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## Electron Microscopy in Disease Diagnosis

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Understanding the pathogen and the pathogenesis at cellular levels are imperative in the studies of disease causing organisms. With its very high resolving and magnifying powers, Electron Microscopy has opened up new vistas in studying the ultra structure and has become an indispensable tool in understanding many of the diseases and their etiological agents. The limitations of Light Microscopes, low magnifying and resolving powers (1000 x magnification and a resolution of 0.2 micrometers) paved the way for the development of electron microscopes. Electron Microscopes are instruments that use a beam of highly energetic electrons to examine objects on a very fine scale and function exactly like their optical counterparts. Present day electron microscopes are capable of giving magnifications up to 1000000 X and 800000 X and a resolving power of 0.1 nm and 0.4 nm in T E M and S E M respectively. Transmission electron microscopy (TEM) can reveal the ultra structural details at cellular levels, whereas Scanning electron microscopy (SEM) can show the morphology of minute structures/organisms in its three-dimensional state. Combining the TEM and SEM, it has become possible to study and classify the viruses and virus like organisms.

Commonly employed methods for disease diagnosis include histology, serology, microbiology, molecular diagnostics and electron microscopy and each method has its own advantages and disadvantages.

| **Pathogens** | **Size** | **Microscopy** |
|---|---|---|
| Helminth | mm - cm | Light microscopy |
| Helminth eggs | 50 mm and above | Light microscopy |
| Fungi | 5 mm and above | L M & E M |
| Protozoa | 2 mm and above | L M & E M |
| Bacteria | 0.2 mm and above | L M & E M |
| Rickettsias | 0.3 – 0.6 mm | L M & E M |
| Virus | 0.01 – 0.4 mm (10 – 400 nm ) | Electron microscopy |

Among these diagnostic techniques, electron microscopy remains the most important tool to establish a viral etiology in the case of disease outbreaks without any previous history, and stands out as the only technique, which can visualize and record viral pathogenesis at cellular levels.

Histology uses light microscopy and is still an invaluable tool in disease diagnosis. It does not require sophisticated instruments and is useful in many disease conditions. However, misleading

observations may make confirmatory diagnosis difficult. In the case of viral infections, one can find lesions or inclusions, which are only suggestive of a specific viral infection through histopathology. TEM provides information about the morphology of pathogens, sub cellular changes / particles / structures etc. Moreover, due to the limited magnification and resolution, ultra structural / sub cellular changes and minute pathogens/stages cannot be observed.

Sero-diagnostic methods play an important role in disease diagnosis, especially in field conditions. Serology still remains the mainstay of viral diagnosis. The tests are normally based on specific antibodies (immunoprobes) and can detect sub clinical / latent / carrier states of infection. However, the draw backs of serological tests are (a) highly variable sensitivity & specificity (b) many viruses often produce clinical disease before the appearance of antibodies (c) Less useful in the case of latent viruses (d) antigenic cross-reactivity between related viruses may lead to false positive results and (e) less effective in invertebrates which does not produce antibodies.

Microbiological methods are widely used for the diagnosis of bacterial infections and involve culture, isolation and identification of the pathogens. But the procedure is tedious and time consuming and may even take weeks in some cases.

Molecular biology tools involve the detection of genetic material of pathogens using molecular probes. Advantages of Molecular tools include (a) extremely high sensitivity (b) easy to set up and (c) fast turnaround time. Disadvantages are (a) expensive (b) extremely liable to contamination (c) high degree of operator skill required (d) quantitative assay difficult and (e) difficulty in interpreting positive results, especially with latent viruses and (f) though they are more sensitive, are only capable of identifying the presence of genomic material for previously identified agents.

Electron microscopy can be an important adjunct to conventional culture and serologic techniques in diagnosing viral illnesses. Though detection of viruses by E M requires relatively large numbers of virions, and provides no information regarding specific serotypes within a virus family, it has the distinct advantages of being simple and rapid. Also, infectious particles are not required. Some viruses do not grow in tissue culture or grow only after special manipulation, and may not survive if transportation conditions to the lab are not optimal. Naturally, culturing would miss these agents. Additionally, a wide variety of agents can be visualized by E M; because specific reagents such as antibodies, antigens, or nucleic acid and protein probes are not required, one is not limited to the availability of these reagents, and prior knowledge of the virus identity for reagent selection is not required. Diagnostic electron microscopy has two advantages over enzyme-linked immunosorbent assay and nucleic acid amplification tests. After a simple and fast negative stain preparation, the undirected, “open view” of electron microscopy allows rapid morphologic identification and differential diagnosis of different agents contained in the specimen.

The biggest advantage of electron microscopy lies in the fact that it provides direct visual evidence of various pathogens/biological processes, while most of the other techniques are indirect and in some instances non-specific. Electron microscopic diagnosis is uniquely suited for rapid identification of infectious agents. A specimen can be ready for examination and an experienced virologist or technologist can identify, by electron microscopy, a viral pathogen morphologically within 10 minutes of arrival in the electron microscopy laboratory. Once the histopathological observations using light microscopy provides primary information on the target tissues, electron microscopy can

be employed to visualize the the pathogens and study its morphology. Electron microscopy can also provide information on the ultrastructural modifications/changes at sub-cellular levels caused by the pathogen.

So compared with other methods, E M benefits from an "open view", which means that as a "catch all" method it also reveals double infections and the presence of agents that might not otherwise have been considered. Finally, since the test entails the visualization of the virus itself, rather than a color change or agglutination reaction, false positive tests resulting from cross-reactions of reagents with similar materials are not likely. Hence electron microscope can be considered as the ultimate tool in identifying the etiology of emerging diseases.

Two types of preparations are primarily used for routine EM virus identification, negative staining and thin sectioning, although specialized research techniques such as scanning E M, specific antibody aggregation or labeling with electron-dense tags, *in situ* labeling, cryomicroscopy, and high-voltage microscopy have been used to classify viruses and describe virus-host relationships. With the simple negative staining preparation available, E M allows the rapid and direct detection of an etiological agent on a sample from a patient, or from diagnostic cell cultures.

Negative staining of liquid samples is very rapid, and can provide an answer within a few minutes to a couple of hours.. It enables the examiner to view cell particles and organelles in isolation. The isolated cell/particle is placed in a "puddle" of staining material, usually uranyl acetate or phosphotungstic acid, and is then supported on a thin, plastic film. The stain molecules deposit into surface crevices in the specimen during the drying process and typically produce a "ghost" image in which the specimen appears light against a dark background. Sensitivity and specificity of E M may be further enhanced by immuno electron microscopy, which includes classical immunoelectron microscopy and solid phase immuno electron microscopy.

In classical immuno electron microscopy, the sample is treated with specific anti-sera before being put up for EM. The viral particles present will be agglutinated and thus congregate together by the antibody, making them easily visible.

In solid phase immuno electron microscopy the grid is coated with specific anti-sera. The virus particles present in the sample will be absorbed onto the grid by the antibody thus enhancing the visibility under the microscope.

**Advantages:** The most important among the benefits offered by the electron microscope is undoubtedly the very high resolution. Since timely and accurate diagnosis forms the first step in the health management of farmed fishes and shellfishes, the right diagnosis defines the very success of disease control. Though E M has an important role in the diagnosis of viral infections, it is equally useful in the diagnosis and understanding the pathogens as well as the pathological changes caused by various other pathogenic organisms. As a confirmatory diagnostic method for many of the existing and emerging diseases, especially of viral origin, electron microscopy still remains an indispensable tool in the field of disease investigation and control. To exploit the potential of diagnostic electron microscopy fully, it should be quality controlled, applied as a frontline method, and be coordinated and run in parallel with other diagnostic techniques.

**Disadvantages:** However, the disadvantages of E M in the diagnosis of infections are (a) detection of viruses by E M requires relatively large numbers of virus particles (b) possibility of false negatives, if concentration is very low (c) provides no information regarding specific serotypes within a virus family and (d). Factors like high cost of operation and infrastructure, need for skilled technical personnel, laborious and time-consuming procedures, thorough knowledge needed for interpretation etc. restricts the use of electron microscopy as a routine diagnostic tool.

## Suggested Reading

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