

## Collection of saliva samples Protocol

### *Important*

Guidelines and methods of saliva collection depend on the type of research. Please consult the literature and always confirm with your supervisor which guidelines are to be followed. Should these deviate from this protocol, then you should discuss this beforehand with the research technicians at SOLO.

### *Supplies*

- Saliva collection tubes;
- Labels;
- Gloves;
- Disinfectant.

### *Guidelines for saliva collection*

- Always wear **gloves** when touching saliva tubes and other material that has been in contact with saliva;
- **Always label the samples.** Do not leave behind any unlabelled samples! Label the tubes **before** collecting any saliva. Use labels that can be frozen, so that they do not fall off the tubes in the freezer;
- **Disinfect** the outside of the tubes with an alcohol wipe after securing the lid and before they are stored;
- Follow the instructions provided with the sample collection equipment.

### *Sample contamination*

Instructions for preventing contamination of the sample:

- No alcohol consumption for 12 hours prior to the saliva collection;
- No food or drink, except for water, for a minimum of 1 hour (preferably 2 hours) beforehand;
- Ideally no water directly before collection of the saliva sample. If necessary, the participant may rinse his/her mouth with water. Wait at least 10 minutes after rinsing before collecting the saliva to prevent thinning of the sample;
- Document the participant's consumption of alcohol, caffeine, nicotine and medication.

The samples can also be contaminated by blood. This can be problematic, as many of the values that are tested for can be found in the blood in higher concentrations.

Instructions to prevent contamination of the sample with blood:

- Ensure the participant does not brush his/her teeth for at least 45 minutes before the sample is taken;
- No visits to the dentist for a minimum of 48 hours prior to the saliva sample being collected;
- Screen the participant beforehand for oral health and oral damage/injuries;
- Saliva samples that are visibly tainted by blood must not be used for analysis.

### *Guidelines for storage of samples*

- Think carefully about how and where the sample will be stored before the start of the study and before any saliva samples are collected. Some substances cannot be kept at room temperature. The guidelines on freezing/storing samples depend on the type of research. Consult the literature and always confirm the guidelines to be followed with your supervisor;

- Generally, it is advisable to freeze saliva samples as soon as possible after collection at a temperature of  $-20^{\circ}\text{C}$  or below. If this is not possible, keep the samples cooled ( $4^{\circ}\text{C}$ ) and place them in a freezer as soon as possible (ideally within 2 hours);
- Make sure the samples do not defrost and subsequently refreeze;
- If you keep samples in the  $-80^{\circ}\text{C}$  freezer, do not touch the freezer without wearing protecting gloves, not even the doors or the drawers.

### *Saliva collection methods*

There are different methods for collecting saliva. These methods can broadly be divided into passive drool, and absorbent material.

Below a number of examples are given of collection methods with different types of equipment. Please note: there are different collection supplies on the market. The method to be used depends on the equipment used; always check the instructions provided with the equipment. The method also depends on the type of research to be done and the substances that are to be measured.

Put all the supplies out on a tray. Ensure that the collection tube can be partially or completely upright after collection. Wear gloves when collecting the sample and allow the participant to wipe his/her mouth with a cloth afterwards if they want to, drink some water, and possibly wash their hands.

### *Passive drool technique*

- Take the top off the saliva tube;
- Give the participant the tube and ask them to spit into the tube.
- Afterwards, replace the cap on the tube.

### *Absorbent material*

- Swab stick: use with babies
  - Take the swab out of the wrapper.
  - Put the stick in the baby's mouth for around 1 minute. It is important to keep monitoring the baby's mood. If the baby becomes fractious, you should stop;
  - Put the stick in the tube.
- Salivette cotton swab: use on adults
  - Remove the cap from the tube and take the sponge out of the tube;
  - Get the participant to keep the sponge in their mouth for at least 1 minute;
  - Ensure that the sponge is put in the innermost tube afterwards;
  - Put the cap back on the tube afterwards.