HCMV Stock 11/1987/ modified 1/2011

1. Set up 9-12 Roller bottles (RB) of diploid human fibroblasts (HF)
2. Infect at ~moi = 0.001 in 10 ml DME10%serum/ RB for 1h
3. Add medium to ~100 ml / RB
4. Decant medium and refeed twice / week. (Note in one experiment, refeeding with DME-2% serum yielded comparable virus titer vs. using DME10%.)
5. When cells are really almost all coming off the plastic, harvest by shaking the cells loose (or use sterile glass beads).
6. Collect medium, cells, and debris into centrifuge bottles
7. Spin 15000 x g (e.g. 10000 rpm in JA14 rotor) in high speed centrifuge 30 min, room temp.
8. Decant and resuspend pellets in 2 ml medium + 2 ml sterilized skim milk (prepared from powdered milk at 1 x and autoclaved - cooled three times) – so 4 ml total / RB
9. Sonicate on wet ice, 3 x 10" with 10" pause between pulses
10. Spin to clarify – e.g. 2500 rpm in table top centrifuge, 5'.
11. Save supernatant, aliquot and freeze at -80d.

Note added 4/11/14

1. We now often make smaller stock in 5 -10 15 cm dishes rather than roller bottles.