

Virus Identification by Electron Microscopy

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Electron microscopy is clearly the best way to look at enteric viruses, many of which do not grow in tissue culture, and those that can be so coaxed, do so under special conditions that are not routinely found in the culture lab. Biochemical identification (e.g., immunological kits, PCR, Western Blots) require a specific reagent to recognize the virus, and if the right reagent is not used, the viruses will be missed (e.g., if you run a test for rotavirus, you will miss adenovirus, etc.). Furthermore, there are not biochemical reagents for all viruses. With electron microscopy, one can see a wide variety of viruses by doing a negative stain of an aqueous extract of stool.

We perform some 1000 examinations each year for viruses using electron microscopy. The following are ways to look at viruses, as well as a summary of virus types and references of virus identification, by electron microscopy.

Two Ways to Look at Viruses by Electron Microscopy

Negative staining.

- Used with liquid samples.
- Shows whole virus (size, surface structure important).

Thin sectioning.

- Used with cells and tissues.
- Shows a slice of an infected cell.
- Shows relationship of viruses to cell organelles (internal structure and cell organelle association important).

Location within the cell is a clue to nucleic acid type.

DNA viruses are usually seen in the nucleus (some exceptions).

RNA viruses are usually seen in the cytoplasm (some exceptions).

Exceptions:

Naked viruses get out of the cell by lysis; if the cell is very sick, there may be virions everywhere, e.g., adenovirus (DNA).

Poxviruses are DNA viruses, but are constructed in the cytoplasm.

Hepatitis B core particles (DNA) may be in the nucleus and cytoplasm. Dane particles (complete viruses) are only cytoplasmic.

Some paramyxovirus (RNA) nucleocapsids, not the complete virion, can be seen in the nucleus (e.g., measles virus).

Enveloped viruses are associated with cell membranes. The type of cell membrane (plasma membrane, nuclear membrane, endoplasmic reticulum, vacuoles) can be a clue to virus identification.

Summary of Virus Types (based on morphology)

Naked viruses.

All human naked viruses are icosahedral (spherical).

There are 3 size ranges from 20-35 nm, 45-55 nm, 70-90 nm. Examples:

Small: parvovirus, enterovirus, rhinovirus.

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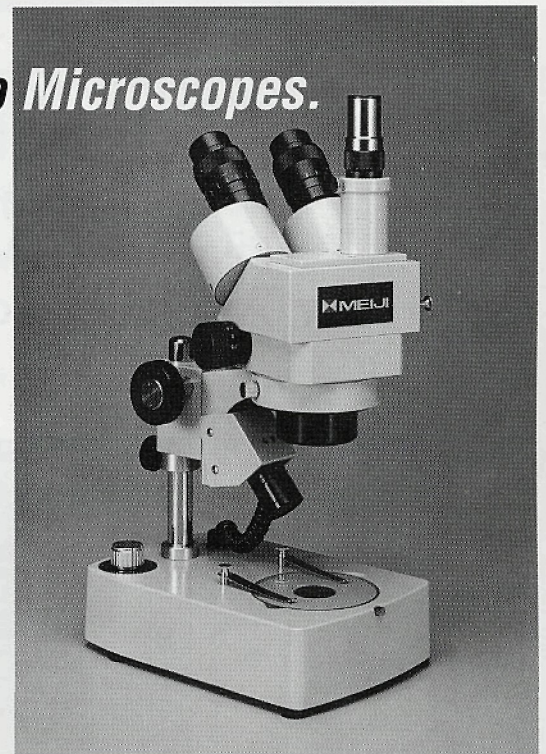
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Medium: polyomavirus, papillomavirus.

Large: rotavirus/reovirus, adenovirus

Enveloped viruses.

All have a lipoprotein membrane on the outside of the nucleocapsid. Most derive this layer by budding through cell membranes; this pliable layer makes the virion pleomorphic. Poxviruses synthesize their shell *de novo*; sometimes they pick up an extra covering by budding. They are not pleomorphic, but different varieties may be more ovoid, others, brick-shaped.

Most range in size from 40-400 nm. (Filoviruses are 80 nm by up to 1400 nm.)

The envelope has proteins on the outside that may look like spikes or fuzz (e.g., influenza virus, coronavirus).

Sometimes the spikes are too short to be distinguished by EM, making the virion appear smooth (e.g., herpesviruses, rubella virus).

The nucleocapsid shape can be:

- a. icosahedral, like the naked viruses; however, some are morphologically nondescript as seen by EM;
- b. filamentous, like a "slinky."
- c. complex (poxvirus cores look like dumbbells with lateral bodies).
- d. morphologically nondescript (just dense without any particular shape).

Enveloped DNA viruses (except pox) construct their nucleocapsids in the nucleus.

Enveloped RNA viruses construct their nucleocapsids in the cytoplasm. (Measles nucleocapsids can sometimes be seen in the nucleus).

References on Virus Identification by Electron Microscopy

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Notes on above references:

- #s 2,5, 9, & 11-14 = Methods and micrographs
- #s 2 & 14 = Excellent atlases
- #s 7 & 8 = Brief synopses of technique and basis of virus ID
- # 15 = Good general reference, but don't memorize!! Lists all viruses, not just human.

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