

Prepared by: Marijke Gordijn	SOP – TAKING SALIVA SAMPLES FOR MEASUREMENT OF MELATONIN
Revised by:	March 5th, 2007

PURPOSE:

The following procedure contains the minimum requirements necessary to take saliva samples for measurement of melatonin levels.

EQUIPMENT:

Labelled collection tubes (e.g. Salivette® **51.1534** with cotton swab, no citric acid, Sarstedt AG & Co).

RESPONSIBILITY:

The collection of saliva is a simple non-invasive method. Subjects can do this by themselves. If correctly instructed, this could even be done at home. However some precautions are needed (see procedure).

PROCEDURE:

- a) The period starting 1h before sampling until the last sample is taken is further called the “sampling period”.
- b) Environmental lighting needs to be maintained at < 8 lux throughout the sampling period. In the home environment subjects should be instructed to keep light levels as low as possible, not to sit near a lamp, not closer than 3 m in front of the T.V. and to close the curtains. Light levels and the intensity/contrast of computer screens should be decreased as much as possible, so that reading is just possible.
- c) Subjects are required to minimise their movement during the sampling period.
- d) During the 10 min before each sample is taken, subjects are required to remain in their designated, controlled posture position. For example, subjects may be in a sitting position during waking hours and in a recumbent position when they are sleeping.
- e) Subjects should refrain from eating during the sampling period as much as possible and at least finish their meal or snack 30 min prior to the collection time. Eating bananas and chocolate on the collection day should be avoided.

- f) During the sampling period, subjects are allowed to drink beverages without artificial colorants, and without caffeine, only when finishing it 30 min prior to the collection time. Drinking coffee, cola and alcohol is prohibited throughout the sampling period. Drinking water is allowed until 10 min before collection of each sample.
- g) Subjects should rinse their mouth with water 10 min prior to collection of each sample. No toothbrushing with toothpaste is allowed during the sampling period.
- h) Subjects should not stimulate saliva flow e.g. by chewing gum.
- i) The use of Aspirin or aspirin and ibuprofen containing drugs (Algifor, Brufen, Dismenol, Dolocyl, Ecoprofen) is prohibited on the collection day.
- j) The number and timing of collecting the samples depends on the protocol of the study. Some general rules: (1) To measure melatonin onset a sampling interval of maximal 1 h is required starting 5 hours prior to habitual sleep onset until 1 hour after habitual sleep onset. (2) When a 24-h curve is needed, sampling during wake time should have an interval of 1h, during sleep a 2-h interval is allowed.
- k) In the lab: centrifuge saliva samples and store them at -20°C. At home: keep the samples in the refrigerator (2-8°C) for a maximum of 3 days and send them in a special box to the lab. If they are not send within 3 days they should be placed in a freezer. Samples should not be send just before a weekend. Bühlmann tested the stability of melatonin in saliva using their RIA (RK-DSM) as well as their ELISA (EK-DSM) using samples either kept at -20°C or room temperature for 7 days, without observing an effect on the melatonin concentration.