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Diagnosis of viral infections

The laboratory diagnosis of viral infections based on three general approaches

1. **Direct detection** of viral components either in cells derived from infected tissues or free in fluid specimens.
2. **Isolation of viruses**, usually in cell cultures, followed by identification.
3. **Demonstration of a significant** increase in serum levels of antibodies to the etiological virus during the course of the illness or the presence of antibodies of class [IgM] specific to the etiological virus.

Specimens in viral infection

1- **Timing:** Specimens for virus isolation & direct detection as well as blood samples must be collected within the first few days of the illness.

2- **Choice of the specimens:** This done by knowing the pathogenesis of the virus e.g. Polio virus infection, the neurological samples are not important in the diagnosis, but throat wash is important, because the virus start replication in the oropharynx.

3- **Clinical data:** Because each type of virus needs special type of tissue culture for replication, so clinical data is important.

4- **Specimen transport & storage:** Specimens should deliver without delay, kept at neutral pH to avoid affection of viral infectivity. Keep the specimens at 4 °C if delay is expected for four days (refrigeration), If the delay is > four days the specimens should be kept at -70 °C (freezing).

Culture and Isolation

Viral Culture Viruses don't grow on artificial media, they must be cultured on cell cultures or other type of living system.

1- **Emboryonated eggs**

Inoculation of embryonated eggs used for growing of some viruses, (mumps & influenza viruses). Fertilized egg is incubated for (5-14) days after that inoculated. The site of inoculation & the period of incubation is depend on the type of virus.

Then embryonated examined for the evidence of viral replication (as Death of the yolk sac, Examination of the embryonated egg fluid for the viral antigens, or Pocks formation as cytopathic effect on the chorion).

2- **Laboratory animals**
In the past, mice & chimpanzees were inoculated with viruses, but now this has been largely replaced by the use of cell cultures.

3- **Cell cultures** originated from viable host cells & grow into a monolayer on the sides of glass or plastic test tubes.
Cell culture help in:
   a. Isolation & growth of a variety of viruses.
   b. Studying viral replication.
   c. Development of attenuated mutant strains, & vaccine production.

Rapid diagnostic tests

These tests in comparison to the cell culture (required days), few hours required after receiving the specimens to detect the virus.

These tests include:

- Direct EM examination of the specimens e.g. examination of the stool for Rotavirus & Hepatitis A.
- Immune electron microscopy.
- Antigen detection method the antigen of the virus is detected by immunofluorescent technique e.g. detection of the RSV antigen in the respiratory secretions.
- Agglutination method Here antibody is added to the specimen to detect the presence of the viral antigen.
- Molecular technique (Nucleic acid hybridization) Use to detect viral genome & genome sequence in clinical specimens.

Examples of the molecular methods

1- Hybirdization technique use single stranded, complementary nucleic acid probes. It is highly sensitive & specific. This technique used to identify:

a. Adenovirus in nasopharyngeal washings.
b. CMV in urine.
c. HIV in blood of individuals

2- PCR: Increases the sensitivity of nucleic acid hybridization by increases the amount of viral genome obtained from the patient.

Serology

Serological test used to detect antibodies produced against viral antigens.

The aim of the serological tests

1- Detect a significant increase in the titer of antibodies to the etiological virus.
2- 2- The presence of IgM specific antibodies to the etiological virus.

From these serological tests we have

- Radioimmunoassay RIA.
- Enzyme immune assay.
- Indirect immunofluorescent tests.
- Hemagglutination inhibition test, measure antibodies directed against hemagglutinating viruses, such as influenza viruses.
- Complement fixation test.