



54

LABORATORY DIAGNOSIS OF VIRAL INFECTIONS

54.1 INTRODUCTION

Viruses are infective organisms which are responsible for many diseases in humans. Since viruses cannot be grown in artificial media & the tissue culture techniques may require longer periods, serodiagnosis of viral infections is the mainstay of diagnostic virology. Along side other techniques like direct detections of virus in clinical specimens & cultivation of viruses are also practiced. The development of technologies like PCR (Polymerase Chain Reaction) have provided an effective alternative for diagnosing viral infection



OBJECTIVES

After reading this lesson, you will be able to:

- describe the importance of specimen collection
- explain the Methods to preserve and transport the sample
- describe the Various methods of isolation of viruses.
- explain the Molecular techniques used in diagnosis of viral infections.

Lab diagnosis of viral infection begins from the very step of collection of specimen.

54.2 SPECIMEN COLLECTION

Attempts should be made to obtain material from the organs that are infected eg. Skin for cutaneous lesions, direct secretions from respiratory & Gastro intestinal tract that are required in case of respiratory or GI system involvement. In fact since most viruses enter the body through the respiratory or GI tract, the

MODULE

Microbiology

Laboratory Diagnosis of Viral Infections



Notes

most obvious & appropriate specimen is respiratory tract secretions or the GI tract secretions. In case of internal organ involvement where direct organ specimen is difficult to obtain, sampling from multiple sites seems useful. Eg., in case of measles, Skin is most dramatically involved. Yet measles virus may be isolated from respiratory tract or from urine. Similarly Central Nervous System & Cardio Vascular System are commonly affected in serious viral infections, but the virus may be isolated from the upper respiratory tract or Gastro Intestinal Tract.

The optimal specimens for viral culture are aspirates of fluids, exudates or secretion, tissues, washings of upper air ways or stool specimens, swab specimen are acceptable in most situations. Nasopharyngeal washings are generally sufficient for respiratory viruses. Blood may be useful for enteroviral infections in young children & infants.

To culture vesicular skin lesions the skin should be cleaned with an alcohol swab and allowed to dry for at least one minute. The vesicle should then be unrolled with a sterile scalpel, a sterile swab touched several times to the base.

As a general rule the frequency with which virus is recovered decreases as the duration of illness increases and every effort should be made to obtain specimen as early in the infection as possible.

54.3 TRANSPORTATION & STORAGE OF SPECIMEN

All the samples should be transported to laboratory in a sterile, leak proof container. Interval between collection of the specimen & its inoculation should be minimal. In case of some fragile viruses for eg RSV virus inoculation of cell cultures at the bed side has been recommended.

Swabs should be placed in viral transport media swabs, any swabs are not acceptable.

Specimens are refrigerated till they are further processed. The specimens should be maintained at -70°C if they are to be stored for a very long time (weeks or months). When the delay is short (less than 24 hours) specimens are stored at 4°C temperature.



Fig. 54.1: Transport medium

Appropriate specimen collected from patients, preserved and transported to the laboratory in the proper manner along with relevant clinical & epidemiological information. (Table 54.1)

Table 54.1

System	Specimen	Required
	For Isolation	For direct examination
Respiratory	Throat swab, Throat washings, Aspirates	Nasopharygeal aspirate
Central Nervous system	Faeces, Blood, CSF	Brain biopsy, CSF
Cardio Vascular System	Faeces, Macular popular scrapings, ulcer scrapings, throat swab	Vesicular/pustular fluid, Ulcer scraping.
Eye	Conjunctival scraping and swabs.	Conjunctival scraping/ swads
Liver	Blood	Serum
Congenital infections	Throat swab, product of conception	NIL



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54.4 LABORATORY DIAGNOSIS

In the laboratory the following methods are commonly employed, microscopic demonstration of the virus or its inclusion body. Demonstration of virus antigen, or detection of the specific antibody & molecular techniques.

54.4.1 Microscopy

It includes

1. Detection of viral inclusion body by light microscopy
2. Detection of virus or viral particles with the help of electron microscope.

Light Microscopy

Light Microscopy has been traditionally used in directly demonstrating viral infections by detecting the viral inclusion body in smear & tissue.

Inclusion bodies are dense aggregates of stainable substances, usually proteins. They can be either intra nuclear (present inside the nucleus) of infected well or intracytoplasmic (present inside the cytoplasm of the infected cell). Viruses that



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are assembled in the nucleus (usually DNA viruses) like herpes simplex viruses, Varicella zoster virus, Cytomegalo virus, adeno virus & papova viruses forms intranucleus inclusions. While viruses that are assembled in the cytoplasm (usually RNA viruses) like Respiratory syncytial virus, rabies virus & viruses of the pox group forms intra cytoplasmic inclusion bodies. The intracytoplasmic lesions of rabies virus are known as Negri bodies, intracytoplasmic inclusions of the pox viruses known as Guarneri bodies

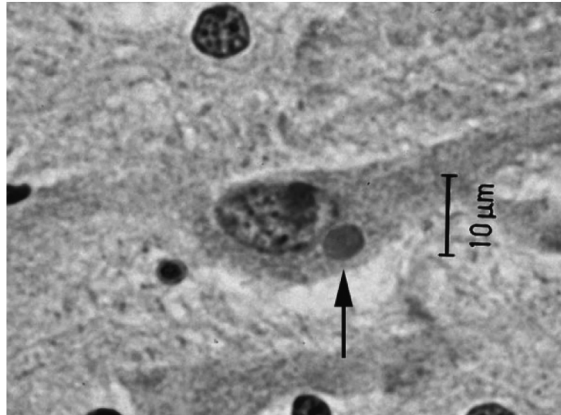


Fig. 54.2: Negri bodies

Electron Microscopy

Electron Microscopy is used in the study of clinical specimens & cell cultures. Electron microscope has been used effectively in the detection of viral agent of gastro enteritis especially those that are not recovered by conventional cell cultures. Electron microscopes are useful in ultra structural observations especially for research purposes.

The diagnosis of important viral infections, such smallpox, can be made quickly & safely with help of Electron microscopy.

54.4.2 Demonstration of virus antigen

Direct detections of virus antigen can be done in cases where the antigen is abundant in the lesions. It is demonstrated by serological methods such as precipitate in gel or immunofluorescence.

Counter immune electrophoresis, radio immune assay & Enzyme Linked immunosorbent assay have found wide application in diagnostic virology for the detection of viral antigens in clinical samples.

Molecular methods like polymerase chain Reaction (PCR), Real time polymerase reaction (RT-PCR), Nucleic acid. Sequence based Amplification (NASBA), Transcription Mediated Amplification (TMA), etc provide rapid sensitive and

specific techniques for detections of viral antigens. They are fast becoming routine diagnostic methods in developed countries.

54.4.3 Isolation of Virus

The method used for isolation of virus depend upon the virus sought. In general they consist of inoculation into animals, eggs or tissue culture. After that isolates are identified by neutralization or other suitable serological process.

Isolation of virus from patient does not always means that it is the causative agent of the patients illness. Many viruses like adeno viruses, entero virus etc. are frequently found in normal individuals. Thus it is imperative to interpret the isolation of virus in light of clinical data.

54.4.3.1 Egg inoculation

Embryonated eggs are among the most useful form of living animal tissue for the isolation of viruses, for titrating viruses & for large quantity cultivation in the production of viral vaccines.

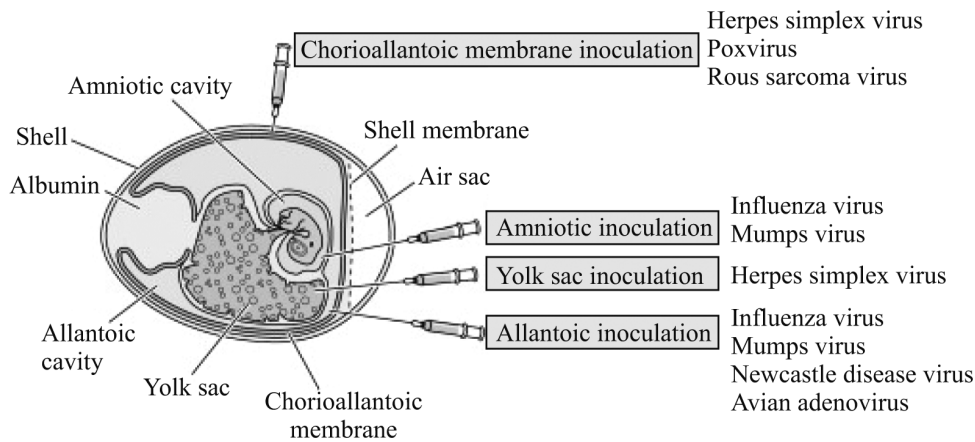


Fig. 54.3

The embryonated egg has various sites namely chorioallantric membrane, Allantoic membrane, Amniotic sac. Yolk sac & the embryo proper. Different virus grow at different above mentioned sites (see table)

Site	Virus grown
Chorioallartic membrane	Variola. Vaccinia
Allantoic membrane	Influenza paramyxo virus
Amniotic sac	Influenza virus
Yolk sac	Chlamydia Rickettsiae



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54.4.3.2 Animal Inoculation

It is the earliest method for the cultivation of viruses causing human diseases. Reed & colleagues F in 1900 used human volunteers for their work on yellow fever. In 1909, Landsteiner & Popper used monkeys to isolate Polio virus. Theiler used white mice which extended the scope of animal inoculation greatly. Rabbit, Guinea pig, mouse, rat are now commonly used in virus cultivation.

It needs to be noted that different animals are used for different viruses & also there exists different routes to inoculation. The growth of virus in inoculated animals may be indicated by death, disease or visible lesions. Disadvantages of animal inoculation are that they may interfere with viral growth and that animals often harbour latent viruses.



Fig. 54.4: mouse

Animal inoculation in particular is useful in the study of pathogenesis, immune response, epidemiology & oncogenesis.

54.4.3.3 Tissue culture

Tissue culture is often a generic term. Three types of tissue cultures are available

a. Organ culture

Small bits of organs are maintained *in vitro* for days & weeks. They are used for cultivation of viruses which can be grown only in specific organ.

b. Explants culture

Fragments of minced tissues is grown as explants embedded in plasma clot. Eg. Adeno tissue explants for adenovirus.

c. Cell culture

It is routinely used based on their origin. Chromosomal characters & the number of generation through which they can be maintained, cell cultures are classified into 3 types



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1. Primary cell culture

It consists of normal cells freshly taken from body & cultured. They are capable of limited growth in culture & cannot be maintained in serial culture. Eg. Monkey, kidney, human embryonic kidney, human amnion & chick embryo cell culture. They are useful for isolation of viruses & for vaccine production.

2. Diploid cell culture

These are used to ensure a continuous supply of cell line. After 50 serial passages, they undergo senescence. They are useful in viral vaccine production. Eg. Human fibroblasts

3. Continuous cell culture

These are single type usually derived from cancer cells that are capable of continuous serial cultivation indefinitely. These cell lines may be maintained by serial sub-cultivation or stored in the cold (-70°C) for use when necessary. They are useful for vaccine production. Eg. Verocell for rabies vaccine, Hela, HEp-2, KB

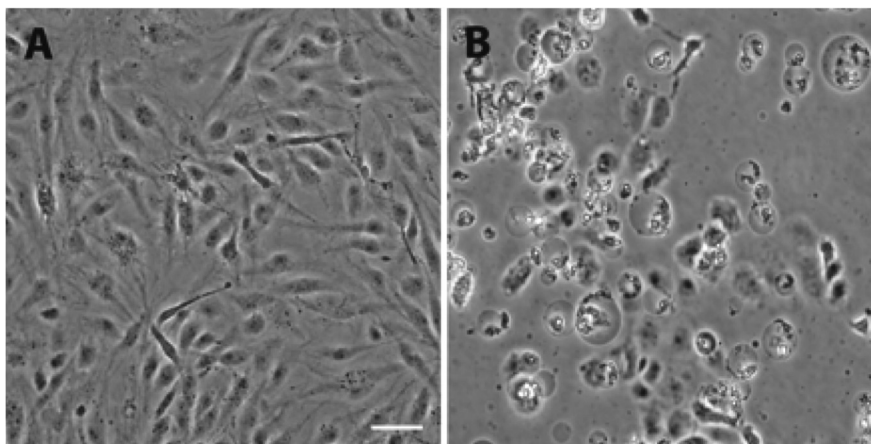


Fig. 54.5: Cytopathic effect. The cytopathic effect was apparent as refractile, rounded, swollen, and semiattached cells, and areas of clearance were compared to those of the uninfected control cells

The growth of virus in the cell culture can be detected by following methods

- i. Cytoplasmic effect
- ii. Metabolic inhibitors
- iii. Hemadsorption
- iv. Interference
- v. Transformation
- vi. Immunofluorescence



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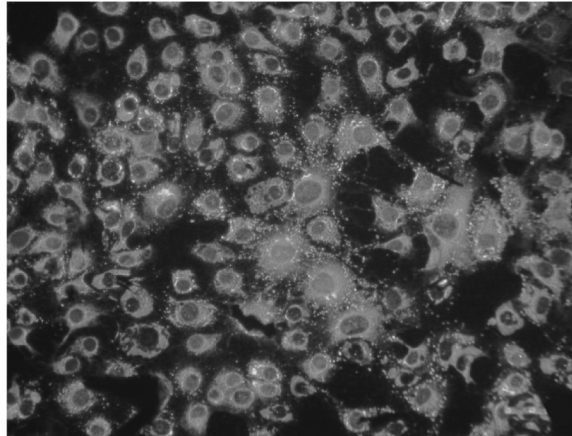


Fig. 54.6: Indirect immunofluorescence microscopy performed on chicken sera with a neutralization titre of 1/80 (diluted 1:1000 in PBS) on West Nile virus infected Vero cells

54.5 SEROLOGICAL DIAGNOSIS

It refers to diagnosis of disease based on reactions in the blood serum. The mere presence of an antibody to a virus is not sufficient to diagnose a viral infection. It only denotes that the person's immune system has been exposed to the viral antigen, besides a current infection by the virus, the antibody may have been formed by the following

- A previous symptomatic / asymptomatic infection
- In response to cross reacting antigen and
- Vaccination

In all these cases the level of the antibody will remain near the same for the entire duration of current illness. However, if the current illness is caused by the virus then the antibody levels would rise during the course of the disease therefore, the rule of "four fold rise" in the titre of antibodies is applied. It means that when serum samples are tested from an individual taken at an interval of ten to fourteen days there should be a fourfold increase of antibody titre, to be a indicative of current infection with the particular virus

Variety of methods is available to diagnose viral infection by antibody detection the choice of the method depends upon the sensitive of the test and intensity of immune response. Various tests available are ELISA, complement fixation test, neutralization, hemagglutination tests.

54.6 MOLECULAR TECHNIQUES

With development in molecular technology diagnosis of viral infections is becoming rapid & specific. Various molecular technique which can be used in

diagnosis of viral infections are

1. Nucleic acid sequence based amplification (NASBA)
2. Transcription mediated amplification
3. Polymerase chain reaction
4. Real time polymerase chain reaction



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Fig. 54.7: RT PCR System

The advantages of molecular techniques are that, they are rapid and are highly sensitive & specific. The only disadvantage is that it requires technical expertise and are resource intensive.



INTEXT QUESTIONS 54.1

1. The specimen collected in case of measles is
2. If delay in transport of specimen is anticipated, specimens to be stored at temperatures
3. Microscopically & are detected
4. Intracytoplasmic lesion of rabies virus is known as
5. Intracytoplasmic lesion of pox virus is known as

**WHAT YOU HAVE LEARNT**

- Viruses are infective organisms which are responsible for many diseases in humans.
- As viruses cannot be grown in artificial media & the tissue culture techniques may require longer periods, serodiagnosis of viral infections is the mainstay of diagnostic virology
- Attempts should be made to obtain material from the organs that are infected
- The optimal specimens for viral culture are aspirates of fluids, exudates or secretion, tissues, washings of upper air ways or stool specimens, swab specimen are acceptable in most situations.
- As a general rule the frequency with which virus is recovered decreases as the duration of illness increases and every effort should be made to obtain specimen as early in the infection as possible.
- All the samples should be transported to laboratory in a sterile, leak proof container. Interval between collection of the specimen & its inoculation should be minimal.
- In the laboratory the following methods are commonly employed, microscopic demonstration of the virus or its inclusion body, Demonstration of virus antigen, or detection of the specific antibody & molecular techniques.
- Direct detections of virus antigen can be done in cases where the antigen is abundant in the lesions. It is demonstrated by serological methods such as precipitate in gel or immunofluorescence.
- The method used for isolation of virus depend upon the virus sought. In general they consist of inoculation into animals, eggs or tissue culture
- Tissue culture is often a generic term. Three types of tissue cultures are available namely Organ culture, Explants culture, Cell culture
- Cell culture are of three types namely Primary cell culture, Diploid cell culture, Continuous cell culture
- Variety of methods is available to diagnose viral infection by antibody detection.
- The choice of the method depends upon the sensitivity of the test and intensity of immune response.
- Various tests available are ELISA, complement fixation test, neutralization, hemagglutination tests.

**Notes**

Laboratory Diagnosis of Viral Infections

- Various molecular techniques which can be used in diagnosis of viral infections are Nucleic acid sequence based amplification (NASBA), Transcription mediated amplification (TMA), Polymerase chain reaction (PCR) and Real time polymerase chain reaction (RT-PCR)



TERMINAL QUESTIONS

1. Enlist the various specimen which can be used for diagnosis of viral infection
2. Describe in brief the precaution one needs to take while preserving and transporting the specimen
3. Describe in brief various methods used for isolation of virus
4. Describe in brief the serological methods used in viral infection
5. Enlist the molecular techniques used for diagnosis of viral infections



ANSWERS TO INTEXT QUESTIONS 54.1

54.1

1. Skin
2. 4°C
3. Viral inclusion & Viral particles
4. Negri bodies
5. Gaurneri bodies

MODULE

Microbiology



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