The Role of Electron Microscopy in Viral Diagnosis

Kleophant Thakerngpol, M.Sc.*
Tinrat Stitnimankarn, M.D.*

INTRODUCTION

Viruses have been implicated in an extensive spectrum of human diseases. Electron microscopy is one of the available techniques for viral diagnosis. The electron microscope (EM) was developed in 1931 in Germany to visualize the structure beyond the resolution power of the light microscope.[1] The first virus to be seen in the EM was tobacco mosaic in 1940 by Stanley and Anderson.[2] In 1948, Nagler and Rake showed by EM that there were differences in the morphology of virus particles in crust and vesicle fluids of lesions from patients with smallpox and chickenpox (varicella).[3] Brenner and Horne[4] first described the negative contrast in 1955, using phosphotungstic acid as a stain.

The negative staining technique is well established and has become the method of choice for the rapid identification of viruses in clinical specimens. EM by negative stain methods is also valuable adjunct to conventional isolation technique because viruses in tissue culture fluids at a concentration of about 10^6 particles/ml can be grouped by morphology.[5,6]

Virus may also be identified by thin section EM. Although not a rapid method, thin section EM allows identification by replicative site as well as by morphology. EM may be of use in diagnosis and confirmed diagnosis of viral infections. In EM study, a positive result can be recorded photographically, thus providing a permanent record of both specimen and implicated virus.[7,8]

Both negative and positive stains combined with immunoelectron microscopy (IEM) allow visualization of virus in clinical specimens.[9,10]

The purpose of this article is to review the basic techniques commonly used in EM laboratory.

Table 1. Amount of various specimen for EM study[11]

Specimen	**Amount**
Blister fluid	5-10 µl
Blood	5-10 µl
Cerebrospinal fluid	1 - 5 ml
Pericardial fluid	1 - 5 ml
Pleural fluid, nasopharyngeal fluid, sptum	1 - 10 ml
Stool	0.5 - 10 ml
Tears	5-10 µl
Urine	5-10 µl
Tissue	1-3 mm^3

Note: The larger the available sample, the more viral agent could be seen.

**Deparment of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700.*

Electron microscopy techniques

Negative staining:

This is one of the most useful methods for visualization of viruses. With negative staining, the virus particle is surrounded by an electron-dense medium which does not stain the particles but forms a dark background in which it is embedded. The mechanism of the staining depends upon the ability of an electron-dense stain, such as phosphotungstic acid, to surround and fill the contours and crevices of a specimen, thereby revealing its shape. By this means, many viruses have been shown to display a definite symmetry either on the surface or in an internal component. Resolution is better in negatively stained preparation than in conventional positive stained thin sections. The disadvantages are the limitation to the study of isolated particles and the requirement of high concentration of particles in the sample.

Specimen support films:

Viruses or particulate specimens lack sufficient contrast in the EM. Specimen support membranes for grids with a very thin film from suitable materials are necessary for examination in the EM. Specimen support membranes should be thin enough to transit electron beams, scatter as few electrons as possible, and resist electron bombardment to prevent drift and shrinkage due to the heating effect of the electron beam. The most commonly used support membrane is 5-20 nm thick and is made from collodion or formvar. Collodion and formvar are organic substances, which are decomposed by electron beam. Therefore, their surfaces are coated with carbon before practical use.[12,13]

Thin section:

Fixed or fresh specimens in the form of tissue blocks and sectioning are used for this technique. Thin section is useful in the diagnosis of viral infections when facilities for culturing and identifying viruses by routine methods are not available. Appropriately chosen fresh tissues may be obtained at surgical biopsy or even at autopsy to establish and corroborate light microscopic diagnoses of viral infections. Confirmation of viral infection by EM on tissue originally processed for light microscopy is frequently useful. For example, virus particles have been seen in thin sections of tissue, which had been stored in formalin over a long period. Paraffin sections can be marked to indicate cells with inclusions and the appropriate areas of the section embedded for electron microscopic examination of the same cells.[10,14]

Table 2. Rapid tissue processing for EM

Procedure	**Time (mins)**
Tissue is cut into a very small pieces and process as follow :	
Pre-fixation in 4% phosphate buffered glutaraldehyde	30
Buffer wash, 3 changes	9
Post-fixation in 2% phosphate buffered osmium tetroxide	30
Distilled water wash	3
Stain with 2% uranyl acetate aqueous solution	20
Ethyl alcohol 70% 2 changes	3
Ethyl alcohol 80% 2 changes	3
Ethyl alcohol 90% 2 changes	6
Ethyl alcohol 95% 2 changes	6
Absolute ethyl alcohol 3 changes	9
Propylene oxide 3 changes	9
Infiltrate in 50:50 mixture of propylene oxide and epoxy resin mixture	30 (at 37°C)
Infiltrate in epoxy resin mixture alone	120 (at 37°C) with changes
Embed in polypropylene capsules	
Polymerize in oven 70°C overnight	

Immunoelectron microscopy (IEM) :

Both negative and positive stains combined with IEM allow the visualization of viruses in clinical specimens. IEM is a concentration step and/or a specific virus identification step. The fundamentals of IEM are the same as those of immunofluoreseence and immunohistochemistry, except that markers which are visible to an EM are used. The choice of the marker for IEM depends primarily upon the location of the sought antigen. Antigens can be localized within tissues or cells, upon cell surfaces, or upon the surface of ultrathin sections of tissues or cells. If the antigen to be localized exists within solid tissues or within cells, the antibody conjugate must be able to penetrate to the site of the antigen.[15,16]

Table 3. Requirements for an EM for virus diagnosis[17]

A magnification step of about 50,000 x.
Stability (i.e. lens currents) does not fluctuate significantly, magnification does not vary, no electron drift
Fine-grain viewing screens
High resolution (0.2 nm.)
Good illumination at the working magnification
Large viewing screen
Good object contrast (from a small objective aperture)

In conclusion, it is important to know the morphological characteristics of each virus in the examined sample when viewing it under the EM. Negative stained samples should be sent to the EM laboratory unfixed and undiluted. Tissue samples should be fixed as soon after removal as possible in buffered glutaraldehyde. It is of interest to note that most of the specimens examined by negative staining are infective materials, and are pathogenic. Aseptic precautions must be taken at all times with such specimens. Ultraviolet irradiation kills most viruses and has no effect on morphology. In contrast to glutaraldehyde, the use of formaldehyde or hypochlorite may alter the morphology of viruses. It is desirable to keep a container of disinfectant on the workbench to dispose of contaminated materials.

REFERENCES

1. Peven DR, Gruhn JD. The development of Electron Microscopy. Arch Pathol Lab Med 1985; **109:** 683-91.
2. Stanley WM, Anderson TF. A study of purified viruses with the electron microscope. J Biol Chem 1941; **139:** 325-38.
3. Nagler FPO, Rake G. Use of the electron microscope in diagnosis of variola, vaccinia and varicella. J Bacteriol 1948; **55:** 45-51.
4. Brenner S, Horne RW. A negative staining method for high resolution electron microscopy of viruses. Biochim Biophys Acta 1959; **34:** 103-10.
5. Palmer EL, Martin ML. Electron microscopy in viral diagnosis. Boca Raton, FL : CRC Press, 1988.
6. Hayat MA, Miller SE. Negative staining. New York : Mc Graw Hill Publishing, 1990.
7. Yunis EL, Hashida Y, Haas JE. The role of electron microscopy in the identification of viruses in human discase. In : Sommer SC, ed. Pathology Annual. New York : Appleton Century Crofts, 1977.
8. Almeida JD. Rapid viral diagnosis by electron microscopy. Scan J Infect Dis 1982; **36(Suppl):** 117-20.
9. Hayat MA. Basic technique for transmission electron microscopy. New York : Academic Press, 1986.
10. Miller SE. Detection and identification of viruses by electron microscopy. J Electron Microscopy Technique 1986; **4:** 265-301.
11. Miller SE. Evaluation of electron microscopic information available from clinical samples. In : De la Maza LM, Peterson EM, eds. Medical Virolgy. New York : Plenum Press, 1991: 21-54.

12. Mclean DM, Wong KK. Same-day diagnosis of human virus infections. Florida : CRC Press, 1984.
13. Oshiro LS, Miller SE. Application of electron microscopy to the diagnosis of viral infections. In : Lennette EH, ed. Laboratory diagnosis of viral infections. 2nd ed. New York : Marcel Dekker, 1992: 45-68.
14. Miller SE. Electron microscopy of viral infections. In : Lennette EH and Smith TF, eds. Laboratory diagnosis of viral infections. 3rd ed. New York : Marcel Dekker, 1999: 45-70.
15. Doane FW. Electron microscopy and immunoelectron microscopy. In : Spector S, Lancz GL, eds. Clinical Virology Manual. New York : Elsevier, 1986: 71-88.
16. Robinson G, Gray T. Electron microscopy 2 : Practical procedures. In : Bancroft JD, Stevens A, eds. Theory and practice of histological technique. London : Churchill Livingstone, 1996: 585-626.
17. Madeley CR. The search for viruses by negative contrast. In : Papadimitriou JM, Henderson DW, Spagnolo DV, eds. Diagnostic ultrastructure of non-neoplastic diseases. London : Churchill Livingstone, 1992: 141-54.