Title: *Hybridoma Generation**: Animal Boosting*** *with Inactivated Gradient-Enriched Coronavirus (CV) or Adenovirus (Ad) expression vector-spike (Ad-S) or Ad-nucleoprotein (Ad-N)*

No: RTLP-GL-Ab-14

Location:	Approval Date:	Supersedes Date:
Old CCRC Tripp Lab	10 September 2004	

Materials:

- Lab coat
- Gloves
- Inactivated gradient-enriched coronavirus (CV)
- Adenovirus (Ad) espression vector-spike (Ad-S)
- Adenovirus (Ad) espression vector-nucleoprotein (Ad-N)
- 1 mL Leur- lock syringes
- 25 gauge needle
- Adjuvant: TiterMax Gold
- Phosphate Buffered Saline (PBS)
- Pipettes
- Pipetteman
- Pipette Aid
- Pipetteman tips

Antigen Preparation:

- For Ad-S or Ad-N: re-suspend to 2 x 10^7 pfu/ml in PBS
- For CV proteins: re-suspend 1-50 mg in PBS.

Procedures:

I. Animal Boosting

1. Using a 1 mL Leur-lok syringe, draw up CV, Ad-S, or Ad-N protein solution.
2. Prepare adjuvant (TiterMax Gold) per manufacturers instructions, then draw up an equal volume of adjuvant in **a separate** syringe
3. Attach 3-way stopcock,and emulsify the CV protein and adjuvant.
4. Transfer CV-emulsification to a single 1 mL syringe.
5. Eye/tail bleed naïve mice for baseline sera screening.
6. Inject CV-emulsification intra-peritoneally into naïve mouse using 0.2 ml emulsification/mouse
7. Let the mouse rest 14 days.

8. At day 14 post-treatment, eye/tail bleed mouse for sera screening.
9. Repeat steps 1-5 and boost animal with the same dose as in step 7.
10. Let mouse rest 7 days.

11. At day 21 post-boost, eye/tail bleed for sera screening.
12. Boost animal with the same dose as in step 7.
13. At day 24, harvest spleen for fusion.

Author	Management Approval/Date	Effective Date