

SUPPLEMENTARY FILE 2: Study operations manual: collection and storage of saliva



Chapter 7. Collection, storage and transport of human tissue

It should be recorded which samples were taken, where they were stored and under what conditions.

Method, documentation and responsibilities

Collection of saliva:

Each patient will be instructed to refrain from drinking, eating, chewing, brushing teeth and smoking for 90 minutes before each visit. To minimize diurnal variation, all appointments for each patient will be at the same time of day and in the same room (temperature $21 \pm 2^\circ\text{C}$, humidity 50-60%).

- Collection of unstimulated whole saliva: Participants will spit out saliva that has spontaneously accumulated in the mouth without stimulation into a pre-weighed 25 ml plastic collection vessel (with blue screw cap) every 30 seconds for exactly five minutes. In this way, unstimulated saliva production can be determined. The collection tube is placed in a container with ice. After collection, the tube is weighed again to determine the amount of saliva (ml/min, 1 g = 1 ml).
- Stimulated whole saliva: Subsequently, participants will be asked to chew on a square inch of parafilm and spit out all saliva collected in the mouth into a pre-weighed collection vessel for exactly 5 minutes while the parafilm remains in the mouth. The chewing rate is controlled at 60 strokes/min by a metronome. This reflects a normal chewing rate. In this way, stimulated saliva can be collected and the saliva secretion rate can be determined. The collection tube is placed in a container with ice.
- Stimulated parotid saliva: Patients will expectorate stimulated parotid saliva into Lasley Cups placed on the orifices of the parotid ducts (both left and right). The cups remain attached by an underpressure created by applying suction on the circular outer chamber with the syringe attached to the cup. This underpressure is maintained by placing an artery clamp on the tube. Taped tubes and the artery clamp are attached to clothing of the participant. Then, 2% w/v citric acid is applied to the borders of the tongue, saliva is collected for 5 min and the pre-weighed collection tube is placed in a container filled with ice.

Immediately after collection, each collection tube is closed and placed in the refrigerator with an anonymous patient code (1 to 10), date, measurement time (T0, T1, etc) and type of saliva. After weighing the collection vessels, saliva will be transferred to Eppendorf tubes (Eppendorf AG, Hamburg, Germany) and centrifuged for 5 min at 10,000 g at 4°C as described by Silletti et al. (2007). After centrifuging, the saliva supernatant will be decanted and stored at -80°C in plastic containers (Cryogenic Vials Nalgene tubes).

The -80°C freezer is located in the laboratory of the Department of Oral and Maxillofacial Surgery and Oral Pathology in the O2 building (floor 8, room 65). These tasks have been delegated to trained and calibrated master students (see training and delegation log).

DIT DOCUMENT EN INHOUD ZIJN HET EXCLUSIEVE EIGENDOM VAN VUMC. DIT DOCUMENT MAG NIET WORDEN GEREPRODUCEERD IN WELKE VORM DAN OOK ZONDER TOESTEMMING VUMC.

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