Quantitatively Depositing Viruses onto TEM Grids

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Purpose: This protocol describes how to quantitatively deposit viruses onto TEM grids using an Airfuge (an air-driven ultracentrifuge made by Beckman). The purpose of using this protocol is to prepare aquatic samples to obtain viral assemblage characteristics (e.g. morphotypes, capsid widths, tail lengths) using transmission electron microscopy (TEM).

Note: An ultracentrifuge can be used in place of an Airfuge, but will require much greater volume of your sample because of the larger area over which the viruses are being deposited. If an ultracentrifuge is used, follow steps 1-8 of the FVIC Protocol using 80,000*g* for 90 minutes in step 7 (Borsheim et al., 1990).

Materials required:

- glow discharge apparatus
- EM grid-grade forceps (2 or more pairs)
- Beckman Airfuge and EM-90 rotor
- EM grids (formvar coated 200 mesh copper)
- double-sided tape
- razor blade
- Spinkote
- Beckman silicone vacuum grease
- Kimwipes

Steps:

CAUTION!: The Airfuge and its rotor are expensive and the rotor can easily be damaged. Make sure you get proper training before operating the Airfuge.

**Before you start anything, check that the stator pad in the Airfuge is not worn and that the bushing at the bottom of the rotor is not damaged. If they are damaged, then they need to be replaced before proceeding to avoid expensive damage to the rotor and Airfuge.

1) On the same day as grid prep, use glow discharge to render grids hydrophylic

2) Use Spinkote to prep the threads where the top and bottom of the EM-90 rotor screw together (this prevents wear caused by metal-on-metal grinding)
    a) put a tiny amount of Spinkote on a Kimwipe
    b) rub the Spinkote into the metal threads on the bottom half of the rotor
    c) wipe off all of the Spinkote from the threads with a Kimwipe

3) Secure grids in the rotor chambers
    a) cut ~1 mm X ~4 mm strips of double-sided tape with a razor blade
    b) stick the strips to the top, interior, distal surface of the rotor chambers (Figure 1A)
    c) attach the grids (shiny side facing in) to the tape such that only the rim of the grids is attached to the tape strips (Figure 1B)

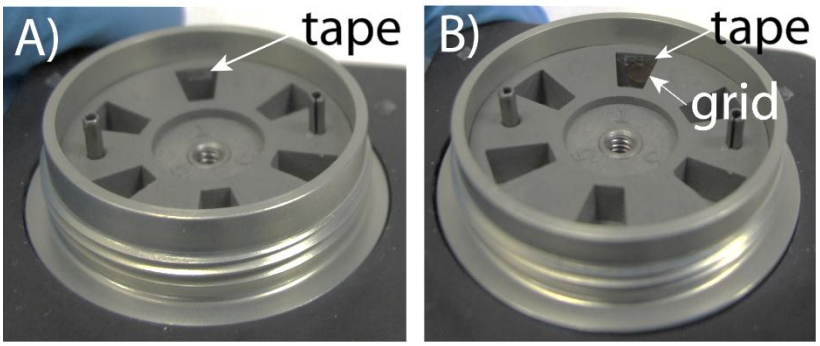

Figure 1. Inserting the tape and grid into the rotor chambers.

4) Lubricate the rotor gasket (this step ensures that the rotor gasket will seal against the rotor chambers, preventing your sample from leaking out of the chambers)
    a) using a Kimwipe, rub a small amount of Beckman silicone vacuum grease into the rotor's gasket, then rub away ALL of the grease

    CAUTION this vacuum grease evaporates in a TEM which dirties the inside of the TEM, therefore be sure to wipe away all of the grease and do not allow any of the grease to enter the rotor chambers in order to prevent it from being deposited onto the grids

5) Add ≤100 µl of your sample to the rotor chambers
    a) the rotor MUST be balanced exactly
        - this means that 2, 3, or 6 of the chambers must have tape, a grid, and the same volume of liquid
    b) the volume of sample required depends entirely on the concentration of viruses in your sample (e.g. 400 µl of surface seawater at $5x10^6$ viruses ml^{-1} is required to deposit enough viruses onto the grid)
        - if >100 µl is required, do multiple Airfuge runs on the same grid, replacing the sample in the rotor chambers in between each run

6) Assemble the rotor
    a) put the gasket on top of the rotor chambers, lining up the pegs with the holes (Figure 2A)
    b) put the hard disk on top of the gasket, lining up the pegs with the holes (Figure 2B)
    c) tightly screw down the rotor lid (Figure 2C)

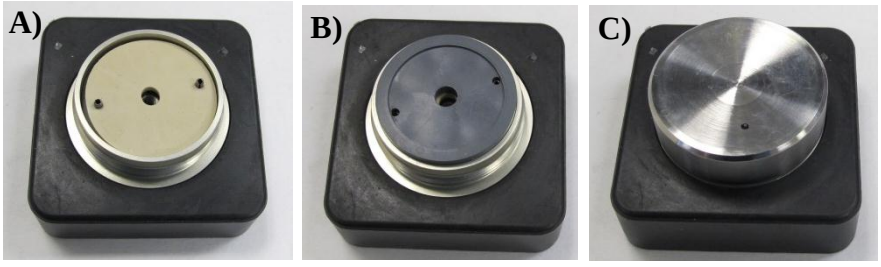

Figure 2. Assembling the rotor

7) Centrifuge your samples in the Airfuge **CAUTION!!! This is where you can break things!
   a) turn on the air attached to the Airfuge
   b) open the lid of the Airfuge
   c) remove the stator pad and use the level to make sure the Airfuge is level (Figure 3A), then put the stator pad back
      - if it is not level, turn the knobs on the bottom of the Airfuge until it is level
   d) gently place the rotor in the Airfuge chamber such that the rod in the chamber is inserted into the bottom of the rotor (Figure 3B)
   e) close the Airfuge lid but DO NOT TIGHTEN IT!
   f) turn the dial on the Airfuge to 25 minutes
   g) slowly (and I mean slooooowly) tighten the knob on top of the rotor chamber
      - as you tighten the knob, the pressure will increase and the rotor will start to spin
      - continue to slowly tighten until the pressure is at 30 psi
      !!CAUTION!! If the rotor begins to wobble, turn the knob on top of the Airfuge counterclockwise *a bit* and allow the rotor to stabilize. Then start tightening the knob again (slooowly).

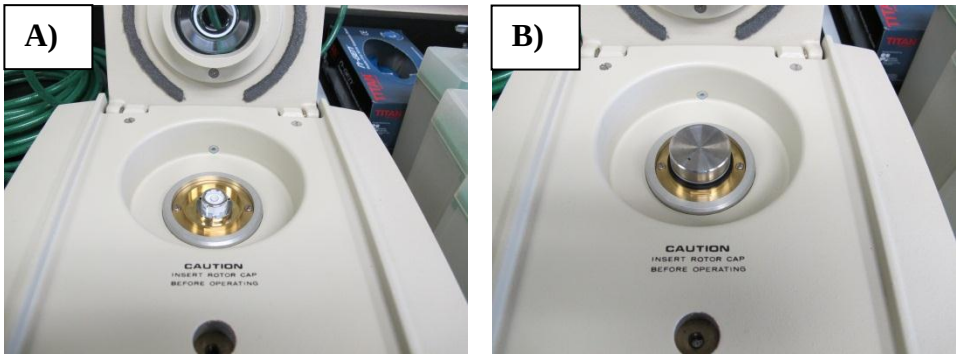

Figure. 3. Making sure the Airfuge is level (A), and inserting the rotor into the Airfuge chamber.

8) At the end of the 25 minutes, the Airfuge rotor will start to slow down

9) Wait until the rotor has stopped COMPLETELY

10) Loosen the knob on top of the chamber and open the Airfuge

11) Open the rotor and *carefully* remove your grids from the tape
   - this may require 2 pairs of forceps so you don't mangle your grid

12) Now stain your grids using the protocol "Positive and Negative Staining of Viruses on TEM Grids"

Applicable References:

Hammond et al., 1981 – Original description of the use of the EM-90 rotor to deposit viruses onto grids.

Brum and Steward, 2010 - Reference to cite for this protocol.

Brum et al., 2013 – Reference to cite for stating that this protocol is *quantitative*.

Ackermann and Heldal, 2010 – An excellent reference for learning about imaging of viruses.

Ackermann, H.-W., and Heldal, M. (2010) Basic electron microscopy of aquatic viruses. In *Manual of Aquatic Viral Ecology*. Wilhelm, S.W., Weinbauer, M.G., and Suttle, C.A. (eds): ASLO, pp. 182-192.

Borsheim, K.Y., Bratbak, G., and Heldal, M. (1990) Enumeration and biomass estimation of planktonic bacteria and viruses by transmission electron microscopy. *Applied and Environmental Microbiology* **56**: 352-356.

Brum, J.R., and Steward, G.F. (2010) Morphological characterization of viruses in the stratified water column of alkaline, hypersaline Mono Lake. *Microbial Ecology* **60**: 636-643.

Brum, J.R., Schenck, R.O., and Sullivan, M.B. (2013) Global morphological analysis of marine viruses shows minimal regional variation and dominance of non-tailed viruses. *The ISME Journal* doi:10.1038/ismej.2013.67

Hammond, G.W., Hazelton, P.R., Chuang, I., and Klisko, B. (1981) Improved detection of viruses by electron microscopy after direct ultracentrifuge preparation of specimens. *Journal of Clinical Microbiology* **14**: 210-221.