FLORIDA INTERNATIONAL UNIVERSITY

SOP #: 702.01

Title: **SOP -** Processing and Submission of Laboratory Specimens

Approvals:

Attending Veterinarian _____ **Date:** 10/11/12

Assistant Director LAR _____ **Date:** 10/11/12

1. Purpose

1.1 To provide guidance for collection and submission of laboratory specimens at Florida International University

2. Responsibility

2.1 Veterinary Care Staff, Principal Investigators, Laboratory Technicians

3. Definitions

3.1 CBC & Differential: Complete Blood Count & Differential White Blood Cell count is a test panel that gives information about the cells in a patient's blood.

3.2 EDTA: Ethylenediamine tetra-acetic acid

3.3 PCR: Polymerase Chain Reaction is a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence

4. Guidelines

4.1 Regardless of the type of submission, a detailed case history should be included with the samples to assist laboratory personnel in determining a diagnosis.

4.2 The information should include: owner, species, breed, sex, age, animal identification, clinical signs, gross appearance (including size and location) of the lesion(s), previous

treatment (if any), time of recurrence from any previous treatment, and morbidity/mortality in the group.

4.3 If a zoonotic disease is suspected, this should also be clearly indicated on the submission sheet to alert lab personnel. The submission form should be placed in a waterproof bag to protect it from any fluids that might be present in the packaged materials. Waterproof markers should be used when labeling specimen bags and containers.

4.4 Hematology:

4.4.1 Routine studies require anticoagulated whole blood and several blood smears.

4.4.2 Blood smears should be prepared immediately after the sample has been collected to minimize cell deterioration.

4.4.3 CBC & Differential: EDTA is the anticoagulant of choice for a CBC because it best preserves the cellular components of the blood and prevents platelet aggregation.

4.4.4 Coagulation testing: blood should be collected into a blue top tube, which contains sodium citrate. After mixing, the sample should be centrifuged for 5 min, and then plasma should be removed and transferred to a clean tube without anticoagulant. The plasma should be kept frozen until the time of analysis.

4.4.5 Whole blood should not be frozen because this causes cell lysis and gross hemolysis, which interfere with testing. Anticoagulated blood should be kept refrigerated; blood smears should not.

4.5 Clinical Chemistry:

4.5.1 Most clinical chemistry tests require serum, but an occasional test may require plasma.

4.5.2 Anticoagulants present in plasma may interfere with tests; therefore, serum should always be submitted unless plasma is specifically requested.

4.5.3 For serum samples, the blood should be drawn into a red top tube or a separator tube (SST). The sample should be held at room temperature for 20-30 min to allow complete clot formation and retraction. Incomplete clot formation may cause the serum to gel due to latent fibrin formation.

4.5.4 The clot should be separated from the glass by gently running an applicator stick around the tube walls ("rimming"). The sample should then be centrifuged at high speed (~1,000 g; 2,200 rpm) for 10 min. Rough handling of the sample or incomplete separation of erythrocytes from serum may promote hemolysis, which can interfere with certain tests. If the sample has been collected into a serum separator tube, centrifugation will cause a layer of silicone gel to lodge between the packed cells and the serum. The gel layer should be inspected to ensure the integrity of the barrier, and re-centrifugation is recommended if there is a visible crack in this layer. If a red top tube has been used, the serum should be removed and transferred to a clean tube.

4.5.5 Serum should be refrigerated or frozen until analyzed.

4.6 Serology:

4.6.1 Serology generally requires serum, but plasma is often satisfactory. Samples should be collected as described for clinical chemistry tests and should always be free of hemolysis. In some instances, paired samples may be required for an adequate diagnosis. The acute sample should be collected early in the course of the disease and frozen. The convalescent sample should be collected 10-14 days later, and both samples should be forwarded to the laboratory at the same time.

4.7 Cytology:

4.7.1 Air-dried smears are usually acceptable. Rapid air drying of smears minimizes cell distortion, thereby enhancing diagnostic quality. However, depending on the method of staining used, some laboratories prefer alcohol-fixed smears.

4.7.2 Samples can be obtained by fine-needle aspiration or by scraping. Imprints (touch preparations) of external lesions can also be used, although these tend to have a greater degree of contamination.

4.7.3 Aspirated material should always be smeared before air drying. Smears of fluid can be prepared using a traditional blood smearing technique.

4.7.4 Highly cellular fluids may be smeared directly; fluids of low cellularity should be centrifuged to concentrate the cells. Thick material or viscous fluid is more readily smeared using a squash technique in which a second glass slide is placed over the aspirated material and then slid rapidly and smoothly down the length of the lower slide.

4.7.5 Blood or cytologic smears should never be mailed to the laboratory in the same package with formalin-fixed tissues because formalin vapors will produce artifacts in the specimen.

4.8 Fluid Analysis:

4.8.1 Body cavity effusions should be analyzed. Fluid analysis usually includes determination of protein content and total cell count and cytologic examination.

4.8.2 A sample of effusion should be collected into an EDTA (purple top) tube for routine analysis.

4.8.3 A second sample should be collected into a serum (red top) tube if any biochemical analyses (eg, triglyceride, cholesterol, lipase) are to be performed or if a bacterial culture is desired.

4.8.4 Smears for cytologic examination should be prepared immediately after the sample has been collected to minimize cell deterioration and other in vitro artifacts.

4.9 Microbiology:

4.9.1 Available tests include bacteriologic culture, virus isolation, in-situ hybridization, PCR, fluorescent antibody tests, latex agglutination tests, and ELISA. Therefore, it is critical to obtain specific instructions from the diagnostic laboratory on sample collection and handling.

4.9.2 Usually, unfixed specimens are submitted and should be collected aseptically, as soon as possible after death.

4.9.3 If PCR testing is to be performed, it is particularly important to avoid cross-contamination between multiple animals in a submission; this applies to tissues, fluids, and even dissection instruments.

4.9.4 Tissues for most microbiologic assays may be frozen before shipment, but freezing is undesirable if samples can be chilled and delivered directly to the laboratory in a short period. Adequate refrigerant should be provided so that samples will remain chilled until they reach the laboratory.

4.10 Histology:

4.10.1 Microscopic examination of adequately prepared tissue sections is a valuable aid to diagnosis. Numerous immunohistochemical tests can be applied to formalin-fixed tissue.

5. References

5.1 Merck Veterinary Manual – 9th Edition