**EM Staining Protocol:**

The solutions and workspace is located in the Vogl Lab. We do not need to mix these toxic chemicals ourselves.

Setup: lay a piece of parafilm down in a large glass petri dish (with lid), sticking the edges down to the glass. You should have a piece of filter paper with a small glass petri dish to ready to put your stained grids down on. You will also need three scintillation vials with ddH2O, a wash bottle filled with ddH2O, and a beaker for the washes.

Get the container of Uranyl Acetate solution (glass volumetric flask covered in tin foil, in 4˚C fridge in Vogl lab). – CAREFULLY carry to the work station. You will need to filter the Uranyl Acetate solution, so that undissolved particles don’t wreck your grids. Fold a filter paper and filter into a scintillation vial. Transfer UA using a Pasteur pipette. You need one drop per grid, but some is lost during filtration, so you’ll have to filter a bit more.

Using the Pasteur pipette, put several drops of UA onto the parafilm in the large petri dish (one drop per grid).

STAINING – for conventional EM (no immunogold): Each grid is on the stain for 4 min.

* Put the grid on shiny (sample) side down and turn on the ‘count up’ function on the timer.
* At the 2 min mark, put the next grid on
* At the end of the first grid’s staining time, you will put the 3rd grid on, and take the 1st off at the same time. Continue with this sequence for each subsequent grid.
* After the stain, the grid is rinsed for 25s in the first ddH2O vial, 25s in the 2nd ddH2O, and 25s in the 3rd ddH2O. The grid is then dried gently with a filter paper, and put (sample side up) on the sample holder to dry.
* At this point, the 2nd grid should be done, and the 4th grid can be added as the 2nd is removed for the washes. It’s an assembly line process that works well.

**STAINING – for immunogold samples**: Each grid is on the stain for 2 min.

* Put the grid on shiny (sample) side down and turn on the ‘count up’ function on the timer.
* At the 1 min mark, put the next grid on
* At the end of the first grid’s staining time, you will put the 3rd grid on, and take the 1st off at the same time. Continue with this sequence for each subsequent grid.
* After the stain, the grid is rinsed for 10s in the first ddH2O vial, 10s in the 2nd ddH2O, and 10s in the 3rd ddH2O. The grid is then dried gently with a filter paper, and put (sample side up) on the sample holder to dry.
* At this point, the 2nd grid should be done, and the 4th grid can be added as the 2nd is removed for the washes. It’s an assembly line process that works well.

After all grids are through this process, mop up the drops of UA with a filter paper, and throw out the filter paper and parafilm. Lay out a new sheet of parafilm on the glass, and spread some sodium hydroxide pellets around the parafilm sheet. Empty out the rinse vials and get fresh ddH2O for them. Get the lead citrate solution (also in a glass volumetric flask covered in tin foil, in the Vogl fridge), and CAREFULLY bring it over to the workstation. You do not need to filter this, but do not take from the very bottom of the container. Again, using a Pasteur pipette, put one drop per grid on the parafilm. When the lead citrate solution is exposed to air (ie. When the dish is uncovered or the stock solution is open) NO BREATHING or TALKING allowed. You don’t want to inhale lead.

STAINING: Follow the same assembly line timelines as for the UA stain (4 min on, 3x 25s washes for conventional EM; 2 min on, 3 x 10s washes for immunoEM). Once all grids are through the process, give them some time to dry on the filter paper while you clean up.

Again, use a filter paper to mop up the lead citrate, toss out the filter paper and parafilm, and put the used Pasteur pipettes in glass garbage. Sodium Hydroxide pellets can be rinsed down the sink with lots and lots of water.