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NEGATIVE STAIN TRANSMISSION ELECTRON MICROSCOPY OF VIRUSES AND VIRUS-LIKE PARTICLES

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Negative stain transmission electron microscopy (NS-TEM) is a powerful tool for identifying pathogens both directly in body fluids (Fig. 1), and indirectly after successful culture (Fig. 2). Negative stain electron microscopy is also important for monitoring cell-culture adapted viruses and recombinant virus-like particles (VLPs) that are produced for biological and pharmaceutical purposes.

Cell-culture adapted viruses and VLPs are produced to make diagnostic immunoassay reagents, vaccines, pharmacological vectors, or biological control agents. They also may be used for basic studies of virus structure and formation. Monitoring structural character and purity is crucial to their successful use. Quality of viruses and VLPs cannot always be determined by visual interpretation of protein band locations after gradient centrifugation or gel electrophoresis. Electron microscopy (EM) including scanning EM, transmission EM (TEM), and scanning probe microscopy may also be used to assess the quality of virus/VLP production and purification. TEM processing may include vitreous ice embedding, chemical fixation-plastic embedding and thin-section, or negative staining as preparation for EM. NS-TEM of viruses and VLPs is performed routinely within our laboratory for quality/quantity assessment. Evaluations are done for preparation purity, particle structural quality, and quantity. Examples of viruses and VLPs we have tested in the past are listed below; some of which will be presented in more detail.

Conventional Culture
Arenavirus (Fig. 2)
Bacteriophage: biological control agents; (Figs. 4-6)
West Nile virus
Coronaviruses: SARS CoV, HCoV-43, HCoV-229E and others
Paramyxovirus: Mumps, Parainfluenzavirus 1, 2
Metapneumovirus
Adenovirus: various types
Coxsackievirus: various types
Enterovirus: various types
Rotavirus A
Hepatitis A, B, E viruses
Influenzaviruses A, B
Mimivirus

Recombinant VLPs
Rotavirus C (Fig. 3)
Rotavirus B
West Nile virus
Western Equine Encephalitis virus
Papillomavirus 8
Papillomavirus 11
Papillomavirus 16
Sapporovirus
Norovirus
Hepatitis E virus
Human Bocavirus

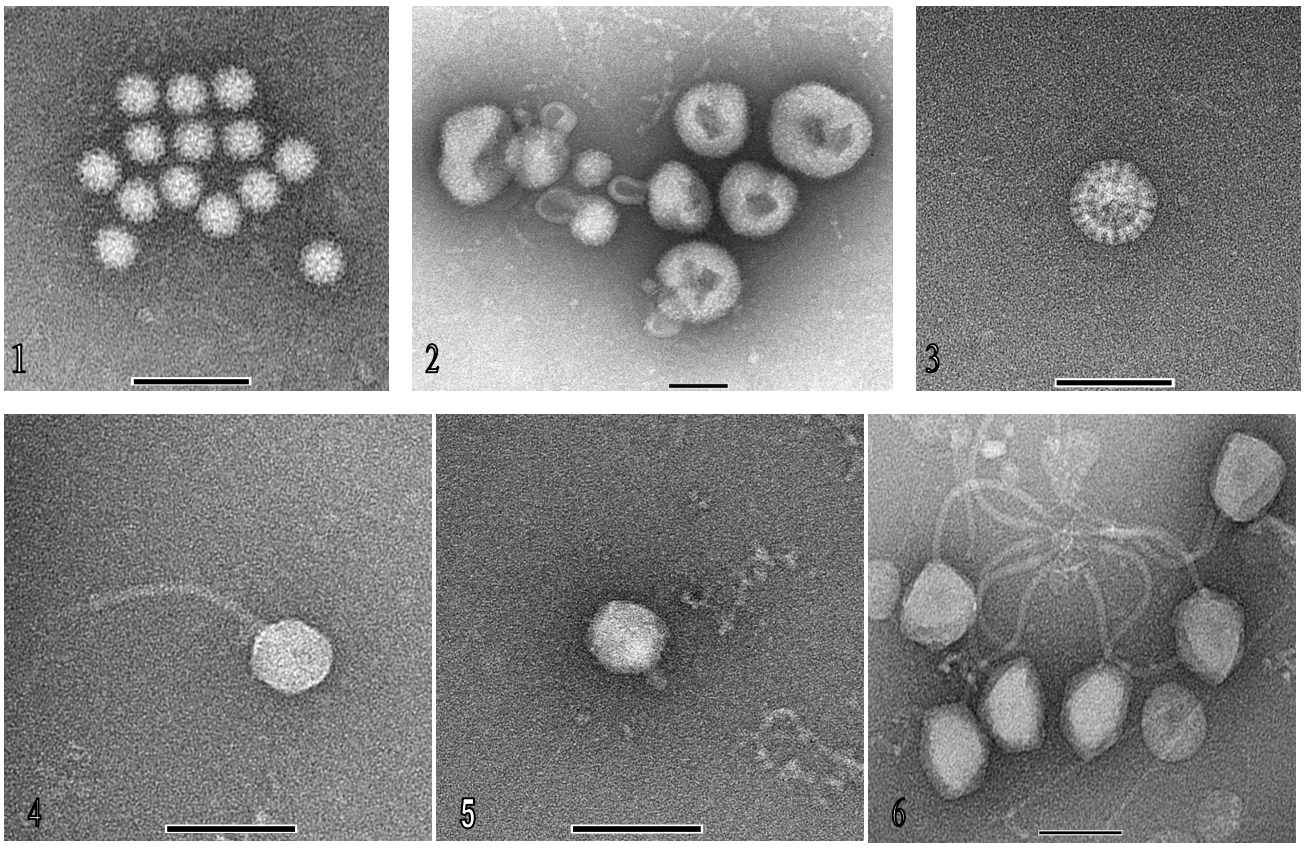

FIG. 1 Norovirus aggregate observed in human stool. Bar = 100 nm.
FIG. 2 "Old World" arenavirus grown in cell culture. Bar = 100 nm.
FIG. 3 Rotavirus C recombinant VLP formed by combination of virus protein 6 and virus protein 7. Bar = 100 nm.
FIG. 4. *Siphoviridae* bacteriophage; a potential biological control agent against *Pseudomonas aeruginosa*. Bar = 100 nm.
FIG. 5. *Podaviridae* bacteriophage; a potential biological control agent against *Pseudomonas aeruginosa*. Bar = 100 nm.
FIG. 6 *Siphoviridae* bacteriophage; a potential biological control agent against *Proteus mirabilis*. Bar = 100 nm.

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