



**Title:** *Purification of HMPV using discontinuous sucrose gradient* No: RTLP-HMPV-4

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Location:  
*AHRC 202*

Approval Date:  
Aug 2006

Supersedes Date:

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**Materials:**

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- HMPV infected cultures of LLCMK2 or VeroE6 ~ 20 ml
- 20 % sucrose(filter sterilized)
- 60 % sucrose(filter sterilized)
- handheld sonicator
- UltraCentrifuge tubes
- UltraCentrifuge cell scrapers
- cryovials
- Pipettes
- Pipetteman
- Pipette Aid
- Pipetteman tips
- lab coat
- gloves

**Tips:** Sterile filter (0.2  $\mu$ M) sucrose solutions prior to use. Keep all solutions, tubes and virus stocks on ice.

**Procedure:**

1. Harvest supernatants with scraped monolayer and clarify by centrifugation (300 x g for 10 minutes). Sonicate pellet 90% duty cycle continuous x 30 seconds at 4°C. Recentrifuge, and take supernatant only.
2. 20ml of the solution is then layered onto a discontinuous sucrose gradient of 20% and 60% sucrose solutions. Layer each 38ml Ultraclear centrifuge tube with 5ml 60% sucrose over which 10ml of 20% sucrose is laid, followed by 20ml of sample. Centrifuge tubes must be balanced in pairs (1-4, 2-5, 3-6) on triple beam balance.
3. Samples are then centrifuged using a Sorvall AH629 rotor at 26,000 rpm at 4°C for 90 minutes.
4. The 1-3ml interface between sucrose solutions is then collected and flash frozen in dry ice and stored at -80°C, until ready to titer.