Virology

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## Reference Books

- 1. Introduction to Modern Virology (7<sup>th</sup> Ed.) Wiley-Blackwell Publication
- 2. Principles and Practices of Clinical Virology (5th Ed.) Wiley-Blackwell Publication
- **3. Basic Virology (3rd Ed.)** Wiley-Blackwell Publication
- 4. Clinical Virology Manual (4th Ed.) ASM Press
- 5. Fundamentals of Molecular Virology (2nd Ed.) By Nicholas H. Acheson
- 6. Introduction to Modern Virology (6th Ed.) Blackwell Publishing

# Laboratory Diagnosis of Viral Diseases

#### Q. State the methods of laboratory diagnosis of viral diseases.

**Methods of laboratory diagnosis of viral diseases:** There are five approaches to the diagnosis of viral diseases by the use of clinical specimens:

- 1. Identification of the virus in cell culture.
- 2. Microscopic identification directly in the specimen.
- 3. Serologic procedures to detect a rise in antibody titer or the presence of IgM antibody.
- 4. Detection of viral antigens in blood or body fluids.
- 5. Detection of viral nucleic acids in blood or the patients' cells.

#### Specimen collection:

- 1. Blood
- 2. Sputum
- 3. Nasal swab
- 4. Throat washings
- 5. Stool, urine, CSF
- 6. Biopsy
- 7. Autopsy materials

**Identification in cell culture:** Viruses can be cultured in cell culture media and detected by seeing cytopathic effect (CPE). CPE is not specific, i.e., many viruses cause it.

**Microscopic identification:** Viruses can be detected and identified by direct in pseudo pictual minimation of clinical specimens by three procedures:

- 1. **Light microscopy:** Reveals characteristic incluies a locies or multinucleated giant cells.
- 2. UV microscopy: Used for fluorescere ant budy staining of the visited in fected cells.
- 3. Electron microscopy: Detects virus particles.

### Serological techniques or Detection of antibodies

- A use in the titer of antibody the virus can be used to diagnose current infection.
- Presence of IgM can be used to diagnose current infection.
- Presence of IgG indicates either current infection or past infection or vaccination.
- An antibody titer that is fourfold or greater in the convalescent serum sample compared to the acute serum sample can be used to make a diagnosis.
- Antibody titer can be determined by CIE, RIA, ELISA immunodiffusion test.

#### Detection of viral antigens:

• Viral antigens are detected by ELISA, RIA. For example, p24 of HIV and hepatitis B surface antigen are commonly used in diagnosis.

#### Detection of viral nucleic acids:

- Detection of viral DNA or RNA is increasingly becoming the 'gold standard' in viral diagnosis.
- Labeled probes are highly specific and results are rapidly obtained.
- An important example is the 'viral load' assay of HIV RNA.

#### Q. Name three viral culture media.

Viral culture media: Cell cultures are of 3 types:

1. **Primary cells:** Prepared directly from animal or human tissues and can be subcultured only once or twice e.g., primary monkey or baboon kidney.

Characteristics	Killed vaccine	Live vaccine
1. Number of doses	Multiple	Single
2. Need for adjuvant	Yes	No
3. Duration of immunity	Shorter	Longer
4. Effectiveness of protection (more closely mimics natural infection)	Lower	Greater
5. Immunoglobulins produced	IgG	IgA and IgG
6. Mucosal immunity produced	Poor	Strong
7. Cell mediated immunity produced	Poor	Strong
8. Residual virulent virus in vaccine	Possible	No
9. Reversion to virulence	No	Possible
10. Excretion of vaccine virus and transmission of non-immune contacts	No	Possible
11. Interference by other viruses in host	No	Possible
12. Stability at room temperature	High	Low
13. Interruption of transmission of virulent virus	Less effective	More effective

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HHV-8	Uncertain	Uncertain	Kaposis's	Sexual or organ transplantation.
			sarcoma	

#### Q. Classify herpes viruses with diseases produced by them.

Herpes viruses	Diseases
1. Herpes simplex virus type-1 (HSV-1)	Gingivo-stomatitis
	Recurrent herpes labialis
	Keratoconjunctivitis
	• Encephalitis
	Genital ulcer
	Finger infections (whitlows)
	Eczema herpeticum
2. Herpes simplex virus type-2 (HSV-2)	Genital herpes
	Neonatal herpes
	Cervical and vulvar carcinoma
	• Encephalitis, acute meningitis or transverse
	myelitis.
3. Varicella Zoster virus (VZV)	• Varicella (chicken pox) is the primary disease
	of children.
	• Zoster (Shingles) – recurrent form in adults.
4. Cytomegalovirus (CMV)	• Cytomegalic inclusion disease (especially
	congenital abnormalities) in neonates.
	• Infectious mononucleosis (heterophile
	antibody-negative)
	• Hepatitis
	• Disease in immunocompromiser patients:
	retinitis, encephantis, preumonitis, nepatitis,
5 Epstein Barr virus (EBV)	
5. Epstelli ball vilus (EDV)	Precticus mononucleosis
	Durkin s ly bhoma
SrOW '	• Natophe v graf carcinoma
	ther lumphomas
6 Human Carbo Jury 6	Exapthema subitum, shildhood disease with fever and
	skin rashes
7. Human herpes virus 7	Latest, non-pathogenic and may be helpful against
	HIV.
8. Human herpes virus 8	Kaposi's sarcoma
	Primary effusion lymphoma
	• Multicentric Castleman's disease.

#### Q. Why repeated reactivation occurs in Herpes virus infection?

Herpes viruses establish latent infections mostly in the sensory ganglia. In these sites of latent infection, immune system cannot attack and destroy them. As a result, herpes viruses persist indefinitely in infected hosts and are frequently reactivated in immunosuppressed hosts.

#### Q. Name the herpes viruses associated with malignancy.

#### Herpes viruses associated with malignancy:

Herpes viruses	Malignancy
1. Herpes simplex virus type-2 (HSV-2)	• Cervical and vulvar carcinoma.
2. Epstein Barr virus	<ul><li>Burkit's lymphoma</li><li>Nasopharyngeal carcinoma</li><li>Other lymphomas</li></ul>
3. Human herpes virus 8	Kaposi's sarcoma

4. PCR: PCR is the most sensitive test for HSV diagnosis.

Treatment: Drugs effective against HSV infections include acyclovir, valacyclovir and vidarabine.

#### **Prevention:**

- 1. Cesarean section is recommended for women who are at term and who have genital lesions or positive viral cultures.
- 2. Circumcision reduces the risk of infection by HSV-2.

#### Q. How can you differentiate between HSV-1 and HSV-2?

#### Differentiation between HSV-1 and HSV-2:

- HSV-1 and HSV-2 are structurally and morphologically indistinguishable. They can however, be differentiated by the restriction endonuclease patterns of the genome DNA and by type-specific monoclonal antisera.
- HSV-1 and HSV-2 are distinguished between by two main criteria: Antigenically and location of lesions. Lesions caused by HSV-1 are, in general, above the waist, whereas those caused by HSV-2 are below the waist.

#### Comparison of diseases caused by HSV-1 and HSV-2:

Site	Disease caused by HSV-1	Disease cause by HSV-2
Skin	Vesicular lesions above the waist.	Vesicular lesions below the waist
		(especially genitals).
Mouth	Gingivostomatitis	Rare
Eye	Keratoconjunctivitis	Rare
Central nervous system	Encephalitis	Rare
Neonate	Rare <sup>1</sup>	Skin lesions, encipalitis and
		disseminated infection
Dissemination to viscera and	Yes	Rate
immunocompromised patients	late	
<sup>1</sup> Infection acquire after birth from HSV-1 – infected period		
<sup>2</sup> Infection acquired during passage through in heanal. <b>Preve Page 27 0 8 0</b>		

Varicella Zoster Virus

Disease: Varicella (chicken pox) is the primary disease; zoster (shingles) is the recurrent form.

#### Q. Hoe chicken pox spread?

Transmission of chicken pox: In case of chicken pox/varicella, the virus is transmitted by -

- Respiratory droplets
- By direct contact with lesion.

#### Q. Write down the pathogenesis of varicella or chicken pox:

Viruses infect the mucous membrane of the upper respiratory tract or the conjunctiva.

Initial replication in the regional lymph nodes.

↓ Primary viremia

Replication in liver and spleen

Secondary viremia involving infected mononuclear cells transport virus to skin, where the typical rash develops.  $\downarrow$ 

# Parvoviruses

#### Human diseases associated with B19 Parvovirus:

- 1. Erythema infectiosum (slapped cheek syndrome, fifth disease)
- 2. Transient aplastic crisis
- 3. Pure red cell aplasia
- 4. Hydrops fetalis

#### **Important properties:**

- 1. Parvovirus B19 is a very small non-enveloped virus with single stranded DNA genome.
- 2. Capsid: Icosahedral.
- 3. There is no serotype.

#### Transmission:

- 1. B19 virus is transmitted primarily by the respiratory route.
- 2. Transplacental transmission.
- 3. Blood transfusions.

**Reservoir of infection:** Humans are the natural reservoir; animals are not a source of human infection.

#### Pathogenesis:

- B19 virus primarily infects two types of cells: red blood precursors (erythroblasts) in the type marrow, which accounts for the aplastic anemia and endothelial cells in the blood vessel. Wich accounts, in part, for the rash associated with erythema infectiosum.
- Immune complexes composed of virus and IgM or IgG pathwest the pathogenesis of the rash and arthritis.
- Hydrops fetalis manifests as massive edge a of the retus.

Immunity: Infection provides lifting inhunity against pil fectio

### Laborator u a me

- 1. Fifth disease and aplastic anemia are usually diagnosed by detecting IgM antibodies.
- 2. B19 virus can be isolated from throat swabs, but this is not usually done.
- 3. In immunocompromised patients, viral DNA in the blood can be assayed by PCR.
- 4. Fetal infection can be determined by PCR analysis of amniotic fluid.

#### Treatment and prevention:

- There is no specific treatment of B19 infection.
- Pooled immune globulins for chronic B19 infection in patients with immunodeficiencies.
- There is no vaccine or chemoprophylaxis.

# Orthomyxoviruses (Influenza Viruses)

The Orthomyxoviruses (influenza viruses) are a major determinant of morbidity and mortality caused by respiratory disease. Influenza viruses are important human pathogens because they cause both outbreaks of influenza that sicken and kill thousands of people each year as well as infrequent devastating worldwide epidemics (pandemics).

#### Q. What are the myoxviruses? Why are they so called?

Myxoviruses include Orthomyxoviruses and Paramyxoviruses. Myxo means mucins. These viruses attack mucin layer. So they are called myxo viruses.

#### Q. Classify Orthomyxoviruses.

Classification of Orthomyxoviruses: Influenza viruses are the only members of the Orthomyxovirus family. Influenza virus has three immunologic types:

Influenza virus	Diseases	
1. Influenza A	Worldwide epidemics (pandemics) of influenza.	
2. Influenza B	Major outbreaks of influenza.	
3. Influenza C	Mild respiratory tract infections but does not cause outbreaks of influenza.	

- Q. What are the important characteristics of Orthomyxoviruses? esale.co.uk Important properties of Orthomyxoviruses: 1. Virion: Spherical of nucleocapsid 1. Virion: Spherical, pleomorphic, felo
  - 2. Genome: Single strand, VINA, segmented (eight n O ecules), negative sense.

  - Proteins: A proteins, one monotorial.
     Enclore: Contains viral hemorgal (tange HA) and neuraminidase (NA) proteins.
  - 5. replication: Nuclear transcription.
  - 6. Outstanding characteristics:
    - Genetic re-assortment common among members of the same genus. ٠
    - Influenza viruses cause worldwide epidemics.

#### Q. Mention the comparisons between Orthomyxoviruses and Paramyxoviruses.

#### Comparisons between Orthomyxoviruses and Paramyxoviruses:

	Properties	Orthomyxoviruses	Paramyxoviruses
1.	Viruses	Influenza A, B and C viruses.	Measles, mumps and respiratory
			syncytial and parainfluenza viruses.
2.	Genome	Segmented (eight pieces) single	Non-segmented single stranded RNA of
		stranded RNA of negative polarity.	negative polarity.
3.	Viral RNA	Yes	Yes
	polymerase		
4.	Capsid	Helical	Helical
5.	Envelope	Yes	Yes
6.	Size	Smaller (110 nm)	Larger (150 nm)
7.	Surface spikes	Hemagglutinin and neuraminidase in	Hemagglutinin and neuraminidase on
		different spikes.	the same spike.
8.	Giant cell formation	No	Yes
9.	Antigenic stability	Unstable	Stable

children prior to 9 months of age, because maternal antibody in the child can neutralize the virus and reduce the immune response.

#### O. A girl of 9 years old presented with rash in the whole body. She had fever for last few days. What would be your differential diagnosis?

#### Differential diagnosis of fever with rash:

- 1. Viral exanthems (rubella, measles, varicella, mumps, human herpes virus 6, enteroviruses).
- 2. Chikungunya
- 3. Dengue
- 4. Spotted or typhus group rickettsiosis.
- 5. Typhoid fever
- 6. Parvovirus
- 7. Meningococcal meningitis.

### **Mumps Virus**

Disease: The virus causes mumps, a systemic viral infection characterized by parotid gland swelling. It occurs primarily in childhood (5-9 years of age).

Transmission: Mumps virus is transmitted via respiratory droplets.

**Pathogenesis:** The virus infects the upper respiratory tract and then spreads through the blood to infect the parotid glands, testes, ovaries, pancreas and in some cases, meninges. Alternatively, the virus may ascend from the buccal mucosa up Stensen's duct to the parotid gland.

Immunity: Infection of mumps gives lifelong immunity. Mumps occur only once. Maternal artico placenta and provides protection during the first 6 months of life.
Clinical findings:

Incubation period: 18 to 21 days. passes the

- 2. Fever, malaise and anorexia.
- 3. Tender swelling of the way tid glands, either unifitera
- 4. Characteristic il cocose in parotid pain when drinking citrus juices.
- The ista e is typically benig the s spontaneously within 1 week. 5.

Q. Mention the complications of mumps.

#### **Complications of mumps:**

Frequent	Rare
<ul> <li>Orchitis</li> <li>Oophoritis</li> <li>Pancreatitis</li> <li>Abortion</li> </ul>	<ul> <li>Bilateral orchitis leading to sterility</li> <li>Nerve deafness</li> <li>Arthritis</li> <li>Labyrinthitis</li> <li>Myocarditis</li> <li>Meningitis</li> <li>Encephalitis</li> <li>Hydrocephalus</li> </ul>

Laboratory diagnosis: The diagnosis of mumps is usually made clinically, but laboratory tests are available for confirmation.

- 1. The virus can be isolated from cell culture from saliva, spinal fluid or urine.
- 2. A fourfold rise in antibody titer in either the hemagglutination inhibition or CFT is diagnostic.
  - Antibody to S antigen indicates current infection.
  - Antibody to V antigen indicates past infection.

#### Main reservoirs of rabies virus:

- 1. Dogs (99% cases in Bangladesh).
- 2. Mongoose
- 3. Jackal
- 4. Fox
- 5. Wolf
- 6. Cat
- 7. Hyena
- 8. Vampire bat

#### Q. Mention the condition on which the incubation period of rabies depends.

Incubation period of rabies: The incubation period (IP) varies, according to the location of the bite, from as short as 2 weeks to 16 weeks or longer. It is shorter when bites are sustained on the head rather than on the leg, because the virus has a shorter distance to travel to reach the central nervous system.

#### Nice to know:

Clinical findings: The disease is an acute, fulminant, fatal encephalitis. The clinical spectrum ca be divided into three phases:

- 1. A short prodromal phase (2 10 days): Malaise, anorexia, headache, photophobia, nausea and vomiting, sore throat, fever, abnormal sensation around the wound site.
- 2. An acute neurologic phase (2 7 days): Signs of nervous system dysfunction such as a nervousness, apprehension, hallucinations and bizarre behavior. General sympathetic over activity is observed, including lacrimation, pupillary dialatation, increased salivation and perspiration. The act of swallowing precipitates a painful spasm of the throat muscles. This results in hydrophobia (fear of water). There may be aerophobia (fear when feeling a breeze).
- 3. Coma: Convulsive seizures or coma and death. The major cause of death is risplant
  Q. Give a laboratory diagnosis of rabies.
  Laboratory diagnosis of rabies:
  Rabies in animals can be diagnosed by: paralysis.

- nipation of train tissue by using call of fluorescent antibody to rabies virus or histologic staining of it posts in the hippocar rate of note. Examination 1.
- The virus can be isolated from the animal brain by growth in the cell culture, but this takes too long to be 2 useful in the decision of whether to give the vaccine.

#### Rabies in humans can be diagnosed by-

- 1. Fluorescent antibody staining of a biopsy specimen, usually taken from the skin of the neck at the hairline.
- 2. Isolation of the virus from sources such as saliva, spinal fluid and brain tissues.
- 3. A rise in titer of antibody to the virus.
- 4. Negri bodies can be demonstrated in corneal scrapings and in autopsy specimens of the brain.

#### Q. What do you mean by a street virus?

Street virus: Freshly isolated virulent virus from rabid animals in the laboratory is called street virus.

#### Criteria:

- 1. The virus is excreted in the saliva of the affected animals. The virus, as it occurs in nature is known street virus
- 2. The virus can also be isolated from human cases. '
- 3. It is pathogenic to all animals.
- 4. It can multiply in the neuronal and extra-neuronal tissues.
- 5. It has long and variable incubation periods (usually 21 60 days in dogs).
- 6. It regularly produces intracytoplasmic inclusion bodies.

#### Q. What do you mean by a fixed virus?

A. An early acute stage: Usually begins 2 to 4 weeks after infection. Fever, lethargy, sore throat and generalized lymphadenopathy occur. A maculopapular rash on the trunk, arms and legs (but sparing the palms and soles) is also seen.

#### **Characteristics:**

- 1. Leukopenia
- 2. CD4+ cell count is usually normal.
- 3. A high level viremia typically occurs.
- 4. The infection is readily transmissible.
- 5. Typically resolves spontaneously in about 2 weeks.
- 6. Antibodies to HIV typically appear 10 to 14 days after infection.
- B. A middle latent stage: In the middle stage, a long latent period, measured in years, usually ensues. In untreated patients, the latent period usually lasts for 7 to 11 years.

### **Characteristics:**

- 1. The patient is asymptomatic during this period.
- 2. Viremia is low or absent.
- 3. A large amount of HIV being produced by lymph node cells but remains sequestered within the lymph nodes. As a result, viremia is low.
- 4. AIDS related complex (ARC) can occur during the latent period.
- **C.** A late, immunodeficiency stage: The late stage of HIV infection is AIDS, manifested by a decline in the number of CD4+ cells to below  $400/\mu$ L and an increase in the frequency and severity of opportunistic infections.

#### Q. Write short note on: AIDS related complex.

AIDS related complex (ARC): A syndrome called AIDS related complex can occur during the latent period of HIV infection. ARC often progresses to AIDS.

Cause of ARC: ARC is caused by damage to the immune system, not by the opportunistic infection and cancers associated with AIDS.

Clinical features of ARC:

1. Unexplained diarrhea lasting longer than emorph
2. Fatigue.
3. Malaise.
4. Loss of more that opercent body weight
5. Fave.

- 5. Fare.
- 6. Night sweats.
- Other middle opportunistic infections such as oral thrush, generalized lymphadenopathy or enlarged 7. spleen.

Patients from high risk groups who have two or more of these manifestations (typically including generalized lymphadenopathy), and who have a decreased number of T helper lymphocytes are considered to have AIDS related complex.

#### Nice to know:

**Opportunistic infections in AIDS:** The predominant causes of morbidity and mortality among AIDS patients are opportunistic infections, i.e., severe infections induced by agents that rarely cause serious disease in immune competent individuals. Opportunistic infections usually do not occur in HIV infected patients until CD4+ T cell counts have dropped from the normal level of about 1000 cells  $\mu$ L to less than 200 cells/ $\mu$ L.

Causes of opportunistic infections in AIDS: HIV infects CD4+ T cells. As a result, there is loss of T cell response to form cytotoxic T cell and proliferation and differentiation of B cells. So both the cell mediated and antibody mediated immunity is lost. For this reason, opportunistic pathogens cause severe disease.

#### Q. Write down the laboratory diagnosis of AIDS.

Lab diagnosis of AIDS:

Principle: AIDS is diagnosed by serological test for antibody detection against the HIV. Nucleic acid based techniques are used in special cases.

Specimen collection: Blood for serological test and culture.

#### **Procedure:**

- 1. Serological tests or detection of antibody:
  - ELISA: ELISA detects anti HIV antibodies and gives presumptive diagnosis of HIV infection. There are some false positive results with this test.
  - Western blot: The most widely used confirmatory test is the Western blot technique, which detects antibodies to HIV proteins including p24, gp41, gp120 or gp160.

(Most individuals with HIV infection will have detectable antibodies within 6-12 weeks after infection, whereas virtually all will be positive within 6 months. HIV infection for longer than 6 months without a detectable antibody is very uncommon.)

- 2. Detection of viral RNA by PCR: PCR detects viral RNA and is very sensitive and specific. PCR also can detect viral RNA load which is an important marker of disease progression and prognosis.
- 3. **Isolation of virus:** HIV can be cultured from lymphocytes in peripheral blood culture for research purpose.
- 4. **Detection of viral antigen:** Low levels of circulating HIV-1 p24 antigen can be detected in the plasma by EIA soon after infection.
- 5. Immune function test: By counting CD4+ cells, e.g., cell count below 200/mm<sup>3</sup> indicates cell mediated immunity severely suppressed and the patient is suffering from opportunistic infection.

Preventive measures for HIV: No vaccine is available. Prevention consists of taking following measures:

#### A. Prevention of sexual transmission:

- Public awareness campaigns for HIV.
   Easily accessible or discreet testing centers.
   Safe sex practices (avoiding penetrative intercourses the aping sexual debut, condom use, fewer sexual partners).
   Targeting safe sex product of the approximation of the
- 5. Targeting safe sex method
- 6. Control for STIs 5 T
- Effective treatment of HIV infe 7.

#### Rost is real exposure prodon of parenteral transpission:

- Blood product transmission: Donor questionnaire, routine screening of donated blood, blood 1. substitute use.
- Injection drug use: Education, needle/syringe exchange, avoidance of shooting galleries, 2. sharing and support for methadone maintenance programs.

### C. Perinatal:

- 1. Routine opt out antenatal HIV antibody testing.
- 2. Preconception family planning if HIV serotype.
- 3. Measures to reduce vertical transmission.

### D. Occupational:

- 1. Education or training: Universal precautions, needle stich avoidance.
- 2. Post exposure prophylaxis.

In adults: Inadequate CD8+ cell response.

Chronic carriage is more likely to occur when infection occurs in a newborn than in an adult. Approximately 90% of infected neonates become chronic carriers. Chronic carriage resulting from neonatal infection is associated with a high risk of hepatocellular carcinoma.

#### Nice to know:

**Immunity:** Lifelong immunity occurs after the natural infection and is mediated by humoral antibody against HbsAg. Antibody HbsAg (HBsAb) is protective because it binds to surface antigen on the virion and prevents it from interacting with receptors on the hepatocytes.

#### Q. Name the sero-markers of HBV with interpretations.

Lab diagnosis of HBV infection or Seromarkers of HBV: HBV contains several antigens to which infected persons produce antibodies; these antigens and their antibodies are important in identifying HBV infection.

#### Antigens of HBV in the serum:

- HBsAg
- HBeAg

#### Antibodies of HBV in the serum:

- Anti HBc IgM.
- Anti HBs IgG.
- Anti HBe IgM.

#### Interpretation of serological markers:

- 1. HBsAg:
- Ag:
  HBsAg is an indicator of active infection and a negative test f CHBsAg markers HBV infection very unlikely.
  - In acute liver failure from hepatitis **In the five damage** is mediated by viral clearance and so HBsAg is negative.
  - Prolonged presence (it east 5 months) of HBsAg indicates carrier state and the risk of chronic hepatitis are hyperic carcinoma.
- 2. **HBAG**: The HBAG reflects active call ation of the virus in the liver. Its presence indicates a high like mood of transmissibility. Fnate, FreeAg positive patients are highly infectious.
- 3. Detection of anti HBsAg IgG in blood indicates either a previous infection, in which case anti HBc is usually also present, or previous vaccination, in which case anti HBc is not present.
- 4. Detection of anti HBc IgM in blood indicates surest marker of active infections.
- 5. Detection of anti HBe IgM in blood indicates recovery and low transmissibility.
- 6. The detection of viral DNA in the serum is strong evidence that infectious virions are present.

#### Q. Define window period. Discuss its importance in relation to HBV and HIV infection.

**Window period:** It is the period of time between the disappearance of a specific antigen and the appearance of antibody against that particular antigen.

**Importance of window period in HBV infection:** In case of HBV infection, there is a period of several weeks when HBsAg (the most important and readily detectable seromarker) has disappeared but anti HBsAb is not yet detectable. This is the window phase. At this time, the anti HBc IgM Ab is always positive and can be used to make the diagnosis.

**Importance of window period in HIV infection:** In case of HIV infection, there is a period of several weeks when HIV antigen has disappeared but antibody is not yet detectable, and therefore diagnosis becomes difficult.

## Q. A patient of jaundice tested for HBsAg and was found negative. Do you think that the patient is not infected with hepatitis B virus? What will be your next step? Justify.

HBsAg negative does not indicate that the patient is free from hepatitis B virus infection, because patient may be in window period when HBsAg is not found.

### Classical dengue fever (first exposure of dengue virus):

Dengue infection by one of the four serotypes

Antibody is formed

Formation of immune complex and activation of complement

Increased vascular permeability and thrombocytopenia.

Dengue hemorrhagic fever or dengue shock syndrome: Hemorrhagic shock syndrome is due to the • production of large amounts of cross reacting antibody at the time of a second dengue infection.

The patient recovers from classic dengue caused by one of the four serotypes and antibody against that serotype is produced.

Patient infected with another serotype of dengue virus.

An anamnestic, heterotypic response occurs.

Large amount of cross reacting antibody of the first serotype are produced.

There are two hypotheses about what happens next-

Immune complex composed of virus and antibody are formed that activate complement, causing increased vascular permeability and thrombocytopenia.

Antibodies increase the entry of virus into monocytes and macrophages with the consequent liberation of a large Dic fever. amount of cytokines.

In either scenario, shock and hemorrhage result.

Q. Write down the laboratory diagnosis of dengue here

Laboratory diagnosis of dengue hemo

Specimen collection:

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Serological test or antibody detection. The diagnosis is confirmed by detection of IgM antibody or a fourfold or greater rise in antibody titer in acute and convalescent sera. Antibodies can be detected by-

- 1. ELISA
- 2. ICT (Immunochromatography test)
- 3. Immune blot (Western blot)

Detection of NS1 dengue antigen: ELISA kits to detect the NS1 (non-structural protein) dengue antigen, although less sensitive than PCR, are becoming more widely available in endemic area.

Isolation: Isolation of dengue virus from blood by cell culture can confirm diagnosis (available in specialist laboratories).

Nucleic acid based technique: Detection of dengue virus RNA by PCR can confirm diagnosis (available in specialist laboratories).

#### **Routine blood test:**

- Platelet count: Thrombocytopenia (<100,000/mm<sup>3</sup>) •
- Hematocrit: Increased.
- Prothrombin time: Increased.

Q. A 12-year old girl presented with rash in whole body. She had fever for last few days. What would be the differential diagnosis?

#### Differential diagnosis of fever with rash: