Post-Imbedding Immunoelectron Microscopy

I use a sheet of parafilm pressed onto a 96 well plate to make indentations for the drops of solutions on which the grids are floated. Place the 96 well dish in a plastic box with wet filter paper on the bottom to humidify the interior. I use drops of 50 µl volume. A self-closing, non-capillary forceps is best. I don't dry the grid between the different steps. All buffers below are filtered through a 0.45 µm filter.

- Washing may be done with a gentle stream or "jet" instead of transfers between drops.
- TBS may work better than PBS with some antibodies.

1. (Optional) Etch: 10 - 60 min at room temperature (RT) with freshly prepared 10% NaIO₄. (I usually use 15 minutes.)
2. Wash 3 X 2 minutes with water.
3. Block 15 - 30 minutes with PBS + 1% BSA + 0.01% TX-100 + NaN₃.
4. Incubate with antibody for 1 - 2 hours at RT. Dilute antibody 1:2 to 1:50 in PBS + 1% BSA + 0.01% TX-100 + NaN₃. Use monoclonal supernatant without dilution.
5. Wash 1 X 1 minutes + 4 X 5 minutes with PBS + 0.1% BSA + 0.01% TX-100 + NaN₃. (The wash buffer has a lower BSA concentration.)
6. Incubate with colloidal gold secondary antibody for 1 hr at RT. Dilute antibody 1:10 to 1:25 in PBS + 1% BSA + 0.01% TX-100 + NaN₃.
7. Wash 1 X 1 minutes + 4 X 5 minutes with PBS + 0.1% BSA + 0.01% TX-100 + NaN₃.
8. Wash 4 X 2 minutes in PBS.
9. Fix with 2% glutaraldehyde in PBS for 10 minutes at RT. This step may not be necessary depending on the effectiveness of the staining.
10. Wash 3 X 2 minutes with PBS.
11. Wash 3 X 2 minutes with water.
12. Stain 5 minutes at RT with freshly made 1% uranyl acetate (pH = 4 - 4.5).
13. Wash 3 X 2 minutes with water.
14. Air dry completely
15. View at 60 kV for maximum contrast.