Immediately wash the grid with at least 10 drops of distilled or deionized water to remove all traces of the washing solution. This step may be omitted if the previous washing solution consisted only of water or of water containing bacitracin.

Wash the grid with 2 or 3 drops of negative stain. This will remove any remaining water and insure a uniform distribution of stain. Use a dry corner of the blotting paper to remove as much of the stain as possible; leaving only a thin film. Allow the grid to dry before removing it from the tape. Exercise care in pulling the grid from the tape or the substrate may be damaged (many parallel tears in the substrate are usually indicative of this type of damage).

The grid is now ready for storage or examination in the electron microscope. If the grid has been stained with a uranyl compound, then it may be safely stored for extended periods with no special precautions. However, as some stains such as PTA are hygroscopic, it is sound procedure to routinely store all of the grids in a desiccator (using Drierite, silica gel, or calcium chloride as the adsorbent). Grids have been stored for years in this manner with no noticeable deterioration.

Negative Staining of Immunosorbent Materials.

Immunosorbent electron microscopy is a term that covers several different techniques that increase the number of virions adsorbed to the surface of a specimen grid, and/or aid in the process of identifying a particular virus infection. It is beyond the scope of this bulletin to cover immunosorbent electron microscopy (ISEM) in depth; however, it may be useful to briefly outline grid preparation procedures as we have adapted them for use with ISEM. The materials that are needed for ISEM are mostly the same as those that are needed for the regular leaf dip procedure (see the materials in Appendix 1). Additionally, appropriate antisera and a supply of protein A are needed.

1. The Derrick Method (Protein A Modification). Place a droplet of an aqueous solution of protein A (10%, w/v) on a grid attached to double-sided cellophane tape as described before, and allow it to stand for 15 min. Wash the grid with 20 drops of SP buffer (0.01 M KPO_4, pH 7.2).

Immediately remove most of the buffer and wash the grid with 1 droplet of antiserum diluted with SP buffer. The precise amount of dilution should not appreciably affect the number of antibodies adsorbed to the grid as long as the dilution is not too great. We have used dilutions ranging from 1:20 to 1:1000 with good results. Washing the grid with a drop of the antiserum diluted to the proper strength precludes any further diluting effect by the buffer. Apply a