Immunogold labelling on ultra-thin frozen sections

1. Cut ultra-thin sections.

2. Make solutions (especially PVA/lead citrate ---start chilling water).

3. Sections are sitting on 10% Fetal Calf Serum (FCS) (or can use 1% BSA) in PBS, pH 7.4, 0.1M.

4. Quench for 10 minutes with 0.01M glycine in 10% FCS (or 1% BSA) in PBS. Wash in PBS/10% FCS (or 1% BSA) for five minutes over three puddles. (Quenching binds the free aldehyde groups. The step is not needed when a Nakane fix is used.)

   Or wash in PBS/0.1% BSA many times over 30 minutes to get rid of all sucrose, and then wash in PBS/1% BSA 2x 2 minutes.

5. Add first antibody. Incubate for one hour. Antibodies are diluted in 10% FCS (or 1% BSA) in PBS.

6. Wash with 1% FCS (or 0.1% BSA) in PBS for 10 to 15 minutes. Go over a total of eight puddles, the first three to four puddles are passed over quickly. Then place in two 10% FCS (or 1% BSA) puddles, two min EA.

7. If a bridge antibody is needed, it may be added here for 30 minutes to one hour. The antibody is diluted in 10% FCS (or 1% BSA). This step is followed by a wash similar the same as is explained in step six.

8. Centrifuge the gold conjugate for five minutes in the cold room. The gold conjugate is diluted in 10% FCS (or 1% BSA) and administered onto parafilm in 10 to 20 microliter droplets which are dispensed from the upper portion of the Eppendorf tube, using care to avoid the bottom of the tube. Incubation with the gold conjugate continues for 30 minutes. (Keep in mind that the larger the gold particle, the harder it is for the gold to enter the cells.)

9. Wash in the same manner as was explained in step six, except, if you are only adding one antibody probe, the last washing step in done in plain PBS and then you can jump to step 15.

   (If you did not begin making PVA in the beginning, you must start making it now )

10. If a second antibody is to added, it would be done here, and incubated for one hour.

12. This gold conjugate step is the same as is explained in step eight. Incubate for one half hour.

13. Wash as explained in step 9.

14. Post-fix for 10 minutes in 2% Glutaraldehyde in PBS. Make sure you change your forceps for this step.

15. Wash with ddH2O in a petri dish (or if you have been using BSA, wash in PBS 2x 2 min and then one petri dish of ddH2O). Bring petri dish to hood.

16. Fix for 20 minutes with a 50:50 mixture of 4% OsO4 and ddH2O in a covered petri dish under the hood (This incubation could be prolonged for up to one hour.)

17. Contrast with 2% Uranyl Acetate in ddH2O for 20 to 30 minutes in an uncovered petri dish (to avoid humidity). Wipe the forceps after touching each grid.

18. Incubate for five minutes in polyvinyl alcohol (PVA) through two changes, dipping the forceps in alcohol and drying them in between each grid. The PVA recipe is 2.2 g PVA in 100 ml ddH2O + 0.0025 g lead citrate. (The PVA dehydrates the specimen and acts as an embedding medium.)

22. Pick up grids with a loop and suck off excess PVA with filter paper. Let air dry for about five minutes.

23. Pick grids out of loops with forceps.

24. Examine on TEM.