Protocol for TEM Grid Staining

This protocol demonstrates two different methods for staining TEM grids with bacteriophage samples: the Formvar method and the Valentine method. The initial steps for preparing the phage sample are the same, but the staining methods are different, and are subdivided accordingly. Note that, while the Valentine method takes more effort, the results are often cleaner when performed correctly. Typically, both methods are employed for each sample to increase the likelihood of obtaining a good image.

Supplies:

- 2 % w/v uranyl acetate* (recommended to spin down 1 min at ≥14,000*xg* to avoid any precipitation; observe all local rules about waste collection)
- 5 mM $MgSO_4$ (TEM diluent buffer)
- Parafilm
- Precision EM forceps
- Formvar method: copper grids, 300 or 400-mesh
- For Valentine method: carbon-on-mica (often an imaging center can assist with this material) and uncoated copper grids, 300 or 400 mesh
- Glow discharge cleaning system (to clean EM grids)
- Phage sample at ≥10^9 pfu/ml

Before staining, clean grids should be prepared. Pre-coated grids should be glow-discharged, which removes residual hydrocarbons and makes the copper grid less hydrophobic. After cleaning, the grids are good to be used for several hours. To perform cleaning, please follow instructions for using the specific system at your laboratory.

Preparing phage sample

1. Label two sterile microcentrifuge tubes appropriately to your sample name.
2. Take a 1 mL aliquot of sterilized phage sample and transfer it to a labeled tube.
3. Centrifuge this aliquot for 10 minutes at ≥14,000*xg* to pellet debris.
4. Transfer 500 µL of the supernatant into the second labeled tube. Use the contents of the second tube for the following EM staining steps.
5. Lay a paper towel out on the bench.
6. Cut off a 6” strip of parafilm, remove the paper backing, and place it on the paper towel (with the side that faced the paper backing up).
7. Collect a piece of filter paper, grid forceps, and uranyl acetate stain.
8. Gently pipette or ‘flick’ your phage tube to evenly suspend the phage particles.
9. Place a 10 µl drop of phage lysate on the parafilm. Add 40 µl of TEM diluent buffer (5mM $MgSO_4$) solution to obtain a 5x dilution of the phage; mix by slowly pipetting.
10. Next to each drop of phage, place 40 µl of 2% w/v uranyl acetate on the parafilm.
    a. Use care when pipetting the uranyl acetate solution to avoid splashes or droplet formation.

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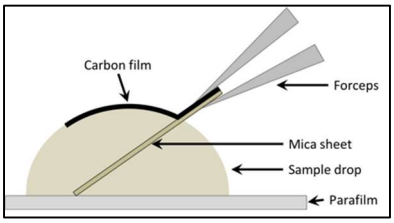
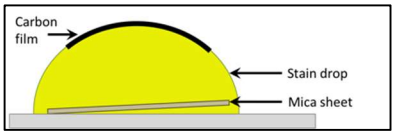
Method 1 – Valentine-based staining[1]

1. Gather the clean, uncoated copper grids and carbon-on-mica.
2. Next, cut off a square piece of the carbon-on-mica, slightly larger than the size of the copper grid. Determine which side is mica and which is carbon. This step takes practice, and it is often best to perform a test in a droplet of water to see which side bubbles up (that is the carbon film).

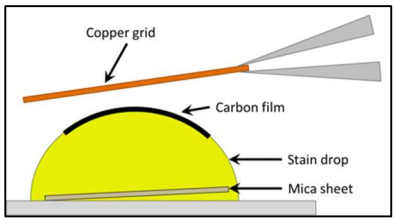
3. Holding near the edge of the carbon-on-mica piece with the forceps, place the carbon side up and at a ~45-degree angle dip the mica ¾ths of the way into the sample drop. The carbon should float off of the mica and rest on top of the drop, but remain attached to the mica at the unsubmerged area. Hold the carbon film in the sample for **1 minute**. *Stabilize your hand to avoid shaking and breaking up the carbon film*.



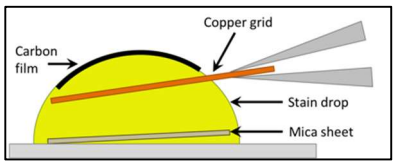
4. Transfer the mica to the stain drop, but this time let the mica fully submerge in the liquid and let go with the forceps. The carbon film should detach from the mica and float on top of the stain for **~10-15 seconds**.



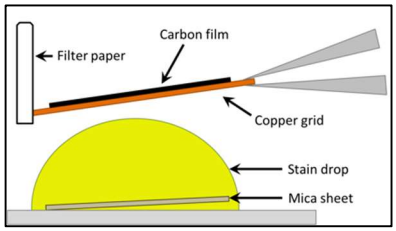
5. Use the grid forceps to pick up a new, clean TEM grid, dull side up.



6. After the carbon film has been floating on the stain for the appropriate amount of time (about 10-15 seconds), dip the grid into the drop and pick up the film with the grid from the bottom.



7. Lift the grid from the drop and check that at least some of the carbon film has adhered smoothly to the grid. Remove the excess stain by lightly touching the *edge* of the grid with a small piece of absorbent filter paper. Place the grid on a grid mat carbon side up or in a grid box and allow to air dry. **Be sure to record the grid position in your notebook! Allow the grid to fully dry before imaging.**



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Method 2 – Formvar-based staining

Note that Formvar Grids have two sides---a shiny side and a darker-looking carbon side. Make sure you can tell the difference. The dark side should contact your phage.

1. Set a timer for one minute or position yourself to watch the clock.
2. Pick up a Formvar carbon grid with grid tweezers (try not to bend the grid) and carefully float it, carbon-side (darker side) down, on the drop of sample for **1 min.**
3. Pick up the grid and float it on top of the drop of uranyl acetate, carbon side up this time. Let it sit for **1 min**.
4. Remove the grid from the uranyl acetate and gently touch the *edge* of the grid to sterile filter paper to wick off the excess stain.
5. Place the grid, carbon side up, onto a grid mat/in a grid box and allow to dry.

References

1. Regulation of glutamine synthetase. XII. Electron microscopy of the enzyme from Escherichia coli, R. C. Valentine, B. M. Shapiro, and E. R. Stadtman, Biochemistry **1968** 7 (6), 2143-2152 DOI: 10.1021/bi00846a017