Negative staining of viral samples

Scope:
Negative staining is a quick method to study the morphology and structure of particular specimens (example viruses). The specimen is stained with a heavy metal such as phosphotungstic acid or Uranyl Acetate and the difference between the specimen and background provides the contrast to view the sample with an electron microscope.

Reference:

Hayat, M.A. 2000. Principles and techniques of Electron Microscopy. Biological Applications. 4th edition. Cambridge University Press.

Bozzola, J.J. and L.D. Russell. 1999. Electron Microscopy. Principles and Techniques for Biologists, 2nd edition. Jones and Bartlett Publishers, Boston.

Chemicals:
3% Phosphotungstic acid [PTA] or 4% Uranyl acetate [UA]

Method:

<u>Scabs/skin/tissue/plant material</u>:

Place sample in a mortar dish.
Cut the sample into smaller pieces with the scalpel and add a few drops of dist H_2O.
Use the pestle to grind the pieces of tissue into a paste.
Add more dist H_2O and mix into the paste – withdraw this solution with Pasteur pipette into an eppendorf tube & centrifuge @ 3000 rpm for 15 min.
Keep supernatant & centrifuge @ 13 000rpm for 45 min – decant supernatant & mix one drop of dist H_2O to mix with the pellet. One drop of this mixture is mixed with one drop of PTA or UA – leave for 30 seconds, place formvar coated grid on the mixture, leave for 30 seconds, blot dry by holding the grid upright against paper towel.

[Can also: Keep one drop of the original mixture and do a direct stain without centrifugation]

<u>If pellet is minute</u> – rinse very carefully, add PTA/UA directly to pellet, mix, place grid on a drop of this mixture.