The University of Georgia	Ralph Tripp Lab Protocols	*STANDARD OPERATING PROCEDURES*

Title: *Tissue Preparation Protocols* No: RTLP-SARS-7

Location:	Approval Date:	Supersedes Date:
Old CCRC Tripp Lab	16 August 2005	
Materials:		Page 1 of 2

•Lab coat •Gloves •Collection Tubes (K-EDTA) • Cryovials •Trizol	•Phosphate Buffered Saline (PBS) •DMEM-5% FBS (if tissue prep) •Pen/Strep (if tissue prep) •L-Glutamine (if tissue prep)	•-80°C Storage •dH_2O •MicroChem Plus •Homogenizer (if tissue prep)	•Pipettes •Pipetteman •Pipette Aid •Pipetteman tips •microfuge •microfuge tubes

Blood and Serum Collection for $TCID_{50}$ Assays and RNA Extraction

1. Blood should be collected from animals in both K-EDTA (Purple top) and serum (Red top) collection tubes from the animals at the desired time points.

2. Whole blood is to be used for $TCID_{50}$ assays and RT-PCR. Whole blood (~0.5 ml) for $TCID_{50}$ assays should be placed into labeled cryovials and stored at -80°C. For RNA isolation, 100 µl of whole blood should be added to 900 µl of Trizol LS and the mixture stored at -80°C. ***Liberally spray the tubes with 10% Microchem Plus prior to removal from the BSL-3.***

3. Blood collected in serum tubes (Red top) should be allowed to clot for ~30 minutes, centrifuged at ~3,000 rpm for 10 minutes, and the serum removed and placed in a labeled cryovial for storage at -80°C.

Handling of Nasal Wash/Flush Samples for $TCID_{50}$ Assays and RNA Extraction

1. Nasal washes/flushes will be performed on animals and the fluid collected at the time of necropsy.

2. Nasal wash fluid is to be used for $TCID_{50}$ assays and RT-PCR. The collected fluid (~0.15 ml) for $TCID_{50}$ assays should be placed into labeled cryovials and

stored at -80°C. For RNA isolation, 100 µl of nasal wash fluid should be added to 900 µl of Trizol LS and the mixture stored at -80°C. ***Liberally spray the tubes with 10% Microchem Plus prior to removal from the BSL-3.***

Tissue Preparation (Lung, Kidney, Intestine) for $TCID_{50}$ Assays

1. Frozen tissues should be sectioned, weighed, and 100 mg added to 1ml of DMEM containing phenol red, 5% FBS, P/S, L-glutamine. Tissue samples should then be homogenized with disposable grinders.

2. The homogenate should them be transferred to sterile microcentrifuge tubes, and centrifuged at ~2,000 rpm for 5 minutes.

3. Remove the homogenate fluid, place into 4 labeled cryovials, and store at -80°C.

Tissue Preparation (Lung, Kidney, Intestine) for Total RNA Extraction

1. Frozen tissues should be sectioned and weighed into 100 mg samples. Tissue samples should be homogenized using disposable grinders and ~1ml of Trizol reagent added to the tube (after homogenization). The sample volume should not exceed 10% of the volume of Trizol reagent used for homogenization.

2. Incubate the homogenized sample for 5 minutes at 15-30°C to permit complete dissociation of nucleotide complexes. Aliquot the sample into 4 labeled cryovials. Store at -80°C until transferred to Georgia Ali for RNA extraction. ***Liberally spray the tubes with 10% MicroChem Plus prior to removal from the BSL-3. Immediately transfer the samples to or store at -80°C.***