Direct detection of viral infection

Most viruses are small enough to be at the limit of resolution of even the best light microscopes, and can be visualized in liquid samples or infected cells only by EM (electron microscopy).

Electron microscopy is widely used in virology because viruses are generally too small for a direct inspection by light microscopy. Analysis of virus morphology is necessary in many circumstances, e.g.,

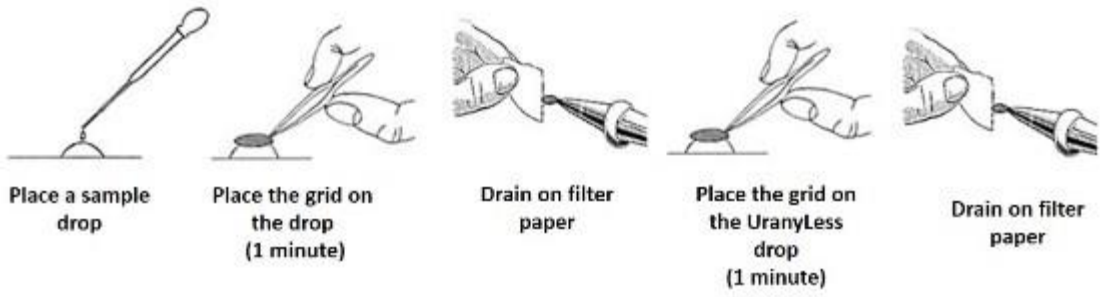
- For the diagnosis of a virus in particular clinical situations
- the analysis of virus entry and assembly.
- quality control of virus particle integrity is required if a virus is propagated in cell culture, particularly if the virus genome has changed.

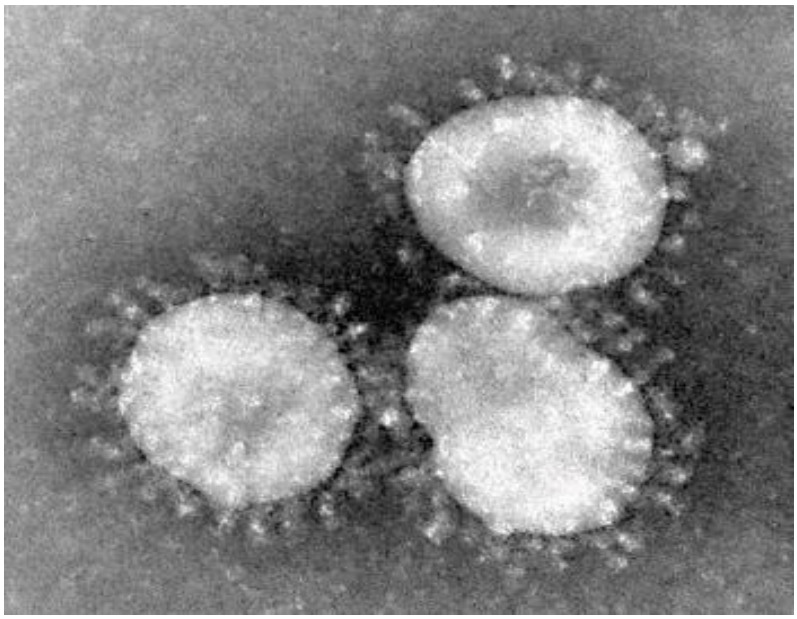
In most cases already the basic methodology for transmission electron microscopy, i.e., negative staining and ultrathin sectioning, is sufficient to give relevant information on virus ultrastructure.

Negative staining

This technique uses of heavy metals salts to provide contrast to viruses. In EM viruses will appear translucent, while the electrondense stain forms a dense, highly detailed halo. Phosphotungstic acid (PTA) is probably the most commonly used negative stain within diagnostic microbiology, but there are others that are used also. We used routinely 2 % ammonium molybdate and 2 % aqueous uranyl acetate. Two grids were used for each virus suspension, negatively stained one with 2 % ammonium molybdate and with 2 % uranyl acetate. Because uranyl acetate and ammonium molybdate differ in staining properties both stains were applied in parallel of every sample. The specimens were processed in a biohazard

hood in compliance with the biological safety level regulations. The grids were examined under an electron microscope at a magnification of 100000×.





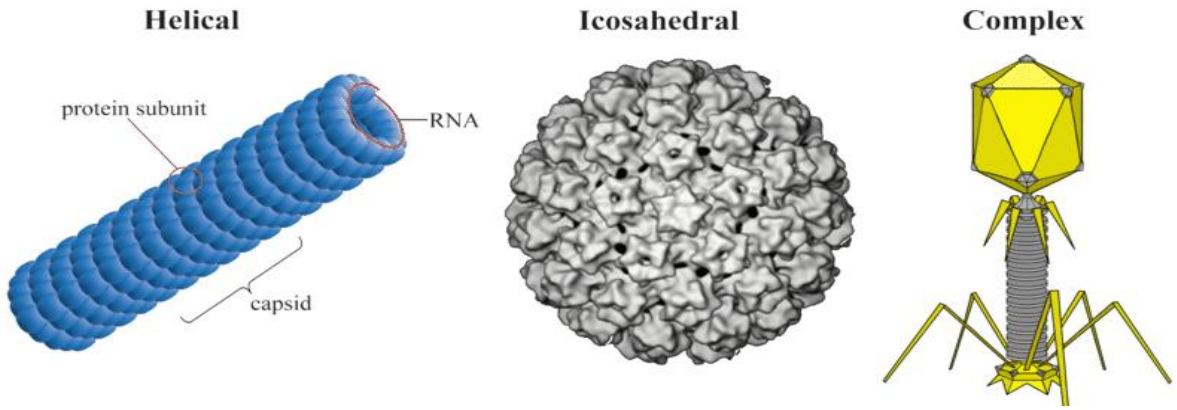
DETECTION OF VIRUSES IN VARIOUS CLINICAL SAMPLES

Direct EM detected viruses in a wide range of clinical specimens. It was made possible by the nonselective nature of the method and morphological differences between virus families. Viruses come in whole range of size and shapes, but into three morphological group characterized by

(1) helical symmetry

(2) cubic or icosahedral symmetry, and

(3) other or complex symmetry



Viruses are taxonomically grouped in families and genera by morphological criteria, i.e. by size, shape, fine structure of viral capsid and the presence or absence of an envelope and surface projections. Members of the same family or genus have a similar morphological appearance and can originating from various clinical specimens, but they are antigenic distinct. EM detects not only complete but also incomplete viruses and their morphological variants, e.g., empty capsids that are found in 30–40 % cells after virus replication. Antibody-coated viruses or viruses still enclosed in cells are also can be seen by EM.