Appendix 9.b
Standard Operating Procedure (SOP) for Samples Preparation and Processing: Buccal swab and oral rinse

Version 3.3 – June 2022

1 Introduction

The purpose of this SOP is to describe the instructions for the collection, processing and storage of buccal swab and oral rinse samples for the HEADSpAcE study.

2 Objective

The purpose of this SOP is to define the procedure and establish the basic quality guidelines with respect to the collection of buccal swab and oral rinse samples to be processed and stored at the participant institutions until DNA extraction for further genetic/epigenetic analysis.

3 Preparation

- If possible, avoid taking a buccal swab for at least 30 minutes after eating or drinking. Please make a note of this on the REDCap form , if this is the case.
- If the patient is suffering from mucositis, has bleeding from the gums, mouth ulcers, or is on chemotherapy, postpone the collection until they are resolved.
- **IMPORTANT NOTE:** COVID-19 is transmitted through aerosols, therefore, to prevent transmission to clinical and lab staff this process must not be undertaken unless the donor is currently COVID-19 negative or has been shielding or in isolation for at least 10 days prior to testing. If there is a possibility the donor is carrying COVID-19, **DO NOT** take the samples.

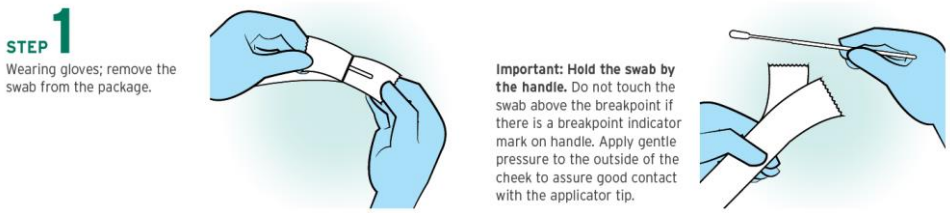
4 Required equipment/material

- Foam swabs (Cat. No. 25-1506 1PF BT, Puritan)
- Centrifuge
- Fridge and cold packs for transport (4°C)
- Pipettes and tips
- Oral rinse (per subject)
- 50 mL Conical Centrifuge Tubes
- 1.5 mL collection tubes with cap
- 0.9% sterile saline solution
- 1.8 mL cryotubes (color coded with **orange and purple tops**)
- 1X sterile Phosphate Buffered Saline (1X PBS) (diluted from 10X PBS, Cat. No. 1002416941, Sigma-Aldrich)
- Protease inhibitor cocktail (Cat. No. P8340, Sigma-Aldrich)

5 Buccal swab collection

5.1 Collection protocol

- Ask the subject to thoroughly rinse their mouth twice with water.
- Break the seal on the buccal swab casing and remove the swab stick.



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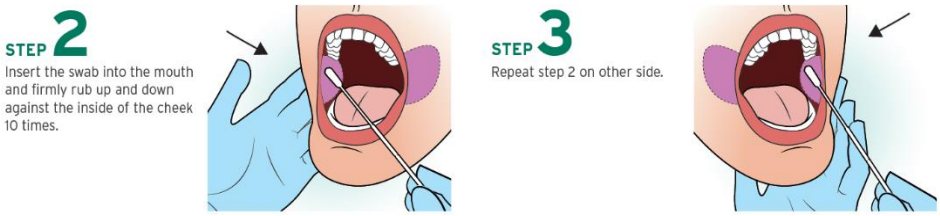
- Collect buccal cells by rolling the swab firmly and slowly on the inside of the cheek, approximately 10 times on each side, whilst moving the brush over the entire cheek.



- Place swab into the casing.

5.2 Processing protocol

Buccal swabs should be processed under the following protocol:

1. After a sample is taken, **air-dry the swab for 10 minutes** prior to putting it back in the tube.
2. The small end of the tube in which the swab handle sits can be used as a stand by bending back the plastic. Bend the swab slightly upwards so as not to touch the bench.
3. Once dry, place the swab back into the tube.
4. Place the tube into the sample collection bag and transport it in the designated collection point. If the weather is very hot (e.g. >35°C), samples should be transported with cool packs.

Label each tube with appropriate barcoded label. Labels will be provided by IARC, and include readable subject's full identification number, the type of sample, and a barcode.

5.3 Long-term sample storage

Buccal swabs are stable for a week at room temperature, for 1 month at 4°C, and for a year at -20°C. **Record the number and the type of stored sample** in the database provided by IARC (see logsheet).

6 Oral rinse collection

6.1 Collection protocol

- Add 10 mL of 0.9% sterile saline solution, that will be used for oral rinse, into 50 mL conical centrifuge tubes.
- Specimen collection is accomplished with a 60 seconds oral rinse and gargle with the 10 mL of 0.9% sterile saline solution and then returned into the 50 mL tube.

Note: Patients should be reminded not to expectorate mucus or phlegm while collecting the oral rinse sample.

- Sample should be refrigerated at 4°C until processing.

6.2 Short-term storage and transport

Oral rinse tubes must be stored and transported at 4°C until processing, for no longer than 3 hours after collection. **Ideally, oral rinse should be processed within 2 hours.**

6.3 Yield

The protocol below describes the processing of oral rinse for storage of supernatant and cell pellet.

From 10 mL of oral rinse in saline solution, the following should be obtained:

- 1 tube of cells pellet (in **orange top** 1.8 mL cryotubes)
- 2 tubes of supernatant (in **purple top** 1.8 mL cryotubes)

6.4 Processing protocol

Oral rinse will be processed and frozen under the following protocol:

1. Centrifuge the 50 mL conical centrifuge tubes containing the biological sample for **10 minutes at 2500 x rpm and 4°C.**
2. Collect 4 mL of **supernatant** and transfer it into the two 1.8 mL cryotubes with **purple top**. The remaining supernatant will be discarded.
3. Add 2 µL of protease inhibitor cocktail (Cat. No. P8340, Sigma-Aldrich) to each cryotube containing the supernatant and store at -80°C.
4. Resuspend the cell pellet with 200 µL of 1X sterile PBS and transfer to a fresh 1.5 mL collection tube.
5. Centrifuge for **1 minute at 13000 x rpm and 4°C**.
6. Discard the supernatant and resuspend the **cell pellet** into 50 µL of 1% sterile PBS.
7. Transfer the content into one cryotube with **orange top** and store at -80°C.

Label each tube with appropriate barcoded label. Labels will be provided by IARC, and include readable subject's full identification number, the type of sample, and a barcode.

6.5 Long-term sample storage

All samples should be stored at -80°C in the white **carton boxes** provided with the tubes. **Record the number and the type of stored sample** in the database provided by IARC (see logsheet). Enter storage location in your local tracking system.