Tissue Protocols for PCR

General Considerations: Upon occasion, tissues are the sample available for PCR rather than EDTA whole blood. VBDDL can process tissue, although due to the extra processing steps, an additional fee is added to the charge for PCR. For PCR to detect genera other than *Bartonella* spp, fresh tissue is preferred over formalin fixed or fixed and embedded tissues. Prolonged formalin fixation cross links and degrades the quality of DNA that can be obtained leading to false PCR results.

- Fresh tissue can be shipped in sterile saline in a red top tube on ice packs for overnight delivery, packed in accordance with standard federal shipping regulations.
- Paraffin embedded tissue --the whole block is preferred over tissue scrolls, but for genera other than Bartonella spp. scrolls can be accommodated as well, shipped in packaging that protects from extreme high temperatures.

Sampling from paraffin blocks for *Bartonella* spp PCR--Research performed by VBDDL associated doctoral student, M. Varanat, demonstrated that the sampling of paraffin blocks for *Bartonella* PCR using a microtome may result in DNA carry over and cross contamination, unless very stringent cleaning procedures are followed. This is particularly risky if clinics or veterinary hospitals routinely handle *Bartonella* reservoir host animals such as cats or cattle. Send whole blocks, if possible shipped in packaging that protects from extreme high temperatures.

If whole blocks cannot be sent, sampling from formalin fixed paraffin embedded tissues must follow the procedure outlined substituting sterile scalpel blades for microtome.

1) Scrape off the tissue from the paraffin block using a No 10 single use sterile scalpel blade and collect on a sterile piece of paper (can use kimwipes). Make sure to scrape a uniform layer from the entire face of the block to prevent damage to the block and also this will ensure a more representative sample. We need only about 25mg of the tissue for the extraction (something like a 50 μm thick section).

2) Transfer the tissue in to a sterile 1.5 ml microcentrifuge tube and label with the block number and date.

3) Use new gloves, transfer paper and blades for each sample. Complete the procedure on a clean surface each time. Do not allow scrapings from one case to contact the bench/working surface and then potentially transfer to the next case material. Work on a clean disposable surface between each block or aggressively clean the surface between blocks. If blocks were kept in contact with each other, clean the surface of the block with alcohol and new Kimwipes each time to remove surface contaminants.

Reference: Varanat M, Maggi R, Linder K, Horton S, Breitschwerdt E. Cross-contamination in the molecular detection of Bartonella from paraffin-embedded tissues. Vet Pathol. 2009 May 9. [Epub ahead of print].