Electron Microscopy in Diagnosis of Infectious Diseases

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Outline Part 1

A. Virology
   1. Advantages of using EM in diagnostic virology
   2. Limitations of using EM in diagnostic virology
   3. Workarounds
   4. Virus structure
      a. In negative stains
      b. In thin sections
Outline Part 1

B. Other organisms
   1. Bacteriology
   2. Mycology
   3. Photology (algae)
   4. Protozoology

Questions/Discussion
C. Virus look-alikes

1. Examples of confusing things in fluids
2. Examples of cell organelles that resemble viruses in tissues
Outline Part 2

D. Real cases

1. Examples of organisms diagnosed from patients
2. Quiz for fun
A. Virology

1. Advantages of using EM in diagnostic virology
2. Limitations of using EM in diagnostic virology
3. Workarounds
4. Virus structure
   a. In negative stains
   b. In thin sections
A. Virology
Advantages of EM in Virus Diagnosis/Identification

• Fast
• Does not require living organisms
• Does not require special reagents (antibodies, nucleic acid standards, protein standards)
• Can visualize other organisms: odd agents, odd locations
• Works when other modalities are not enough (mutant genomes may not react in PCRs)
• No false positive results (cross-reactions with similar things)
• What you see is the real thing
Limitations of EM in Virus Diagnosis/Identification

- Less sensitive than some other tests
- Requires expensive instruments (EM)
- Requires virology knowledge
Ways To Get Around Limitations

- Ultracentrifugation
- Antibody concentration
- Confocal microscopy of wet tissue slabs
- Multiple tissue locations
- Semi-thin sections of epoxy-embedded tissues
- Where to look in tissues: inflammation, nucleated cells, necrosis edge, unusual ultrastructure for the tissue type), syncytia
Virus Terminology

Naked

Capsid → Nucleocapsid

Enveloped

Capsid → Nucleocapsid → Envelope

Virion (complete virus)
Identification of Viruses in Fluids

**Naked:** Icosahedral  
**Enveloped:** Pleomorphic

**Representative Viruses Visualized by Negative Staining**

- **Naked viruses**
  - 30-35 nm
  - 45-55 nm
  - 70-90 nm
  - [Diagram of naked viruses]

- **Enveloped viruses**
  - 150-300 nm
  - "smooth" surface
  - Isometric nucleocapsid
  - Surface spikes
  - Helical nucleocapsid
  - [Diagram of enveloped viruses]

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Identification of Viruses in Fluids, Con’t.

**Enveloped:** Not Pleomorphic

Negative Staining of Naked and Enveloped Viruses

**Enteric Viruses**
- Rotavirus
- Adenovirus
- Astrovirus
- Coronavirus
- Calicivirus
- Picornavirus

**Polyomavirus from Urine**

**Influenzavirus**
Negative Staining of Spherical and Helical Nucleocapsids

Herpesvirus

Measlesvirus
Identification of Viruses in Tissues

Naked (Icosahedral)
- 3 size ranges
- Paracrystalline arrays

Location in cells
- DNA--nucleus
- RNA--cytoplasm

Enveloped (Pleomorphic)
- Membrane-associated
  - “Smooth”
  - Fringed
- Nucleocapsids
  - Spherical
  - Helical

Representative viruses visualized by thin sectioning

DNA Viruses: Usually produced in the nucleus

RNA Viruses: Usually produced in the cytoplasm
Nuclear Membrane Budding

100 nm

Budding virus

Vesicular Budding

Plasma Membrane Budding

Hantavirus

nc

HTLV

Herpesvirus
Spherical Nucleocapsids

Helical Nucleocapsids

Herpesvirus

Measlesvirus

nc

100 nm

1 µm
Bioterrorism: the intentional release or dissemination of biological agents such as bacteria, viruses, or toxins to create disease and fear.
Laboratory Response Network (LRN)

- **Collaboration:** FBI & Association of Public Health Laboratories
- **Started:** August, 1999
- **Includes:** State & local public health labs, veterinary, agriculture, military, environmental, & water- & food-testing labs
- **Function:** rapid testing, timely notification, & secure results reporting from public health emergencies (biological/chemical terrorism & emerging diseases)
LRNs and Electron Microscopy

• About half the LRNs are paired with electron microscopy laboratories.

• The Duke Electron Microscopy Service (DEMS) is the EM partner of the NC State Public Health Laboratory in the NC LRN.

• If asked to process biohazard sample, read Dos and Don’ts article.
References for Protocols:

Negative Staining Electron Microscopic Protocol for Rash Illness.  
http://www.bt.cdc.gov/labissues/  
Then click on title above.

Electron Microscopy for Rapid Diagnosis of Emerging Infectious Agents.  

Bioterrorism and electron microscopic differentiation of poxviruses from herpesviruses: dos and don’ts.  
Ultrastruc Pathol. 2003;27:133-140.

Modern uses of electron microscopy for detection of viruses.  

Detection and identification of viruses by electron microscopy.  
Class A Viral Agents of Bioterrorism

Smallpox virus
• Easily disseminated
• Easily weaponized
• High mortality rate, survivors badly scarred
• Previously mass-produced
• Believed to have been distributed after USSR collapse

Hemorrhagic fever viruses
• Filoviruses (Marburg, Ebola)
• Arenaviruses (Lassa, Machupo)
Because of ID speed, EM is on the front line in bioterrorism surveillance (e.g., smallpox).

Poxvirus lesions are most likely to be confused with varicella-zoster virus (VZV) (a herpesvirus) lesions.
EM in Surveillance of Bioterrorism

1. Orthopoxvirus

2. Herpesvirus

Palmer E, Martin ML. CRC.
EM in Surveillance of Emerging Diseases

Viral emerging diseases identified first by EM
- Parvovirus B-19
- Monkeypox
- SARS coronavirus
- Metapneumovirus
- Morbilivirus
- Nipah virus
EM was crucial in identifying the coronavirus in the SARS outbreak.

Costs (11/02 – 7/03):
774 Deaths, 9.6% fatality (China, Hong Kong, Taiwan, Canada)
$11 billion (Asia alone)
Thin Section of SARS Coronavirus

Courtesy of Cynthia Goldsmith, CDC, Atlanta.
EM was crucial in identifying the monkeypox virus outbreak.

Number of likely monkeypox cases rises to 33 in Midwest

BY NICOLE ZIEGLER DIZON
Associated Press

CHICAGO — Federal health officials investigating an outbreak of monkeypox that apparently spread from pet prairie dogs to people in three Midwestern states said Monday the number of possible cases has risen to at least 33.

The Centers for Disease Control and Prevention and state officials have been working to contain the outbreak, which has affected people in Illinois, Indiana, and Missouri.

For the average citizen, I would not necessarily be concerned at this point of being exposed to monkeypox.

"I would not necessarily be concerned at this point of being exposed to monkeypox."

The human mortality rate from monkeypox in Africa has ranged from 1 percent to 10 percent, but the virus may be less lethal in the United States because people are typically better nourished and medical technology is far more advanced here.

A giant Gambian rat (like the one shown above) likely has infected prairie dogs with monkeypox disease. The illness has infected 33 people in Midwestern states.
Thin Section of Monkeypox Virus Infected Cell

Negative Stain
Summary: Identification of Viruses

• In fluids (negative stains)
  o Naked icosahedral: size, surface structure
  o Enveloped: size, surface structure of core/envelope

• In tissues (thin sections)
  o Naked
  o Enveloped (usually budding)
  o Location: nucleus/cytoplasm