

Medical laboratory techniques

Assist lect: Shahad A.alrahman + Ola Abbas Khdhair + Zahraa Sami



Direct Examination



Diagnostic tests can be grouped into 3 categories:

- (1) direct detection
- (2) indirect examination (virus isolation)
- (3) serology. In direct examination

Note: the clinical specimen is examined directly for the presence of virus particles, virus antigen or viral nucleic acids. In indirect examination, the specimen into cell culture, eggs or animals in an attempt to grow the virus: this is called virus isolation.

Direct Examination of Specimen

- Electron Microscopy morphology
- ➤ Light microscopy histological appearance e.g. inclusion bodies
- ➤ Antigen detection immunofluorescence, ELISA etc.
- ➤ Molecular techniques for the direct detection of viral genomes

2. Indirect Examination

- Cell Culture cytopathic effect
- Eggs pocks on CAM haemagglutination, inclusion bodies
- Animals disease or death confirmation by neutralization

3. Serology

- Complement fixation tests (CFT)
- Enzyme linked immunosorbent assay
- Immunofluorescence techniques (IF)
- Particle agglutination



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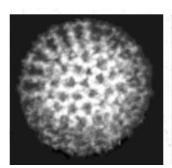
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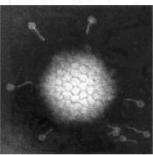
Electron Microscopy (EM)

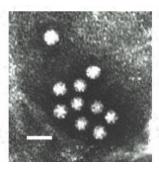
- Virus particles are detected and identified on the basis of morphology. A
 magnification of around 50,000 is normally used. EM is now mainly used for
 the diagnosis of viral gastroenteritis by detecting viruses in faeces e.g.
 rotavirus, adenovirus.
- The sensitivity and specificity of EM may be enhanced by immune electron microscopy, whereby virus specific antibody is used to agglutinate virus particles together and thus making them easier to recognize, or to capture virus particles onto the EM grid.
- The main problem with EM is the expense.

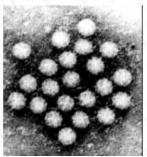
Types of electron microscope:

- **1- transmission EM (TEM):** is used to image the interior of cells, the structure of protein molecules and he organization of molecules in viruses
- 2- scanning EM (SEM): provides detailed images of the surfaces of cells of virus











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> Light Microscopy

- Replicating virus often produce histological changes in infected cells. These
 changes may be characteristic or non-specific. Viral inclusion bodies are
 basically collections of replicating virus particles either in the nucleus or
 cytoplasm.
- not sensitive or specific, serves as a useful adjunct in the diagnosis of certain viral infections.

> Viral Genome Detection

Methods based on the detection of viral genome are also commonly known as molecular methods. It is the future direction of viral diagnosis. These techniques may allow for the quantification of DNA/RNA present in the specimen.

Detection of a Virus

Regardless of the method of cultivation, once a virus has been introduced into a whole host organism, embryo, or tissue-culture cell, a sample can be prepared from the infected host, embryo, or cell line for further analysis under a brightfield, electron, or fluorescent microscope. **Cytopathic effects (CPEs)** are distinct observable cell abnormalities due to viral infection. CPEs can include loss of adherence to the surface of the container, changes in cell shape from flat to round, shrinkage of the nucleus, vacuoles in the cytoplasm, fusion of cytoplasmic membranes and the formation of multinucleated syncytia, inclusion bodies in the nucleus or cytoplasm, and complete cell lysis

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