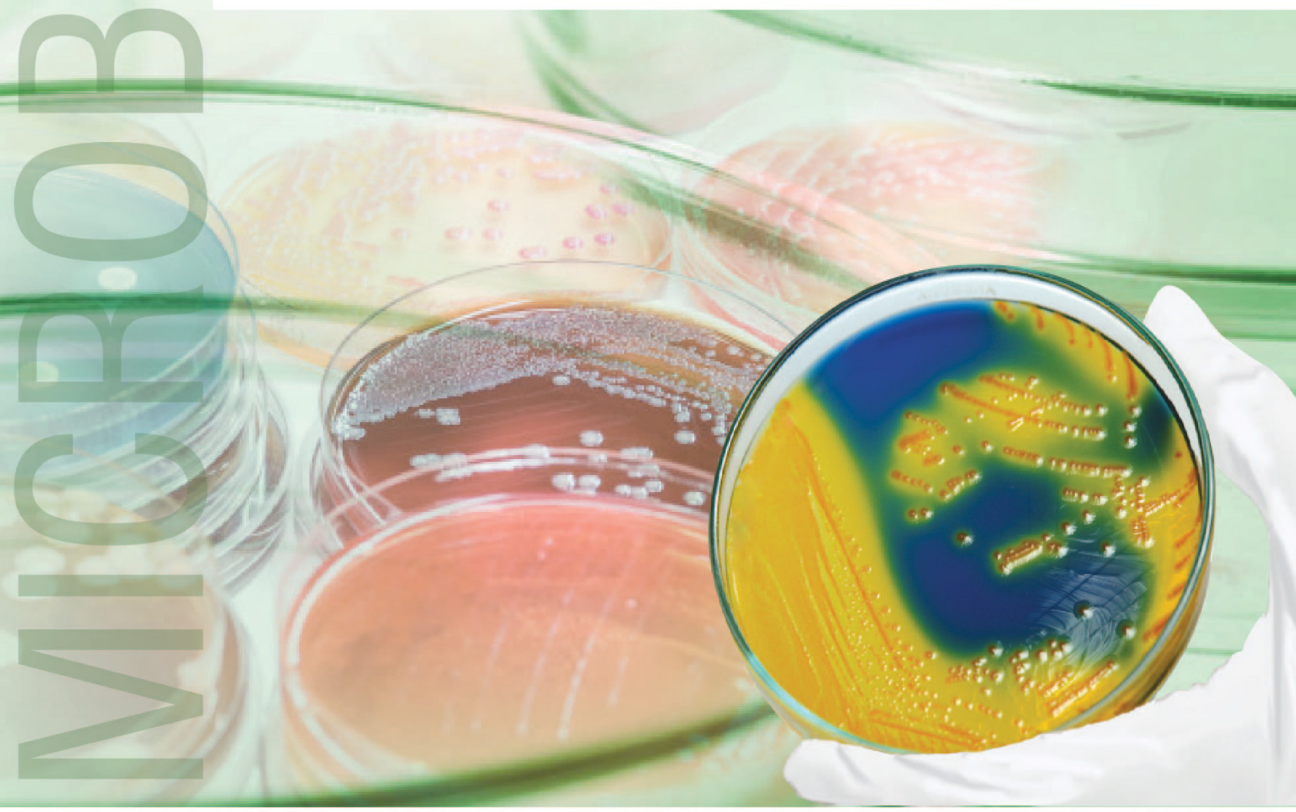
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MICROBIOLOGY

Second MBBS BUSTER MICROBIOLOGY



Deepak Gautam

3rd Edition

JAYPEE

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CHAPTER

4

Virology

LONG QUESTIONS

Q.1. Describe in brief the laboratory diagnosis of viral diseases.

Introduction: Viruses, being obligate intracellular parasites, cannot be grown on any inanimate culture media which makes it difficult in diagnosis or invites some special methods for its diagnosis. The different methods employed for the diagnosis of viral diseases are discussed below:

I. **Collection of the specimen**

The collection of the specimen depends on the clinical presentation of the patient.

- Diarrhea and vomiting—feces
- Vesicular rash—vesicular fluid
- Warty lesions—Molluscum bodies
- Meningitis—CSF
- Systemic infections—Blood and serum

II. **Direct detection of virus, viral antigens or viral genome**

- **Detection of virus**

  The detection of virus can be done by examination of the stained smears prepared from the materials obtained. On the basis of their distinctive appearances, most of the viruses can be assigned to correct family. The following methods can be used for the direct demonstration of the viruses:
  - Electron microscopy
  - Immunoelectron microscopy
  - Fluorescence microscopy
  - Light microscopy

These methods can also be used for the identification of virus isolates in cell cultures.

- **Detection of viral antigens**

  These may be detected by direct and indirect ELISA, radioimmunoassay and latex agglutination tests.
- **Detection of viral genome**

  Enzyme labelled or radiolabelled nucleic acid (DNA or RNA) sequences complementary to unique regions in nucleic acid sequences of most viruses can be

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manufactured commercially and detected. The viral DNA extracted from a very small number of virions or infected cells can also be amplified to the point where it can be readily identified. The following methods are used for the detection of viral genome:

- Nucleic acid probe
- *In situ* hybridization
- PCR

III. **Virus isolation**

The specimen is collected properly and transported with least delay to the laboratory. As most viruses are heat labile, refrigeration is essential during transport. After processing the specimens to remove the bacterial contaminants, it is inoculated into animals, egg or tissue culture. The isolates are identified by neutralization and other suitable serological procedures. The results of isolation are interpreted in the light of clinical data as there are many viruses which are found in normal individual.

IV. **Detection of specific antibody by serological diagnosis**

Demonstration in rise of titer of antibodies to a virus during a course of disease is a strong evidence of that particular viral disease. The paired sera can be collected in two forms:

- 'Acute sample' collected early in the course of disease
- 'Convalescent sample' collected 10–14 days later

The serological techniques employed are neutralization test, CFT, ELISA and hemagglutination test.

V. **Cytological or histological examination of the cells from the site of infection**

Virus-induced histopathology (multinucleated giant cells and inclusion bodies) may be recognized by light microscopy. Tissue section or smear of infected cells is stained with special stains and seen under the light microscope.

Q.2. A boy was bitten by a dog while he was going to school, the dog died within a week. What may be the probable viral infection that may be transmitted through the bite? How do you proceed towards diagnosis? What prophylactic measures will be provided to the boy for prevention of such viral disease?

Presenting complaint: Boy bitten by a dog.

Relevant information: The dog died within a week.

Probable viral infection: The probable viral infection that might have been transmitted by the bite is rabies virus.

Laboratory diagnosis

I. **Collection of the specimen**

- Corneal or conjunctival smears
- Saliva
- Skin biopsy from the face or neck
- CSF and blood
- Brain of the dead dog (along with the hippocampus and cerebellum).

The brain is divided into two portions one of which is preserved in 50% glycerine saline and the other in Zenker's fixative.

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II. **Demonstration of the Negri bodies**
The section or impression smears of brain are stained by Seller's technique. Microscopy reveals intracytoplasmic, oval to round (3–27 mm), eosinophilic cytoplasm with basophilic inner granules called the inclusion bodies (known as negri bodies in case of rabies). These inclusion bodies are mainly seen in pyramidal cells of Ammon's horn, Purkinje cells of hippocampus, brainstem and cerebellum.

III. **Demonstration of rabies antigen**
The rabies antigens can be demonstrated by direct immunofluorescence technique in smears prepared from different specimens using specific antibodies.
- Antemortem—salivary, corneal or conjunctival smears or skin biopsy from nape of neck.
- Postmortem—impression smears of cut surface of salivary gland, hippocampus, brainstem or cerebellum.

IV. **Detection of genomic RNA and viral mRNA**
Genomic RNA and viral mRNA can be detected by PCR or dot-blot hybridization with P-32 labelled circular DNA probes on skin biopsy, corneal impression or saliva.

V. **Isolation of the virus**
The virus can be isolated by following methods:
- **Mouse inoculation:** The collected specimen (saliva, CSF, urine) can be inoculated into the suckling mice. The mouse will die in 7–21 days or is killed after 5 days and the brain smears stained with fluorescent tagged antibody.
- **Isolation in cell culture:** The virus can be grown in following cell cultures:
  - Baby hamster kidney
  - Mouse neuroblastoma
  - Human diploid lung fibroblasts
  - Chick embryo fibroblasts
  - Vero monkey kidney cells

18–24 hours after inoculation the cells are examined. It reveals no cytopathic changes but the rabies antigens can be detected by fluorescent antibody staining.
- **Amplification of rabies virus**
The rabies viral genome can be amplified from fixed or unfixed brain tissue by reverse transcription—PCR technique.

VI. **Serology**
Rabies antibodies can be detected in the serum and serum of the patient by ELISA.

Prophylactic measures

To prevent the boy from acquiring rabies, the following preventive measures are adopted:
- First aid treatment to eliminate the virus from the site of infection by prompt cleansing the wound with plenty of soap and water or solutions or quaternary ammonium compounds.
- Human rabies immunoglobulin 20 IU/kg body weight, half dose administered intramuscularly in the gluteal region and remaining half of the dose infiltrated in around the bite.
- A potent cell culture vaccine, 1 ml of it given intramuscularly in the days 0, 3, 7, 14, 30 and 90 in the deltoid region.
- Adequate tetanus prophylaxis in addition to above.

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Q.3. A child had an attack of fever followed by flaccid paralysis of left lower limb. What is the probable etiological agent? How will you proceed to diagnose the case? What preventive measures will you take to prevent the disease?

Presenting complaint: A child with an attack of fever

Relevant information: Flaccid paralysis of the left lower limb

Inference: Considering the presenting complaint and the relevant information given, the fever is followed by paralysis of the limb suggesting that the child is suffering from poliomyelitis.

Etiological agent: The etiological agent for the illness is poliovirus, an enterovirus.

Laboratory diagnosis

I. **Collection of the material**
- Stool
- Throat swab
- Pharyngeal aspiration (if presented within 3–5 days)

II. **Direct demonstration**
The poliovirus can be demonstrated directly in stool by electron microscopy or immune electron microscopy.

III. **Virus isolation**
The virus can be cultivated in following cell cultures:
- Monkey kidney
- Human amnion
- HeLa
- HEp-2

A cytopathic effect is usually seen within 48 hours which consists of:
- Cell retraction
- Increased refractivity
- Cytoplasmic granularity
- Nuclear pyknosis
- Differentiation of wild virus from live attenuated strain (vaccine virus)

A wild virus can be differentiated from attenuated virus strain by:
- Virulence tests in monkeys
- Nucleic acid hybridization

IV. **Serotyping**
The identification of the serotypes (poliovirus 1, 2 and 3) can be done by neutralization tests.

V. **Serology**
The antibodies against poliovirus can be identified from the paired sera by neutralization tests and CFT.

Preventive measures

The following vaccines are given so as to prevent the disease:
- **Salk's killed polio vaccine:** The vaccine is given by deep subcutaneous or intramuscular injections. Three injections are given in an interval of 6–8 weeks between

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the first and second doses and 4–6 months between second and the third dose. It produces long lasting immunity to all the three poliovirus types.

- **Sabin's live attenuated vaccine (OPV):** The vaccine is administered orally 2 drops each time immediately after birth (if possible) and at 6, 10 and 14 weeks. Booster doses are given at 18 and 24 weeks. It produces immunity against one serotype at a time.

Q.4. A 15-year-old boy presented with mild fever, malaise, loss of appetite and yellowish discoloration of eyes. He has a history of injury 2 months back treated by injections and wound repair in a clinic. What is/are the possible etiological agent? How will you establish the laboratory diagnosis? What prophylaxis could have been offered to prevent the condition?

Presenting complaint: A boy with mild fever, malaise, loss of appetite and yellowish discoloration of eyes.

Relevant information: History of injury two months back which was treated by injections and wound repair.

Inference: On considering the presenting complaint with the relevant information provided (long incubation period), the boy has been suffering from viral hepatitis that had been transmitted via blood (during injections and wound repair).

Possible etiological agents

The possible etiological agents are:

- Hepatitis B virus (with or without hepatitis D virus)
- Hepatitis C virus

Laboratory diagnosis

I. **Collection of the specimen**
   - Blood
   - Liver biopsy material

II. **Detection of immunological markers**

Detection of immunological and viral markers is the mainstay in diagnosis of the disease. The following viral markers are detected in the blood. These can be detected by sensitive and specific tests like ELISA and RIA.

- HBs antigen: Presence of this antigen denotes infection with hepatitis B (present or past).
- Anti-HBs antibody: Presence of this antibody denotes immunity against HBV (post-exposure or vaccination).
- Anti-HBc antibody: Presence of it denotes recent (acute) infection if the antibody is of IgM type, and post (chronic) infection if the antibody is of IgG type. Here the boy is giving a history of injury of 2 months back so presence of IgM anti-HBC antibody is likely.
- HBe antigen: Presence of this antigen denotes high infectivity.
- Anti-HBe antibody: Presence of this antibody denotes low infectivity.
- Anti-HCV antibody: Presence of this antibody denotes infection with hepatitis C virus.

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- Detection of HBc antigen (core antigen): It can be detected in the nuclei of hepatocytes (liver biopsy material) by orcein stain, presence of which denotes infection with HBV.

III. Detection of viral genomes

The viral genomes can be detected in the blood or the biopsy specimens. The newer and sophisticated techniques like PCR, IF and *in situ* hybridization are employed in the diagnosis.

- HBV DNA
- HCV RNA

IV. **Liver function tests (indirect evidences)**

- **Blood**
  - Bilirubin: Increased
  - SGOT: Raised
  - SGPT: Raised
  - Alkaline phosphatase: Raised
  - Total protein: Decreased
  - Albumin: Globulin ratio: Altered
  - PT and aPTT: Prolonged
- **Urine**
  - Urobilinogen: Present in high amount
  - Bilirubin: Present (conjugated type).

Prophylaxis

The following prophylactic measures could have been offered so as to prevent the condition:

- Use of sterile disposable syringes while giving the injections.
- Use of sterile needles by the medical personnels during the wound repair.
- Passive immunization with hepatitis B immunoglobulin (HBIG) soon after the injections and wound repair (preferably within 48 hours). It is given in the dose of 300–500 IU intramuscularly. A second dose is usually given 4 weeks after the first dose.
- Active immunization with hepatitis B vaccine (plasma-derived or recombinant yeast extract) alone or in combination with HBIG. A total course of three doses is given at 0, 1 and 6 months, intramuscularly in the deltoid region.

Q.5. An epidemic of high fever with severe headache and progressive drowsiness within a few days of illness in several areas in the district of Burdwan has started. Lumbar puncture in such cases shows clear CSF. Name the probable diagnosis and the etiological agent. Write down the mode of spread and laboratory diagnosis of such cases.

Presenting complaint: An epidemic of high fever with severe headache and progressive drowsiness.

Relevant information

- Lumbar puncture showed clear CSF
- The epidemic occurred in the district of Burdwan

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Probable diagnosis
On considering the presenting compliant and the relevant information with the region affected (i.e. Burdwan district), it might be an epidemic of Japanese encephalitis.

Etiological agent
It is caused by an encephalitis virus, Japanese B encephalitis virus, an arbovirus which is most common in the Burdwan district (West Bengal).

Mode of spread
It is transmitted by the biting of the infected mosquito *Culex tritaeniorhynchus* that breeds in the irrigated rice fields. It acts as an amplifying vector and transmits the virus to man from water birds and pigs.

Laboratory diagnosis

I. **Collection of the specimen**
- Specimen of blood and CSF collected during the early acute phase of the disease
- Brain tissue of the patients died of the disease

II. **Examination of CSF**
- Physical
  - Pressure: Normal or increased
  - Appearance: Clear (given)
- Cytology
  - Mostly lymphocytes 10–500/ cu mm
- Biochemistry
  - Sugar: Normal
  - Protein: Normal to slightly raised (60–120 mg%)

III. **Isolation of the virus**
The collected specimen can be subjected to grow in susceptible tissue cultures or in egg embryos. The virus can be identified by:
- Neutralization test
- Hemagglutination inhibition test with standard antisera to determine the group

IV. **Serology**
Paired sample of the sera showing rising titer (≥ 4 fold rise) of hemagglutinating and neutralizing antibody is diagnostic.

V. **Animal pathogenicity**
The brain tissue of dead patients when inoculated intracerebrally into suckling mice, the animal will develop fatal encephalitis.

Q.6. A six-year-old boy is brought to the pediatric OPD with complaints of fever, rhinitis and cough. On examination, there are characteristic maculopapular rashes over the forehead, neck and the chest as well as back. His mother says that he has not received any vaccines after he had last at 6 months of age. What is your provisional diagnosis? Name the etiological agent. How will you diagnose the case? How could have been this disease prevented?

Presenting complaints: A six-year-old boy with fever, rhinitis and cough.

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Relevant information

- Presence of characteristic maculopapular rashes over the forehead, neck, chest and back
- Nonreceipt of any vaccines after 6 months of age

Provisional diagnosis: On considering the presenting complaints and the relevant information given, the boy is probably having viral rashes most likely measles.

Etiological agent

The disease is caused by measles virus, a morbillivirus (genus) of the family paramyxoviridae.

Diagnosis

The diagnosis of the case is done on the following basis:

A. **History**

The patient had not received any vaccines since 6 months of age, i.e. he is deprived of measles vaccine which is given at the age of 9 months in India.

B. **Clinical features**

The diagnosis of measles is almost clinical—characteristic maculopapular rashes over the forehead, neck, chest and the back. The rashes first appear on the neck and then spread to rest of the body. This characteristic appearance of the rash is seen in measles.

C. **Laboratory diagnosis**

I. **Collection of the specimen**

- Nasal secretions
- Throat washings
- Nasopharyngeal aspirate
- Blood
- Urinary sediment
- Lymphoid tissue biopsy

II. **Smear preparation and staining**

Smears are prepared form the nasal secretions and the other materials collected above and stained with Giemsa's stain. It reveals multinucleated giant cells and intranuclear/intracytoplasmic inclusion bodies.

III. **Detection of virus antigen**

Viral antigen can be directly detected in the specimens collected by using specific fluorescent tagged antibody.

IV. **Isolation of the virus**

The virus can be isolated by cultures in following tissue cultures:

- Monkey kidney
- Primary human embryo kidney
- Human amnion cells

The appearance of multinucleated giant cells containing numerous acidophilic inclusions in cytoplasm and nuclei in the cultured cells suggests the presence of

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measles virus. Final confirmation is done by immunofluorescent staining with monoclonal antibody.

V. **Serology**
Measles antibody in the patient's serum can be detected by IgM capture ELISA. A rise in antibody titer is diagnostic. CFT test can be carried out on acute and convalescent sera.

Prevention of the disease
This disease could have been prevented by following vaccines:
- Measles vaccines at the age of 9–12 months, or
- MMR vaccine at the age of 15 months followed by the booster dose at the age of 4–6 years.

SHORT QUESTIONS

Q.1. Classify viruses.

Introduction: On the basis of the presence of the genetic material either DNA or RNA, viruses are classified as follows:
- **DNA viruses**
  - Herpes—Herpes simplex, varicella, EBV, CMV
  - Adenoviruses—Mastadeno virus, Aviadeno virus
  - Hepadnaviruses—Hepatitis B virus
  - Poxvirus—Variola virus, Vaccinia virus
  - Papovavirus—Papilloma virus, Polyoma virus
  - Parvovirus—Parvovirus, Adenosatello virus
- **RNA viruses**
  - Togaviruses—Alphavirus, flavivirus, rubivirus, pestivirus
  - Reovirus—Reovirus
  - Rhabdovirus—Rabies virus
  - Retrovirus—HIV virus
  - Orthomyxovirus—Influenza virus
  - Picornavirus—Poliovirus, coxsackie virus, echovirus, rhinovirus
  - Paramyxovirus—Parainfluenza virus, measles, mumps
  - Arenavirus—Lassa virus
  - Astrovirus—Astrovirus
  - Coronavirus—Human murine virus, avian murine virus, calicivirus
  - Flavivirus—Flavivirus
  - Filovirus—Filovirus

Q.2. What are the fundamental differences between bacteria and viruses?

The fundamental differences between the bacteria and viruses are as follows:

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Points	*Bacteria*	*Viruses*
1. Size	In micrometer	In nanometer
2. Growth on nonliving media	+	–
3. Independent metabolism	+	–
4. Cellular organism	+	–
5. Binary fission	+	–
6. Nuclear material	DNA, RNA	Either DNA or RNA
7. Ribosomes	+	–
8. Sensitivity to antibiotics	+	–
9. Nucleic acid	Noninfectious	Infectious
10. Sensitivity to IFN	–	+

Q.3. How does pulse polio help in the eradication of polio infection?

Routine immunization alone cannot eradicate poliomyelitis since outbreaks have been reported in countries even with good coverage. This is because of clusters of unimmunized or incompletely immunized children and lower rate of seroconversion in the endemic countries like India. So a newer activity is needed to supplement it. Pulse polio immunization (synonymous to mass campaigns) is helpful for it. Here all the target children, i.e. up to 5 years are immunized in 1 to 3 days irrespective of the previous immunization status so as to cover the deficits. It helps in the eradication of the disease in following ways:

- By flooding the environment with vaccine virus so that the probability of encountering the wild virus is low.
- By rapidly increasing the population immunity.
- By increasing the rate of seroconversion.

SHORT NOTES

Q.1. Modes of infection in viral diseases.

There are different modes of transmission of viral diseases which are as follows:

I. **Respiratory tract**

- Respiratory viruses
  - ➢ Influenza virus A, B, and C
  - ➢ Parainfluenza virus 1 and 4
  - ➢ Respiratory syncytial virus (RSV)
  - ➢ Rhinovirus
  - ➢ Corona virus
  - ➢ Coxsackie virus
  - ➢ Adeno virus
- Systemic virus
  - ➢ Mumps, measles, rubella
  - ➢ Varicella zoster virus
  - ➢ Cytomegalovirus (CMV)
  - ➢ Epstein-Barr virus (EBV)

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II. **Alimentary tract**
- Enteroviruses
  - Polio
  - Hepatitis A virus
  - Adeno virus
  - Norwalk and related virus
  - Corona virus, astro virus, echo virus, coxsackie virus A and B

III. **Skin**
- Abrasions—Papilloma, molluscum contagiosum, herpes simplex virus, hepatitis B
- Insect bite—Arboviruses
- Animal bite—Rabies, hepatitis B
- Injection—Hepatitis B, hepatitis C, HIV 1 and 2, EBV, CMV, Ebola virus

IV. ***Genital tract*—**Papilloma, HSV, HIV, HBV, HCV, HTLV

V. ***Conjunctiva***—Adeno virus, measles

VI. ***Transplacental*—**Rubella, CMV, Avian leukosis virus

VII. ***Breast milk*—**Murine mammary tumor virus

Q.2. Inclusion bodies.

Introduction: A characteristic morphological change in cells infected with certain viruses is the formation of inclusion bodies within the cells which can be seen by light microscope after fixation and staining.

Composition of inclusion bodies: It consists of aggregates of products of viral replication such as virus particles ready for release, overproduced particular viral proteins and some aberrant cellular structure.

***Types*:** On the basis of the site inclusion bodies are formed in, they are of following types:

- **Cytoplasmic inclusion bodies:** Mainly RNA viruses yield in racytoplasmic inclusion bodies.
  Examples:
  - Rabies virus—Negri bodies
  - Vaccinia virus—Guarnieri bodies
  - Fowl pox virus—Bollinger bodies
  - Molluscum contagosium virus—Molluscum bodies
  - Paramyxovirus and reovirus
- **Intranuclear inclusion bodies:** Mainly DNA viruses yield intranuclear inclusion bodies.
  Examples:
  - Herpes virus
  - Adeno virus
  - Parvo virus
- **Both intracytoplasmic and intranuclear inclusion bodies**
  Some viruses like measles and CMV produce both intracytoplasmic and intranuclear inclusion bodies.

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Inclusion bodies can also be classified on the basis of number (single or multiple), size (large or small) and shape (round or irregular).

Stain: Inclusion bodies can be stained by Eosin and Hematoxylin stains (E and H stains). Accordingly they may be:

- Acidophilic (stained by eosin)
- Basophilic (stained by hematoxylin)

Q.3. Tissue culture

Introduction: Viruses are obligate intracellular parasites. They cannot be grown on any intimate culture medium. Tissue culture is employed for the cultivation of viruses. Three types of tissue cultures are available as follows:

- **Organ culture:** Small bits of organs are maintained *in vitro* preserving their original architecture and function.
  Example: Tracheal ring organ culture employed for the isolation of coronavirus (respiratory pathogen).
- **Explant culture:** Here the minced tissue are grown as explants embedded in plasma clots.
  Example: Adenoid tissue explant culture employed for the isolation of adenoviruses.
- **Cell culture:** It is the widely used method. The tissues are dissociated into component cells by the action of proteolytic enzymes such as trypsin and mechanical shaking. The essential constituents of growth like essential amino acids, vitamins, glucose, salts, buffers (bicarbonates and 5% carbon dioxide) are added in physiological amounts. Antibiotics may be added. There are three types of cell cultures:
  - **Primary cell culture:** These are normal cells freshly taken from body and cultures. They have the capability of only limited growth. *Examples:* Monkey kidney, Human embryonic kidney, human amnion, chick embryo cell.
  - **Diploid cell culture:** These are cells of a single type that retain the original diploid chromosome number and karyotype during serial subcultivation for a limited number of times. *Example:* Human fibroblasts.
  - **Continuous cell culture:** These are cells of single type (usually derived from cancerous cells) that are capable of continuous indefinitely. *Examples:* HeLa, Hep-2, KB cell lines.

Q.4. Interferons (IFN).

Introduction: These are a family of natural glycoproteins induced by many viruses, some bacteria and protozoa as well which render the cells resistant to infection. These act as regulatory proteins inhibiting the viral replication.

Classification

- **IFN-α:** These are produced by leukocytes following viral infections. It has got 20 subtypes.
  Function: It has got antiviral activity by inhibiting protein synthesis.
- **IFN-β:** These are produced by fibroblasts and epithelial cells following viral infections. It has got only one subtype.

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Deepak Gautam MBBS MS (Ortho, AIIMS) is an Assistant Professor of Orthopedics at All India Institute of Medical Sciences (AIIMS), New Delhi, India. He did his master of Surgery in Orthopedics from AIIMS, New Delhi. He did his fellowship in Computer Assisted Navigated Hip and Knee Arthroplasty from Pius-Hospital, Oldenburg, Germany. He was an Asia Pacific Orthopaedic Association Joint Replacement Fellow, Thailand (2015); Synthes Trauma Fellow, South Korea (2016), and Johnson and Johnson Travelling Fellow of Indian Orthopedic Association (2016). He is a talented writer, editor of 3 books and 14 chapters in various books. He is an author of over 40 articles, 28 abstracts and 25 posters. He worked as a Research Associate in the Department of Orthopedics at AIIMS, New Delhi (2013–2016). He worked as a Clinical Research Fellow, India Alliance at AIIMS, New Delhi.

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