

## **Laboratory for Advanced Electron and Light Optical Methods**

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### **Negative Staining with Phosphotungstic Acid (PTA)**

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**Modified from:** M.A. Hayat and S.E. Miller. 1990. Negative Staining. McGraw-Hill Publishing Co., NY. (Page 216)

- 1. Applications and Objectives:** Negative staining with slightly alkaline PTA is commonly used for many types of particulate samples. Nermut (1982) reports that this stain is most appropriate for the proteinaceous capsomeres of viruses while uranyl acetate at acidic pH is superior for the membranous components and glycoprotein spikes associated with the surfaces of enveloped viruses. The objective is to produce a finely electron-dense coating over the entire surface of a film-coated grid bearing particulate materials, with only slight stain pooling around the particulates, allowing easy visualization of surface structures of the particulate samples (viral capsomeres, bacterial pili or flagella, protozoan cell surface scales, etc.).

**The optimum concentrations of particulates is determined empirically, but  $10^6$  to  $10^8$ /mL is good for viruses and bacteriophage;  $10^{10}$ /ml is good for bacteria.**

#### **2. Materials Needed:**

sodium phosphotungstate  
bacitracin (powder)  
1 N NaOH  
100 ml stock bottle  
distilled water

Pasteur pipets

pH meter

Formvar-coated grids

concentrated particulate sample suspended in a small volume  
of fluid

forceps

Whatman #1 filter paper (9 cm disks)

### **3. Preparation of Staining Solution:**

3.1. Add 0.1 g of sodium phosphotungstate (PTA) powder to 10 ml of distilled water in a shell vial. Check the PTA solution pH with the pH meter and adjust the pH to 7.2-7.4 with the 0.1 N NaOH

3.2 Add 500 µg bacitracin powder to the phosphotungstic acid solution

### **4. Staining Procedure:**

4.1. Pick up a Formvar-coated grid with forceps and push the forceps locking ring down so that grid is held firmly. Lay forceps down on Petri dish lid with tips extending over the edge with the grid held coated-side up.

4.2. Add one drop of concentrated particulate suspension (see section on preparing viral samples for negative staining) to grid with Pasteur pipet.

4.3. After 3-5 min, remove suspension by touching ragged torn edge of filter paper to the edge of the forceps jaws where they contact the grid) until the grid surface is nearly dry. Never let the grid surface totally dry out because it will produce a coating of culture or body fluids and yield an excessively dirty grid.

4.4. Add one drop of PTA/bacitracin solution to the grid.

4.5. After 1 min, dry the grid as before with the ragged torn edge of filter paper except, this time, dry the grid quickly and completely. Immediately touch the sample-side of the grid to a clean piece of filter paper in the bottom of a Petri dish. Slide a fresh piece of filter paper down between the forceps jaws to push the dried grid out of the forceps tips and onto a clean, dry part of the filter paper in the Petri dish.

4.6. Let the grid dry for 15 min in the Petri dish (covered) and then examine with a TEM.

**5. Results expected:** The grid will have a finely granular electron density over the entire surface, with slightly darker regions surrounding the particulate sample, but the stain will be excluded from intact particulate materials.

**6. Cautionary Statements:** Negative stains are all heavy metals, so should be handled as the toxic and hazardous materials that they are. Dried droplets of stain should be handled with care to avoid inhalation. All wastes should be disposed of according to the institutional guidelines for hazardous wastes. **If working with pathogenic viruses or bacteria, it should be noted that negative staining does not kill all pathogens. Thus, the infectious agent should be inactivated prior to negative staining or the grid should be sterilized by gas or ultraviolet radiation prior to being handled or put into an electron microscope.** If the viability of a pathogen is in question, proper safety precautions should be employed and, following negative staining, the grid should be tested for pathogen viability before determining the necessary safety procedures to be employed in future studies of the agent.

Some older papers and procedure manuals suggest the use of nebulizers to produce a fine mist of viral suspension that is directed toward a coated grid. This procedure should not be used under any circumstances since atomizing a suspension of viral particles does not conform to safe laboratory practices.

**The negative stain solution keeps 1-3 weeks at 4° C.**

**References:**

- Hayat, M.A., and S.E. Miller. 1990. Negative staining. McGraw-Hill, Inc., NY. 253 p.
- Nermut, M.V. 1982. Advanced methods in electron microscopy of viruses. IN: C.R. Howard (ed.), New developments in practical virology. Alan R. Liss, NY. 343 p.