Saliva Sampling Participant Guide

Provided by: Anna Biller

Date: 10/03/2024

Version: 1.2

General information

- Saliva samples are collected in regular sampling intervals (every 30min), starting 5 hours before your normal bedtime = before you usually go to bed on Tuesday evening
- The last of the 11 evening samples is taken when you go to bed on Tuesday
- After waking up, you take another 4 samples on Wednesday morning straight after waking!
- The saliva samples are obtained by using Salivettes (cotton swabs) which remain in your mouth and cheeks for 1-3 min (max. 5 min) until the swab is well soaked after which you place it back in the tubes
- They are then temporarily stored in your fridge (4-7°C)
- On Thursday morning, you will bring back your samples to the laboratory in a cooling bag where they are centrifuged (= spinned very quickly) and frozen
- We then analyse your sample to determine circadian phase and amplitude, that is your biological rhythm.

Provided material issued on Monday morning

• Labelled Salivette tubes

- **11 white ones** for the Tuesday evening saliva collection
- 4 blue ones for the Wednesday morning saliva collection
- Grey cooling bag
- 2 white cooling pads
- please keep the samples in the grey bag and close it so no UV light reaches the tubes



15 Salivette Tubes (11 white Salivettes = evening samples and 4 blue ones = morning samples)





Grey Cooling bags and white cooling pads

Sampling intervals

- 11 samples are taken every 30 minutes on <u>Tuesday evening using the WHITE</u> SALIVETTES
- On Tuesday morning, plan your day and think about your bedtime for the evening
- Start <u>5h before your normal bedtime</u> which results in 11 times points

Example: if your usual bedtime is 23:00 on Tuesday, you will collect saliva at these 11 time points for the evening $\rightarrow e = evening$)

- 18:00 (e1)
- 18:30 (e2)
- 19:00 (e3)
- 19:30 (e4)
- 20:00 (e5)
- 20:30 (e6)
- 21:00 (e7)
- 21:30 (e8)
- 22:00 (e9)
- 22:30 (e10)
- 23:00 (e11)
- The next morning (Wednesday), start your saliva collection immediately after getting up using the <u>BLUE SALIVETTES</u>! It is very important to do this quickly after waking so we can capture your cortisol awakening response which we will miss if you start collection too late

Example: if you get up at 7:00, start data collection at 7:00 and repeat every 30 minutes for 4 times resulting in the following time points with **m = morning**:

• 07:00 (m1)

- 07:30 (m2)
- 08:00 (m3)
- 08:30 (m4)

How to collect saliva samples?

Preparation – Start 5h before your usual bedtime

- stop eating and drinking at least 10 min before the (next) sample
- rinse your mouth with water (no toothpaste or chemical mouth rinse solutions!)
- sit comfortably for 3-5 min before taking each sample
- prepare all the Salivette tubes (white for evening, blue for morning)
- have a water-resistent pen ready to label the tubes

Sample collection

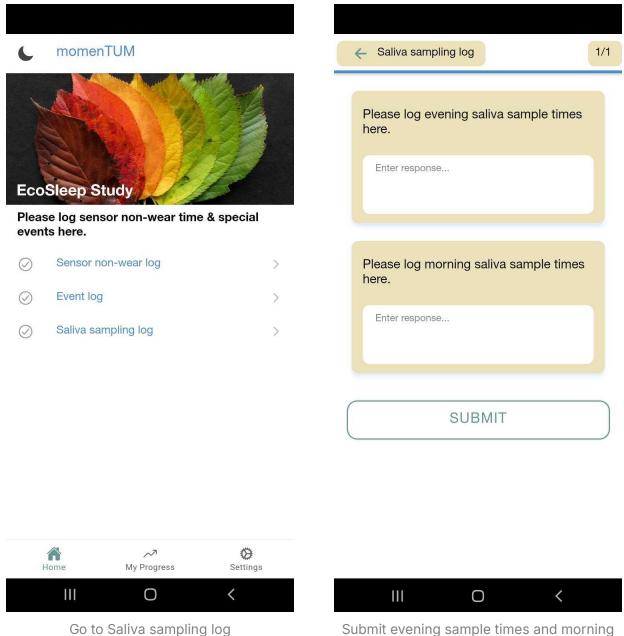
- !!Make sure you choose the correct Salivette:!!
 - white ones for evening samples: e1, e2, e3..., e11 are used for the time points when you collect the samples (e = evening, 1 = sample number 1 etc.)
 - blue ones for morning sample: m1, m2, m3, m4 (m = morning, 1 = sample number 1)
- open the lid of the correct Salivette tube
- glide the Salivette = cotton piece in your mouth without touching it
- move the Salivette = cotton piece gently inside your mouth on both sides of the cheeks until the cotton piece is well soaked for 1-3 min, max. 5 min (no chewing!)
- Salivette well soaked?

- then after 1-3 (max. 5 min) minutes glide it back into the tube, again without touching it
- close the lid



Procedure for saliva collection.

- double check for the correct time and sample number → for this write down the sample time on each tube immediately after sampling und put the time down in momenTUM under the "saliva sampling log"
- alternatively, you can note the times down somewhere else and submit all times together for the evening and morning in a batch in momenTUM. It's just important that you keep track of your sampling times and submit them in momenTUM + write them down on the tubes.
- store the samples in the fridge at home (not freezer!)



sample times separately

This procedure is repeated every 30 minutes for 5 hours until you go to bed

• in between, you may go about your usual activities

- In case you produce only little saliva, drink some water and keep it a little bit in your mouth to rinse it slightly but do this ahead of time to your next sample
- please do NOT drink or eat anything at least 10 minutes before the next saliva sample
- if you eat something in between, **rinse your mouth immediately** with water (please do not use toothpaste, toothbrush or mouthwash)

Do NOT

- no food or drinks at least 10 min before each sample
- no lipstick during the sampling period and no chewing gums and bonbons/drops
- no bananas or chocolate immediately before and during the sampling period
- no coffee consumption
- no saliva collection while sleeping, snoozing, lying down, doing sports etc. → body position affects the result
- there are several known medications which interfere with this measurement: beta-blockers, selective serotonin reuptake inhibitors (SSRI), monoamine oxidase inhibitors (MAOs), and nonsteroidal anti-inflammatory drugs (NSRIs). Please tell the study team in case you take any of these medications or start taking time during the course of the study.

After sample collection

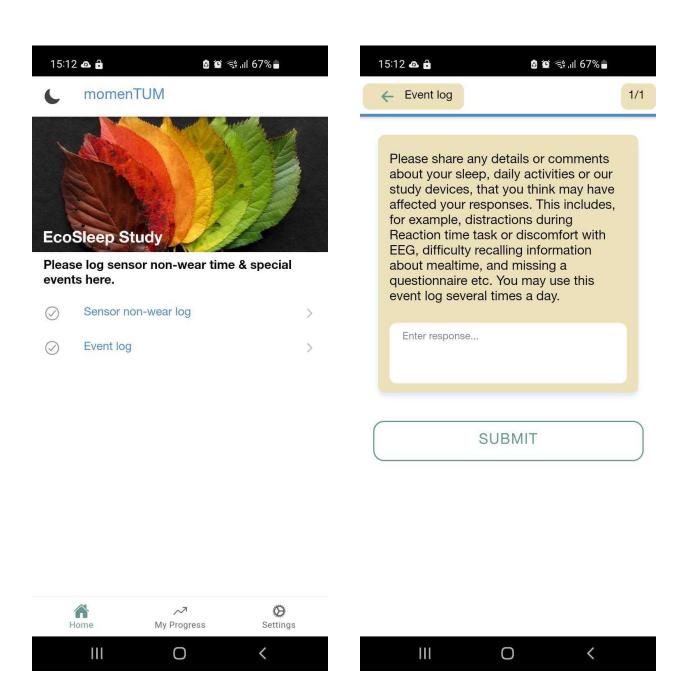
- Make sure the correct sampling time is on the correct tube
- Log the sampling time(s) in the momenTUM App
- Store the samples in your fridge
- Make sure that the white cooling pad is in your freezer
- On Thursday morning before coming to the lab, place the white cooling pad in the grey cooling bag, then insert the saliva tubes, then place another cooling pad on top. Close the cooling bag.

• Bring the cooling bag for the measurement sessions in the lab at the prevention centre on Thursday morning.

Important things to consider

It Make sure any deviations are noted down in the momenTUM App under "Event log".

Inform study team if one or more sample(s) have not been collected or returned !!



Where to download the momenTUM App for logging special events and logging saliva sample times?

• Apple Appstore:

https://apps.apple.com/de/app/tum-momentum/id6444047291

• Google Playstore

https://play.google.com/store/apps/details?id=de.tum.sg.momentum&pli=1