messenger RNA.

2. Translation:- The mechanism by which a particular base sequence in messenger RNA results in production of a specific amino acid sequence in protein.

MICROSCOPY

Although the majority of viruses cannot be seen by ordinary microscopic methods, the light microscope nevertheless has some important applications in virology.

The large viruses of the pox group are just above the limit of resolution with ordinary light and can be demonstrated by the use of staining procedures which, as a result of deposition of stain on the surface of the particle, increase apparent size.

The fluorescent antibody technique has been of considerable value for the demonstration of viral antigens in tissues. Antigenic components of the smallest viruses can be demonstrated by this technique.

For the recognition of virus induced degenerative changes (cytopathogenic effect, CPE) in infected tissue cultures.

For the recognition of characteristic histological and cytological changes in infected animals or man e.g. Poliomyelitis glandular fever and the characteristic myositis produced in body mice by coxsackie viruses.

The greatest advances of recent years in our knowledge of the structure of virus particles have been achieved through the use of the electron microscope. In this instrument a beam of electrons is used instead of light rays and because of the very short wave length of high velocity electrons, the limit of resolution is enormously increased. The electron microscope is capable of resolving particles with diameters of less than ι nm and its use has consequently brought even the smallest viruses into visible range.

Electron microscopy has also been used for counting the number of virus particles in a suspension. For this purpose the suspension mixed with a suspension of polystyrere latex particle of a known concentration. From the relative numbers of latex particles and of virus particle seen in droplets of the mixture the concentration of virus in the original suspension can be estimated.

CHEMICAL COMPOSITION OF VIRUSES

Viral Protein: The structured proteins of viruses have several important functions. They serve to protect the viral genome against inactivation by nucleases, participate in the attachment of the virus particle a susceptible cell and are responsible for the structural symmetry of the virus particle. Also, proteins determine the antigenic characteristics of the virus. The structural proteins of many viruses could be studied by dissociating the proteins of the virus particle with a detergent (sodium dodecyl sulfate) and then separating them by electrophoresis through polyacrylamide gel matrix.

Virus structural proteins may be very specialized molecules designed to perform a specif task (a) vaccinia verus carries many enzymes within its particle to perform certain functions early in infection cycle; (b) some viruses have specific proteins for attachment to cells e.g. influenza virus hemagglutinin and (c) RNA tumor virus contain enzyme, reverse transcriptase that makes a DNA copy of virus RNA, which is important step in transformation by these viruses.

Viral Nucleic Acid: Viruses contain a single kind of nucleic acid, either DNA or RNA, that encodes the genetic information necessary for the replication of the virus. The RNA or DNA genome may be single-stranded or double stranded. Most major families of RNA-containing animal viruses have single-stranded RNA genomes except reoviruses, which have double-stranded RNA. NOTE Major families of DNA-containing animal viruses have double-stranded DNA genomes with exception of single-stranded DNA-containing parvovirus. The nuleic acid can be determined using either the intact virus particle or the free nucleic acid. Both the type of nucleic acid and the strandedness can be determined in the fluorescence microscope by staining with acridine orange and the nucleic acids identification by color reaction and enzymes digestion tests.

Viral Lipids: A number of different viruses com lipids as part of their structure. Lipid-contain Nucleic Ac*viruses are sensitive to treatment with ether and other organic solvents, indicating that* disruption or loss of lipid results in loss of infectivity. Nonlipid-containing viruses are generally resistant to the action of ether.

Viral Carbohydrates: The viral envelope also has a significant amount of carbohydrate mainly in glycoproteins. These glycproteins may contain flucosamine, fructose, galactose, and mannose. The glycoproteins are important components of viral antigenic

determinants. Glycoprotein synthesis may be partially controlled by the virus but is determined also by the host cell genome.

CLASSIFICATION OF VIRUSES

Basis of Classification

The following properties, listed in the order of importance, have been used as a basis for the classification of viruses. The amount of information available in each category is not uniform for all viruses:

1. Nucleic acid type: RNA or DNA, single-stranded or double-stranded i.e. strategy of replication.
2. Size and morphology including type of symmetry, number of capsomeres and presence of membranes.
3. Susceptibility to physical and chemical agents, especially either.
4. Immunologic properties.
5. Natural method of transmission
6. Host, tissue and cell tropism.
7. Pathology, including inclusion body formation.
8. Symptomatology.

Classification of viruses into family based on chemical and physical properties

Nucleic Acid Core	*Virion-Envelope or Na Red*	*Ether Sensitivity*	*Virus Family*
DNA	Naked	Resistant	Parvoviridae Papovaviridae Adenoviridae
	Enveloped	Sensitive	Herpetoviridae
	Complex coats	Resistant	Poxviridae
RNA	Naked	Resistant	Picornaviridae Revoviridae Togaviridae
	Enveloped	Sensitive	Togaviridae Arenaviridae Coronaviridae Retroviridae Bunyaviridae Orthomycoviridae Paramyqoviridae Rhabdoviridae

*Some resistant (majority) but few sensitivity

Classification by Symptomatology

The oldest classification of viruses is based on the diseases they produce, and this system offers certain conveniences for the clinician. However, it is not satisfactory for the biologist because the same virus may appear in several groups, since it causes more than one disease depending upon the organ attacked.

I. Generalized Diseases: Disease in which virus is spread throughout the body via the bloodstream and in which multiple organs are affected. These include smallpox, vaccinia measles, rubella, chickenpox, yellow fever, dengue, enteroviruses, and many others.

II. Diseases Primarily Affecting Specific Organs: The virus may spread to the organs through the bloodstream, along the peripheral nerves or by other routes.
   1) Diseases of the nervous system – poliomyelitis, aseptic meningitis (polio – coxsackie – and echoviruses), rabies, arthropod-borne encephalitides, lymphocytic chromeningitis, herpes simplex meningoencephalitis of mumps, measles, vaccinia and "slow" virus infections.
   2) Diseases of the respiratory tract – Influenza, parainfluenza, RJ pheumonial and bronchiolitis, adenovirus pharyngitis, common cold (caused by many viruses).
   3) Localized diseases of the skin or mucous membranes – Herpes simplex, molluscum contagiosum, warts, harpangina, herpes zoster and others.
   4) Diseases of the eye-Adenovirus conjunctivitis, New-castle virus conjunctivitis, herpes kerato conjunctivitis and epidemic hemorrhagic conjunctivitis (enterovirus – 70).
   5) Diseases of the Liver – Hepatitis type A (infectious hepatitis) and type B (serum hepatitis), yellow fever. In neonate, enteroviruses, herpes v is used and rubella virus.
   6) Diseases of the salivary of glands – mumps and cytomegalovirus.
   7) Diseases of gastrointestinal tract – Gastroenteritis A virus and gastroenteritis B virus (rotavirus), poliovirus, Hepatitis A, Hepatitis B.

Classification by Biologic, Chemical and Physical Properties

Viruses can be clearly separated into families on the basis of the nucleic acid genome and the dice shape, substructure and mode of replication of the virus particle within each family, general are usually based on antigenicity.

DNA-containing Viruses

1. PARVOVIRIDAE FAMILY – The members of this family are very small viruses. They contain single-stranded DNA. They have no

envelope and are either-resistant. Replication and lapsed assembly take place in the nucleus of the infected cell. Genus PARVOVIRUS includes the autonomously replicating parvoviruses of harmster, rats, mice and swine. Genus ADENOSATELLOVIRUSES consist of the adeno-associated satellite viruses which are defective and cannot multiply in the absence of a replicating adnovirus which serves as a "helper virus". Herpesvirus can act as a partial helper. Genus DENSOVIRUS include the arthropod parvoviruses.

2. PAPOVAVIRIDAE FAMILY: these are small, either-resistant viruses containg double-stranded circular DNA. The human representative are the pilloma or wart, virus and SV40-like viruses. Isolated from the brain tissue of patients with progressive multifocal leukoeniaphalopathy (PML) (JC virus) or from the urine of immunosuppressed renal transplant recipients (BK virus). Ethers are papilloma viruses of rabbits and cattle, polyoma virus of mice and vacuolating viruses of monkeys (SV40) and of rabbits. These viruses have relatively slow growth cycles characterized by replication within the nucleus. Papovaviruses produce latent and chronic infections in their natural hosts.
3. ADENOVIRIDAE FAMILY: These are medium-sized viruses containing double-stranded DNA. They are not enveloped and are ether-resistant. Thirty-one types are known to infect humans. They have predilection for mucous membranes and may persist for years in lymphoid tissue. Some of these agent cause acute respiratory diseases, febrile catarrhs, pharyngitis and conjunctivity. Human adenoviruses rarely cause disease in laboratory animals.
4. HERPETOVIRIDAE FAMILY: These are medium sized viruses containing double-stranded DNA. Latent infections may occur and last for the life span of the host even in the presence of ciruculating antibodies.

   In human, herpes simplex virus types 1 and 2 cause oral and genital lesions (cold sores). Varicella – zoster viruses causes zoster and chickenpos. Cytsmegalovirus causes cytomegalic inclusion disease.
   Amd EB virus causes infections mononucleosis. EB virus has also been associated with several human malignanties. Other members occur in monkey, rabbits, cattle, horses, pigs, dogs, frogs, shrews, fowl and snakes.
5. POXYVIRIDAE FAMILY: These are relatively large, brick-shaped or ovoid viruses containing a double-stranded DNA genome and protein, enveloped by double membranes. This is the major DNA-containing virus family that replicates solely within the cytoplasm.

This family includes members that are chiefly pathogenic for the skin in human (small pox, vaccine, milluscum contagiosum) and in animals (e.g. cowpox, monkeypox and myxoma of rabbits). Some of the animal poxvirus (e.g. cowpox and monkeypox) can infect humans.

1. PICORNAVIRIDAE FAMILY: The genera whose members commonly infect humans are ENTEROVIRUS and PHINOVIRUS. At least to human enteroviruses are known – these include polio, coxsackie and echo-viruses. Other types exist throughout the animal kingdom. More than 100 rhivoviruses exist and are the most common cause of colds in humans. Two other genera are APHTHOVIRUS (foot- and mouth-virus of cattle) and CARDIOVIRUS (encephalomyocarditis virus, which rarely infects humans).
   Picornaviruses are small, ether resistant viruses that contain single-stranded.
2. REOVIRIDAE FAMILY: Reoviruses were the first organisms shown to have double-stranded RNA. They are ether-resistant viruses. Reovirus strains from animals are similar to those of humans. Diseases associated with them not really clear.
   The family Reoviridae contains two genera. REOVIRUS and ORBIVIRUS. A probable new genus is ROTAVIRUS, the agent of infantile gastroenteritis. Rotaviruses are major pathogens of humans, causing infantile diarrhea in all parts of the world. This is one of the most common childhood illness and in developing countries is a leading cause of infant death.
3. TOGAVIRIDAE FAMILY: This family includes most arbiviruses of antigenic groups A and B, rubella virus, and LDH (Lactic dehydrogenase) virus of mice. These viruses possess a lipid-containing, ether-sensitive envelope and have a genome of single-stranded RNA. Togaviruses include 4 genera:
   a) Alphavirus (group A arboviruses), with sindbis virus as the type species.
   b) Flavivirus (group B arboviruses), with yellow fever virus as the type species.
   c) Rubivirus, with rubella virus as the type species and (d) Pestivitus, with mucosal diarrhea virus as the type species.
4. CORONAVIRIDAE FAMILY: This members are enveloped and contains a genome of single-stranded RNA. The human coronaviruses have been isolated from patients with acute upper respiratory tract illness.
5. BUNYAVIRIDAE FAMILY: They are ophevilal and enveloped particles. These viruses replicate in the cytoplasm and acquire an envelope by budding through the cytoplasmic membrane. They are sensitive to ether, acid and heat.

6. ORTHOMYXOVIRIDAE FAMILY: These are medium-sized enveloped viruses containing single-stranded RNA. The orthomyxoviruses are sensitive to diactinomycin.
   A;; arthomyxoviruses are influenza viruses. They are classed as types A, B or C on the basis of these RNA antigen, which does not cross-react between types.
7. RHABDOVIRIDAE FAMILY: Members of this family have enveloped virion that are rod-shaped, resembling a bullet. The genome is single-stranded RNA. Virus particles are formed by budding from the cell surface membrane. Members include abies virus and other viruses of cattle, fish and plants.
8. Other Viruses: For some viruses there are insufficient data to permit their classification. These include viruses that cause hepatitis (hepatitis A, and hepatitis B) and viruses responsible for certain immune complex disease and for neurologic disorders with a long laent period ("slow" virus diseases).
9. Viroids: A class of infectious agents smaller than viruses, termed viroids. Viroids exhibit the characteristics of nucleic acids in crude extracts i.e. they are insensitive to heat and organic solvents but sensitive to nuclease, and they do not appear to possess a protein coat. Presently known viroids consist solely of a short strand of RNA.

CULTIVATION; QUANTIFICATION, INCLUSION BODIES; CHROMOSOME DAMAGE

CULTIVATION OF VIRUSES

In the early years of virus research, the use of animals was mandatory for the recognition of viruses, and rapid, quantitative results were often difficult. For example, poliomyphitis research was limited as long as the presence of the virus could be detected only by monkey inoculation. At present, many viruses can be grown in cell cultures or infertile eggs under strictly controlled conditions. Growth of virus in animals is still used for the primary isolation of certain viruses and for the study of pathogenesis of viruses.

I. CHICK EMBRYO: Virus growth in an embryonated egg may result in the death of the embryo (e.g. encephalitis virus), the production of plaques on the chorioallantoic membrane (e.g. herpes, smallpox, vaccinia), the development of hemagglutinins in embryonic fluids or tissues (e.g. influenza) or the development of infective virus (e.g. poliovirus type 2).

Inoculation may be into the allantoic or amniotic cavities into the yolk sac or on to the chorioallantoic membrane, the precise route used depending on the particular virus being cultivated. The amniotic route

is the method of choice for the isolation of the influenza and mumps viruses. The alantoic route, which is technically the simplest, is used mainly for the passage of influenza viruses that have already been established in embryo. Yolk sac inoculation is of particular value for the propagation of rickettsince. Inoculation on to the chorioallantoic and characterization of the poxviruses and herpes simplex.

Tissue Cultures: Most of the humans pathogenic viruses may be propagated in tissue cultures derived from a variety of animal species. Since, however, a number of common viruses e.g. the enteroviruses and the adenoviruses will grow only in the cells of primate tissues, the tissue cultures used in diagnostic virology are generally derived from monkey or human sources.

Tissue culture techniques may be divided broadly into three groups: (1) Fragment cultures, (2) cell cultures and (3) organ cultures.

1. Fragment Cultures: The simplest form of fragment culture is Maitland type of culture which consists of fragments of tissue suspended in a fluid medium. The cell remain viable for several days-sufficiently long to permit virus growth but do not multiply. In plasma clot cultures, the tissue fragments are fixed by a plasma clot to the sides of tubes on rattler; new cells, mostly fibroblast, then grow out from the tissue fragments. Fragment cultures have no application in routine diagnostic virology.
2. Cell Cultures: These are prepared from cell suspensions obtained from intact tissue or from a prior tissue culture. Dispersal of cells is usually achieved by treatment of the tissue or tissue culture with a proteolytic enzyme usually trypsin or with the chelating agent versene (EDTA, ethylenediamine tetra-acetic acid, sequenstrene). This results in the release of single cells and small aggregates of cells capable of initiating growth. Cell culture may be prepared as suspended cultures or as monolayer cultures.

    (a) Suspended cell cultures resemble Maitland type culture in that the cells are simply suspended in nutrient medium. They are used for metabolic inhibition tests which may, in diagnostic work, be applied to the estimation of antiviral antibody. Uninfected cells metabolize and actively produce acid. This causes a colour change in an appropriate pH indicator incorporated in the nutrient medium. If the cells are infected by virus, their metabolism is interfered with and the indicator change does not occur. If however the virus is neutralized by antibody the indicator change occurs as in a normal uninfected culture.

(b) In monolayer cell cultures, the cells are allowed to settle on the sides of a tube or flat bottle and are covered with nutrient medium. Antibiotics are incorporated in order to control, bacterial and fungal contamination. After two to seven days incubation at 37°C the growing cells will have formed into a continuous sheet or monolayer, no called because it is one cell thick, adherent to the glass of the tube or bottle. The cell growth medium is then removed and replaced with a maintenance medians which is nutritionally less rich than the initial growth medium but adequate to maintain the viability of the tissue cells. Then the virus inoculation is introduced and culture again incubated. During incubation tubes are maintained in a slightly sloped and bottles in a horizontal position. Many viruses when propagated in a monolayer cultures produce degenerative changes in the tissue cells readily visible under the lower power objective or if the area involved is sufficiently large, to the naked eye. This is known as a cytopathogenic effect (cytopathic effect, CPE). The type of cellular change produced differs with different viruses.

Cell cultures may be divided into three types according to the damage history of cells used for their preparation:

i) Primary and secondary cell cultures – primary cell cultures are prepared from cells obtained directly from the tissues. A secondary cell culture is the first subculture of a primary cell culture, the cells of which are dispersed by treatment with typsin or versene for the preparation of the secondary culture. This procedure is particularly convenient for the preparation of monkey Kichey cell cultures.

ii) Continuous cell lines – As a rule when tissue cultures are prepared directly from an animal tissue, the cultures cannot be serially propagated, the cells dying out after a few subcultures. A number of lines of mammalian cells are, however, available (mainly derived from fetal and malignant tissues) which can serially be propagated more or less indefinitely. These established cells lines have the advantage of the reccorsity for procuring fresh animal tissue for each set of cultures. They have the disadvantage, on the other hand, that on prolonged subculture the tissue is liable to undergo spontaneous changes in its susceptibility to infection. The established cell lines most frequently employed are Hela cell culture which was derived originally from a human cervical caocinoma, and the HEp-2 cell derived from a carcinoma of the larynx.

iii) Diphoid cell cultures – The cells of certain tissues, notably human embryo cells can be serially propagated for about 50 subcultures

without transformation. Genetically, they differ from continuous cell lines and resemble normal cells. Diphoid cell cultures are highly susceptible to infection by viruses such as the H rhinoviruses and the cytomegalovirus which may be difficult to propagate in other systems.

3. Organ Culture: Organ culture has been recommended for the isolation of cold viruses which cannot be propagated by other procedures. This is done by use of tissue from appropriate organ for the isolation of viruses.

III. Animal Inoculation: Of the ordinary laboratory animals the mouse is the most generally useful and depending on the virus, mouse can be infected by nasal instillation or by intraperitoneal (stomach) or intracerebral inoculation. The presence of virus in the inoculated material is shown by the development of appropriate symptoms, diagnostic pathological changes or specific antibody.

Quantification of Virus

I. PHYSICAL METHODS: Virus particle can be counted directly in the electron microscope by comparison with a standard suspension of the latex particles of similar small size. However, a relatively concentrated preparation of virus is necessary for the procedure and infectious virus particles cannot be distinguished from noninfectious ones. HemiAGGLUTINATION – The red blood cells of humans, chickens and animals can be agglutinated by many different virus. Hemagglutination by viruses has led to rapid and inexpensive quantitative methods of virus assay. Since both non-infective and infective viruses give the reaction, the test measures the total number of virus particles present.

The orthomycoviruses contain a hemagglutinin that is an integral part of the viral envelope. Once these viruses have agglutinated with the cells, spontaneous dissociation of the virus from the cells can occur. The dissociated cells can no longer be agglutinated by the same virus species, but the recovered virus is able to agglutinate fresh cells. This is due to the destruction of specific mucopolysaccharide receptor sites on the surface of the erythrocyte by the enzyme neuraminidase of the virus particles.

The reaction of red blood cells with virus can also indicate the growth of paramyxovirus in tissue cultures. The erythrocyte hemadsorb to the infected cells and can be observed visually.

Poxviruses also agglutinate red cells, but the hemagglutinin is separable from the intact, infective viruses particle. The hemagglutinin, a phospholirid-protein complex, is smaller than the virus.

The third group of viruses (arbiviruses and others) have hemagglutinin that appear to be identical with the virus. The union between hemagglutinin and red blood cells is irreversible.

II. BIOLOGIC METHODS: Quantal assays depend on the measurement of animal death, animal infection, or cytopathic effects in tissue culture upon end point dilution of the virus being tested. The tilted is expressed as the 50% infectious dose (ID_{50}), which is the reciprocal of the dilution of virus that produces the effect in 50% of the cells or animals inoculated. Precise assays require the use of a large number of test subjects.

Pack assays, quantification of the number of pocks produced (leisions) on chorioallantoic membranes of embryonated eggs inoculated with dilutions of virus, can be used to determine the amount of infectious virus for those viruses which produce such lesions e.g. herpes, vaccinia, smallpox.

The most widely used assay for infectious virus is the plaque assay. Monolayers of host cells are inoculated with suitable dilutions of virus and after adsorption are overlaid with medium containing agar or carboxymethylcellulose to prevent virus spreading. After several days, the cell initially infected have produced virus that spreads only to surrounding cells, producing a small area of infection or plaque. Under controlled conditions a single plaque can arise from a single infectious virus particle, termed a plaque-orming unit (PFU). The cytopathic effect of infected cells within the plaque can be distinguished from uninfected cells of the monolayer, with or asthant suitable staining, and plaques can usually be counted macroscopically.

INCLUSION BODY FORMATION

In the course of virus multiplication within cells, virus-specific structure called inclusion bodies may be produced. They become far larger than the individual virus particle and often have an affinity for and dyes (e.g. eosin). They may be situated in the nucleus (herpesvirus), in the cytoplasm (poxvirus), or both (measle virus). In many viral infections, the inclusion bodies are the site of development of the virions (the virus factories).

The presence of inclusion bodies may be of considerable diagnostic aid. The intracytoplasmic inclusion in the nerve cells the NEGRI body, is peculiar for rabies infection.

CHROMOSOME DAMAGE

One of the consequences of infection of cells by viruses is derangement of the karyotype. Most of the changes observed are random. Frequently, breakage, fragmentation, rearrangement of the chromosomes, abnormal chromosomes, and changes in chromosome number occur. Chromosome break have been observed in leukocytes from cases of chickenpox or measles. These viruses, as well as rubella virus, cause similar aberration when inoculated into cultured cells. Cells infected with or transformed to malignancy by SV40, polyoma or adenovirus type 12 also exhibit random chromosomal abnormalities.

PATHOGENESIS OF VIRUS DISEASES

Virus implantation and multiplication occur in different tissues as the infectious agent travels to the target organs from the portal of entry. In the target organ, virus multiplication must reach a critical level before cell necrosis occurs and disease become manifest. Where polymorphonuclear teukocytes form the principal cellular response to the acute inflammation caused by pyogenir bacteria, infiltration with mononuclear cells and lymphocytes characterizes the inflammatory reaction of uncomplicated viral lesions.

In poliomyelitis, virus enters by way of the alimentary tract, multiplies locally at the initial sites of viral unplantation (torsils) or the lymph nodes which drain these tissues and begins to appear in the throat and in the feces. Secondary virus spread occurs by way of the bloodstream to other susceptible tissues, namely, other lymph nodes, brown fat and the central nervous system (CNS). Within the CNS the virus spreads along nerve fibers. If a high level of multiplication occurs as the virus spreads through the CNS, motor neurons are destroyed and paralysis occurs. Secondary spread to the CNS is readily interrupted by the presence of antibodies, induced by prior infection or vaccination.

Persistent Viral Infection & Immune Complex Disease

Certain viruses do not invariably kill the cells they infect. The immunologic response of the host to these viruses may be responsible for the observed pathologic changes and the clinical illness.

Persistence ("slow") viral infections may play a far-reaching role in human disease. Persistent viral infections are associated with leukemias as well as progressive degerative disease of CNS of human.

Modes of Transmission of Viruses

Viruses may be transmitted in the following ways:

1. Direct transmission from person to person by contact, in which dooplet or aerosol infection may play the major role (e.g. influenza, measles, smallpox).
2. Transmission by means of alimentary tract (intimate association with carrier, food and drink) (e.g. enterovirus infection, infectious hepatitis).
3. Transmission by bite (e.g. rabies).
4. Transmission by means of an arthropod vector (e.g. arboviruses).

Virus Vaccines

Although the use of different vaccines is dealing with specific virus families and diseases, but certain general principles, however, apply to most virus vaccines for use in the prevention of human disease.

Neither vaccination nor recovery from natural infection always results in total protection against a later infection with the same virus. This situation holds for diseases for which successful control measures are available, including polio, smallpox, influenza, rubella, measles, mumps and adenovirus infections. Control can be achieved by limiting the multiplication of virulent virus upon subsequent exposure and preventing its spread to target organs where the pathologic damage is done (e.g. polio and measles viruses kept from the brain and spinal cord; rubella virus from embryo).

Inactivated Virus Vaccines Inactivated vaccines prepared from whole virus generally stimulate the development of circulating antibody against the coat proteins of the virus, conferring some degree of resistance. For some diseases, inactivated vaccines are currently the only ones available. The following disadvantages have been inherent in the use of inactivated vaccines.

1. Extreme care is required in their manufacture to make certain that no residual live virulent virus is present in the vaccine.
2. The immunity conferred is often brief and must be boosted, which not only involves the logistic problem of repeatedly reaching the persons in need of immunization but also has caused concern about the possible effects (hypersensitivity reactions) or repeated administration of foreign proteins.

Live Attenuated Virus Vaccines

Attenuated vaccines have the advantage of acting like the natural infection with regard to their effect on immunity. They multiply in the host and tend to stimulate longer-lasting antibody and also to induce antibody and resistance at the portal of entry. The disadvantages of live attenuated vaccines include (1) The risk of reversion to greater virulence during multiplication within the vaccine. Although reversion has not proved to be a problem in practice, its potential cannot be overlooked. The approved vaccines should be utilized fully, but alert monitoring should be continued. (2) The storage and limited shelf life of attenuated vaccines present problems, but this can be overcome in some cases by use of viral stabilizers (.e.g $MgCl_2$, for poliocaccine).

Collection and Storage of Specimen for Laboratory Diagnosis or Identification of Viruses

1. Blood specimen for serological Test:- since it is necessary to demonstrate significant increase in antibody titer, an acute phase and convalescent phase specimen is mandatory. Acute phase specimen is taken within 7 days after the onset of illness. The convalescent phase specimen is taken 14 days or longer after collection of the acute phase specimen. Aseptically collect 10mL whole blood, allow to clot, separate serum clot, sore at -20^{o}C. DO NOT FREEZE WHOLE BLOOD.
2. Nasa Washing:- Collect aseptically within 3 days or no longer than 7 days after onset of illness. Instill 4-5mL sterile saline into each nostril. Collect wash in sterile glass screwcap container. Add 1% borine sermon albumin to preserve virus.
3. Throat washings:- Collect aseptically within 3 days or no longer than 7 days after onset of illness. Gargle with 10mL. Sterile bacteriological culture medium and collect in sterile screwcap glass vial.
4. Eye Excidate:- Use sterile cotton swab to collect exudates. Place swab in tube with 2-3mL tissue culture medium.
5. Stools:- Collect 2 specimens 24-48 hours apart. Place 2-4 grams in screwcap glass jars. Freeze and store at -70^{o}C.
6. Urine:- Collect clean-voided urine in a sterile screwcap glass jar. Add penicillin (500unit/Ml), streptomycin (500µg/ml) and mystatin (200units/mL).
7. Spinal Fluid:- Place 3-5mL in sterile screwcap glass vial. Freeze and store at -70^{o}C.
8. Autopsy-Biopsy Specimens:- Collect small sections of tissue and place in a sterile screwcap glass jar. Keep at 4^{o}C or freeze and store at -70^{o}C.

Identification of Viruses

I. CULTIVATION OF VIRUSES

1. Chick embryo – Virus culture in embryonated chick egg results in death to the embryo, production of pocks or plaques on the choriallantoic membrane or the development of hemagglutinin or the development of the infective virus.
2. TISSUE CULTURE – Virus growing in tissue culture can be followed by determining neucrosis, inability to produce acid, appearance of either a hemagglutinative or complement-fixing antigen, and the ability to adsorb erythrocytes on the infected cells.

II. SEROLOGICAL PROCEDURES

1. NEUTRALIZATION TESTS – These consist of injecting susceptible animal with a mixture of the suspected virus and serum containing the specific antibodies should the animal survive or be not infected, the presence of neutralizing antibodies is proved.
2. Complement-Fixation Tests – Antiviral serum has complement-fixing abilities and has been used in the serologic detection of many viruses.
3. Hemagglutination-Inhibition Tests – There are a number of viruses that have the ability to agglutinate erythrocytes. If a serum containing specific antibodies is introduced, this ability of hemagglutination is inhibited.

PURIFICATION OF VIRUS PARTICLES

For purification studies, the starting material is usually large volume of tissue culture medium, body fluids or infected cells. The first step involves concentration of the virus particles by precipitation with ammonium sulfate, ethanol, or polyethylene glycol or by ultrafiltration. Once concentrated, virus can be separated from host materials by differential centrifugation and density gradient centrifugation, other methods could be by column chromatography and electrophoresis.

Rate-zonal centrifugation: A sample of concentrated virus is layered onto a performed linear density gradient of sucrosis or glycerol and during centrifugation the virus sediments as a bond at a rate determined primarily by the size and weight of the virus particle. Samples are collected by piercing a hole in the bottom of centrifuge tube. The band of purified virus may be detected by

optical method, by radiolabelling the virus or by assaying for infectivity.

Equilibrium Density Gradient Centrifugation: Viruses can also be purified by high-speed centrifugation in density gradients of cesium chloride (C_sCl), potassium tartrate, potassium citrate or sucrose. The gradient material of choice is the one that is least toxic to the virus. Virus particle migrate to an equilibrium position where the density of the solution is equal to their buoyant density and the virus particles form a visible band. Virus band are harvested by puncture through the bottom of the plastic centrifuge tube and assayed for infectivity. C_sCl is the material of choice for this procedure because of its high density and low viscosity. Certain viruses are unstable in C_sCl and some of these have been successfully banded in potassium tartrate and citrate and in sucrose.

IDENTIFICATION OF A PARTICLE AS A VIRUS

When a characteristic physical particle has been obtained, it should fulfill the following criteria before it is identified as a virus particle:

1) The particle can be obtained only from infected cells or tissues.
2) Particles obtained from various sources are identical, regardless of the cellular species in which the virus is grown.
3) The degree of infective activity of the virus varies directly with the number of particles present.
4) The degree of destruction of the physical particle by chemical or physical mean is associated with a corresponding loss of virus activity.
5) Certain properties of the particles and infectivity must be shown to be identical, such as their sedimentation behavior in the ultracentrifuge and their pH stability curves.
6) The absorption spectrum of the purified physical particle in the ultraviolet range should coincide with the ultraviolet inactivation spectrum of the virus.
7) Antisera prepared against the infective virus should react with the characteristic particle and vice versa. Direct observation of an unknown virus can be accomplished by electron microscopic examination of aggregate formation in a mixture of antisera and crude virions suspension.
8) The particle should be able to induce the characteristic disease in vivo (if such experiments are feasible).
9) Passage of the particles in tissue culture should result in production of progeny with biologic and serologic properties of the virus.

ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

Although the first patients reported were homosexuals, it has now become clear that AIDS is not exclusively a venereal disease. Soon afterwards, other at risk groups were identified. These groups included intravenous dong abusers, recipients of blood and blood products, and heterosexual partners of AIDS patients. HIV (Human Immunodeficiency Virus) causes AIDS. HIV I & II.

Classification of HIV Infection

Group I – Acute Infection: As with other infectious diseases, when an individual meets the causative organism the immune system responds. This response can be measured soon afterwards by detecting specific antibody to the infecting agent. When an individual is first infected with HIV, he sometimes suffers an acute illness similar to glandular fever immediately before antibody to HIV can be detected in his blood. This is known as the SEROCONVERSION illness. Patients are considered particularly infectious during this stage. The range of symptoms associated with seroconversion is wide, ranging from a wild flu-like illness to a severe meningitis or encephalitis (headache, fever, nausea, diarrhea, transient rash, etc.). The majority of patients, however are symptomatic during this period.

Group II – Asymptomatic HIV Infection: These patients are infected with HIV and yet experience no symptoms signs. Some may become symptomatic within a few months and others may remain symptom-free for many years perhaps for decades. What the future holds for them in the long term is not yet known. At present time, all of these asymptomatic patients should be regarded as every bit as infectious as the patient suffering from AIDS.

GROUP III – Generalized Lymphasdenopathy (PGL): Most patients in this group are symptom-free and may remain so for many years. Palpable lymphadenopathy (lymph node enlargement) is noticed in the absence of concurrent of illness.

GROUP IV – Other Disease: This group osf patients may or may not have lyphademopathy in addition to other clinical features of HIV infection. Classification into group IV depends on the type of symptoms such as one or more of the following fever persisting for more than one month, involuntary weight loss of greater than 10% of baseline, diarrhea persisting for more than 1 month. As well Laboratory feature could be HIV antibody positive, elevated immunoglobulin anaemia, thrombocytopenia, elevated ESR, etc.

Prevention of Community Spread of HIV

1. HIV is easily transmitted by sexual contact
2. HIV is easily spread by blood-blood contact
3. HIV is frequently passed on from an infection mother to her unborn fetus
4. HIV is NOT spread by casual everyday non-sexual contact

The rate of spread of HIV epidemic is a reflection of the involvement of large number of sexual partners. The first stage of any prevention programme must be to educate each nation so that individual can identify whether they are in an 'at-risk' group. Secondly, they should be given easily understood accurate information on how to modity their behavior so that they can protect themselves and others. Homosexual have extremely decreased and this would also help the heterosexual professional like prostitutes if each nation comes into action (i.e. education).

The second stage of prevention programme must be to educate the majority of the population on sexual behavior like one partner, use of condom.

2. The spread via blood is secondary in epidemic of HIV. The risk of acquiring HIV via transfused blood or plasma was formally high. The risk is now minimal due to screening of donors blood, although theoretically an occasional HIV carrier could be missed who is antibody-negative. A much greater problem that has emerged is the rapid spread of the virus in some areas among the drug addict population, due largely to the sharing of needles and syringes. Clearly the ideal situation is to stop drug addiction at every step by the government.
3. HIV-infected mothers are capable of passing the infection to their unborn children. The majority are drug addicts, but some have acquired their infection heterosexually and others by receiving a blood transfusion. The result of a positive HIV antibody test in a child born to an infected mother may be difficult to interpret. Maternal IgG which has crossed the placenta will result in a positive test in the child during the first few months of life. This persist beyond four months it reflects genuine infection in the child.

At present time, all HIV-infected women are advised not to become pregnant. If already pregnant the mother should be asked to consider termination.

RESPIRATORY TRACT VIRUSES

Symptoms attributed to the common cold can result from infection by any one of a hundred serotype of adenoviruses, four different serotypes of parainfluenza viruses, respiratory syncytial virus and three serotypes of reoviruses. Thus, the effectiveness of a vaccine seems highly unlikely. Adnoviruses produce a somewhat more severe upper respiratory infection than that we think of as a common cold. In addition, certain serotypes of adenoviruses have been shown to induce cancer in newborn hamsters. There are three serologic types of influenza virus, but epidemics are most frequently caused by type A. This virus undergo periodic mutations resulting in antigenic changes which allot it to spread throughout the world in an essentially non-immune population. There is only one antigenic type of mumps or measle virus. A living attenuated vaccine is routinely used to prevent both measle (rubeola) and mumps.

Rubella is a very mild infection which seriously only inject pregnant women. In these cases, it can cross the placental wall, infect the fetus, and result in many congenital defects in the child. A living attenuated vaccine is now available that appear to stimulate immunity. Chickenpox (variella) is a moderately mild childhood disease caused by a herpesvirus. Apparently, in some, if not all, cases, after recovery from chickenpox, the virus continues to exist for many years as a latent infection. This latent infection is sometimes activated in adults to produce a sensory nerve infection called zoster or stingles. Smallpox virus is a complex, brick-shaped, DNA virus. The disease has possibly been eliminated from the world.

Discuss viruses that can cause the symptoms we call the common cold syndrome.

ENTERIC VIRUSES

The enteric viruses include polio viruses, coxsackie viruses and echoviruse. All are spread via fecal-oral route. Hepatitis A is also spread via a fecal-oral route or rarely by the transfusion of infected blood. Hepatitis B also may be acquired via a fecal-oral route, but is more commonly spread via infected blood. Hepatitis B carriers can now be determined by the presence of HBS in their blood.

Herpes simplex type I is a cause of fever blisters on the oral mucous membranes. Other herpesviruses acquired via oral route include cytomegatovirus, a major cause of birth defect, and EB virus, the tiologic agent of infectious mononucleosis and a possible cause of a human malignancy called Burkitt's lymphoma. Rotaviruses are a common cause of infant diarrhea.

Rabies Virus

Rabies is usually caused by the bite of a rabid animal. There is no satisfactory treatment once symptoms have appeared, but individuals can frequently be successfully immunized with killed virus vaccine, during the long incubation period (1-3 months) of the disease.

Prominent symptoms of the disease are headache, nervousness, fever malaise and paralysis. Delirium, convulsions and coma may follow painful spasms of the swallowing muscles cause the patient to fear both food and water which is the basis for the common wave of hydrophobia.

Characteristic inclusion bodies usually are seen in the brain cells of infected animals. These inclusions, called NEGRI BODIES, are ova or globular and approximately the size of a red blood cell.

Control of rabies is based on the elimination of the disease in the wild animal and dog population.

YELLOW-FEVER VIRUS

The yellow fever virus is a very small spherical particle. The agent belongs antigenically to the Group B togaviruses. It can be grown in chick embryos, cell cultures, and certain animals, particularly monkeys. There is only one major antigenic strain of yellow fever virus, so a single vaccine is effective.

Yellow fever is an acute infectious disease that is characterized by severe liver damage. Symptoms include headache, backache, fever, prostration, nausea and vomiting. The disease may be milk or severe. As a result of the liver damage, jaundice may be evidence as early as the fourth or the fifth day of the illness. Following recovery, a person is permanently immune to reinfection. The incubation period is usually three to six days.

Clinical grounds are usually sufficient for diagnosis during periods of epidemics but mild cases may be difficult to diagnose. Various laboratory tests include the inoculation of blood into various animals, particularly mice, and the demonstration of neutralizing antibodies in the patient.

The eradication of *Aedes aegytius* mosquitoes in urban areas is a very effective control measure.

There is a living attenuated vaccine for yellow fever (17D strain virus) which is grown in chick embryos and has proved to be very effective in stimulating active immunity to the disease. There is no specific treatment.

DENGUE FEVER VIRUS

Dengue fever virus is also very small particle which is transmitted from person to person by several species of *Aedes* mosquitoes.

Dengue fever is an acute infection which is usually manifested by headache, backbone, fatique, stiffners, loss of appetite, chillness, and occasionally a rash. Probably the most characteristic symptom is emphasized by another name for this disease i.e. break-bone fever.

The incubation period is usually 5-8 days but seems to be influenced by the amount of infecting virus and may vary from 3 to 15 days. Humans and *Aedes* mosquito are the recognized reservoir and vector for this disease.

Serologic test that are usually as diagnostic aids include complement fixation hemagglutionation and neutralization. The major types of the viruses are adapted for growth in mice, and it is this source that is used for the preparation of the specific antigen.

Control measures are directed toward the elimination of the *Aedes* mosquitoes. A vaccine has been effective in experimental work, but it is not yet available for general use. There is no specific treatment that is effective against the virus.

Envelope – Some viruses are enveloped e.g. Influenza virus

- Chemical property of a virus is dependent on the type of nucleic acid a virus is possessing and the protein coat
- A virus can only possess either of ribonucleic acid (RNA) e.g. *Cucumber mosaic virus* (CMV) genus *Cucumovirus* or deoxyribonucleic acid (DNA) e.g. *Maize streak virus* (MSV).

Other chemical properties include

- Coat protein
- Lipid
- Carbohydrates
- Water
- Others such as possession of metallic ions

Bacteriophages

- Bacteria viruses are known as bacteriophages
- Two types are known
- -the lytic phages
- - the temperate phages
- The lytic types attack the host's cell, distrupt its metabolism and cause lysis of the host cell

- While the temperate phage integrates its nucleic acid into the host's bacteria chromosome in a process called LYZOGENIZATION e.g. Lambda (λ) phage

Viruses and genetic engineering

- The integration of foreign genes into the genomes of a cell is called Genetic engineering
- Some viruses can integrate their genomes into the host's genome e.g. lambda phage
- When this happens, they become latent and get reproduced alongside the host's genome Allowing their nucleic acid to be transmitted to each daughter cell from generation to generation
- This method has been found useful in genetic engineering
- For example, transferring of β-galactosidase gene from lactose positive strain of *Escherichia coli* to the negative strain through specialized transduction (β-galactosidase is needed for assimilation of lactose)

Plant viruses endemic in Africa

- Some of the plant viruses endemic in Africa include:
- *Cowpea aphid-borne mosaic virus* (CAbMV) genus *Potyvirus*
- *Africa cassava mosaic virus* (ACMV)
- *Cucumber mosaic virus* (CMV) genus *Cucumovirus*