Positive and Negative Staining of Viruses on TEM Grids

Jennifer Brum, Tucson Marine Phage Lab
April, 2011

Purpose: This protocol describes how to negatively or positively stain viruses that have been deposited onto TEM grids so that they can be imaged using transmission electron microscopy (TEM). Positive staining stains the virus itself such that the virus is dark against a lighter background (Figure 1A). This is the easiest staining to do and yields results suitable for analyzing the morphological diversity of viruses from aquatic samples. Negative staining relies on some stain remaining around the edges of a virus such that the details of the virus are clearly defined (Figure 1B). Negative staining is more difficult and is used to obtain publication quality micrographs of viruses.

Note: To deposit viruses onto the grids prior to staining, use either the "Adsorbing Viruses onto TEM Grids" protocol or the "Quantitatively Depositing Viruses onto TEM Grids" protocol. Grids should be stained immediately after depositing viruses onto them.

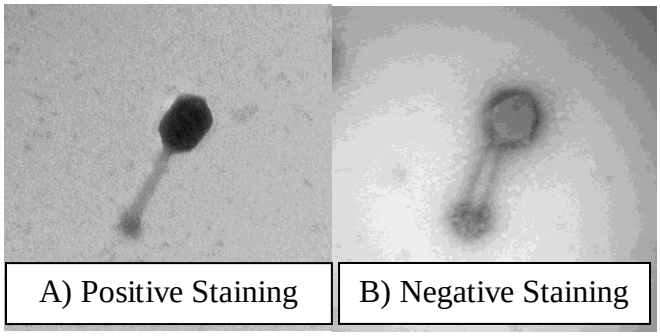

Figure 1. Examples of a positively stained virus (A) and negatively stained virus (B).

Materials required:

- EM grid-grade tweezers (2 or more pairs)
- uranyl acetate
- ultra-purified water (e.g. Milli-Q water)
- petri dish
- 4x 2 ml microcentrifuge tubes with screw caps
- 4 ml plastic culture tube with cap
- small stir bar
- stir plate
- filter paper (cut into wedges)
- 0.02 µm syringe filter and syringe (3-5 ml)
- lab coat, respiratory protection (mask), eye protection
- timer
- waste container for uranyl acetate

Positive Staining of Viruses on Grids:

This is the easiest staining to do. It is "quick and dirty". It provides less detail of your viruses, but will allow you to measure their dimensions. It is not the proper staining method if you want really pretty micrographs of your viruses.

1) Make 2 ml of 2% uranyl acetate with ultra-purified water in a 4 ml culture tube using a stir bar to mix the sample on a stir plate for ~30 minutes to 1 hour (do not turn on the heat!)
(0.04 g uranyl acetate + 2 ml Q-water = 2% uranyl acetate)
*Caution: uranyl acetate is toxic so read the MSDS and use a lab coat, eye protection, respiratory mask, and gloves when handling it in powdered form

2) Filter the uranyl acetate solution through a 0.02 µm syringe filter into a 2 ml screw-cap tube
- this removes any particles of uranyl acetate that have not fully dissolved

3) Fill 3x 2 ml screw-cap tubes with ultra-purified water

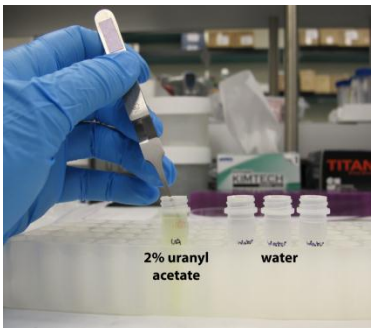

Figure 2. Immersing the grid in uranyl acetate.

4) Immerse the grid into the 2% uranyl acetate solution for 30 seconds (Figure 2)

5) Immerse the grid into the 1st tube of ultra-purified water for 10 seconds

6) Immerse the grid into the 2nd tube of ultra-purified water for 10 seconds

7) Immerse the grid into the 3rd tube of ultra-purified water for 10 seconds

8) Wick away liquid from the grid using a wedge of filter paper placed at the edge of the grid (Figure 3)

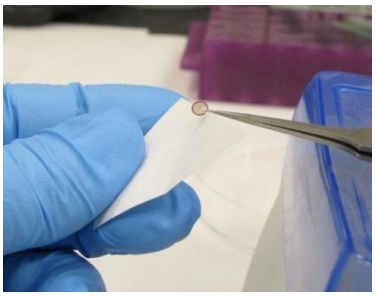

Figure 3. Wicking liquid from the grid.

11) Place the grid in a grid box and allow to dry overnight, then store the grid in a desiccator until analysis (do not put wet grids in a desiccator immediately as this can create "cracking" of the stain)

Negative Staining of Viruses on Grids:

Negative staining is equal parts magic, art, luck, and skill. If done correctly, it will yield detailed views of your viruses and if you are very lucky, it will yield a few pretty micrographs of your viruses.

1) Make 2 ml of 2% uranyl acetate with ultra-purified water in a 4 ml culture tube using a stir bar to mix the sample on a stir plate for ~30 minutes to 1 hour (do not turn on the heat!)
   (0.04 g uranyl acetate + 2 ml Q-water = 2% uranyl acetate)
   *Caution: uranyl acetate is toxic so read the MSDS and use a lab coat, eye protection, respiratory mask, and gloves when handling it in powdered form

2) Filter the uranyl acetate solution through a 0.02 µm syringe filter into a 2 ml screw-cap tube
   - this removes any particles of uranyl acetate that have not fully dissolved

3) Hold the grid in EM grid-grade tweezers

4) Using a pipettor, drip 3 drops of uranyl acetate solution onto the shiny side of the grid, allowing each of them to run off into the uranyl acetate waste container

5) Add a third drop of uranyl acetate solution to the shiny side of the grid and allow it to sit for 45 seconds (Figure 4)

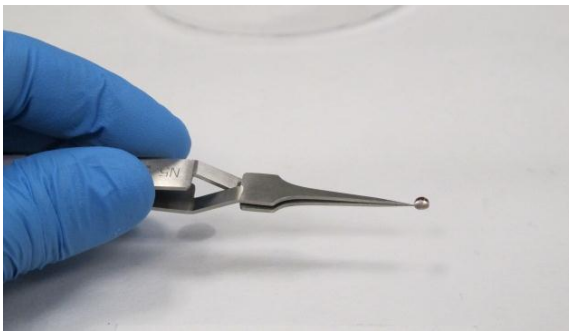

Figure 4. Staining the grid with a drop of uranyl acetate.

6) Wet a wedge of filter paper in ultra-purified water – it should be wet, not soaking wet

7) Using the wedge of wet filter paper, wick away the stain from the grid but *leave a thin sheen of stain on the grid*
   - This is the magic/skill part
     - too much stain left on the grid and all you will see in the TEM is stain
     - too little stain left on the grid and your viruses will not be negatively stained

8) Set the tweezers with the grid on a petri dish such that the grid does not touch anything (Figure 5)

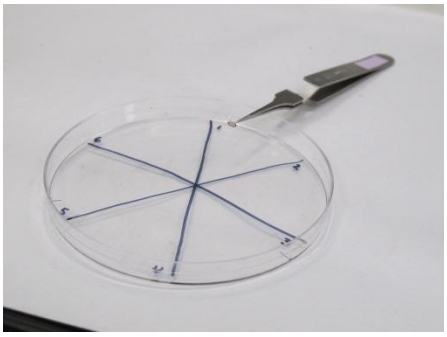

Figure 5. Drying the grid.

9) Wait until the grid is dry (~1hr) (this step is optional - the grid can dry in the grid box if you are in a hurry - just heed the warning in step 10)

10) Place the grid in the grid box and store the grid box in a desiccator
    - WARNING: if you put the grid in the desiccator before it has dried, the stain will look "cracked"

Applicable References:

Ackermann and Heldal, 2010 – An excellent reference for learning about imaging of viruses.

Brum and Steward, 2010 – A reference for the positive staining protocol.

Ackermann, H.-W., and Heldal, M. (2010) Basic electron microscopy of aquatic viruses. In *Manual of Aquatic Viral Ecology*. Wilhelm, S.W., Weinbauer, M.G., and Suttle, C.A. (eds): ASLO, pp. 182-192.

Brum, J.R., and Steward, G.F. (2010) Morphological characterization of viruses in the stratified water column of alkaline, hypersaline Mono Lake. *Microbial Ecology* **60**: 636-643.