

# Exposome project for health and occupational research

Protocols for collection, pre-processing and storage of biological samples (WP3, Task 3.1)

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WP (number and title)	WP3- Internal exposure and effect assessment using biomonitoring, omics and minimally invasive biomarker development
Deliverable Number	D3.1
Deliverable Title	Protocol for the collection, pre-processing and storage of biological samples in WP6, WP7
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Lead beneficiary	6 - KUL
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## Collection, pre-processing and storage of saliva

#### Introduction

Steroid hormones (including glucocorticoids, androgens and progestogens) play important roles in key human functions. Their concentrations might be altered by night shift. Melatonin is a hormone mainly released by the pineal gland at nighttime. The main role of melatonin is the regulation of the sleepwake cycle, although it plays additional roles such as antioxidant, body weight regulation or reproduction. Saliva is a well-established matrix for melatonin determination. Quantification of salivary melatonin is especially useful for capturing sharp changes in the pineal activity. Saliva will be used as a non-invasive matrix to measure steroid profile concentrations and melatonin concentrations in shift workers from WP7. Concentration will be measured at multiple timepoints.

#### Materials needed

Collection:	<ul> <li>-Cotton Salivettes<sup>®</sup> Cortisol (blue screw cap) (Starstedt, Nümbrecht, Germany)</li> <li>-Tap water</li> <li>-Labels: participant ID – sample ID (including date and time of collection)</li> <li>-Datasheet for information on time of sample collection and covariates (duration of sleep, time of awakening, alcohol/coffee consumption, smoking, medication use and working/non-working day, and sex)</li> </ul>
Pre-processing:	-Centrifuge -1.5 mL polypropylene tubes (Eppendorf, Hamburg, Germany) -Labels: country ID – participant ID – sample ID
Storage:	-Storage box for 1.5 mL polypropylene tubes -Fridge/Freezer (-80 °C)

### Collection of saliva

Participants are asked to refrain from drinking, eating, brushing their teeth and smoking before collection.

The tubes are labeled.

The participant removes the swab from the Salivette<sup>®</sup> and places it in the mouth.

After chewing the swab for 1 minute, the swab is placed back in the tube and the tube is closed immediately with the blue screw cap. This process is repeated for a second swab.

After collection of the first sample, the participants should rinse their mouth with tap water 15 minutes before each next sample is collected.

The tube is stored in the fridge (2-8 °C) until pre-processing.

More details for saliva collection timepoints will be available after discussion and agreement with WP7 leaders.

Pre-processing and storage of saliva at site of collection

Samples are centrifuged (2000 x g, 5 minutes, at 20 °C).

After centrifugation, saliva samples are transferred to 1.5 mL polypropylene tubes and labeled. The samples are stored at -20  $^{\circ}$ C.

