Title: *Propagation of Respiratory Syncytial Virus* No: RTLP-GL-VP-2a

Location:	Approval Date:	Supersedes Date:
Old CCRC Tripp Lab	09 August 2006	12 December 2005

- Lab coat
- Gloves
- T-150 Flasks
- Vero E6 cells
- HEPES buffer
- D-MEM hi glucose
- 37°C, 5% CO_2 incubator
- CO_2 Incubator
- -70 °C Storage
- Centrifuge
- 1mL Freezer Vials
- Pipettes
- Pipetteman
- Pipette Aid
- Pipetteman tips

Procedure (per flask):
(aseptic technique mandatory)

1. Infect one T-150 cm^2 flask of Vero E6 cells (~80% confluency) at a multiplicity of infection of 0.5 (moi = 0.5), in a total volume of 5 ml of serum-free D-MEM (Hyclone Cat. # 10-013-CV), and incubate for 2 hour at 37°C, 5% CO_2.
2. Following incubation, add 8 mL of 5% FBS diluted in D-MEM and incubate at 37°C, 5% CO_2.
3. At day 4 P.I. the cells should develop extensive syncytia. Remove all but 2 ml of the media/flask.
4. Scrape the cells and collect all the fluid into a 50ml tube. Distribute 5ml cells per tube.
5. Sonicate the cells in the tubes 3 times , 5 sec per time @ a setting of 25W till all cells become debris. Do this on ice.
6. Remove the cell debris by centrifugation at 3000 rpm for 7 minutes at 4°C.
7. Remove the supernatant and store on ice.
8. The virus is in the supernatant and can be stored at -80 °C at this stage or expanded to a working stock as indicated below:

Passaging RSV from Stock

9. To develop a working stock, the supernatant can be used to infect Vero E6 cells (80% confluency) by diluting the supernatant at the ratio of 1:25 in serum-free media.
10. The procedure to follow for infection is indicated above – essentially add a total volume of 5 ml of the diluted virus per flask and incubate for 2 hour at 37^{o}C, 5% CO_2.
11. Following incubation, add 8 mL of 5% FBS diluted in D-MEM and incubate at 37^{o}C, 5% CO_2.
12. At day 3 or 4 P.I. the cells should develop extensive syncytia. Decant all but 2 mls of the media/flask.
13. Scrape the cells into a 50ml tube. Distribute 5ml cells per tube.
14. Sonicate the cells in 50ml tubes for 3 times , 5 sec per time @ at a setting of 25W.
15. Keep the material cold.
16. Remove the cell debris by centrifugation at 3000 rpm for 7 minutes at 4^{o}C.
17. Remove the supernatant and store on ice.
18. The virus is in the supernatant and can be stored at -80 ^{o}C at this stage.

** of note: the cell pellet also contains substantial infectious virions, thus the pellet can also be stored and used as a backup to recover virus in case of emergency