Sample protocol UU

# Sample kit

In order for the participating vet to collect the necessary samples they should have the following:

1 Streck tube

2 Cryotubes with ~1.3 ml RNAlater

1 Consent form

1 Phenotype information slip

# Sample collection (veterinarian)

**Prior to surgery:**

* Signing of consent form
* Collect 10 ml of blood in Streck tubes with a maximum of 1.6 ml / kg including any samples taken for paraclinical tests. Keep sample refrigerated until further processing. The sample should be processed within 3 days and *must* be processed within 7 days. Preferably it should be processed the same day.

**After/during surgery:**

* Before fixating the tumor in formalin take 1-2 tumor samples ~(0.3 x 0.3 x 0.3 cm) and put in the cryotubes with RNAlater provided. These samples must not compromise margins/diagnosis. The samples should be kept refrigerated until further processing.

**Follow up:**

* The dog should have a follow-up visit performed every 2 months for a year and then after 18 months. At these visits a clinical examination should be performed and a new blood sample should be collected in a streck tube ( same procedure as the first one).

# Sample processing

Materials:

1000 µl pipette

Pipette tips – RNAse, DNAse and pyrogen free, sterile, and with aerosol barrier (Fisher-sci product # 11973466)

1 10 ml streck tube

1 15 ml conical centrifuge tube that can withstand 16 000 g (Eppendorf™ 0030122208)

2 or more cryotubes

For additional details see QIAamp ® MinElute ® ccfDNA Handbook 08/18.

Procedure

1. Collect Streck tubes (10ml) from the hospital. Keep sample refrigerated until further processing the sample. The sample should be processed within 3 days and *must* be processed within 7 days. Preferably it should be processed the same day.
2. Centrifuge the blood sample for 10 min at 1900 x g with temperature set to 4°C.
3. Carefully aspirate the plasma/supernatant from the tube without disturbing the buffy coat layer.
4. Transfer aspirated plasma into the 15 ml eppendorph conical-bottom tube.
5. Aspirate the buffy coat to a cryotube and reserve.
6. Centrifuge the plasma sample for 10 min at 16 000 g with temperature set to 4°C.
7. Using a pipette, carefully transfer the supernatant from the eppendorph tube into cryo-tube(s) without disturbing the pellet.
8. Freeze supernatant at –80°C (this is the cfDNA tube)
9. Transfer pellet to cryotube with buffy coat and freeze at –80˚C (this cryor tube is your normal sample)