Electron microscopy for virus identification and virus assemblage characterization

Kenneth M. Stedman, Kate Porter, and Mike L. Dyall-Smith

Abstract

This protocol is based on Schleper et al. (1992) as modified by Stedman et al. (2003).

This is a protocol from:

Stedman, K. M., K. Porter, and M. L. Dyall-Smith. 2010. Chapter 6: The isolation of viruses infecting Archaea. Manual of Aquatic Viral Ecology. Waco, TX:American Society of Limnology and Oceanography. doi:10.4319/mave.2010.978-0-9845591-0-7

Please see the published manuscript for additional information.

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Guidelines

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Generally this method is successful only if there is an indication for the presence of virus, for instance a halo on a lawn. Even when halos are formed, finding viruses by TEM can be challenging; often supernatants are concentrated 10- through 1000-fold by ultrafiltration or ultracentrifugation (Rice et al. 2001).

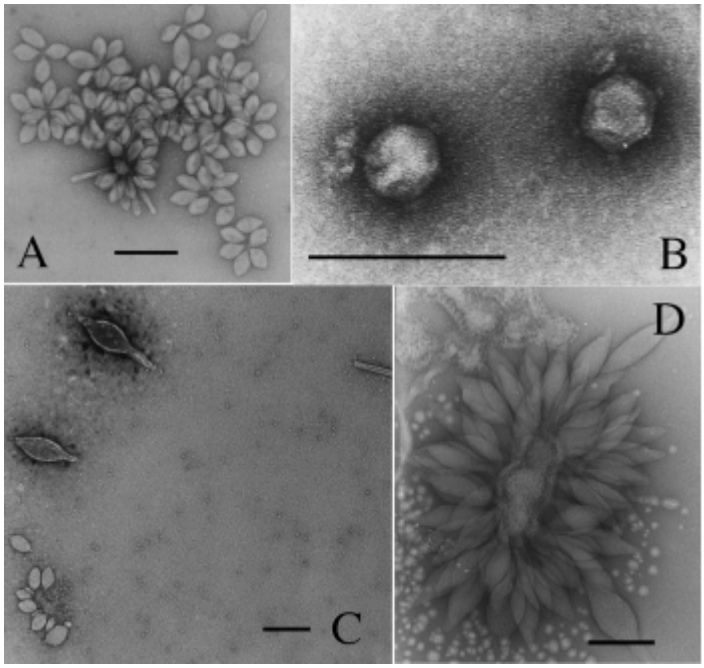

Fig. 2: TEM of *Sulfolobus* viruses and VLPs.
(A) *Sulfolobus* spindle-shaped virus SSV-I2 particles.
(B) *Sulfolobus* turreted icosahedral virus (STIV).
(C) Three different VLPs from an enrichment culture from Amphitheater Springs, Yellowstone National Park, USA. Note end of a *Sulfolobus islandicus* rod-shaped virus (SIRV)-like particle in upper right of image).
(D) Virus-like particles from Amphitheater Springs. All scale bars 200 nm. Negative stain with uranyl acetate.

Protocol

Step 1.
5µL of an enrichment culture, or 0.2 µm filtered and centrifuged (10 min at 3000g) cell-free supernatant, is spotted onto carbon/formvar-coated electron microscope grids (Ted Pella or EM Sciences).

DURATION
00:10:00

Step 2.
It is then allowed to absorb for 2 min.

DURATION
00:02:00

Step 3.
Remove sample from grid by slowly bringing a small (ca. 1cm^2) piece of filter paper perpendicular to the grid to the side of the grid. The sample will be removed by wicking.

Stain grid by placing on a 5µL drop of 2% Uranyl Acetate (or Phosphotungstate) for 15-30 seconds. Remove stain by wicking as above. Air dry for at least 10 minutes

NOTES
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VLPs can generally be discerned at ×16,000–20,000 magnification (Fig. 2 in guidelines).

Step 4.
Samples are examined by transmission electron microscopy (TEM), e.g., JEOL 100 cx, operated at 100 keV.

NOTES

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VLPs can generally be discerned at ×16,000–20,000 magnification (Fig. 2 in guidelines).