S.O.P Collection of osteosarcoma tumor sample for the Greyhound Health Initiative-funded study entitled "Developing tumor infiltrating lymphocytes as a future treatment for canine osteosarcoma"

1.0 PURPOSE

1.1 To collect fresh (not formalin fixed) osteosarcoma tissue for studies on living tumor cells and tumor-infiltrating lymphocytes.

2.0 PROCEDURE

2.1 **NOTE**: Ensure the sample is collected in a sterile manner.

2.2 **NOTE**: It is important that harvesting the tumor tissue for this study does not jeopardize the routine histopathological evaluation and diagnosis for the patient – i.e., Do **NOT** place the entire tumor in the transport liquid, as sufficient tissue must be left for routine histopathology. This study does not pay for routine histopathology.

2.3 **NOTE**: Tissue should be harvested as quickly as possible after the leg is removed and placed in the transport liquid provided (ideally within 15-20 minutes of the amputation).

2.4 Complete the label in the collection kit with patient details as indicated. Apply the label to the tube containing the transport media.

2.5 Once the limb is removed from the patient, the area over the tumor will be shaved, the skin will be scrubbed and prepared for tumor harvest.

2.6 A large incision is made in the skin over the boney lesion using a sterile scalpel blade and the underlying tissues are dissected in a sterile manner to reveal the boney lesion.

2.7 Using a fresh sterile scalpel blade (not the one used for the skin incision), an accessible portion of the tumor is located and a ~1-2cm^3 block of tissue is removed in a sterile manner. It is important that removal of this piece of tissue does not compromise/jeopardize subsequent histopathological diagnostics for the patient. It is also important to avoid heavily necrotic or hemorrhagic tumor tissue.

2.8 The tumor tissue removed is placed on a sterile drape and cut into smaller (<0.5cm^3) pieces. All pieces of tumor tissue are placed in the transport liquid provided.

2.9 The lid is placed on the transport liquid tube and parafilm is used to seal the top of the tube to prevent leakage during shipping.

2.10 The plastic transport liquid tube is placed in the provided plastic bag, sealed and placed in the shipping container with the consent form signed by the owner and the attending veterinarian.

2.11 The container is prepared for overnight shipping and the pre-paid FedEx return label is attached to the package. FedEx is called for the same day pick up. It is of the utmost importance that the sample is shipped overnight to arrive at the processing laboratory the next day. Delay will lead to death of the immune cells within the tumor.

2.12 Please send an e-mail to nmason@vet.upenn.edu to inform us that the sample will be arriving the next day.

2.13 Please scan the QR code for a brief video describing the above process: