



THE FROZEN ARK PROJECT

Saving the DNA and viable cells of the world's endangered species

COLLECTION PROTOCOLS – ZOO AND AQUARIA ANIMALS

1. TISSUE SAMPLES

Take a **tissue section** of approximately **1cm in diameter**. Split this into 2 parts – a smaller section (approximately $\frac{1}{4}$ of the tissue section) and a larger section (approximately $\frac{3}{4}$ of the tissue section).

For the smaller part ($\frac{1}{4}$) place the tissue in the buffers. **Label the tube** and send to repository if applicable.

For the larger part ($\frac{3}{4}$) place the tissue in a screw top tube (clear screw top tube), label the tube and **freeze immediately** (ideally at -80°C but failing this -20°C will suffice). At the earliest opportunity the specimen should be transported (either on dry ice or in liquid nitrogen) to a -80°C freezer in your institution or the local Frozen Ark repository. The specimen must always remain frozen!

For **skin samples** please shave the area first and wipe with an antiseptic wipe. Please avoid taking tissue samples from internal organs – while these provide large amounts of DNA the DNA can become degraded too quickly to make them viable for sample collection.

2. BLOOD SAMPLES

Place a maximum of **125 μl of blood** onto a **Whatman[®] paper/FTA card**. Avoid 'puddling' the sample as it will overload the chemicals on the card – dispense blood slowly and evenly across the card. Also do not rub or smear the blood onto the card. Allow the card to dry (approximately 1 hour but this will depend on conditions), **label the front of the card** and then store in a **dry place**.

Place the remainder in a **blood collection tube** (purple capped tube), **label the tube and freeze immediately** (ideally at -80°C but failing this -20°C will suffice). At the earliest opportunity the specimen should be transported (either on dry ice or in liquid nitrogen) to a repository. The specimen must always remain frozen! Depending upon the volume of blood collected fill between 1 and a maximum of 10 tubes.

3. SAMPLE NAMES AND SAMPLE COLLECTION SHEET

For each animal, all samples taken (all tubes, Whatman[®] papers, etc.) at each occasion should be **labelled with a single code**. This ensures that all samples collected from an animal at each occasion have the same unique code. E.g. Twycross Zoo use the code TWY with specimens labelled TWY1, TWY2 etc. Complete the sample data collection sheet and send a paper copy of this with the sample to your chosen biobank facilities.

Labelling Specimens:

Label tubes using a permanent marker pen on the exterior of the collection tube.

Specimen Preparation:

It is essential that **aseptic conditions** are used during sample preparation. In particular, it is extremely important that fresh sterile scalpel blades, syringes, needles, etc. are used for each specimen when taking samples of tissue in order to avoid cross contamination.