

Laboratory for Advanced Electron and Light Optical Methods

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Ultracentrifugation Technique for Viral Sample Preparation

1. **Applications and Objectives:** This technique is utilized to concentrate viral particles from infected cell cultures, tissues, or body fluids. For this technique to be highly successful, the number of viral particles needs to be fairly high.
2. **Materials Needed:**
 - Pasteur pipets
 - 15 ml clinical centrifuge tubes
 - ultracentrifuge tubes
 - clinical centrifuge
 - ultracentrifuge
 - distilled water
3. **Procedure:**
 - 3.1. Collect sample, dilute about 1:10 with PBS; store frozen if necessary. Do not sonicate cell samples or scrape mucosal linings to release viral particles from cells. Sometimes, it is useful to freeze (at -20°C) and thaw infected cell cultures several times to release viral particles.
 - 3.2. Centrifuge sample at approximately 1,000 relative centrifugal force (rcf) for 5 min or until clarified on a clinical centrifuge.
 - 3.3. Centrifuge supernate from step (3.2) at 10,000 rcf for 30 min.
 - 3.4. Centrifuge supernate from step (3.3) at 40,000 rcf for 60 min.
 - 3.5. Discard supernate from step (3.4) and resuspend the remaining pellet in about 0.1 ml of distilled water or PBS. If pellet can be seen after the final centrifugation, remove all but about 0.1 ml of the supernate very gently with a Pasteur pipet.
 - 3.6. Negative stain the resuspended pellet material (or 0.1 ml droplet) according to the instructions for the negative stain of your choice.
4. **Results Expected:** Viral particles will be visible after negative staining procedures.

5. **Cautionary Statements:** All normal precautions for dealing with potential viral pathogens should be observed and all cautions for handling and disposal of the heavy metal negative stains should be followed. **Sonication is not recommended at any time for cells containing viral particles**, since membranes tend to become disorganized and many will re-anneal with each other to produce extremely small vesicles that can be confused with some viral particles. Freeze-thawing will release virions from cells without producing vast numbers of small vesicles.

Reference:

Dykstra, M.J. 1993. *A manual of applied techniques for biological electron microscopy*. Plenum Press, NY.