Lab.2/ Virology

B. Serological techniques:

1. Neutralization test:

Neutralization of virus is defined as the loss of infectivity through reaction of the virus with specific antibody.

Virus and serum are mixed under appropriate conditions and then inoculated into cell culture, eggs or animals. The presence of unneutralized virus may be detected by reactions such as cytopathic effect, haemadsorption, haemagglutination, plaque formation, disease in animals.

2. Haemagglutination assay:

Is a common non-fluorescence protein quantification assay specific for influenza. It relies on the fact that haemagglutinin, a surface protein of influenza viruses, agglutinates red blood cells (i.e., causes red blood cells to clump together).

In this assay, dilutions of an influenza sample are incubated with a (1%) erythrocyte solution for (1)hr. and the virus dilution at which agglutination first occurs is visually determined. This assay takes ~ 1-2 hours to complete and result can differ widely based on the technical expertise of the operator.

The haemagglutination inhibition assay is a common variation of the haemagglutination assay used to measure flu-specific antibody levels in blood serum-

In this variation, serum antibodies to the influenza virus will interfere with the virus attachment to red blood cells. Therefore haemagglutination is inhibited when antibodies are present at a sufficient concentration.

3. ELISA assay:

ELISA may be used to detect and measure either antibody or antigen.

There are many variations or types of ELISA assays but can generally be classified as either indirect, competitive, sandwich or reverse.
The indirect ELISA technique

Well is precoated with antigen
↓
Test serum is added—antibodies bind
↓
Labeled antiglobulin is added
↓
Enzyme substrate is added
↓
Color change

The antibody sandwich ELISA

Well is precoated with capture antibody
↓
Test antigen is added
↓
Detection antibody is added
↓
Labeled antiglobulin is added
↓
Color changes after enzyme substrate is added
5. Immunofluorescent test.

C. Electron microscopy:

Electron microscopes are of two general types, the transmission electron microscope (TEM) and scanning electron microscope (SEM). Virus diagnosis by electron microscopy relies on the detection and identification of viruses on the basis of their characteristic morphology. A major advantage of virus diagnosis by (EM) is the ability to visualize the virus. Speed is another advantage of (EM) as the specimen can be processed within minutes of receipt. On the other hand the main disadvantage of (EM) is its inability to examine multiple specimens coincidentally. Secondly, there must be a minimum number of virus particles present (around 10^6 virus particles per ml for detection). Some viruses may give a non distinct morphological appearance which may make detection very difficult.

Finally (EM) is a very expensive service to provide and requires highly skilled personnel.

There are two types of (EM) methods: Direct or immunoelectron microscopy (IEM). With direct methods, negative staining is normally used which requires little special equipment, in contrast to thin sectioning techniques.

(IEM) is a means of increasing the sensitivity and specificity of (EM) and is particularly useful in the following situations:

1. The number of virus particles present in small.

The competitive ELISA assay
2. Many different viruses have different morphology.
3. In an outbreak situation where the pathogens responsible has been identified, so that it may be useful to go back to look at the negative specimens again with IEM.

   There are two types of IEM, simple IEM, where the specimen is inoculated with specific antibody before staining in the hope that the antibody will agglutinate the specimen, and solid phase IEM (SPIEM), where the copy grid is coated with specific antibody which is used to capture virus particles from the specimen.

D. Cytology and Histology:

   This involves the morphologic study of cells or tissue, respectively. Viral inclusion are intracellular structures formed by aggregates of virus or viral components within an infected cell or abnormal accumulations of cellular materials resulting from viral induced metabolic disruption. Inclusions occur in single or syncytial cells.

   Syncytial cells are aggregates of cells fused to form one large cell with multiple nuclei. Pap or Giemsa attained cytologic smears are examined for inclusions or syncytia.

   Inclusions resulting from infection with CMV, adenovirus, parvovirus and papilloma virus are detected by histological examination of tissue stained with hematoxylin and eosin or pap.

   Less commonly, inclusions characteristic of measles and rabies viruses are detected by examining stained tissues. Rabies virus inclusions in brain tissue are called Negri bodies.