

Plant Virus Identification using Transmission Electron Microscopy (TEM)

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Overview



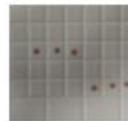
I. Grinding symptomatic plant tissues in buffer



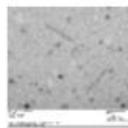
II. Low-speed centrifugation (keep the supernatant)



III. High-speed centrifugation (keep the pellet)



IV. Preparation of grids for TEM



V. TEM screening

Fig.A. Steps in the process of sample preparation for viral detection by TEM. Icons by Pixabay, Delapouite, and Pypaertv. Photos by Tatiana Lenskaia.

I. Grinding plant tissues in buffer

- Grind plant tissues thoroughly in liquid nitrogen:
<https://www.youtube.com/watch?v=AI1SsJ4JwXc>
- Add buffer and continue grinding:
<https://www.youtube.com/watch?v=OeIRziBNn6c>
- Filter through a cloth to remove plant remnants:
<https://www.youtube.com/watch?v=X4z3hC06wAQ>

II. Low-speed centrifugation

This type of centrifugation is used for a crude separation of particles. This step allows us to remove most of the remaining plant tissues and have virus particles released into the liquid buffer.

After the centrifugation,
the **supernatant is kept**
(liquid fraction in the tube),

and the pellet is discarded
(solid fraction at the bottom of the tube).

III. High-speed centrifugation

This type of centrifugation is used for a fine separation of particles. This step allows us to remove most of the remaining buffer and have virus particles concentrated as a pellet at the bottom of the tube.

After the centrifugation,

the pellet is kept
(solid fraction at the bottom of the tube),

the supernatant is discarded
(liquid fraction in the tube).

IV. Grid preparation

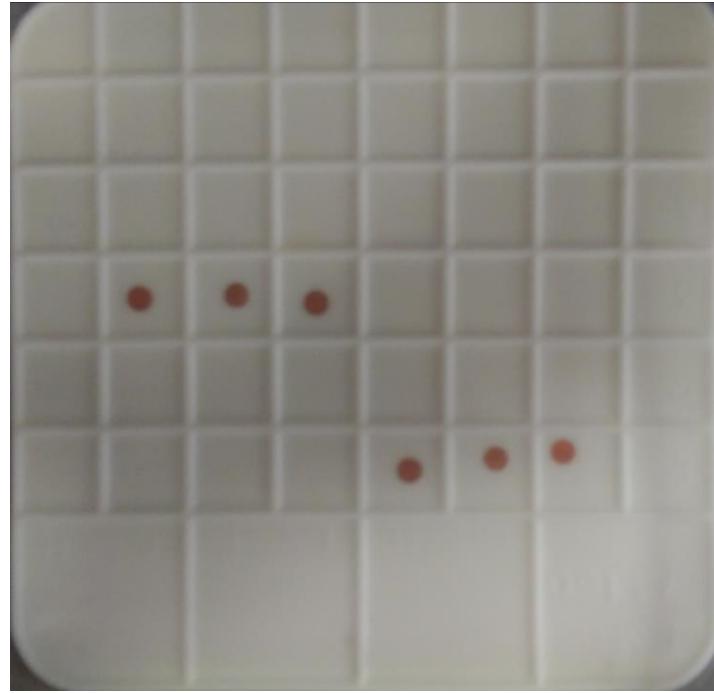


Fig.B. Example of the TEM grids.
Photo by Tatiana Lenskaia.

For detail:

<https://www.sciencelearn.org.nz/resources/500-preparing-samples-for-the-electron-microscope>

V. TEM screening

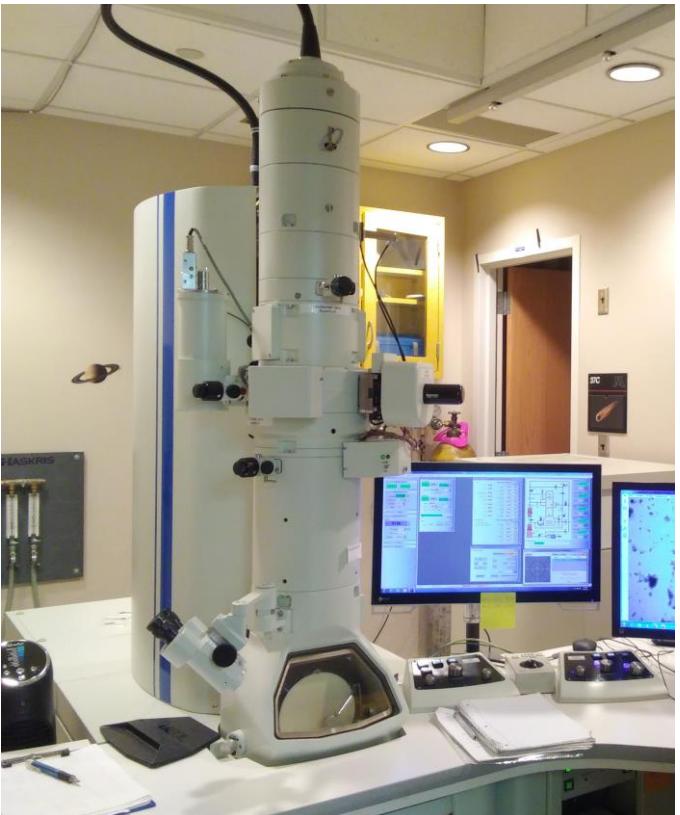


Fig.C. An Example of a transmission electron microscope. Photo by Tatiana Lenskaia

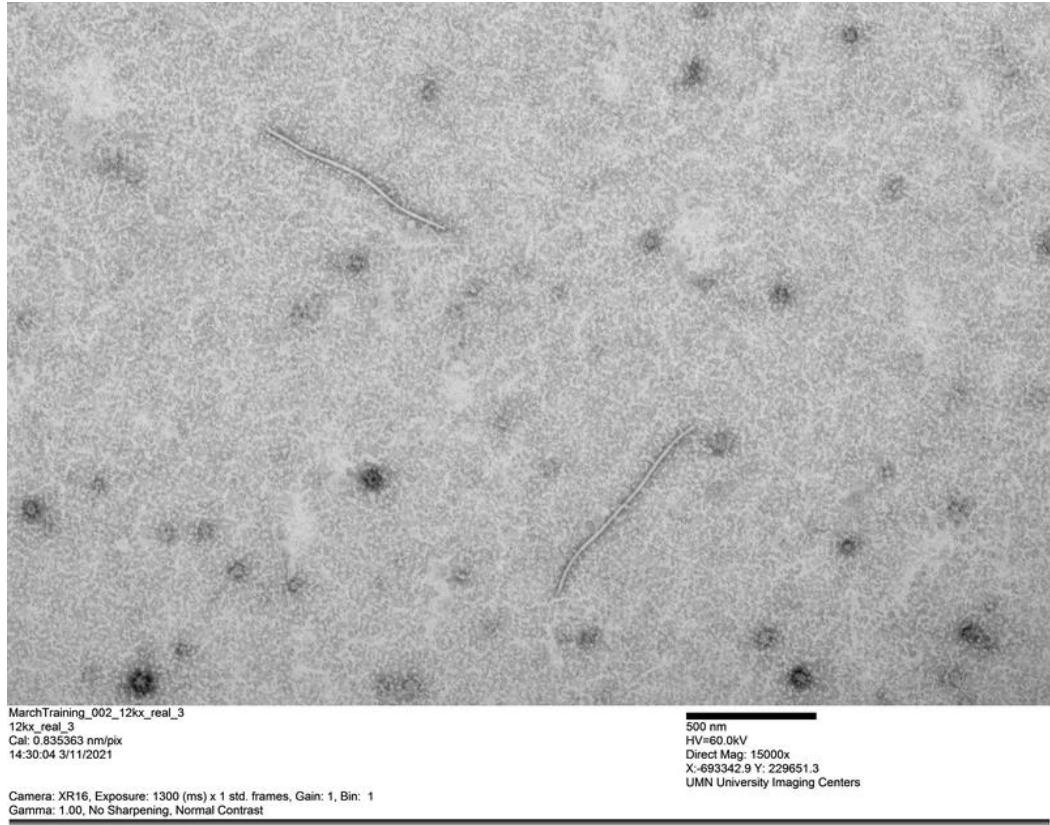


Fig.D. Example of an image of viral particles taken during TEM screening.
Microscopist: Tatiana Lenskaia.