

# Collecting and Handling Saliva Samples for Analysis of Novel Salivary Biomarkers

Ongoing research is identifying many molecules in saliva that are of interest as biomarkers for various research and diagnostic applications. Limited information exists, however, on the best ways to collect and handle saliva for the analysis of many of these molecules. In contrast to better-known salivary analytes such as the steroid hormones, which are not affected by the location of collection in the mouth or by saliva flow rate, there may be little published data on such matters for these novel analytes. Researchers are therefore advised that factors that could potentially affect levels of these analytes in saliva must be controlled and documented, in order to be able to look for possible associations within study results. The following are examples of factors that require particular attention:

- **Saliva flow rate should be measured.** Data on saliva flow (mL/min) should be collected in order to be able to express assay results as a function of time ( $\mu\text{g}/\text{min}$ ), should it prove to be helpful.
- **Location in the mouth where samples are collected should be standardized.** Consistency of collection location is significant for some salivary proteins (e.g., alpha-amylase, SIgA), and it is likely to be significant for other novel analytes. Oral biologists advise the collection of unstimulated whole (mixed) saliva for general studies due to differences in the composition of saliva from the various salivary glands or to changes in composition that may occur with stimulation.
- **Sample collection should be carried out at standardized times.** Some established salivary analytes exhibit diurnal rhythms, and novel analytes may have similar rhythms. Consistency in the time of collection is therefore important.

- **Use of absorptive collection devices (swabs) should be avoided until their use is thoroughly assessed for the novel analyte(s).** Swabs sometimes affect assay results for certain analytes, and they may complicate the estimation of saliva flow rates. Under certain conditions swabs may also possibly collect glandular saliva rather than whole saliva.
- **Storage conditions should be tightly controlled.** Many analytes in saliva are sensitive to degradation and require refrigeration and/or freezing. Effects of repeated freeze-thaw cycles on analyte stability should be investigated.
- **The following conditions should be assessed and documented:**
  - Presence of oral injuries, dental work, and oral diseases in research participants
  - Presence of blood contamination in samples (by visual inspection scale or transferrin assay)
  - Consumption of alcohol, caffeine, nicotine, and prescription and over-the-counter medications by participants
  - Physical activity level of participants

The importance of maintaining consistency in saliva collection procedures cannot be understated. By following a few basic suggestions given here, it should be possible to reduce the amount of variation in data that is due to procedural differences and to identify relationships that may exist between the data and various saliva collection issues. As a result, researchers will be able to work with higher quality data, which should aid them in their exploration of the significance and utility of emerging biomarkers in saliva.

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