



## SAMPLING PROTOCOL FOR DEAD MONTSERRAT MOUNTAIN CHICKENS

Sampling will be carried out indoors in an easily disinfected area, ensuring biosecurity.

### Preparing for your sampling

Work with a team of, at least two people to minimise the risk of contamination when you're collecting samples.

The role of each person will be:

Person 1 (clean):

- Take pictures of the frogs
- Write the labels for each of the preserved specimens
- Hold the tube of the swab for sampling
- Write the labels and notes of the swabs tubes for chytrid

Person 2 (contaminated):

- Handling, sampling and preserving frogs (dead or alive)
- Handling any contaminated material

### Before you start sampling

You need:

**Bucket with lid, plastic zip bags or bags you are able to tie, strong bin bags, pencil, paper, disposable vinyl or latex gloves, plastic ruler, newspapers swab tubes and disinfectant solution (commercial bleach), spring balance, Scissors or scalpel blades, 10% formalin**

- a. Prepare the labels that will be inside the second plastic bag for each of the preserved frogs.
  1. Write the information with a pencil



2. Use a strong paper to avoid the humidity or water inside the bag could deteriorate the paper
3. Write the next information:
  - i. Species name
  - ii. Sex if it's possible to determine
  - iii. Locality and transect where was found
  - iv. Date and Time when was found
  - v. Name of the person who collected
- b. Keep a rubbish plastic bag where you'll put all the used gloves and rest of material that could be contaminated. This bag should be close to your working area but not in contact with any equipment and frogs. Ideally put the bag inside a plastic bin to facilitate the deposit of material.
- c. Cover the working table with a plastic bag (a garbage bag opened is good enough)
- d. For each of the frogs put a couple of pages of newspaper that will help to retain any water from the samples/bags

## Data collection

**Contaminated person activities: a, b, c,**

**Clean person: activities: c, e**

- a. Take out the preserved specimens from their bags and place them on the table
- b. Opens bags and place separately the frogs for the sampling
- c. Collects the rubber gloves from the boxes, remove from the hands and discard them on the garbage bag
- d. Holds the swap from the tube for chytrid test, take the sample and brings it inside the tube again
- e. Puts the frog back to a single bag and close it.
- f. Puts the level inside the second bag with the first bag of the preserved specimen
- g. Put the gloves (instructions on how to use them attached). If the frogs are preserved on individual bags take the weight of each of them inside the bag. The bag and frog are weighed with a spring balance, and the weight of the bag subtracted.
- h. Open the bag, take one frog and place it on one of the newspapers.
- i. Take the weight of the empty bag now. **Note: Careful to don't contaminate the spring balance with gloves that touched the frog (spring balance is held by clean person).**
- j. Record the weight of the bag and frog and subtract the bag.
- k. Take pictures of the dorsal, ventral, detail of legs (dorsal and ventral) and details of any injuries or lesions that may provide. **Note: take pictures of any unusual aspect on the frog that you consider important to highlight. Add a plastic ruler or an element that helps to determine the size of the frog.**

## Proceed with the swabbing



**Contaminated person: a, b, c.1, d, e.1, g, h**

**Clean person activities: c.1, e.1, f**

Clean gloves should be used for every frog and disposed of appropriately in the garbage bag / bin.

- a. Place the frog showing the ventral surface.
- b. Change the pair of gloves for a new one.
- c. Clean person holds the tube of swab for the contaminated person to take from the lid..
- d. Proceed with the swabbing as follows:
  - Swab the inner thigh with a firm continuous action. The aim is to dislodge loose skin, sporangia or zoospores. Repeat three times.

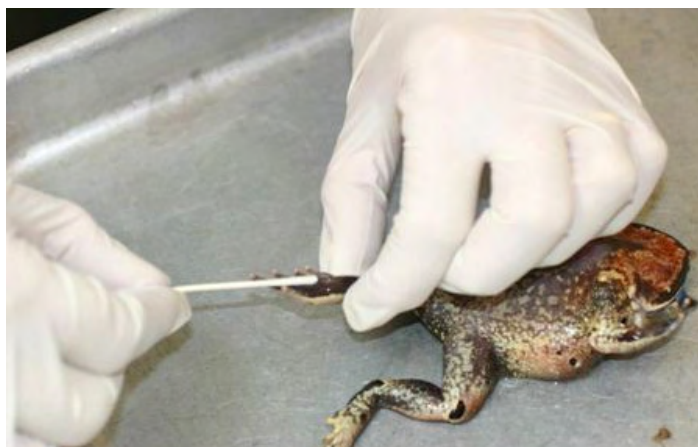


- Repeat the swabbing procedure on the lower hind leg. Repeat three times.





- Repeat the swabbing procedure on the underside of the foot/toes. Repeat three times.



- Repeat the swabbing procedure on the 'drink patch', this found just above the pelvis. Repeat three times.



- e. Clean person holds the tube and the contaminated person places the swab inside the tube.



- f. Clean person writes the key information on the swab tube (Species name, site of collection, date of collection, sex, adult/juvenile/metamorphosed, date of sampling and name of the persons who sampled). These details should be copied in a notebook too.





## Other alternative of swabbing



The swabs should then be kept at 4°C (in fridge) prior to dispatch.

Every other frog you handle should be preserved frozen and the rest in formalin.

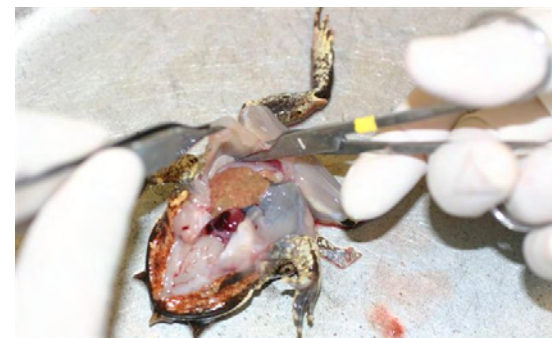
For freezing:

- g. Contaminated person puts the frog back into the plastic zip bag. And drops it into a clean bag held by the clean operator. Clean operator closes the bag, puts it into another bag containing the label, closes it and freezes it.
- h. Put all the specimens in bags from the same day of collection on a common bag for the site.



For formalin:

- i. Contaminated person opens the coelomic cavity with scissors as shown in pictures.
- J. Clean person takes pictures of coelomic contents.
- k. Contaminated person drops the frog into a container with 10% formalin clean operator adds the label and closes the lid and wipes the outside of the pot with disinfectant.





The formalin fixed bodies can be kept at room temperature

Change sheets of newspapers between frogs.

When all the frogs have been sampled and it's the end of the sampling session all disinfection procedures should be followed:

- Collect all garbage in the plastic bag/bin and close it with a tight knot. Clean operator holds a second bin bag open while dirty operator drops the first bag into it. Ensure there is no cross contamination. Clean operator closed the second bag and sprays it with disinfectant.
- Bags are incinerated asap.
- Disinfect the table with diluted commercial bleach at 5% or other disinfectants (Vetcide, Trigene...)
- Protective clothing is placed into plastic bag to take to a hot wash (60°C) as soon as possible.
- Both operators wash hands thoroughly as in attachment