**Molecular Virology Support Core QPCR Sample Submission Guidelines**

**A. Sample Preparation**

**General Notes:** Sarstedt 2mL screwcap tubes (No. 72.694 series) or other gasketed screwcap tubes of identical dimensions are required for plasma and other cell-free samples for viral load assays. These same tubes are required for other sample types that are also processed by the QiaSymphony robot, as indicated below. For safety these same tubes are preferred for other sample types as well. Please do not submit samples in cryovials.

**Plasma:**

Plasma should be prepared using either EDTA or citrate (ACD) as anticoagulant. Avoid the use of heparin. Sample volumes must be at least 0.65 mL and not exceed 1.2 mL. For samples where 0.65 mL is unobtainable, please QS to 0.65 mL with buffer (e.g., PBS). Samples must be submitted in Sarstedt 2mL screwcap tubes (No. 72.694 series) or other gasketed screwcap tubes of identical dimensions. Samples should be kept frozen at -80°C.

**TC SN:**

Sample volumes must be at least 0.65 mL and not exceed 1.2 mL. For samples less than 0.65 mL please QS to 0.65 mL with buffer (e.g., PBS). Samples must be submitted in Sarstedt 2mL screwcap tubes (No. 72.694 series) or other gasketed screwcap tubes of identical dimensions. If you are submitting virus stock or a sample you expect will be very high titer (i.e., greater than 1x107/mL) please submit a 10 L aliquot diluted to 0.65 mL with buffer (e.g., PBS). Alternatively, if a larger volume must be submitted please make a note in the comment section so that we will know to reduce the volume of sample assayed in order to be within the range of the standard curve. Samples should be kept frozen at -80°C.

**Cell Pellets**:

1. **DNA ONLY:** Place a maximum of 3 x 106 nucleated cells per sample tube. Pellet cells by brief spin in microfuge (e.g. 15 sec. full speed). Remove supernatant. Freeze cell pellets at -80°C (avoid leaving large amounts of liquid on cells). If using cell counts from an automated counter (e.g., Coulter) use WBC counts not lymphocyte (or other subgroup) counts.
2. **RNA, or both RNA and DNA:** cells must be lysed immediately following isolation (no frozen pellets!) in TRI reagent. Pellet 2.5 x 106 to 1 x 107 nucleated cells by centrifugation at 900 x g for 4 min. Remove supernatant and add 1 mL cold TRI reagent (0o to 4oC). Pipet up and down and/or vortex vigorously to suspend and lyse cells. Flash spin, then transfer to a lysing matrix tube (Lysing Matrix D, #116913100 from MP Biomedicals or Roche or SPEX). If available process immediately with a bead beater (best results), 2 x 30 sec with incubation on ice in between. Flash spin and freeze immediately at -80oC.

**Blood**:

Place a maximum of 0.3 ml whole blood, collected with EDTA or citrate, in a tube of at least 1.5mL capacity. Avoid the use of heparin. Freeze samples at -80°C.

**Urine:**

Place a maximum of 1.5 ml urine in 2ml Sarstedt tube. Freeze samples at -80°C.

**Buccal swabs:**

Collect sample and deposit swab immediately into 1 ml buccal swab solution (HBSS containing 2% FBS and 50 µg/mL gentamycin). Centrifuge the samples at 1000 x g, 10 min, 4 °C to pellet cells and debris. Transfer supernatant to 2 ml Sarstedt. Freeze samples at -80°C.

**Tissues:**

1. Large tissues are samples ranging from 100-800 mg. Large tissue samples should be stored in 15 ml round-bottom polycarbonate (PC) tubes for large sample processing. The tissue chunks should be frozen to the side of the tube, leaving space for liquid to pass through, as large pieces at the tube bottom may not be effectively pulverized.
2. Small tissues are samples below 100 mg. Small tissue samples should be stored in 2 ml green cap tubes (Lysing Matrix D, #116913100 from MP Biomedicals or Roche or SPEX) containing 1.4 mm ceramic spheres for small sample (<100 mg tissue) processing.

To prevent nucleic acid degradation, always store tissues frozen at -80°C.

**IMPORTANT: Rhesus macaque tissue samples may contain infectious agents, such as SIV, RhCMV (depending on the research study), and may also contain rhesus macaque endogenous agents, such as Herpes B virus, simian retrovirus (SRV), and others. Therefore, work is conducted at BSL-2 or 2+ as appropriate for the particular agents. Always wear appropriate personal protective equipment (lab coat, single or double gloves, safety glasses), take all appropriate precautions, and follow the safety procedures outlined in your institution’s biosafety procedures.**

**B.** **Sample Documentation**

Please fill out the appropriate sample submission form completely, being sure to include a date by which you need the results. Please provide an actual date. Copy/paste your sample information into the appropriate MVSC sample submission form in the order in which you would like the samples run. Note, we use the provided sample ID and sample date for sample tracking so please be brief with descriptions and avoid redundancies (redundancies will be treated as replicates of the same sample by the data analysis software). Enter the sample volume or cell number (numbers only, no text or symbols) for each sample. Email a copy of the completed form to the MVSC.

**C. Local Delivery of Samples**

For SIV PVL Samples place your samples in MVSC SIV PVL dropbox in the MVSC -80°C freezer in the same order as listed in the sample submission form, left to right. Beware there may be other samples already in the dropbox so please open carefully! Also, please minimize the time the drop box is at room temperature. Contact the Core to let us know the samples have been delivered.

For all other samples please bring the samples in a Revco freezer box and hand the samples off in-person.

**D. Intercampus Delivery of Samples**

Place your samples in a Revco freezer box in the same order as listed in the sample submission form, left to right. Send samples on dry ice, or with the appropriate temperature control, to the Core by campus courier. Include a printed copy of the sample submission form within the shipment container. Contact the Core to let us know the samples are being delivered.

**E. Offcampus Delivery of Samples**

Place your samples in a Revco freezer box in the same order as listed in the sample submission form, left to right. Ship your samples on dry ice, or appropriate temperature control, according to your institution’s shipping regulations (most likely category B). Include a printed copy of the sample submission form within the shipment container. Please contact the Core approximately one week prior to sending samples. Also please provide the Core with a courier tracking number.